CATECHOLAMINES, MOOD,

AND CARDIOVASCULAR CONTROL

Catecholaminen, Stemming,

en Cardiovasculaire Regelmechanismen

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Contents

CHAPTER 1

GENERAL INTRODUCTION

Alterations in mental and physical human behavioral states are associated with modulation of the activity of the sympatho-adrenomedullary system. Activation of the sympatho-adrenomedullary system, often resulting in increased plasma concentrations of noradrenaline and adrenaline, occurs in response to stressful or demanding situations, physical activity, or postural changes (i.e., Ward et al., 1983; Robertson et al., 1979), and is also believed to be of importance in the pathogenesis of affective disorders, anxiety disorders, and hypertension (Cameron & Nesse, 1988; Davis et al., 1988; Floras, 1992). Yet, interpretations regarding relationships between concentrations of plasma or urinary catecholamines, cardiovascular activity, and mood states, are often obscured by the complex interplay between central, autonomic (sympathetic and parasympathetic), and metabolic mechanisms.

The research presented in this thesis addresses the above mentioned issue on the basis of four psychophysiological experiments. These experiments were aimed at separately manipulating concentrations of circulating adrenaline and noradrenaline by means of infusions of catecholamines, pharmacological interventions, or psychological (mental stress) challenges in healthy subjects, during studies of the effects on cardiovascular activity and subjective mood. In particular, the dynamics of the cardiovascular system were evaluated in relation to circulating noradrenaline and adrenaline by employing the method of spectral analysis of haemodynamic variables. In order to increase our understanding of the metabolism and renal excretion of catecholamines, special attention was paid to urinary catecholamine metabolites, in relation to altered plasma catecholamine concentrations.

This chapter starts with a synopsis of the modes of action of catecholamines in the central and autonomic nervous system. The technique of spectral analysis and its potential use for the assessment of sympathetic and parasympathetic mechanisms in cardiovascular control is presented subsequently. Some of the approaches to study relationships between catecholamines, cardiovascular activity, and mood states are briefly mentioned, after which the aims of our studies are described. The term catecholamines as used in this thesis strictly refers to adrenaline (epinephrine) and noradrenaline (norepinephrine).

Catecholamines: synopsis of the modes of action

Catecholamines in the central and the autonomic nervous system

The cell bodies of the noradrenergic neurons in the central nervous system are localized almost exclusively within the brainstem and hypothalamus. Approximately 70 % of all brain noradrenergic cell bodies are localized in the locus coeruleus in the dorsolateral pons. Smaller amounts of noradrenergic neurons have been identified in the lateral tegmental complex (comprising among others

the vasomotor center, the nucleus tractus solitarius and the dorsal vagal nucleus). Adrenergic neurons are present in the brainstem; however, not much is known about the role of adrenaline in the brain. Central adrenaline is considered to be of minor importance in comparison with noradrenaline (Nieuwenhuys, 1985).

Noradrenaline and adrenaline exert their effects via α_1/α_2- and $ß_1/ß_2$-adrenoceptors at presynaptic and postsynaptic sites. $ß_1$-Adrenoceptors are distributed throughout the brain, with the exception of the cerebellum, where $ß_2$-adrenoceptors are predominant. However, the localization of ß-adrenoceptors does not strictly correlate with the distribution of presynaptic noradrenergic terminals (Janowsky & Sulser, 1987). In relation to the mechanisms of action of noradrenaline in the central nervous system, the primary focus has been on the α-adrenoceptors. α_2-Adrenoceptors are located in the noradrenergic neurons of the locus coeruleus and other areas (i.e., amygdala, lateral tegmentum). The presynaptic α_2-adrenoceptors regulate the release of noradrenaline through a negative feedback mechanism (with stimulation resulting in an inhibition of noradrenaline release) (Langer, 1981). Regulation of the locus coeruleus has been shown to occur predominantly by means of this form of autoinhibition (Andrade & Aghajanian, 1984). Postsynaptic α_2-adrenoceptors are located on neurons at the level of the lateral tegmentum and thoracic spinal cord and at the level of the hypothalamus. Postsynaptic α_1-adrenoceptors have also been identified at the level of the lateral tegmentum (van Zwieten, 1988).

Central noradrenergic fibers ascend into two bundles. The ventral bundle collects fibers from many nuclei of noradrenergic origin and distributes mainly to the hypothalamus. The dorsal bundle collects from only one nucleus, the locus coeruleus, and distributes to the neocortex, the cerebellum and also to the limbic system. Locus coeruleus neurons receive input from many different neurotransmitter systems and the firing rate and sensitivity to other incoming stimuli can be regulated by these other neurotransmitter systems. These modulating systems include inhibitory input from the 5-HT, opioid, gamma-aminobutyric acid (GABA), dopamine, and glycine systems and excitatory input from the corticotrophin releasing hormone (CRH), purinergic, glutamate, substance P, and muscarinic cholinergic systems (Redmond, 1987). These characteristics make it understandable why the noradrenergic system has been assigned a role in the integration of the adaptive central nervous system responses to environmental, behavioral, or physiological challenges.

At the peripheral level, the catecholamines are primarily observed in the sympathetic part of the autonomic nervous system. Cardiovascular, respiratory, gastrointestinal, renal and endocrine, and other systems are regulated by either the sympathetic or the parasympathetic part of the autonomic nervous system,

but mostly by both. The main function of the autonomic nervous system is its involvement in the regulation of the 'milieu interieur' (Claude Bernard) of the body in order to maintain optimal conditions despite disruptions due to environmental interactions. Sympathetic and parasympathetic outflow to the organs may be under coupled reciprocal control, with increasing activity in the one being associated with decreasing activity in the other. However, exceptions to this rule are increasingly observed, indicating that these two parts of the autonomic nervous system may function either coupled or uncoupled (Berntson et al., 1991). Understanding the role of the catecholamines in the cardiovascular system therefore requires understanding of both sympathetic and parasympathetic branches of the autonomic nervous system.

Sympathetic nervous system

Descending pathways from the hypothalamus, ventrolateral medullary reticular formation, nucleus tractus solitarius, serotonergic raphe nuclei, and noradrenergic nuclei project to the efferent preganglionic fibres whose cell bodies lie in the intermediolateral column of the spinal cord at the level of the thoracic and upper lumbar roots. These nerves emerge from the spinal cord through the ventral roots and synapse in the bilateral chain of sympathetic ganglia with postganglionic sympathetic neurons which innervate vascular smooth muscle, heart, kidney, gut, sweat glands, and many other organs. The preganglionic neurons use acetylcholine as transmitter, while the peripheral postganglionic neurons release noradrenaline (with the exception of the sweat glands, which are innervated by cholinergic neurons). Noradrenaline is removed from the synaptic cleft by (presynaptic) re-uptake, by leakage into the blood stream and by enzymatic degradation. Noradrenaline which spills over from the synaptic cleft into the blood stream acts subsequently as a circulating hormone. The adrenal medulla is innervated directly by the preganglionic sympathetic fibers, using acetylcholine as transmitter. The secretory cells of the adrenal medulla may be thought of as postganglionic cells: they are adrenergic, secreting adrenaline and noradrenaline directly into the circulation. In humans, the major part of the catecholamines secreted from the adrenal medulla consists of adrenaline (80%).

The sympatho-adrenomedullary system realizes its influences by an effect on α- and ß-adrenoceptors. In relation to cardiovascular activity, noradrenaline increases stroke volume and cardiac contractility by means of an effect on $ß_1$-adrenoceptors, while it decreases venous capacitance and arterial blood flow to the muscle, splanchnic bed, kidney and skin, and increases systolic and diastolic blood pressure by means of an effect on α_1- and α_2-adrenoceptors. Adrenaline increases heart rate and force of contraction by affecting $ß_1$-and $ß_2$-adrenoceptors; it induces a constriction of most systemic resistance vessels as a result of

affecting α_1- and α_2-adrenoceptors. Adrenaline induces a dilatation of resistance vessels of skeletal muscle and splanchnic area due to an effect on β_2-adrenoceptors, and a constriction of splanchnic capacitance bed by affecting both α- and ß-adrenoceptors (Lees, 1981; van Zwieten, 1988).

Parasympathetic nervous system

Pathways from the hypothalamus and certain suprabulbar centers project to the cell bodies that give rise to preganglionic axons in the parasympathetic nervous system. They are located in two regions: the nuclei of some of the cranial nerves (especially the vagus nerves) and the intermediate horn of the gray matter in the sacral region of the spinal cord. The ganglia lie in the vicinity of the target organs, and the postganglionic fibers are short. Postganglionic parasympathetic fibres from the vagus innervate salivary gland, cardiac, pulmonary, and gastrointestinal organs, while pelvic organs are innervated by the sacral parasympathetic outflow. In humans, both preganglionic and postganglionic neurons in the parasympathetic nervous system are cholinergic.

Functions of the central-noradrenergic/peripheral autonomic nervous system

Animal research has shown that locus coeruleus activity increases during waking (in comparison with slow-wave sleep or anesthesia). Locus coeruleus activity shows sustained responses to repeated presentations of 'noxious' stimuli, while there is a rapid habituation to novel non-noxious stimuli. Furthermore, there exists a consistent association of spontaneous activity of the locus coeruleus with the level of vigilance or arousal (Redmond, 1987).

In general, the central-noradrenergic/autonomic nervous system forms a principal component of the general adaptational responses of an organism to a wide variety of 'stressors' (the other component being the hypothalamic-pituitary-adrenocortical system, which is, however, not the object of research in this thesis). The term 'stressor' is here defined as any perturbation that disrupts homeostasis. The subsequent adaptive responses to stress include the psychological, behavioral and physiological processes that the organism consistently musters in its attempt to reestablish homeostasis in the face of a wide range of stressors (Johnson et al., 1992). In response to external stressors, particularly those which have a threatening character, an emotional, behavioral, and autonomic response pattern occurs which serves to mobilize the organism for flight or fight, i.e., emergency or alarm reactions to cope with the environmental stimuli (fight-flight defence reaction). Apart from the increased vigilance reaction (accompanied by fear or anxiety), the response pattern is characterized by an activation of sympathetic fibers to the heart, splanchnic region and kidneys, central suppression of vagal restraint of the heart, an ensuing blood pressure elevation and increase of blood flow to the skeletal

muscles, mainly caused by the increased cardiac output. The sympathetic activation to the kidneys is associated with increased renin release, and the renin-angiotensin-aldosterone axis as well as glucocorticoids are subsequently mobilized. The work of Cannon (1929) and Hess & Brügger (1943) provided the fundaments for the study of this response pattern. The central regulation of this response involves the limbic system, the hypothalamus, and the brainstem (Hilton, 1982); the locus coeruleus in particular is involved in its peripheral, autonomic regulation (Redmond & Huang, 1979; Charney & Redmond, 1983; Svensson, 1987).

Animals subjected to inescapable, uncontrollable electric shocks show subsequent deficits in learning to terminate the noxious stimulus even when it is escapable. This phenomenon ('learned helplessness') has been used as a model of depression. The learning deficit was considered analogous to some of the mood/cognitive disturbances observed in patients with depression, who frequently report feelings of helplessness or powerlessness to cope with stress. One mechanism underlying the behavioral deficits in animals exposed to inescapable stress, is a depletion of noradrenaline and/or a decrease in tyrosine hydroxylase activity (Weiss et al., 1981).

Interactions with other central nervous system elements ensure that the central-noradrenergic/autonomic nervous system plays a role in information processing, action initiation and goal-directed behavior, as well as the setting of the emotional tone (Mason, 1981). The mesocortical dopamine system which innervates the prefrontal cortex, a brain region believed to be involved in anticipatory phenomena and cognitive function, can function in interaction with activity from the noradrenergic neurons of the locus coeruleus. In addition, the mesolimbic dopamine system, a region closely linked to the nucleus accumbens, which has been implicated in motivational-reinforcement-reward phenomena, can also influence, or be stimulated by, the noradrenergic neurons from the locus coeruleus (i.e., Roth et al., 1988). Gray (1982) proposed a behavioral inhibition system (involving the limbic system, i.e., the amygdala and septohippocampal complex, the Papez circuit and the prefrontal cortex), which functions as a comparator, checking predicted against actual events and then interrupting behavior when the unexpected is encountered. The system receives, among others, its input from ascending noradrenergic neurons which originate in the brainstem locus coeruleus.

Thus, the central-noradrenergic/peripheral autonomic nervous system has been shown to be involved in the mediation and control of exploratory responses to novel environmental stimuli, action initiation, learning and memory, sleep, vigilance or arousal, pain, anxiety or fear, and blood pressure maintenance.

Indices of catecholamines in plasma and urine

In human psychophysiological research, the sources available to evaluate catecholaminergic activity in the brain and the sympathetic nervous system are limited. Concentrations of circulating noradrenaline and adrenaline are usually measured in venous plasma as indices of activation of the sympathetic nervous system and the adrenal medulla, respectively. Plasma concentrations of catecholamines have been considered to reflect sympathetic output more closely than urinary concentrations: a more detailed pattern of catecholamine responsivity over time may be obtained. However, these responses may be related to the time of sampling only, since both plasma noradrenaline and adrenaline have a very short half-life (1-2 min). Furthermore, interpretation of concentrations of venous noradrenaline is hampered by the fact that these concentrations are influenced by local sympathetic nervous system activity at the site of sample collection. The concentrations are also highly dependent on the rate at which noradrenaline is removed from the circulation and not only its release into plasma. When sampling plasma from the antecubital vein in the forearm, muscle activity during performance of a task can have a major influence on noradrenaline concentrations. Venous plasma noradrenaline concentrations may therefore not always accurately reflect global sympathetic activation (Floras et al., 1986; Esler et al., 1988). Venous plasma adrenaline concentrations are less influenced by these factors; arterial plasma adrenaline primarily reflects activity of the adrenal medulla and is, although extracted by the tissues, not secreted in other organs in the periphery (Kopin, 1985). Arterial plasma catecholamine concentrations are clearly to be preferred above venous concentrations, but this is usually not a feasible option in human psychophysiological research. 3-Methoxy-4-hydroxyphenylglycol (MHPG), the main metabolite of central noradrenaline, can enter the circulation through the blood-brain barrier and subsequently it can be measured in plasma. However, plasma free MHPG is derived from both central and peripheral sources (Kopin et al., 1983), which makes plasma MHPG a weak indicator of central noradrenergic activity.

Changes in urinary catecholamine excretion are presumed to reflect changes in the central as well as peripheral metabolism of catecholamines. In contrast with the plasma concentrations, urinary catecholamines provide integrated measures, and responses are relatively nonspecific, i.e., they are less directly related to the experimental manipulations in time. Urinary excretion of MHPG is a weak indicator of central metabolism, because MHPG is also formed in the periphery and because a considerable proportion of MHPG of both origins is converted to vanillylmandelic acid (VMA) in the periphery. The other metabolites, normetanephrine, metanephrine, and VMA, as well as the catecholamines noradrenaline and adrenaline, can not cross the blood-brain barrier, and excretions in urine are consequently of peripheral origin. It appears

that urinary noradrenaline and its metabolite normetanephrine reflect to a certain extent extraneuronal metabolism and are sensitive to changes in sympathetic neuronal activity. VMA, the main metabolite of noradrenaline, is formed intraneuronally and probably reflects more closely the basal metabolism of noradrenaline (Kopin, 1985; Kopin et al, 1983; Maas & Landis, 1971). The manner in which changes in circulating adrenaline are reflected in urinary excretions of adrenaline and metabolites is, however, not clear.

Furthermore, factors such as age, sex, posture, salt intake, phase of menstrual cycle, obesity, food intake, and smoking are known to influence catecholamine release (i.e., Dimsdale et al., 1987; Esler et al., 1981; Frankenhaeuser et al., 1968; O'Dea et al., 1982; Schwartz et al., 1987). Control for these factors is essential in order to obtain interpretable estimates of catecholamines in plasma and urine after experimental manipulations.

Cardiovascular control mechanisms

The manner in which alterations in activity of the central-noradrenergic/peripheral autonomic nervous system contribute to the cardiovascular changes observed during mental and physical human behavioral states, may be studied by focusing on the cardiovascular control mechanisms. Homeostatic cardiovascular regulation is thought to be effected for a large part through the interplay of sympathetic and vagal activity (Malliani et al., 1991). Recently, spectral analysis of the beat-to-beat changes in heart rate and blood pressure has been advocated as a potential tool to assess these changes in sympathetic and vagal modulation (Akselrod et al., 1981,1985). This analysis may provide separate estimates of both sympathetic and parasympathetic activity within the cardiovascular system, thereby allowing a more detailed interpretation of mechanisms behind the cardiovascular changes observed during different human behavioral states.

The cardiovascular system plays a key role in maintaining homeostasis within the body, largely by adjusting the blood supply to various vascular beds in proportion to their metabolic needs. Basically, the nervous system achieves this by maintaining arterial pressure and regulating cardiac output and total peripheral resistance in the face of different behavioral demands through the interplay of reflex inputs and central drives. At present it is clear that arterial pressure is regulated by several interrelated control systems (Guyton, 1991), which may become functional at different time points after a sudden change in pressure. These systems comprise 1) rapidly acting pressure control mechanisms, which are activated within seconds or minutes: the baroreceptor feedback mechanism, the chemoreceptor mechanism, the central nervous system ischemic mechanism, 2) intermediate time-period pressure control mechanisms, which are

activated a few minutes after an acute pressure change: the renin-angiotensin vasoconstrictor mechanism, the capillary fluid shift mechanism, the stress-relaxation mechanism, and 3) long-term mechanisms for arterial pressure regulation, which are activated on a time scale of hours or days: the renal-blood volume pressure control mechanism and the renin-angiotensin-aldosterone system. Within the framework of this thesis, our focus is primarily on short-term cardiovascular control processes, with the arterial baroreceptor reflex as the major determinant under normal physiological conditions.

Baroreceptor reflex pathways

Blood-pressure homeostasis is maintained by a negative-feedback mechanism. Adjustment processes on a time scale of seconds to hours may be regulated primarily by means of the baroreceptor reflex pathways. Changes in blood pressure are detected by baroreceptors in the heart, carotid sinus, aortic arch, and other large vessels. Afferent impulses are subsequently transmitted from these structures via the carotid-sinus nerve and the glossopharyngeal and vagal nerves to the tractus solitarius in the medullary area of the brainstem. From the nucleus tractus solitarius, neuronal connections are made both with efferent pathways (via vasomotor and vagal nuclei) and also with ascending neurons, which carry information to higher structures in the brain. These various nuclei project directly to the interomediolateral nucleus of the spinal cord. Therefore, baroreflex information reaches the preganglionic sympathetic cells of the spinal cord by means of a multisynaptic pathway.

The efferent impulses function to influence the effectors of the baroreflex system (heart rate, total peripheral resistance, stroke volume, venous volume) in such a manner that appropriate adjustments in blood pressure occur. For instance, a sudden increase in blood pressure leads to an increase in discharges of baroreceptor afferents, which diminishes sympathetic discharges to the heart and blood vessels, and diminishes adrenal secretion through inhibition of adrenal nerve discharges, whereas efferent vagal (parasympathetic) discharges to the heart are augmented. On a longer time scale, the reflex decrease in sympathetic outflow to the kidneys diminishes renin release and leads to a reduced formation of angiotensin I, II, and aldosterone. These neural and neuro-humoral mechanisms in concert cause a decrease in cardiac output as the net result of reduced contractility of the myocardium, bradycardia, natriuresis, and pooling of the blood in the venous system, together with a general vasodilator response (Kumada et al., 1990).

Cardiovascular variability and spectral analysis

Homeostatic cardiovascular processes may be reflected in the pattern of variations as observed in heart rate and blood pressure. Spectral analysis can

be employed as a tool to define these cyclic perturbations in beat to beat fluctuations in heart rate (HR) and arterial blood pressure (BP). On a time scale of seconds to minutes, this method may provide estimates of sympathetic, parasympathetic, and neurohumoral activity within the cardiovascular control system (Hyndman et al., 1971; Akselrod et al., 1981,1985; Appel et al., 1989; Malliani et al., 1991). The fluctuations in HR and BP are characterized by three spectral peaks in a frequency range of 0.02 to 0.50 Hz (Hyndman et al., 1971; Sayers, 1973; Kitney & Rompelman, 1980), which are in strength of appearance dependent upon the posture of the subject (supine, standing, sitting): 1) a low-frequency peak around 0.04 Hz: for HR this peak is associated with both parasympathetic and sympathetic activity (Akselrod et al., 1985), while low-frequency BP fluctuations are linked with variations in peripheral vasomotor activity due to thermoregulatory influences (Burton, 1939; Kitney, 1975) or renin-angiotensin system activity (Akselrod et al., 1985). In this frequency range, a low coherence exists between BP and HR time series (de Boer et al., 1985), indicating a dissociation between HR and BP fluctuations. 2) a mid-frequency peak around 0.1 Hz: these fluctuations in BP with a period of approximately 10 seconds (Mayer waves; Mayer, 1876), may be the result of oscillations (Hyndman et al., 1971) or a resonance in the baroreflex control of peripheral resistance at frequencies around 0.1 Hz, while the HR fluctuations at these frequencies represent a reflection of the baroreflex response (Baselli et al., 1988; Madwed et al., 1989; Wesseling & Settels, 1985). Malliani et al. (1991) proposed that the fluctuations in a frequency range of 0.04-0.13 Hz should be considered as a general marker of sympathetic modulation, although it is clear that HR fluctuations in this frequency range may also reflect parasympathetic activity (Pomeranz et al., 1985). 3) a high-frequency peak centered at the respiratory frequency, usually between 0.20-0.35 Hz: for HR these fluctuations primarily reflect respiratory linked variations (respiratory sinus arrhythmia) as a result of centrally mediated vagal control (Anrep et al., 1936; Angelone & Coulter, 1964; Davies & Nielson, 1967). For BP, these fluctuations are proposed to be the result from the direct effect of centrally mediated HR fluctuations (Akselrod et al., 1985), although the mechanical effects of respiration may also contribute substantially to these fluctuations (Saul et al., 1991).

Presently, spectral analysis (as a non-invasive method) is acknowledged as a potential tool to assess changes in sympathetic and parasympathetic components of cardiovascular control. It has been postulated that spectral analysis of heart rate and blood pressure variations may provide a reliable index of a sympathetic-parasympathetic balance (Pagani et al., 1986). However, further research is essential for several reasons:

1) A large part of the studies is based on spectra of HR only. More studies are

necessary, comparing spectra of both HR and BP, in order to clarify previous interpretations. Recent developments of non-invasive blood pressure recording systems offered new opportunities to study continuous fluctuations in BP by means of spectral analysis (Penaz et al., 1976; Settels & Wesseling, 1985).
2) Spectral analysis offers the possibility to compute transfer functions between spectra of HR and BP, which may increase our understanding of the dynamics of the cardiovascular control system. On the basis of these transfer functions baroreflex sensitivity can be estimated without the inference of test methods such as administration of a pressor agent or the neck suction method (i.e., Robbe et al., 1987).
3) The contribution of the sympathetic nervous system in the origination of cardiovascular fluctuations has so far been evaluated primarily on the basis of pharmacological blockade of α- and ß-receptors (i.e., Akselrod et al., 1985; Pomeranz et al., 1985). It is at present not known what the contribution of peripheral sympathetic nervous system activity and adrenal medullary discharge is, and whether there is a difference in the contributions of circulating noradrenaline and adrenaline in cardiovascular homeostasis. In addition, it is not clear whether the method is sensitive enough to reflect differences in sympathetic control at the level of the peripheral sympathetic nervous system or higher control centers.
4) Increases in heart rate and blood pressure as induced by task performance (mental effort or mental stress) may be accompanied by a reduction in variability of heart rate and blood pressure in healthy subjects (i.e., Mulder & Mulder, 1981; Veldman et al., 1985). Although it is likely that a reduction in baroreflex sensitivity and a change in the sympathetic and parasympathetic balance may explain the increased heart rate and the reduced heart rate variability, the manner in which activity of the sympathetic nervous system, and indirectly of the parasympathetic nervous system, attributes to reduced blood pressure variability during task performance is not completely resolved (van Roon et al., 1990).

Catecholamines, cardiovascular activity and psychological function: approaches for research

Activation of the sympatho-adrenomedullary system occurs in healthy subjects in response to stressful or demanding situations, the type and intensity of the responses being dependent, among others, upon the nature of the stressor (Ward et al., 1983), the coping style and emotional state of the subject as well as whether or not the subject is in control of the situation (Henry & Stephens, 1977; Breier et al., 1987). It has been proposed that stress or abnormal stress responses may be important in the development of affective disorders (Anisman

& Zacharko, 1992; Breier, 1989), and that increased activity of the sympathetic nervous system and the adrenal medulla may be part of the mechanisms involved. In both affective and anxiety disorders abnormalities in the functioning of the sympathetic nervous system and the adrenal medulla have been found, primarily based on abnormalities observed in resting concentrations of plasma or urinary catecholamines. For instance, increased venous adrenaline and noradrenaline concentrations have been observed in patients with anxiety disorders (Mathew et al., 1980), increased arterial adrenaline concentrations in panic disorders (Villacres et al., 1987), and increased venous noradrenaline concentrations (Lake et al., 1982; Roy et al., 1985) as wel as increased urinary excretion of adrenaline, noradrenaline and some of their metabolites (Davis et al., 1988) in depressed patients. In addition, cardiovascular changes such as increased blood pressure or even hypertension in depressed patients (Nakagawara et al., 1987; Yates & Wallace, 1987) and increased heart rate, and cardiomyopathy in anxious patients (Taylor et al., 1986; Kahn et al.,1987), also point to increased sympatho-adrenomedullary activity in these patients. Neuroendocrine challenge studies of central noradrenergic activity suggest that there is a functional blunting of the responsiveness of the postsynaptic α_2-adrenergic receptors in subgroups of patients with panic disorder or depression. The strongest support of this hypothesis has come from investigations demonstrating a blunted growth hormone response to clonidine (α_2-adrenoceptor agonist) (Charney & Heninger, 1986; Delgado & Charney, 1991). Challenge testing with yohimbine (α_2-adrenoceptor antagonist) produced increases in plasma MHPG in both panic disorders and controls, with more severe panic patients showing the largest increases (Charney et al., 1984); clonidine produced larger plasma MHPG decreases in panic patients than controls (Charney & Heninger, 1986). These findings suggested that disturbances of the central noradrenergic and peripheral sympathetic nervous system play a key role in the pathogenesis of anxiety and depressive disorders. Still, unequivocal interpretations regarding relationships between concentrations of plasma or urinary catecholamines and their metabolites, cardiovascular activity, and mood state, often can not be made due to inconsistency of findings. These inconsistencies may, among others, be related to 1) the use of heterogenous groups of patients, 2) differences in experimental procedures and the type of parameters measured, and 3) insufficient control of confounding factors which may influence catecholamine release. Different research strategies may be required to help to clarify the complex interplay between central, autonomic, and metabolic mechanisms.

One way to evaluate the role of the sympatho-adrenomedullary system is to infuse adrenaline and noradrenaline and to measure the psychophysiological responses. This method has been applied in many studies using short-lasting

infusions or bolus-injections. Typically, dose-response effects of adrenaline have been studied with an infusion duration varying between 15-40 min per dose level (i.e., Frankenhaeuser & Jarpe, 1963; Cameron et al., 1990; Zijderveld et al., 1992), inducing plasma concentrations of adrenaline ranging from moderately increased concentrations to concentrations outside the physiological range. Effects of infusion of noradrenaline have received much less attention. In general, infusions during a short period of time provide information on the acute reactions to abrupt increases in circulating adrenaline or noradrenaline. Some deleterious effects of stress, however, may be related to changes following chronic stress, which in itself may be of a mild nature (Anisman & Zacharko, 1992). It is at present not known if the acute effects of short-lasting increases in circulating adrenaline and noradrenaline differ from effects to sustained increases, especially of catecholamine concentrations within the physiological range. Adaptive mechanisms may become apparent which may prove relevant for interpretation of the role of the sympatho-adrenomedullary system in affective disorders or hypertension.

Apart from focusing on peripheral sympathetic nervous system activity, central noradrenergic regulatory mechanisms in mood states and sympatho-adrenomedullary activity may be studied, for instance by means of application of centrally active pharmacological agonists or antagonists of the α_2-adrenoceptor. Specifically, clonidine and yohimbine are frequently used as challenge tests in psychiatric disorders such as panic disorder or depression, in order to search for abnormalities in the noradrenergic system (Charney & Heninger, 1986; Delgado & Charney, 1991). Stimulation of α_2-adrenoceptors by clonidine causes a decrease in firing rate of the locus coeruleus, a decrease in sympathetic outflow (blood pressure, plasma MHPG and noradrenaline concentration), and an increase in growth hormone secretion. Yohimbine, as an α_2-adrenoceptor antagonist, increases the firing rate of the locus coeruleus, and increases sympathetic outflow (blood pressure, plasma MHPG and noradrenaline concentration). At the psychological level, clonidine generally causes a sedative type of behavioral effects, while yohimbine generally causes anxiety and behavioral activation. The effects of clonidine and yohimbine are brought about by means of an influence on complex α_2-adrenergic central and peripheral, as well as presynaptic and postsynaptic effects. Dose-response studies are required to explore whether it is possible to delineate patterns in responsivity at the level of the brainstem, the pituitary or the peripheral sympathetic nervous system. If it is possible to discriminate between patterns in responsivity at different dose-levels, abnormalities or altered states of vulnerability in the noradrenergic system can be established more accurately.

A third approach is the study of sympatho-adrenomedullary activity and subjective mood during a stress-inducing situation. In order to characterize these

responses, i.e., how they may lead to pathological states and how they could be antagonized, a standardized laboratory situation is required and a test that is capable to repeatedly induce sympatho-adrenomedullary activation during sequential presentations. Subsequently, specific pharmacological interventions may be applied in order to influence the responses to the test. By means of this approach we may learn more about the mechanisms responsible for the responses to stress-inducing situations. For instance, application of anxiolytic drugs, such as benzodiazepines, may prove relevant to unravel response mechanisms because these drugs induce different pharmacological effects at different dose levels: anxiolysis at low doses and sedation at higher doses (Geller & Seifter, 1960). High densities of benzodiazepine receptors are found in both the limbic and forebrain areas. These areas are relevant for the setting of the emotional tone, and noradrenergic neurons of the locus coeruleus project to them (Gray, 1982). Suppression of sympathetic nervous system activity by benzodiazepines has been observed in man at doses which induce sedation (Duka et al., 1986; Roy-Byrne et al., 1988). However, the effects might be different at doses which induce anxiolysis. It is therefore of interest to study specific dose-dependent sympatho-adrenomedullary effects to anxiolytic drugs in relation to rest levels and stress-inducing performance tests.

Aims of the studies

In the experiments presented in this thesis, plasma catecholamine concentrations were manipulated by means of pharmacological and psychological challenges. The subjects participating in the studies were healthy young male volunteers, recruited by means of advertisements. All volunteers were subjected to a medical, psychiatric and psychological screening to exclude abnormalities in physical and mental health. Careful attention was paid to the control of confounding factors such as posture, food intake, smoking, and drinking. Where appropriate, endocrine and metabolic parameters were monitored in order to control for the possible effects of our manipulations on these parameters.

1) A 6-Hour Infusion of Adrenaline and Noradrenaline

A six-hour infusion of adrenaline, noradrenaline, or placebo was conducted in order to study responses to elevated concentrations of circulating adrenaline and noradrenaline within the physiological range. Since circulating noradrenaline and adrenaline do not pass the blood-brain barrier, the situation created in this study was one of a sustained peripheral increase in circulating catecholamines. This provided the opportunity 1) to investigate if acute changes differ from sustained, possibly adaptive, changes to increases in circulating adrenaline

and noradrenaline; 2) to describe changes in subjective mood and hormonal parameters in a laboratory situation without requirements of physical or mental exertions, during which catecholamine concentrations were similar to those observed during real-life stress or maximal exercise; 3) to unravel the relative contribution of peripheral sympathetic nervous system activity (increased noradrenaline) and adrenal medullary discharge (increased adrenaline) in homeostatic cardiovascular control mechanisms; and 4) to evaluate which changes in urinary excretion of catecholamines and their metabolites can be explained by increased concentrations of catecholamines in the peripheral circulation and to distinguish the role of circulating adrenaline and noradrenaline in such changes.

2) Dose-response Effects of Intravenous Clonidine

Administration of the α_2-receptor agonist clonidine was employed because it induces a variety of central and peripheral effects in the noradrenergic system. Typically, after a single intravenous dose of 2 μg/kg in healthy subjects, clonidine reduces plasma MHPG and noradrenaline concentrations, blood pressure, and heart rate, while it increases sedation and growth hormone secretion. The mechanisms by which these effects are induced comprise complex α_2-adrenergic peripheral and central, both presynaptic and postsynaptic, effects. In this study, dose-response relationships of clonidine were analyzed, using doses lower than previously reported, with the aim 1) to explore whether it is possible to discriminate dose-dependent clusters or patterns in responsivity in the above-mentioned parameters, in order to delineate the mechanisms of action of clonidine at the level of the brainstem, the pituitary or the peripheral sympathetic nervous system; 2) to evaluate the sensitivity of the method of spectral analysis to reflect changes in homeostatic cardiovascular control mechanisms after clonidine administration; and 3) to compare plasma and urinary concentrations of MHPG and noradrenaline after clonidine administration.

3) The Stroop Color Word Test

In this study, the primary aim was 1) to evaluate the Stroop Color Word Test (CWT) as a test for the study of sympatho-adrenomedullary responses due to mental stress, and 2) to determine catecholamine responses to mental stress both in plasma and urine. The CWT was selected because previous research had shown that the CWT specifically induces increases in plasma and urinary adrenaline (Akerstedt et al., 1983; Hjemdahl et al., 1984), and not in noradrenaline. Responses to the CWT were analyzed on the basis of parameters reflecting subjective mood, sympatho-adrenomedullary activity, muscle activity and pituitary adrenocortical activity. To control for habituation effects and

spontaneous changes of basal values over time, a separate control session (rest periods only) was added to the design.

4) Dose-response Effects of Intravenous Lorazepam

In this study, dose-dependent effects of intravenously administered lorazepam on psychological function, cardiovascular activity, and plasma catecholamines during rest and mental stress (CWT) were investigated with the aim 1) to gain insight in the mechanisms responsible for the responses to the CWT, by examining whether a benzodiazepine can affect stress-induced sympatho-adrenomedullary activity, and if so, whether there is a difference in responses to doses which may induce anxiolysis (low doses) or sedation (higher doses); and 2) to analyze the cardiovascular responses to lorazepam with the method of spectral analysis, in order to obtain an integrated image of both the sympathetic and the parasympathetic cardiovascular properties of lorazepam during rest and mental stress.

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CHAPTER 2

A 6-HOUR INFUSION OF ADRENALINE AND NORADRENALINE

Chapter 2.1

Psychological, Cardiovascular, and Endocrine Changes During Six Hours of Continuous Infusion of Epinephrine or Norepinephrine in Healthy Volunteers

Summary

Psychological, cardiovascular, endocrine, and metabolic reactions to a sustained infusion of epinephrine (E) and norepinephrine (NE) were studied in ten healthy male volunteers in a placebo-controlled randomized design. The subjects participated each in three sessions during which they received a 6-hr infusion of either E (82 pmol/kg/min), NE (178 pmol/kg/min), or placebo (PLA) (saline, 5.4 ml/h). Heart rate and intra-arterial blood pressure were recorded continuously. Blood samples for assay of catecholamines, cortisol, prolactin, growth hormone, insulin, triglycerides, and glucose were obtained at regular intervals. Changes in subjective mood were assessed with the Profile Of Mood States (POMS) and the State-Trait Anxiety Inventory (STAI). During infusion of E, arterial plasma epinephrine levels increased 10-fold, which induced significant increases in heart rate, plasma insulin and glucose levels, and decreases in mean arterial pressure (MAP) and diastolic pressure (DAP). NE infusion caused a 5-fold arterial plasma norepinephrine increase and induced a significant decrease in heart rate and increases in MAP, DAP, and glucose levels. The effects were present shortly after initiation of the infusions, remained fairly constant during the 6 hour infusion period and disappeared within one hour after the infusions had been stopped. Changes in subjective mood were not observed during the infusions, nor after the infusions had been stopped. Infusion of E or NE also had no significant effect on systolic blood pressure, plasma prolactin, growth hormone, cortisol, and triglycerides.

Our results show that moderate cardiovascular and metabolic effects can be caused by sustained increases in circulating catecholamines. The psychological parameters were not affected by a prolonged increase in circulating catecholamines in the high physiological range, which extends previous observations concerning the absence of mood changes to short-lasting catecholamine-infusions in healthy subjects.

Introduction

Activation of the sympathetic adrenomedullary system can occur in response to stressful or demanding situations in healthy subjects (1-7), the type and intensity of the responses being dependent upon the nature of the stressor. Stress or abnormal stress responses may be important in the development of affective disorders (8,9) and increased activity of the sympathetic nervous system and the adrenal medulla, resulting in increased peripheral concentrations of norepinephrine or epinephrine, respectively, may be part of the mechanisms involved. One way to evaluate these mechanisms is to infuse epinephrine or norepinephrine and to measure the responses. This method has been applied in many studies using short-lasting infusions or bolus-injections (10-18). Typically, dose-response effects of epinephrine have been studied with an infusion duration varying between 15-40 min per dose level (i.e., 14,16-18), inducing plasma concentrations of epinephrine ranging from moderately increased levels to levels outside the physiological range. Effects of infusions of norepinephrine have received much less attention, although some cardiovascular studies have been done with short-lasting infusions (i.e., 19,20). Cardiovascular studies with longer infusion durations of either 2 hr (21) or 3 hr (22) also have been performed, with dose levels inducing plasma catecholamine concentrations outside the physiological range.

Infusions during a short period of time provide information on the acute reactions to abrupt increases in circulating epinephrine or norepinephrine. Some deleterious effects of stress, however, may be related to changes following chronic stress, which in itself may be of a mild nature (8). Our aim was to study responses to elevated, but physiological, concentrations of circulating epinephrine and norepinephrine and to investigate acute, as well as delayed, possibly adaptive, changes. A long duration of infusions would have been most informative, but in view of practical and ethical limitations, and as a first exploration, the infusions were limited to 6 hours. The infusion rates were chosen in such a way that concentrations of plasma catecholamines within the physiological range were obtained, corresponding to levels that can be observed during real-life stresses or physical exercise. The dependent variables selected were psychological and cardiovascular parameters. Since plasma cortisol, prolactin, and growth hormone are responsive to stress-induction (23,24), we also analyzed the effect on these variables. Special attention was paid to the caloric intake of the subjects, not only because catecholamines play a role in the regulation of carbohydrate metabolism (24), but also to avoid hypoglycemia induced psychophysiological reactions due to the duration of the study. For this reason the caloric intake of the subjects immediately before and during the experiment was kept under control and effects on insulin, glucose, and

triglycerides were measured.

Previously, we reported on some aspects of the cardiovascular responses to the infusions of epinephrine and norepinephrine (25). Here, we present a comprehensive report of the whole experiment, with exception of the data on the urinary excretion of catecholamines and their metabolites which have been reported elsewhere (26).

Methods

Subjects

Ten healthy male volunteers [mean (sd): age 23.6 (4) years, height 181.6 (9) cm, weight 78.3 (7) kg] participated each in three experimental sessions, after they had given written informed consent. The subjects were undergraduate students who were paid for their participation in the study. Before admission to the study, each subject underwent a medical examination (including electrocardiography, blood, and urine testing) to establish his physical health. All subjects appeared to be in good physical condition. Subjects with excessive drinking habits were excluded from the study. Six of the ten subjects were non-smokers; the other four subjects smoked less than 5 cigarettes per day. A psychiatric interview was conducted by a senior psychiatrist to ascertain mental health, while in addition the subjects filled in the following questionnaires: a) the Minnesota Multiphasic Personality Inventory (MMPI) (validated Dutch version, 27), b) the Amsterdamse Biografische Vragenlijst (ABV) (28): a Dutch inventory to assess the Introversion-Extraversion personality dimension and c) the State-Trait Anxiety Inventory (STAI) (validated Dutch version, 29). Extreme (i.e., pathological) levels were not observed on these questionnaires.

Procedure, Recordings and Analyses

The subjects participated each in three experimental sessions, during which they received at random and double blind a six hour infusion of epinephrine (E) (82 pmol/kg/min: i.e., 15 ng/kg/min), norepinephrine (NE) (178 pmol/kg/min: i.e., 30 ng/kg/min) or placebo (PLA) (saline: 5.4 ml/h). The experimental procedures were approved by the Ethical Committee of the University Hospital Rotterdam Dijkzigt.

The three sessions per subject were scheduled at least 10 days apart. Three days before each recording session, the subjects were requested to avoid abnormal physical or psychic excertions (i.e., sport events, examinations) and to keep a regular sleep-wake schedule. On each recording day the subjects had breakfast in the hospital (a standard light meal without tea or coffee). They were requested to void urine, after which the subjects were in a supine position on bed for the rest of the experiment. The recordings were performed from

09.00 hr A.M. till 17.00 hr P.M. Infusions were given from 10.00-16.00 hr. The subjects were not allowed to eat, drink or smoke. During all recordings they received a 5% dextrose solution infusion at a rate of 2 ml/min, in order to avoid hypoglycaemic effects and to standardize caloric intake. The subjects were requested to stay awake. Occipital, parietal and temporal EEG leads were recorded to control for the awake state of the subject. If the subject dozed off, he was kindly reminded to stay awake.

Forty-five minutes before the start of each session a catheter (Venflon, 18G, Viggo AB, Helsingborg, Sweden) was inserted into an antecubital vein of the dominant forearm through which infusions of dextrose and NE, E or PLA took place, while a second venous catheter was inserted in the non-dominant forearm in order to obtain venous blood samples at 8 regular intervals of 1 hour during the whole recording period and 4 times at 5-min intervals directly after the infusions had been stopped. Intra-arterial blood pressure was recorded in the brachial artery of the non-dominant arm after cannulation under local anesthesia with a 2% lidocaine solution. A Teflon catheter, 1.0 mm in diameter (Plastimed, Saint-Leu-La Foret, France), was introduced by the Seldinger technique and was connected to a miniature transducer-perfusion device (Northwick Park Hospital, London, England); the intra-arterial pressure signal and ECG (precordial lead) were monitored continuously and recorded analogue on an instrumentation recorder (Racal Store 14 DS). The presence of an intra-arterial cannulation allowed us to obtain arterial blood samples from the non-dominant arm. Venous and arterial blood samples (10 ml per sample) for assay of plasma catecholamines were obtained (in the arm contralateral to the site of infusion) at the same time intervals and were collected in chilled heparinized tubes containing 19 mg of EGTA and 12 mg gluthathione. The samples were immediately placed on ice and centrifuged within 15 minutes after collection; plasma was frozen at -70°C for assay of plasma catecholamines by means of high-performance liquid chromatography with electrochemical detection (30). In addition, venous blood samples (7 ml per sample) were obtained in heparinized tubes, centrifuged and frozen at -20°C for assay of cortisol and insulin by means of commercially available radioimmunoassay kits (respectively, "Coat-a-count", Diagnostic Products Corporation, Los Angeles, California, USA and Incstar, Stillwater, Minnesota, USA), and for assay of prolactin and growth hormone by means of an immunoradiometric assay (Euro-diagnostics BV, Apeldoorn, The Netherlands). Blood samples for assay of glucose (4 ml in oxalate tubes) and triglycerides (4 ml in heparinized tubes) were collected just before the infusion started (10.00 hr) and after 1 h (11.00 hr) and 5 hr of infusion (15.00 hr). Glucose was measured in whole blood using the hexose-kinase reaction. Triglycerides were assayed according to the method described by Bucolo & David (31).

The ECG and blood pressure recordings were digitized at a sample frequency of 102.4 Hz on a Personal Computer (Olivetti) connected to a Labmaster Analogue/Digital converter. Before A/D conversion, a Schmitt trigger was used to trigger the incidence of the R-waves in the ECG; the output pulses of the trigger were fed into the converter for sampling. The time between the output pulses (the interbeat interval) was measured with a resolution of 10 ms. Systolic, diastolic and mean arterial blood pressure (SAP, DAP and MAP) were defined per R-R interval of the ECG with a resolution of 0.2 mmHg. ECG and blood pressure recordings were checked for presence of artifacts. Collection of the blood samples, which lasted a few minutes every hour, caused temporary increases in arousal as well as movement artifacts. These periods were excluded from analyses. Otherwise, the data proved of sufficient quality for further processing. Mean heart rate (in BPM) and blood pressure (SAP, DAP and MAP) were calculated on basis of these beat-to-beat analyses for 8 selected periods per recording: a 15 min baseline period directly before the infusion started, per hour during the infusion period (i.e., 6 periods) and for a 30 min period one half hour after the infusion was stopped.

Changes in psychological state were assessed by means of two self-rating questionnaires: 1) a shortened version of the Profile Of Mood States (POMS) of McNair et al. (32) which was validated for the Dutch population (33) and 2) the state-version of the STAI (29). Both questionnaires were filled in immediately before the start of the recording session, after three hours of infusion of NE, E or PLA and one hour after the infusion was stopped.

Statistical analyses: $N=10$ for all statistical analyses unless specified otherwise. For the cardiovascular and biochemical data, similarity of pre- and post-infusion levels between the three conditions was analyzed with a multivariate analysis of variance (MANOVA) for repeated measurements. If a significant effect was found, paired Student's t-tests were used for pairwise comparisons. MANOVA's for repeated measurements were employed, using factors DRUG (NE/E/PLA), TIME (infusion period: 6 consecutive hourly periods) and their interaction (DRUGxTIME), to establish the level of significance of the experimental manipulations. When a significant DRUG-effect was found, additional MANOVA's were performed to study specific effects of E or NE versus PLA, all analyses including factor TIME. Wilcoxon tests (34) were applied for the nonparametric psychological data in order to establish significance of changes in mood and anxiety within each condition and between conditions. A p-value of $<.05$ was used to indicate a significant effect.

Results

For all variables presented, pre-infusion levels were similar for the three experimental conditions (NE/E/PLA), with the exception of the plasma insulin levels (MANOVA F-value: 3.69, p=.05).

Table 1. F-values of the MANOVAs on the plasma catecholamines, cardiovascular, endocrine, and metabolic data, for the factors Drug (infusion of E, NE, or PLA) and Time (6 hourly periods)

	MANOVA (F-values)		
	Drug	Time	Drug x Time
Epinephrine			
arterial	261.37***	2.39*	2.25*
venous	28.91***	1.28	1.57
Norepinephrine			
arterial	435.39***	4.47**	2.78**
venous	50.86***	2.18	0.97
Heart rate	5.55**	0.27	1.22
MAP	16.19***	25.77***	0.84
SAP	1.22	26.93***	0.36
DAP	19.88***	21.15***	1.06
Prolactin	2.25	4.26**	0.58
Growth hormone	3.08	2.14	0.61
Cortisol	0.06	2.34	0.44
Insulin	6.43**	11.97***	1.53
Glucose	36.62***	0.18	1.05
Triglycerides	0.11	1.41	1.54

*: $p<0.05$; **: $p<0.01$; ***: $p<0.001$. Interaction-effects between factors Drug and Time are also presented

Plasma Catecholamines

During infusion of E, arterial plasma epinephrine levels increased 10-fold: from 34 (sem:±6) pg/ml pre-infusion to a mean level of 387 (±15) pg/ml during infusion [i.e., from 0.19 (sem:±0.03) to 2.12 (±0.09) pmol/L]. Arterial plasma norepinephrine levels increased 5-fold during infusion of NE: from 183 (±15) pg/ml pre-infusion to a mean level of 1025 (±38) pg/ml during infusion

[i.e., from 1.08 ($\pm$0.09) to 6.07 ($\pm$0.24) pmol/L]. The effects were present from the first till the last hour of infusion, although a further small, but significant, increase over time was observed for the arterial plasma epinephrine levels during infusion of E (versus NE and versus PLA: $p < .05$), and for the arterial plasma norepinephrine levels during infusion of NE (versus E and versus PLA: $p < .01$) (Table 1). Venous plasma epinephrine levels also increased 10-fold during infusion of E (from 21 ± 4 to 230 ± 28 pg/ml), while venous plasma norepinephrine increased 3.5-fold during infusion of NE (from 207 ± 17 to 705 ± 58 pg/ml). Five min after the infusions had been stopped, arterial or venous levels of plasma catecholamines were similar for the three conditions.

During infusion of E or PLA, venous levels of epinephrine and norepinephrine were 40-50% of the arterial levels. During infusion of NE, venous norepinephrine levels were 30-40% of the arterial levels, indicating a different clearance factor for norepinephrine.

Psychological effects

In comparison with pre-infusion values, infusion of E, NE, or PLA did not cause significant changes in state-anxiety as measured by the STAI, or other aspects of mood (vigor, fatigue, tension, anger and depression) as measured by the POMS (Table 2), nor were there significant differences between the conditions. One hour after the infusion of NE was stopped, subjective vigor was decreased significantly in comparison with pre-infusion values ($z = -2.09$, $p < 0.05$).

When they were asked specifically, the subjects were not able to differentiate at all between the three infusions after completion of the recordings.

Cardiovascular effects

During infusion of E, heart rate increased (6.8%) and MAP decreased (6.9%) as compared to the PLA-infusion (Figure 1). Infusion of NE caused a decrease in heart rate (3.9%), while MAP increased (6.6%). These effects were moderate but significant: for heart rate, MAP, and DAP, the MANOVAs indicated significant DRUG-effects (Table 1): infusion of E and NE caused significant changes versus PLA ($p < .05$). A significant increase over time was observed in MAP, SAP and DAP during all three infusion-periods (Table 1, Figure 1). Effects of the infusions on SAP were too small to show a significant DRUG-effect (Table 1).

Thirty min after the infusions had been stopped, post-infusion levels of heart rate and blood pressure were similar for the three conditions.

Table 2. Changes in mood, before, during, and after infusion of E, NE, or PLA

	PLA			E			NE		
	before	during	after	before	during	after	before	during	after
STAI	27.7(5.7)	28.6(4.6)	29.6(7.2)	28.5(4.8)	29.9(5.0)	29.8(4.2)	30.1(6.0)	29.2(6.6)	28.9(4.7)
POMS									
Depression	8.0(0.0)	8.5(0.9)	8.2(0.6)	8.0(0.0)	8.7(1.3)	8.0(0.0)	8.4(1.0)	8.2(0.4)	8.1(0.3)
Anger	7.2(0.4)	7.7(1.6)	7.4(0.8)	7.0(0.0)	7.3(0.7)	7.2(0.4)	7.0(0.0)	7.4(1.0)	7.5(1.6)
Fatigue	7.2(1.9)	7.4(2.0)	8.3(2.9)	7.0(1.1)	6.9(1.2)	8.4(4.1)	6.8(1.5)	6.8(1.3)	7.7(1.6)
Vigor	18.3(4.4)	16.1(6.4)	17.0(4.4)	16.8(3.8)	15.7(3.1)	15.7(3.7)	18.0(3.9)	16.1(5.2)	14.6(5.3)
Tension	6.9(1.0)	7.0(1.3)	7.5(2.5)	7.4(2.0)	6.6(0.5)	7.3(1.5)	7.2(2.0)	6.9(1.3)	7.0(1.6)

STAI: State-Trait-Anxiety Inventory; POMS: Profile of Mood States. Data are presented in mean (SD), for the STAI and per subscale of the POMS

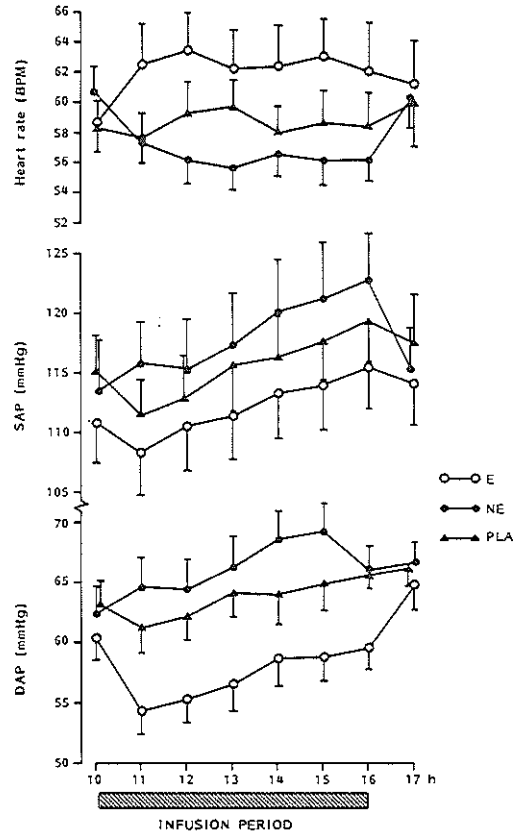

FIGURE 1
Mean and SEM values of heart rate, systolic (SAP), and diastolic (DAP) arterial pressure before, during, and after 6 hr of infusion of E, NE, or placebo (PLA). BPM: beats per minute.

Endocrine and metabolic effects

Plasma prolactin, growth hormone and cortisol levels were not influenced by E, NE, or PLA (Table 1; Figure 2-3). Plasma prolactin levels showed a significant increase over time during all infusions (Table 1; Figure 2). One hour after the infusions were stopped, plasma levels of prolactin, growth hormone and cortisol were similar for the three conditions.

Plasma insulin levels were significantly increased by the infusion of E,

in comparison with PLA ($p<.05$), or NE ($p<.05$); a decreasing trend was observed during all infusions (Table 1; Figure 3). Interpretations of the plasma insulin data during infusion of NE are hampered by the fact that unstable baseline values were observed. Baseline values of the NE-condition were significantly larger than the baseline values of the PLA-condition ($t=-2.34$, $p<0.05$). One hour after the infusions were stopped, post-infusion levels of insulin were similar for the three conditions.

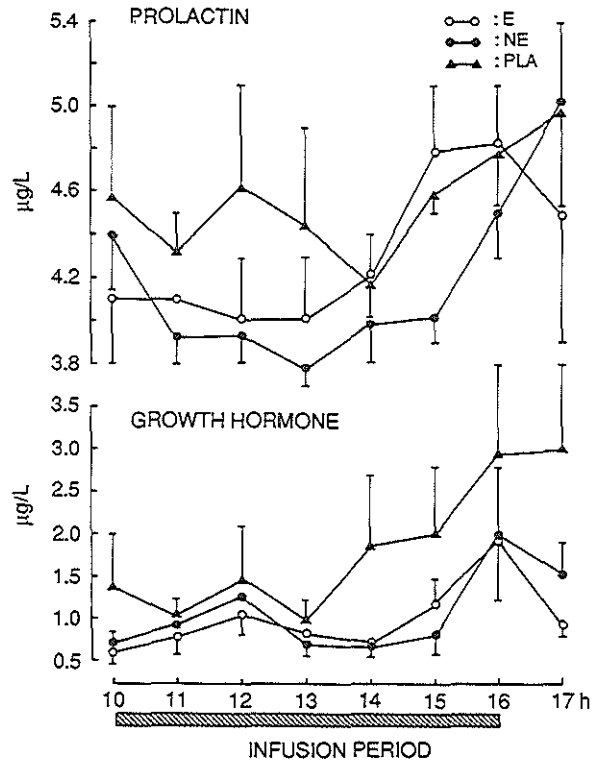

FIGURE 2
Mean and SEM values of plasma prolactin and growth hormone before, during, and after 6 hr of infusion of E, NE, or PLA. Data are presented in µg/liter. For graphical purposes it was not always possible to draw the SEM values. Range of the SEM values of plasma prolactin during infusion of E: 0.17-0.32 µg/liter; range SEM values plasma growth hormone during infusion of NE: 0.09-0.87 µg/liter.

Glucose levels during infusion of E or NE were significantly increased versus glucose levels observed during infusion of PLA ($p < .001$): the effects were stable during the infusion period because no TIME-effects were observed (Table 1,3). Also, the glucose levels during the infusion of E were significantly larger than during infusion of NE ($p < .001$).

Levels of plasma triglycerides were not influenced by the infusion of E, NE, or PLA (Table 1 and 3).

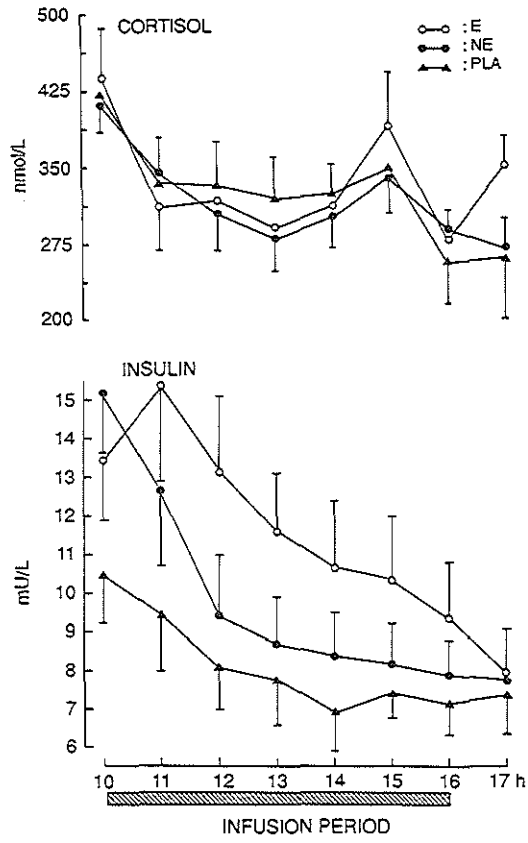

FIGURE 3
Mean and SEM values of plasma cortisol and insulin before, during, and after 6 hr of infusion of E, NE, or PLA. Range of the SEM values of plasma cortisol during infusion of E: 31-53 nmol/-liter.

Table 3. Mean (SD) levels of glucose and triglycerides before and during infusion of E, NE, or PLA

	PLA	E	NE
Glucose (mmol/L)			
Baseline	4.32 (.4)	4.62 (.6)	4.49 (.7)
Infusion: 1 hr	4.54 (.4)	5.30 (.8)	4.81 (.6)
5 hr	4.44 (.3)	5.50 (.5)	4.97 (.5)
Triglycerides (mmol/L)			
Baseline	1.01 (.4)	0.99 (.5)	0.96 (.4)
Infusion: 1 hr	0.98 (.4)	1.06 (.5)	0.90 (.3)
5 hr	0.93 (.4)	0.98 (.5)	0.97 (.5)

Discussion

Circulating catecholamines during infusion of E or NE

In this study, a continuous infusion of 178 pmol/kg/min of NE in healthy males caused a 5- or 3.5-fold increase in arterial or venous plasma norepinephrine levels, respectively, while a continuous infusion of 82 pmol/kg/min of E resulted in a 10-fold increase in arterial and venous plasma epinephrine levels. In most human studies only venous plasma catecholamine levels are reported. Detectable increases in the concentration of circulating epinephrine are found in response to 'discrete' stressful or demanding situations (cognitive stress tasks; cold pressor test; isometric exercise) in the laboratory: healthy subjects show a 1.5- to 3-fold increase in venous plasma levels ranging from moderate to intermediate values (between 50-100 pg/ml) (1-4). During real-life stress (public speaking) these levels may increase upto 300 pg/ml (5). In addition, during maximal exercise (i.e., bicycle exercise) venous plasma epinephrine levels rise to, or exceed, the levels observed in this study (6,16). In patients with panic disorder baseline arterial plasma epinephrine levels of 103 ± 23 pg/ml have been reported (35): a 2- to 3-fold increase in comparison with baseline values of normal controls. This indicates that with the 10-fold increase in this study we induced plasma epinephrine levels in the high physiological range, comparable to the levels observed during acute real-life stress or during physical exercise.

Immediate responses of venous plasma NE to cognitive stress tasks are highly variable and sometimes absent (7). Response-magnitudes to other tasks (such as venipuncture, orthostasis, cold pressor test, isometric exercise) range from a 1.5- to 2-fold increase (1,3). Most consistent are the observations in

healthy subjects of increased levels of venous plasma norepinephrine during situations of increased physical activity (1,6); the 7-fold increase during maximal treadmill exercise as reported by Halter et al. (6) exceeded the norepinephrine levels observed in this study. On the other hand, plasma norepinephrine levels in depressed patiens were found to be about 2 times higher than in controls (36,37). This suggests that in this study we induced plasma norepineprhine concentrations in the moderate to high physiological range. However, the venous plasma norepinephrine levels obtained during or after different tasks or baseline periods may not be directly comparable with the increased levels of norepinephrine as a result of exogenously administered NE (38-40). During sympathetic nervous activity norepinephrine may leak from the synaptic cleft into the bloodstream to behave as a circulating hormone. This process is highly dependent on the rate at which norepinephrine is removed from the circulation, while in addition local sympathetic nervous activity may contribute to the norepinephrine levels observed at the site of sample collection. It is unclear to what extent the increased plasma norepinephrine levels due to exogenously administered NE in this study simulates the increased norepinephrine levels as observed in real-life test situations or in patients.

Within 5 min after the infusion of E or NE were stopped, plasma catecholamine levels returned to the levels observed after infusion of PLA. This finding is in line with previous observations regarding the short half-life of plasma epinephrine and norepinephrine (41,42).

During the infusions, venous levels of epinephrine and norepinephrine were 30-50% of the arterial levels. Differences in this range have been reported before (43,44) and indicate clearance of both catecholamines by the forearm (45,46).

Reactions to the infusions

Cardiovascular. Circulating epinephrine is known to increase heart rate, stroke volume and SAP, while systemic vascular resistance and DAP are lowered (16). On the other hand, norepinephrine increases SAP and DAP, while the baroreflex mediates the slowing of the heart. The magnitudes of the cardiovascular responses to E or NE in this study are in agreement with findings of others who used comparable doses of E or NE during short-lasting infusions or bolus-injections (19,47,48). At the dose-levels we employed, we did not observe a significant effect on SAP during infusion of E or NE. The responses observed in this study resemble the magnitudes of the cardiovascular responses to mild or moderate stressful laboratory tasks in healthy subjects (i.e., a 5-15% increase: 2,4,49).

Endocrine and metabolic. Infusion of E, NE or PLA did not affect plasma levels of prolactin, growth hormone, cortisol or triglycerides. During stressful

situations, the hypothalamus-pituitary-adrenal system may be activated to excrete prolactin, growth hormone or cortisol (23,50). Abnormalities in hormonal excretion also are observed in affective disorders (50,51). However, our results do not seem to indicate a direct role for circulating catecholamines in these changes. Significant increases in blood glucose levels were observed to both E and NE infusions. The increase was most pronounced during the infusion of E. This is in line with findings of Freyschuss et al. (47), who observed increased glucose levels with a short infusion of 18.2 ng/kg/min E. Infusion of E induced a significant increase in plasma insulin levels. We did not have any indication of a - transient - decrease in insulin as observed by Clutter et al. (52). It is clear that sustained metabolic effects were induced by the infusion of E, however, it should be noted that in the present experiment subjects received dextrose during the entire session to avoid hypoglycaemia due to the duration of the experiment.

Psychological. In general, the earlier studies have shown that subjects with a history of anxiety reactions and sympathomimetic symptoms will experience intense anxiety to short-lasting infusions or bolus-injections of E with concentrations above the physiological range (10,12,13,53), while healthy subjects without such a background do not show 'real' anxiety or physiological sensitivity (11,14,15). The absence of psychological reactions was also observed in recent studies on the role of epinephrine in the development of anxiety symptoms in healthy subjects (17) or in patients with pheochromocytomas (catecholamine-secreting tumors) (54). Our data show that increases of both catecholamines during 6 hours in concentrations within the physiological range also do not lead to subjective changes in mood.

In summary, the present sustained infusions of E and NE resulted in plasma catecholamine concentrations in the moderate to high physiological range during the entire 6 hour infusion period. These levels correspond to those observed during acute real-life stress or physical exercise. The cardiovascular effects produced were clear but of mild to moderate magnitude, as were the metabolic effects. All these changes were present immediately after initiation of the infusions and remained fairly constant during the 6 hour infusion period. This indicates that no regulatory mechanisms counteracted the perturbations induced by the sustained infusions of E or NE.

In acute demanding or stressful situations the type and intensity of the psychological, cardiovascular, endocrine and adrenergic responses depend to a large extent on the nature of the stressor, the coping style and emotional state of the subject as well as whether or not the subject is in control of the situation (55,56). Our results indicate that in a neutral environment without requirements of physical or mental exertions, moderate cardiovascular and metabolic effects

may be caused by sustained increases in circulating catecholamines, but subjective mood or hormonal changes are not. The absence of an effect on the psychological parameters during a prolonged increase in circulating catecholamines in the high physiological range extends previous observations on an absence of mood changes due to short-lasting catecholamine-infusions in healthy subjects.

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Chapter 2.2

Spectral Analysis of Hemodynamics during Infusions of Epinephrine and Norepinephrine in Man

Summary

Spectral analysis of fluctuations in hemodynamic parameters is an increasingly popular approach to quantify sympathetic, parasympathetic, and humoral components of cardiovascular homeostatic mechanisms. In order to unravel the relative contribution of peripheral sympathetic nervous system activity and adrenal medullary discharge, we studied heart rate (HR) and arterial blood pressure (ABP) fluctuations by means of spectral analysis during a continuous 6 hour infusion of norepinephrine (NE: 30 ng/kg/min) and epinephrine (E: 15 ng/kg/min) in 10 supine resting normotensive males (mean age: 23.6 years) in a double-blind, randomized, placebo-controlled design. Power spectra were calculated per 5 min periods for HR, systolic (SBP), and diastolic blood pressure (DBP) to yield power values for three frequency bands: low (LFB:.02-.06 Hz), mid (MFB:.07-.14 Hz) and high (HFB:.15-.40 Hz). Baroreflex sensitivity (BRS) was computed as the gain (or modulus) in the MFB between the systolic pressure values and the R-R interval times. We also analyzed the effects on stroke volume (SV), total peripheral resistance (TPR), and respiration, as well as plasma concentrations of catecholamines, renin, and aldosterone. Infusion of E significantly increased plasma epinephrine (by 1000%, versus placebo), renin (54%), and aldosterone concentrations (34%), as well as HR (7%), SV (10%), and LFB power of SBP and DBP (5 and 3%, respectively), and significantly decreased TPR (20 %) and DBP (9%). Infusion of NE significantly increased plasma norepinephrine (500%) and renin concentrations (49%), and DBP (6%); infusion of NE significantly reduced HR (4%) and MFB power of SBP and DBP (7 and 8%, respectively). Our data show that a 5 to 10 fold increase in circulating catecholamines induced only small, though significant, changes in cardiovascular fluctuations. Respiration rate and spontaneous fluctuations in the HFB of HR, SBP, and DBP were not influenced by infusion of E or NE, reflecting a minor role of vagal tone in the observed hemodynamic adjustments. BRS did not change significantly during infusion of E or NE. The increased LFB fluctuations in ABP during infusion of E may

reflect increases in peripheral vasomotor fluctuations probably due to effects of E on TPR and SV. Infusion of NE reduced MFB fluctuations in ABP probably by means of a baroreflex mediated suppression of sympathetic activity, as witnessed also by a tendency of reduced MFB fluctuations in HR. These effects could not be explained by the changes in plasma renin or aldosterone concentrations. Our data signify the need to separate LFB from MFB fluctuations, because these bands may represent different components of baroreflex mediated changes during situations of increased sympatho-adrenomedullary activation under different experimental circumstances. Our results also imply that, under the conditions of the present study (supine rest, 6 hours), changes in plasma catecholamines cannot be unequivocally labelled as indices of altered sympatho-adrenal control of cardiovascular homeostasis.

Introduction

Evidence suggests that in essential hypertension, central sympathetic tone is increased (1), sensitivity of afferent negative feedback baroreflex mechanisms is diminished (2), and peripheral excitatory sympathetic reflex mechanisms are increased (3). It is difficult to delineate these components in overall integrative cardiovascular control, and it is also very hard to find a simple and reliable index of sympathetic outflow.

Recently, spectral analysis has been advocated as a tool to define the cyclic perturbations in beat to beat fluctuations in heart rate (HR) and arterial blood pressure (ABP) on a time scale of seconds to minutes, as a quantitative and qualitative assessment of short-term cardiovascular control, representing estimates of sympathetic, parasympathetic, and neurohumoral activity (4-7). Short-term fluctuations in HR and ABP may be coupled in a closed-loop feedback system, and are found to be characterized by three spectral peaks in a frequency range from 0.02 to 0.50 Hz (8-10): 1) a low-frequency peak around 0.04 Hz: for HR this peak is associated with both parasympathetic and sympathetic activity (5), while low-frequency ABP fluctuations are linked with variations in peripheral vasomotor activity due to thermoregulatory influences (11,12) or renin-angiotensin system activity (5). In this frequency range, a low coherence exists between ABP- and HR-time series, indicating a dissociation of ABP and HR variability (13). 2) a mid-frequency peak around 0.1 Hz: these fluctuations in ABP with a period of approximately 10 seconds (Mayer waves; 14), may be the result of oscillations (8) or a resonance in the baroreflex control of peripheral resistance at frequencies around 0.1 Hz, while the HR fluctuations at these frequencies represent a reflection of the baroreflex response (15-17). Malliani et al. (7) proposed that the fluctuations in a frequency range of 0.04-0.13 Hz depend both on baroreflex mediated negative feedback

mechanisms and positive feedback counteractions of cardiovascular sympathetic afferents, and should therefore be considered as a general marker of sympathetic modulation, regardless of its mechanism. 3) a high-frequency peak centered at the respiratory frequency, usually between 0.20-0.35 Hz: for HR these fluctuations primarily reflect respiratory linked variations (respiratory sinus arrhythmia) as a result of centrally mediated vagal control (18-21). For ABP these fluctuations are proposed to be the result from the direct effect of centrally mediated HR fluctuations (5), although the mechanical effects of respiration may also contribute substantially to these fluctuations (22).

Mancia et al. (23) reported no differences in variation coefficients of HR and ABP between normotensives and hypertensives during ambulation. Others, however, did find an increase in cardiovascular fluctuations around 0.1 Hz in essential hypertensives at rest (24,25), which subsequently has been taken to signify an increase in sympathetic outflow. Some hypotheses on the pathogenesis of essential hypertension relate increases of circulating epinephrine and norepinephrine to over-activity of the adrenal medulla and/or the sympathetic nervous system (1,26). It is at present not known if the pattern in beat to beat fluctuations as observed in hypertensive subjects can be explained in part by increased noradrenergic activity, whether a sustained increase in epinephrine levels is also capable of modulating short-term cardiovascular variability, or whether other factors are responsible. In order to unravel the dynamic role of peripheral sympathetic nervous system activity and adrenal medullary discharge on short-term cardiovascular variability, we analyzed spontaneous HR and ABP fluctuations by means of spectral analysis during a continuous 6 hour infusion of norepinephrine and epinephrine in 10 supine resting normotensive males in a double blind, randomized, placebo-controlled design. The infusion rates resulted in concentrations of arterial plasma catecholamines within the high physiological range. The 6-hour continuous infusion enabled us to evaluate whether time-dependent alterations in short-term hemodynamic adjustments occurred as a result of a prolonged increase of plasma concentrations of epinephrine or norepinephrine. In addition to spectral analysis, we also analyzed stroke volume, total peripheral resistance, respiration, and plasma concentrations of catecholamines, renin and aldosterone. This approach allowed us to interpret our spectral findings in relation to the baroreflex effectors and neurohumoral factors involved in short-term cardiovascular control (27).

Methods

Subjects, procedure, and measurements

Ten normotensive healthy male volunteers (mean age: 23.6 years, age range: 20-31 years) participated in this study, after they had given written informed

consent. The study was approved by the Medical Ethical Review Committee of the University Hospital Rotterdam Dijkzigt. Details of the study protocol and procedures have been described previously (28,29).

According to medical (medical history, physical examination, electrocardiogram, blood and urine testing), and psychiatric examination (interview, personality questionnaires), the subjects appeared in good health. During three days, all days two weeks apart, the volunteers were subjected to either a 6 hour infusion of epinephrine (E: 15 ng/kg/min: i.e. 82 pmol/kg/min), norepinephrine (NE: 30 ng/kg/min: i.e. 178 pmol/kg/min) or placebo (PLA: saline: 5.4 ml/h) in a random and double blind fashion. Measurements were performed from 9 AM till 5 PM, while the subjects were resting supine on a bed in an experimental cabine (ambient temperature: 22±1 °C; sound level in cabine: 36 dB(A)). The subjects were not allowed to eat, drink, or smoke. During all recordings they received a 5% dextrose solution infusion at a rate of 2 ml/min, in order to avoid hypoglycaemic effects and to standardize caloric intake. The E, NE, or PLA infusions were given from 10 AM - 4 PM. During this period no specific requirements were made regarding physical or mental exertions; however, the subjects were requested to keep their eyes open.

Forty-five minutes before the start of each session, two venous catheters (Venflon, Viggo Products, Helsingborg, Sweden) were introduced into an antecubital vein of the forearm: one in the dominant forearm for infusion of E, NE, or PLA, and one in the non-dominant forearm for blood sampling (for assay of catecholamines, renin and aldosterone). From 9 AM - 5 PM heart rate, arterial blood pressure and respiration were monitored on an electroencephalograph (EEG 16 mingograf, Siemens-Elema AB, Solna, Sweden). For off-line computer analyses, all signals were recorded also on a multichannel FM-type analogue recorder (Racal Store 14 DS, Sarasota, Florida, USA). The surface ECG was derived from a precordial lead. Intra-arterial blood pressure was recorded in the brachial artery of the non-dominant arm after cannulation under local anesthesia with a 2% lidocaine solution. A Teflon catheter (Plastimed, Saint-Leu-La Foret, France), 1.0 mm in diameter, was introduced by means of the Seldinger technique and connected to a transducer-perfusion device which allowed calibration and recording of the pressure signal. Thoracic and abdominal respiration were measured by means of two mercury strain gauges placed around the chest at the level of the nipples and the abdomen, respectively.

Analyses

Biochemical assays. Venous and arterial blood samples (10 ml per sample) were obtained at intervals of 1 hour throughout the recording period and 4 times at 5 min intervals directly after the infusions had been stopped for assay of plasma

catecholamines. Blood samples were collected in chilled heparinized tubes containing 19 mg of EGTA and 12 mg of gluthathione; the samples were immediately placed on ice and centrifuged within 15 minutes at 4 °C. Plasma was subsequently frozen at -70 °C until assayed by means of high-performance liquid chromatography with electrochemical detection (30). In addition, venous blood samples (7 ml per sample) were collected in tubes containing EDTA for assay of active plasma renin concentration and plasma aldosterone. The samples were centrifuged and stored at -20 °C until assay. Renin was measured by radioimmunoassay of angiotensin I formed at neutral pH in the presence of saturating concentrations of sheep renin substrate. Results are expressed in μunits of a standard of purified renin from human kidney (Medical Research Council Standard 68/356, National Institute for Biological Standards and Control, London, UK) per ml (31). Plasma aldosterone was measured by a commercially available radioimmunoassay kit (AldoKit, Labservice Benelux, Apeldoorn, The Netherlands).

Physiological analyses. The ECG and blood pressure signals were digitized at a sample frequence of 1024 Hz on a Personal Computer (Commodore PC 60-III) connected to an Analogue/Digital converter (Advantech PC-LabCard model PCL-718). Thoracic respiration was sampled at 51.2 Hz. Per recording, 8 periods were selected for analyses: a 15 min-period (baseline) directly before the infusion started, 6 consecutive periods of one hour during the infusion, and a 30 min post-infusion period half an hour after the infusion had been stopped. Temporary increases in arousal as well as movement artifacts were induced while blood samples were obtained. These periods (a few minutes every hour) were defined and excluded from further analyses.

Spectral analysis of cardiovascular signals: R-R intervals (interbeat intervals: IBIs) in the ECG were detected by means of a computer algorithm with an accuracy of 1 ms. Systolic and diastolic blood pressure (SBP, DBP) were defined per R-R interval of the ECG, with an accuracy of 0.1 mmHg. One subject was excluded from analyses because of frequent supra-ventricular extra beats during all three recording days.

Spectral analysis of time series only yield reliable information if the series can be considered stationary. This requirement can be fullfilled at best if short time periods (between 2-7 min) are analyzed, preferably with controlled respiratory frequency (by means of a metronome). This was not considered appropriate in our study because catecholamines may have an effect on the respiratory parameters (which could have been nullified by the use of a metronome) and controlled respiration is not feasible for prolonged recordings. Per recording, consecutive 5 min-periods (300 sec) of HR-, SBP- and DBP-time series were subjected to a discrete Fourier transform, based on non-equidistant sampling of the R-wave incidences (CARSPAN program, (32,33)), to yield

power spectra of the rhythmic oscillations over a frequency range of 0.02-0.5 Hz, with a resolution of 0.01 Hz. For each time-segment, the power was calculated for the total band (0.02-0.50 Hz), low-frequency band (LFB: 0.02-0.06 Hz), mid-frequency band (MFB: 0.07-0.14 Hz), and high-frequency band (HFB: 0.15-0.40 Hz), in addition to mean HR, SBP, and DBP, variation coefficient of HR, SBP and DBP, stroke volume index (SV), and total peripheral resistance (TPR). Changes in SV were described by means of the pulse contour method, as modified by Wesseling et al. (34). This method proved to be accurate for use on the intrabrachial pulse wave under varying hemodynamic circumstances (34,35), after corrections are made for the pressure dependent compliance of the aorta and for HR to compensate for reflections of pressure waves. TPR was computed as mean blood pressure divided by cardiac output.

Spectral energy was expressed in relative terms, i.e. as fraction of the mean value of the considered signal (squared modulation index, MI^2, to be compared with squared variation coefficient, (36)). Because, per time-segment, the total power equals the squared variation coefficient minus the power of the DC component, total power data are not presented but variation coefficients are. As an index of baroreflex sensitivity (BRS), we computed per time segment the gain (or modulus) in the MFB between the systolic pressure values and the R-R interval times, based on those frequency points within the 0.07-0.14 Hz range with a coherence between the two signals of greater than or equal to 0.5 (37).

Although the experimental circumstances in this study were highly standardized (during all recordings the subjects rested supine on bed), perturbations of stationarity sometimes occurred due to body movements, coughing, or other transient arousals. Each time-segment was scrutinized for presence of these artifacts and deleted when encountered. In this way, a total of 5% (varying between subjects from 2% to 10%) of the time-segments were excluded from the final analysis. The spectral power data were transformed to natural logarithmic values because of skewness of the distributions. The effects of E, NE, and PLA on the parameters were studied by averaging the data of the time-segments per baseline-period, per hour of infusion, and for a post-infusion period of 30 min; this approach reduced a noise-factor due to spontaneous segment to segment fluctuations (38) and allowed a statistical analysis of time-dependent changes over a period of 6 hours.

Respiration. Thoracic respiration was analyzed by means of a software program designed to compute several time- and amplitude-dependent parameters per respiratory cycle. For the purpose of this study only two parameters were considered relevant: respiratory cycle duration (in sec) and respiratory depth. Because tidal volume was not measured, changes in inspiratory amplitude were described as percentage of baseline-amplitude times 100. Artifacts due to

movements were removed before averaging the data per parameter per period. *Statistical analyses.* The data are described as mean (±SEM) for N=10, with the exception of the cardiovascular data (N=9). Similarity of baseline values and post-infusion values of the three sessions was studied by means of a multivariate analysis of variance (MANOVA) for repeated measurements (39). Sustained effects of the infusions on the cardiovascular, biochemical, and respiratory data were analyzed by MANOVA's for repeated measurements, with factors DRUG (PLA/E/NE) and TIME (6 consecutive hourly periods), as well as the interaction-effect between factors DRUG and TIME. If a significant DRUG effect was found, specific effects of E and NE versus PLA were analyzed by means of MANOVA's using pairwise comparisons, all analyses including factor TIME. Effects with a p-value of $<.05$ were considered to be significant effects.

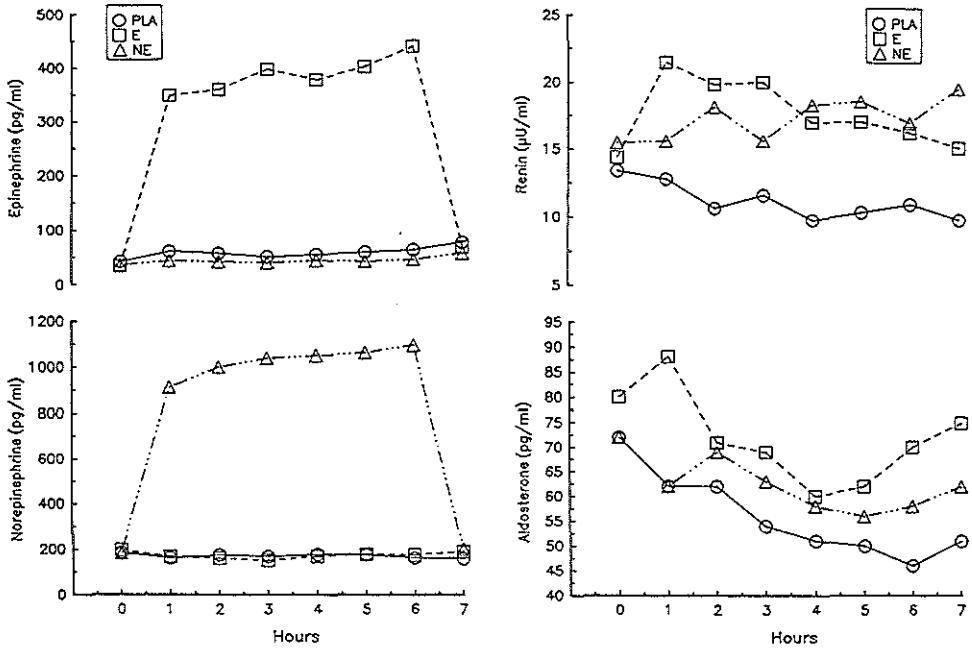

FIGURE 1
Mean arterial plasma epinephrine and norepinephrine concentrations and plasma renin and aldosterone concentrations before (0 hr), during (1-6 hr), and after (7 hr) infusion of PLA, E, and NE.

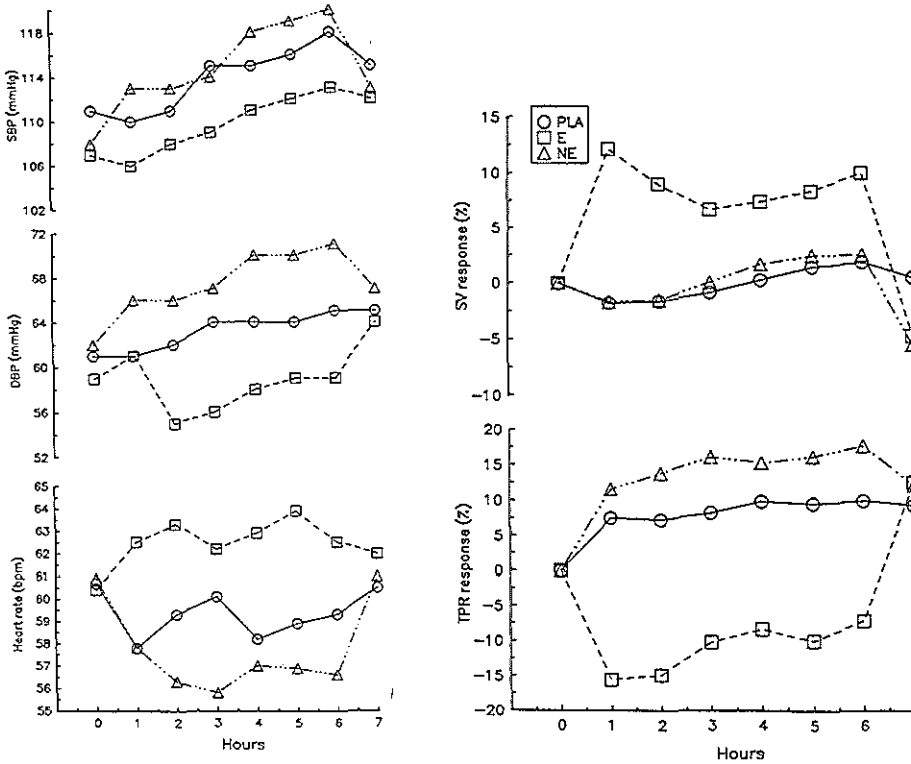

FIGURE 2
Mean SBP, DBP, HR, SV, and TPR before (0 hr), during (1-6 hr), and after (7 hr) infusion of PLA, E, or NE.

Results

Per parameter, pre-infusion levels of the three conditions (PLA/E/NE) were similar (for all parameters: $p > .05$, non-significant effects).

Circulating plasma catecholamines

During infusion of E, arterial plasma epinephrine levels increased significantly versus PLA ($p < .001$); plasma levels rose from 34($\pm$6) pg/ml pre-infusion to 348($\pm$13) pg/ml after one hour of infusion, with a small, but significant, further increase over the entire infusion period ($p < .05$; Figure 1). Infusion of E did not significantly influence plasma norepinephrine concentrations. Infusion of NE induced a significant increase in arterial plasma norepinephrine levels versus PLA ($p < .001$): from 183($\pm$15) pg/ml pre-infusion to 910($\pm$51) pg/ml after one hour of infusion, also with a small, but significant, further increase during the remaining infusion period ($p < .01$; Figure 1). NE had no significant effect on plasma epinephrine concentrations.

Table 1. Mean variation coefficients (VC; %) of SBP and DBP, and mean (SEM) logarithmic power of the high frequency band (HFB) of SBP and DBP, before, during, and after 6 hours of infusion of PLA, E, and NE

	SBP VC			SBP HFB			DBP VC			DBP HFB		
	PLA	E	NE	PLA	E	NE	PLA	E	NE	PLA	E	NE
pre	3.7	3.9	3.6	5.3(.1)	5.5(.2)	5.4(.1)	6.4	6.4	6.1	5.7(.2)	5.9(.2)	5.8(.2)
1	3.8	4.4	3.8	5.5(.1)	5.4(.1)	5.5(.1)	7.1	8.0	6.7	5.9(.2)	6.0(.1)	5.9(.2)
2	4.2	4.4	3.8	5.5(.1)	5.4(.1)	5.5(.1)	7.1	7.7	6.4	5.9(.2)	6.1(.1)	5.9(.2)
3	4.3	4.6	3.9	5.5(.1)	5.4(.1)	5.4(.1)	7.3	7.9	6.3	5.9(.2)	6.0(.1)	5.8(.2)
4	3.9	4.3	3.6	5.4(.1)	5.4(.1)	5.2(.1)	6.8	7.5	6.0	5.9(.2)	6.0(.1)	5.8(.2)
5	4.4	4.3	3.9	5.5(.1)	5.3(.1)	5.3(.1)	7.5	7.2	6.4	6.1(.2)	5.9(.1)	5.9(.2)
6	4.2	4.7	4.2	5.4(.1)	5.4(.2)	5.3(.1)	7.0	7.9	6.8	6.0(.2)	6.0(.1)	5.9(.2)
post	4.0	4.2	4.1	5.4(.1)	5.5(.2)	5.6(.1)	6.9	6.3	6.5	6.0(.2)	5.8(.1)	6.0(.2)

Table 2. Mean HR variation coefficients (VC, %) and mean (SEM) logarithmic power of the LFB, MFB, and HFB of HR before, during, and after 6 hr of infusion of PLA, E, and NE

	HR VC			LFB HR			MFB HR			HFB HR		
	PLA	E	NE	PLA	E	NE	PLA	E	NE	PLA	E	NE
pre	8.0	7.2	7.1	7.6(.1)	7.7(.1)	7.4(.1)	7.1(.2)	7.2(.2)	7.0(.3)	7.3(.4)	7.2(.3)	7.2(.3)
1	8.8	8.9	8.8	7.7(.2)	7.7(.1)	7.7(.2)	7.3(.2)	7.2(.2)	7.1(.3)	7.7(.2)	7.3(.2)	7.5(.3)
2	9.6	9.8	8.5	7.9(.2)	7.9(.1)	7.7(.1)	7.3(.2)	7.2(.2)	7.0(.2)	7.5(.2)	7.4(.3)	7.4(.3)
3	10.4	9.9	9.8	8.1(.2)	8.0(.1)	8.0(.1)	7.3(.2)	7.2(.2)	7.1(.2)	7.5(.3)	7.3(.2)	7.5(.3)
4	9.6	10.6	9.1	8.0(.1)	8.1(.1)	7.9(.1)	7.2(.2)	7.2(.2)	7.1(.2)	7.6(.3)	7.3(.3)	7.3(.3)
5	10.5	9.4	9.0	8.1(.2)	8.0(.2)	7.9(.2)	7.5(.2)	7.2(.2)	7.1(.3)	7.7(.3)	7.2(.3)	7.3(.4)
6	10.4	10.1	10.5	8.2(.2)	8.1(.1)	8.3(.1)	7.5(.1)	7.2(.2)	7.2(.2)	7.6(.2)	7.3(.3)	7.5(.3)
post	10.2	8.6	9.0	8.2(.2)	7.9(.2)	8.0(.1)	7.5(.2)	7.2(.2)	7.2(.2)	7.5(.3)	7.1(.2)	7.1(.2)

After the infusion of E had been stopped arterial plasma epinephrine levels rapidly decreased from 440(±31) to 64(±5) pg/ml after 5 min, and to 76(±11) pg/ml after 20 min. Five min after infusion of NE had been stopped, arterial plasma norepinephrine levels decreased from 1094(±50) to 247(±23) pg/ml, with a further decline to 214(±16) pg/ml after 20 min. During the post-infusion periods (from 4.30 PM - 5 PM), arterial plasma epinephrine and norepinephrine concentrations were similar for the three conditions.

Cardiovascular effects

a. Systolic and diastolic blood pressure (SBP, DBP):

SBP. Averaged over 6 hours, infusion of E induced a small decrease of 4% (mean±SEM during infusion:109.9±4.0 mmHg) and infusion of NE a small increase of 2% (116.0±4.3 mmHg) in SBP, compared to PLA (114.0±3.3 mmHg; Figure 2): these effects were too small to reach significance. During the 6 hour infusion period, SBP showed a significant time-dependent increase during all infusions ($p<.001$; Figure 2). SBP variation coefficients changed significantly ($p<.05$) during infusion of E and NE: E increased and NE decreased SBP variation coefficients to a mean level of 4.5 and 3.9%, respectively (PLA: 4.1%; Table 1). The log power of the LFB during infusion of E was increased significantly by 5% (6.5±0.2), versus PLA (6.3±0.2; $p<.05$; Figure 3). On the other hand, MFB log power during infusion of NE was reduced significantly by 7% (5.2±0.1) when compared with PLA (5.7±0.1; $p<.01$; Figure 3). The LFB and MFB effects were stable over the 6-hour period of infusion (Figure 3). The infusions had no effect on HFB log power (Table 1). Post-infusion levels of SBP, the SBP variation coefficients, LFB, MFB, and HFB log power were similar for the three conditions.

DBP. During infusion of E, DBP decreased by 9% (mean±SEM during infusion: 57.1±2.2 mmHg), while during infusion of NE, DBP increased by 6% (68.4±3.0 mmHg), in comparison with PLA (63.4±2.1 mmHg). The decrease in DBP during infusion of E was significantly different from DBP during infusion of PLA ($p<.01$; Figure 2). A significant DBP increase over time was observed during all infusions ($p<.001$; Figure 2). The DBP variation coefficient during infusion of E (mean: 7.7%) was significantly increased versus PLA (mean: 7.1%; $p<.05$) and NE (mean: 6.4%; $p<.05$; Table 1). In addition, infusion of E induced a small, but significant and sustained, increase of 3% in LFB log power (7.8±0.2), when compared with PLA (7.6±0.2; $p<.05$; Figure 3). In accordance with the SBP results, infusion of NE induced a sustained and significant decrease of 8% in MFB power (6.2±0.2) versus PLA (6.7±0.1; $p<.001$; Figure 3). Infusion of PLA, E, and NE did not affect DBP HFB power (Table 1). Post-infusion levels were similar for all DBP parameters.

b. Heart rate (HR):

The infusions had a significant effect on HR ($p < .05$, Figure 2): during infusion of E mean HR increased by 7% (mean$\pm$SEM during infusion:62.8$\pm$2.8 bpm), while infusion of NE induced a decrease of 4% (56.7$\pm$1.7 bpm), in comparison with PLA (58.8$\pm$1.9 bpm). These effects were present during the entire infusion period and were stable over time (Figure 2). A significant increase in HR variation coefficient, as well as in LFB log power, was observed during the 6 hour recording period, for all infusions ($p < .05$, Table 2); specific effects of E and NE were not found. MFB and HFB log power also were not influenced significantly by infusion of E or NE, although for the MFB there was a trend towards a reduction during infusion of NE (3% decrease versus PLA; $p = 0.08$, NS; Table 2). Post-infusion levels of HR, LFB, and MFB power were similar for the three conditions. Post-infusion levels of the HR variation coefficients and HFB power after infusion of E and NE were significantly lower in comparison with the PLA condition ($p < .05$, Table 2).

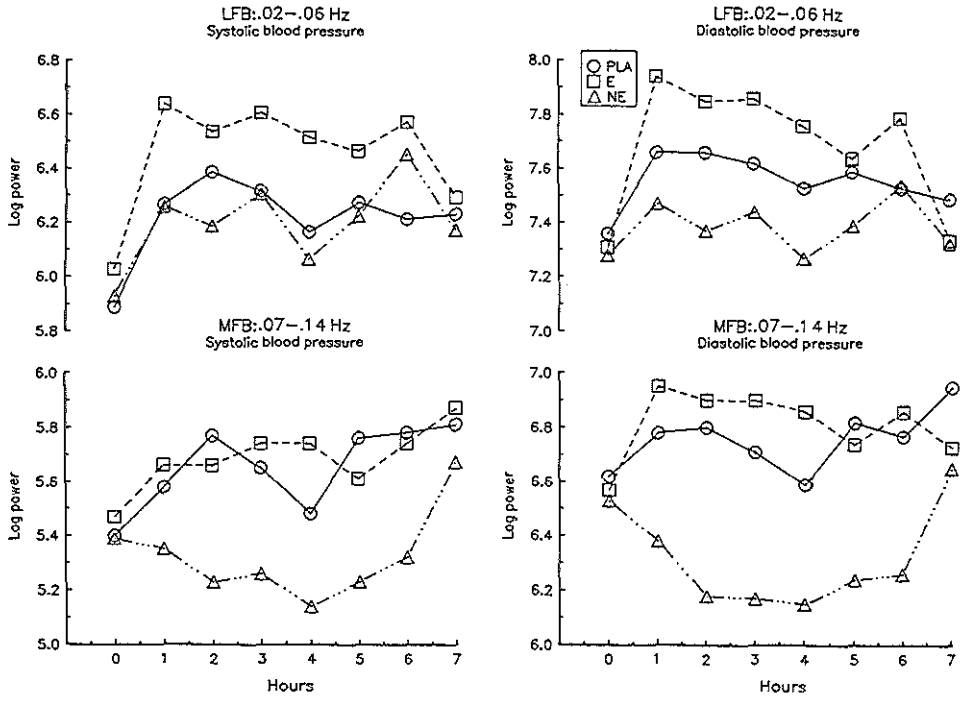

FIGURE 3
Mean logarithmic power values of the low (LFB) and mid frequency band (MFB) of SBP and DBP, before (0 hr), during (1-6 hr), and after (7 hr) infusion of PLA, E, and NE.

c. Stroke volume (SV) and Total Peripheral Resistance (TPR):
SV increased by 10.3% during infusion of E (mean±SEM; % change versus baseline: 10.2±3.4 %) and by 0.7% during infusion of NE (0.6±5.3 %), in comparison with PLA (-0.1±3.9 %; Figure 2). The increase in SV during infusion of E was significant in comparison with PLA ($p < .01$) and was present during the entire infusion period. On average, TPR decreased by 19.8% during infusion of E (mean±SEM; % change versus baseline:-11.1±4.2 %) and increased by 6.4% during infusion of NE (15.1±4.7 %), in comparison with PLA (8.7±5.9 %; Figure 2). The TPR effect during E was significantly different from TPR during PLA ($p < .001$). Although all subjects showed an increase in TPR during infusion of NE, this effect was not significant versus the responses observed during infusion of PLA. The TPR effects sustained during the entire infusion period. SV and TPR returned to baseline values within one hour after the infusions had been stopped; post-infusion values of TPR and SV were similar for the three conditions.

d. Baroreflex sensitivity (BRS):
BRS during infusion of PLA showed an average ratio of 19.4±2.1 ms/mmHg. During infusion of NE this ratio was similar (19.7±2.2 ms/mmHg), while during infusion of E BRS tended to be lower (16.8±1.7 ms/mmHg) (Table 3). However, this effect was not large enough to be significant versus PLA or versus NE. Post-infusion BRS levels were also similar for the three conditions.

Table 3. Mean (SEM) baroreflex sensitivity (BRS) before, during, and after 6 hr of infusion of PLA, E, and NE

	BRS (ms/mmHg)		
	PLA	E	NE
pre	18.1 (1.9)	17.0 (2.6)	17.8 (2.4)
1	22.1 (2.8)	18.4 (1.9)	20.4 (2.6)
2	18.6 (2.1)	17.4 (1.9)	19.9 (2.5)
3	18.4 (2.4)	16.4 (1.8)	20.0 (2.3)
4	19.8 (2.3)	15.9 (1.8)	20.4 (2.2)
5	19.5 (2.5)	15.9 (1.7)	18.5 (2.1)
6	18.0 (2.0)	16.5 (1.9)	19.1 (2.2)
post	16.4 (2.6)	14.4 (1.9)	15.7 (1.5)

Renin and Aldosterone

Mean(range) renin concentrations during infusion of E rose to 17.9(14.1-24.8) μU/ml and during infusion of NE to 17.3(12.0-24.8) μU/ml. Thus, compared with levels observed during PLA (11.6(8.4-15.6) μU/ml), E induced a 54% and NE a 49% increase in renin concentrations. Both increases were significant in comparison with PLA ($p < .001$) (Figure 1). Plasma aldosterone concentrations during infusion of E (mean$\pm$SEM: 70 ± 4 pg/ml) were increased significantly by 35% versus PLA (52 ± 4 pg/ml) ($p < .01$; Figure 1); during infusion of NE plasma aldosterone concentrations (61 ± 6 pg/ml) were not different from PLA. A significant time-dependent decline was present in plasma aldosterone concentrations during all infusions ($p < .001$). Post-infusion plasma renin concentrations after E and NE were significantly increased versus PLA ($p < .01$; Figure 1). Post-infusion levels of plasma aldosterone after infusion of E were significantly increased in comparison with PLA ($p < .01$; Figure 1).

Respiration

Infusion of E and NE caused a small decrease in respiratory cycle duration versus PLA: 3.87 and 3.87 versus 4.08 sec, respectively (i.e. 15.5 and 15.5 versus 14.7 cycles/min) (Table 4). These effects were too small to reach significance. Table 4 suggests an increase in inspiratory depth of 17% during infusion of E. However, this effect was too variable be significant versus PLA or NE (note the increase in SEM-values). Post-infusion levels of respiratory cycle duration and inspiratory depth also were similar for the three conditions.

Table 4. Mean (SEM) respiratory cycle duration and respiratory depth before, during, and after 6 hours of infusion of PLA, E, and NE

	Cycle (sec)			Depth (%b*100)		
	PLA	E	NE	PLA	E	NE
pre	4.2(.3)	4.0(.1)	3.9(.1)	100	100	100
1	4.1(.1)	3.9(.2)	3.9(.1)	93(9)	104(8)	97(5)
2	4.2(.2)	3.8(.2)	3.9(.1)	100(10)	116(17)	93(7)
3	4.1(.1)	3.9(.2)	3.9(.1)	100(6)	117(15)	91(9)
4	4.0(.1)	3.8(.2)	3.8(.1)	86(9)	119(15)	88(8)
5	4.0(.1)	3.8(.2)	3.8(.1)	95(14)	118(17)	93(9)
6	4.1(.2)	4.0(.2)	3.9(.1)	85(10)	118(12)	96(9)
post	3.9(.1)	4.2(.1)	4.1(.1)	102(18)	127(17)	98(11)

Discussion

Circulating catecholamines

Infusion of 15 ng/kg/min of E induced a 10-fold increase in arterial epinephrine concentrations, while infusion of 30 ng/kg/min of NE caused a 5-fold increase in arterial norepinephrine concentrations. These responses showed a time-dependent increment during the entire 6 hour infusion period, and disappeared within 5 min after the infusions were stopped. The catecholamine levels during the E and NE infusions were in the moderate to high physiological range. The corresponding venous catecholamine concentrations have been presented before (28,29); these levels were similar to those observed during real-life stresses (40) or mild to maximal physical exercise (41).

Hemodynamic effects

Infusion of E significantly increased HR (by 7%) and SV (10%) (stimulation of cardiac β1- and β2-adrenoceptors) and significantly decreased TPR (20%; stimulation of vascular β2-adrenoceptors), resulting in a decrease in DBP (9%). During infusion of NE, TPR showed a small increase (6%; stimulation of vascular α1- and α2-adrenoceptors), resulting in a significant increase in DBP (6%) and subsequently a decrease in HR (4%; baroreflex mediated slowing). At a dose level of 30 ng/kg/min, NE had no significant effect on SV or SBP. The HR and ABP responses are similar to other studies based on comparable doses of E or NE during short-lasting infusions or bolus-injections (42-44), although we did not observe a significant increase in SBP during infusion of E or NE. The SBP and DBP data showed a time-dependent increase during all infusions. This was not the case for the SV and TPR data: these effects were stable during the entire infusion period. Post-infusion levels were similar for the three conditions, which is in congruence with the catecholamine data.

Spectral analysis. During infusion of E and NE, distinctive effects on LFB and MFB fluctuations were observed for both SBP and DBP, as well as their variation coefficients. These effects were present from the first till the last hour of infusion and disappeared within one hour after the infusions had been stopped.

The increase in LFB perturbations in SBP and DBP during infusion of E probably is the combined result of an increase in SV and a decrease in TPR and DBP, allowing an increase in peripheral vasomotor fluctuations, which in turn leads to greater blood pressure fluctuations. This increase in vasomotor fluctuations was not translated into an increase in HR fluctuations, thereby confirming previous reports of a dissociation between ABP and HR variations at this frequency range (13). Infusion of E had no significant effect on MFB fluctuations in HR or ABP, nor did it influence BRS. These data confirm that

LFB fluctuations in ABP, but not in HR, reflect changes in peripheral vasomotor fluctuations.

Infusion of NE brought about a significant reduction in MFB fluctuations in SBP and DBP. The small increase in ABP during infusion of NE apparently was enough to activate a baroreflex mediated negative feedback mechanism which resulted in a suppression of ABP oscillations in the 0.1 Hz frequency range. This effect was also reflected in HR. BRS remained unaffected by infusion of NE. NE also had no significant effect on LFB perturbations in HR, SBP or DBP. Thus, our study shows, in accordance with pioneer research in this field (9,10), that it is useful and necessary to separate low frequency fluctuations in a range of 0.02-0.06 Hz from mid frequency fluctuations around 0.1 Hz, because these bands may represent different aspects of baroreflex mediated changes.

Spontaneous HFB fluctuations in HR, SBP and DBP were unaffected by the infusions of E and NE, thereby reflecting a minor role for vagal tone in the observed hemodynamic adjustments. The lack of a significant influence of E or NE on the respiratory parameters is in agreement with this finding. The absence of a significant effect of E and NE on spontaneous variability in HR extends the findings of Pomeranz et al. (45), who showed that in the supine posture and during a situation of standardized breathing, HR variations are primarily under parasympathetic control.

Renin and aldosterone

The renin-angiotensin-aldosterone system (RAAS) is considered to be a slow long-term blood pressure integral controller, yet it may also serve a purpose in the short-term control of cardiovascular homeostasis by controlling low-frequency fluctuations in peripheral vasomotor activity (4,5).

In this study, both infusions of E and NE induced sustained rises in plasma renin concentrations of about 50%. Therefore, the differences observed in cardiovascular variability between infusion of E and NE cannot be attributed to changes in plasma renin concentrations. Infusion of E significantly increased plasma aldosterone concentrations with 35%. The increase in LFB perturbations in SBP and DBP after infusion of E may, as a consequence, theoretically be in part the result of an effect of aldosterone. However, post-infusion levels of LFB, SV, and TPR were similar for the three conditions, while plasma aldosterone concentrations were still significantly increased one hour after the infusion of E had been stopped. This suggests that the increased LFB fluctuations may be specific for E, as a result of the effects of E on HR, SV and TPR, rather than through an effect on the RAAS.

Conclusions. Increased plasma concentrations of epinephrine and norepinephrine

within the high physiological range, which cause distinct but different hemodynamic effects, do not influence respiration patterns and baroreflex sensitivity. Spectral analysis shows that LFB fluctuations in ABP after epinephrine and MFB fluctuations after norepinephrine change in opposite directions. These effects were present from the first till the last hour of infusion and disappeared within one hour after the infusions had been stopped, indicating that no regulatory mechanisms counteracted the perturbations induced by a sustained infusion of low levels of E or NE. Since the responses could not be explained by the effects of the catecholamines on the RAAS, it suggests that norepinephrine and not epinephrine reduced sympathetic activity. Because no changes in HR variability or HFB activity of ABP were observed after either catecholamine, parasympathetic components probably played little role in the observed hemodynamic adjustments. Our data signify the need to separate LFB and MFB fluctuations in ABP under different experimental circumstances. Furthermore, our data also imply that changes in plasma catecholamines cannot be unequivocally labelled as indices of altered sympatho-adrenal control of cardiovascular homeostasis.

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Chapter 2.3

Urinary Excretion of Catecholamines and Their Metabolites in Relation to Circulating Catecholamines

Summary

Some depressed patients have been shown to excrete abnormal amounts of catecholamines and their metabolites in urine. Some studies suggest that hypersecretion of epinephrine by the adrenals and of norepinephrine by the peripheral sympathetic system cause increased excretion of urinary catecholamines and their metabolites in a subgroup of patients. To evaluate the effect of increased catecholamine levels in the peripheral circulation on urinary catecholamine and metabolite levels, we infused healthy volunteers during 6 hours with epinephrine, norepinephrine, or placebo, respectively, in a three-period, double-blind, cross-over design. The results indicate that (1) urinary epinephrine and norepinephrine levels were the most sensitive indicators of increased circulating epinephrine and norepinephrine levels, respectively; (2) changes in circulating epinephrine or norepinephrine levels were not readily reflected in changes in urinary vanillylmandelic acid or 3-methoxy-4-hydroxyphenylglycol levels; and (3) increased normetanephrine excretion was not only induced by infusion of norepinephrine but also by epinephrine. This last finding may be due to activation of the sympathetic nervous system by circulating epinephrine. These results may help to explain the mechanism of adrenal epinephrine and sympathetic nervous system norepinephrine hypersecretion observed in subgroups of depressed patients.

Introduction

In 1965, Schildkraut[1] suggested that depression may be caused by a functional deficit of norepinephrine (NE). Many different measures of catecholamine metabolism have been scrutinized in order to distinguish depressed patients from healthy controls or to differentiate among subtypes of depressive disorders[2-16]. Early studies reported that some depressed patients excreted low amounts of urinary 3-methoxy-4-hydroxyphenylglycol (MHPG), the metabolite which was believed to reflect metabolism of NE in the central nervous system[2,6,7,11]. More recently, the focus has been more on dysfunctions of peripheral noradrenergic

systems. A number of authors have reported increased plasma NE levels or spillover in subgroups of patients with affective disorders[8,9,12,14]. Also, urinary excretion patterns of catecholamines and their main metabolites have been reported in large groups of depressed patients and healthy controls[10,13,15,16].

Maas et al[13] and Davis et al[15] identified depressed patients excreting high levels of epinephrine (E), metanephrine (M), NE, and normetanephrine (NM). These and studies of Schildkraut et al[4,5] and Schatzberg et al[16] suggest that abnormalities in excretion of urinary catecholamines and a number of their metabolites are related. This is in line with high correlations between urinary catecholamines and their major metabolites found in healthy controls[10,17,18] and depressed patients[10,16,18,19]. However, the origin of the abnormalities in metabolites in patients remains obscure.

Changes in urinary excretion patterns could be due to changes in central or peripheral metabolism of catecholamines. 3-Methoxy-4-hydroxyphenylglycol is the main central metabolite of NE, but urinary excretion of MHPG is a weak indicator of central metabolism, because MHPG is also formed in the periphery and because a considerable proportion of MHPG of both origins is converted to vanillylmandelic acid (VMA) in the periphery. The other metabolites, M, NM, and VMA, as well as the catecholamines NE and E, cannot cross the blood-brain barrier and excretions in urine are therefore of peripheral origin. Another distinction can be made between extraneuronal and intraneuronal metabolism. It appears that urinary NE and its metabolite NM reflect to a certain extent extraneuronal metabolism and are sensitive to changes in sympathetic neuronal activity. Vanillylmandelic acid, the main metabolite of NE, is formed intraneuronally and probably reflects more closely the basal metabolism of NE[13,20-22]. Attention has been focused mainly on NE and the sympathetic nervous system, but abnormalities in E and adrenal activity are at least as important because of prominent abnormalities in some depressed patients[13,15].

The present study was designed to evaluate which changes in urinary excretion can be explained by increased concentrations of catecholamines in the peripheral circulation and to distinguish the role of circulating E and NE in such changes.

Methods

Subjects

The subjects were ten healthy male volunteers (mean age [$\pm$SD], 24$\pm$4 years; height, 182$\pm$9 cm; weight, 78.3$\pm$7.0 kg). They gave written informed consent to participate in the study. The protocol was approved by the Medical Ethical Committee of the University Hospital Rotterdam-Dijkzigt, the Netherlands. The

subjects received a medical examination, including laboratory screening and electrocardiogram, and a psychiatric examination to ensure physical and mental health. Also, Dutch versions of the Minnesota Multiphasic Personality Inventory[23] and the State-Trait Anxiety Inventory[24] revealed no abnormal values. Six subjects were nonsmokers, and none reported excessive drinking habits.

Procedure

Subjects had to keep a regular sleep-wake cycle and avoid abnormal physical or psychic activities. Before each experimental session, subjects had a standardized light breakfast without tea or coffee at 8 AM in the hospital, and they voided urine. At 8.15 AM, catheters were inserted into the antecubital veins of both arms. The brachial artery of the nondominant arm was cannulated with a Teflon catheter, introduced by the Seldinger technique, which was connected to a miniature transducer-perfusion device. The intra-arterial pressure signal and electrocardiogram were monitored continuously and recorded analogue on an instrumentation recorder.

From 9 AM until 5 PM, the subjects rested in a supine position and received a 5% dextrose solution, infused intravenously at a rate of 2 mL/min into the dominant arm. They did not eat, drink, or smoke during this period and were kept awake when they dozed off. From 10 AM to 4 PM, epinephrine (82 pmol/kg/min), norepinephrine (178 pmol/kg/min), or a physiological saline solution was infused into the dominant arm at a rate of 5.4 mL/h. Epinephrine and norepinephrine were diluted in physiological saline just before the infusion started. All three infusions were administered to each subject in a three-period, double-blind, randomized, cross-over design. Infusions were at least 10 days apart. The total amount infused during the 6-hour period was 2.31 ± 0.21 μmoles of epinephrine and 5.00 ± 0.45 μmoles of norepinephrine, respectively.

Sample collection

Every hour from 10 AM until 5 PM, a 10-mL venous and a 10-mL arterial blood sample, respectively, were obtained from the non-dominant arm (infusions were into the dominant arm). Samples were collected in chilled tubes containing 19 mg of ethylene glycol-bis[beta-aminoethyl ether] N,N,N^1,N^1-tetraacetic acid and 12 mg of glutathione[25]. Plasma was prepared within 30 min and stored at -70°C until assayed for catecholamines. Urine was collected in three portions: urine sample 1 collected during the infusion from 8 AM to 6 PM; urine sample 2 collected during the next night from 6 PM to 8 AM, which always included the morning voiding; and urine sample 3 collected during the next day from 8 AM to 6 PM. Urine was collected in polyethylene containers over 0.5 g of sodium edetic acid and 0.5 g of sodium metabisulfite and kept at 4°C in the dark[26]. After the collection was complete, aliquots were transferred

to a freezer at -80°C.

Biochemical assays

Catecholamines and metabolites were assayed by high-performance liquid chromatographic techniques, combined with electrochemical detection for determination of plasma catecholamines[25], urinary total MHPG[27], free VMA[28], and total metanephrine values, and combined with fluorescence detection and precolumn derivatization for determination of urinary free catecholamines[29].

Statistics

Differences between the infusion conditions in the urinary excretions of catecholamines and their metabolites were analyzed with repeated-measures analysis of variance, with infusion condition as within-subjects factor. Where appropriate, a square root transformation was applied in order to obtain normal distributions. The analyses were done separately for urine samples 1, 2, and 3 and the 24-hour excretion rates, which was computed by taking the sum of urine 1 and 2.

Differences in plasma catecholamine concentrations were analyzed with repeated-measures analysis of variance, with time and infusion condition as within-subject factors.

Results

Infusion of epinephrine resulted in a 10-fold increase in arterial and venous concentrations of E in the contralateral forearm (Figure). These increases are in the high physiological range and similar to those observed during public speaking[30] or maximal exercise[31,32]. The physiological reactions were moderate, consisting of a decrease of mean arterial blood pressure of 6.9% and an increase in heart rate of 6.8%[33,34]. Infusion of norepinephrine resulted in a 5-fold increase in arterial and a 3.5-fold increase in venous concentrations of NE (Figure). The physiological effects of this infusion were also moderate, consisting of an increase in mean arterial blood pressure of 6.6% and a decrease in heart rate of 3.9%[33,34].

No significant effect on NE plasma concentrations was observed during epinephrine infusion. Infusion of norepinephrine caused a slight but significant decrease in venous and arterial plasma E (both $p < .001$). Five minutes after the infusion was stopped, the catecholamine concentrations had returned to baseline values.

Infusion of norepinephrine resulted in an increase in excretion of NE to 352% in the urine collected during the infusion period (urine sample 1, Table 1). No increase was observed in urine samples collected after the infusion had

Table 1. Urinary catecholamines and their metabolites during and after 6-hour infusion of epinephrine or norepinephrine

	Infusion			
	Placebo	Norepinephrine	Epinephrine	Effect,p
E, nmol				
Urine 1,8-18 h	22.0±7.7(10)	21.0±9.6(10)	149±31.0(9)	<.001
Urine 2,18-8 h	20.1±12.8(10)	16.9±10.6(10)	23.4±10.5(10)	NS
Urine 3,8-18 h	18.9±11.6(9)	30.6±11.9(8)	30.0±11.1(10)	.013
M, nmol				
Urine 1,8-18 h	291±92(10)	298±67(9)	559±186(9)	<.001
Urine 2,18-8 h	325±192(10)	374±209(10)	556±219(10)	<.001
Urine 3,8-18 h	356±263(9)	326±105(8)	385±149(10)	NS
NE, nmol				
Urine 1,8-18 h	82.5±32.1(10)	290±60.3(10)	91.7±33.3(9)	<.001
Urine 2,18-8 h	159±48.3(10)	156±39.7(10)	154±48.9(10)	NS
Urine 3,8-18 h	128±62.6(9)	165±47.2(8)	171±62.0(10)	NS
NM, nmol				
Urine 1,8-18 h	415±106(10)	521±92(9)	518±143(8)	.054
Urine 2,18-8 h	559±165(10)	737±229(10)	743±221(10)	.050
Urine 3,8-18 h	647±397(9)	610±74(8)	699±280(10)	NS
MHPG, μmol				
Urine 1,8-18 h	6.05±2.98(10)	5.35±1.59(10)	4.88±1.15(9)	NS
Urine 2,18-8 h	8.67±3.46(10)	7.73±4.05(10)	7.12±3.95(10)	NS
Urine 3,8-18 h	7.48±5.75(9)	6.11±1.73(8)	6.51±2.79(10)	NS
VMA, μmol				
Urine 1,8-18 h	7.58±2.11(9)	8.40±1.35(10)	8.81±1.72(9)	NS
Urine 2,18-8 h	10.48±4.96(9)	11.20±4.04(10)	10.90±4.70(9)	NS
Urine 3,8-18 h	7.86±2.78(9)	9.29±2.71(8)	10.17±4.75(10)	NS
Creatinine,mmol				
Urine 1,8-18 h	6.9±1.15(10)	6.8±1.05(10)	7.1±1.55(9)	NS
Urine 2,18-8 h	8.2±2.49(10)	8.0±1.70(10)	8.5±2.53(10)	NS
Urine 3,8-18 h	7.1±2.97(9)	7.2±1.35(8)	7.6±2.61(10)	NS
Volume, L				
Urine 1,8-18 h	0.97±0.36(10)	0.95±0.44(10)	0.77±0.24(9)	NS
Urine 2,18-8 h	0.76±0.55(10)	0.58±0.34(10)	0.60±0.51(10)	NS
Urine 3,8-18 h	0.82±0.44(9)	0.77±0.50(8)	0.64±0.33(10)	NS

All values are expressed as mean±SD (number of subjects)

Table 2. Twenty-four-hour urinary excretion of catecholamines and their metabolites after 6-hour infusion of norepinephrine or epinephrine, and comparison with values reported by others

	Present study				Davis et al[15]		Koslow et al[10]	Linnoila et al[17]
	Placebo	Norepinephrine	Epinephrine	Effect,p	Controls	Depressed patiens	Healthy controls	Healthy controls
E#	0.04±0.01	0.04±0.01	0.17±0.04	<.001	0.05	0.07/0.16*	0.08±0.06	0.05±0.04§
M§	0.62±0.27	0.67±0.14	1.13±0.39	<.001	0.47	0.51/0.92*	0.54±0.22	0.79±0.34
NE#	0.24±0.07	0.45±0.05	0.25±0.08	<.001	0.15	0.21	0.16±0.08	0.57±0.21§
NM§	0.97±0.22	1.23±0.25	1.29±0.32	.024	0.60/1.23	0.86/2.14*	1.07±0.37	1.76±0.64
MHPG§	14.7±6.20	13.1±4.30	12.4±4.50	NS	9.6	10.2	12.2±4.60	12.2±3.20
VMA#	18.3±6.50	19.6±5.00	19.9±5.70	NS	14.8	18.3	13.8±7.30	21.1±6.60

NS indicates not significant. Values are in micromoles per day. Values of Koslow et al[10] were recalculated from their table 7, values of Davis et al[15] from their figs 1, 3, and 4.
#: Free (unconjugated); *: Bimodal distribution; §: Total (conjugated and unconjugated)

been stopped. Excretion of NM was increased by 26% in urine sample 1 and by 32% in urine sample 2. These effects of infusion were marginally significant for NM (Table 1).

The 24-hour excretion rate of both NE and NM were significantly increased (Table 2). The increase in NE plus NM excretion compared with placebo infusion amounted to 0.47 μmoles ([0.45-0.24]+[1.23-0.97] μmol; Table 2), which is 9.4% of the 5.00 μmoles of norepinephrine infused.

Infusion of epinephrine resulted in an increase in excretion of E to 677% in urine sample 1, but no increase was observed in urine sample 2. Excretion of M was increased by 92% in urine sample 1 and 71% in urine sample 2, respectively. Infusion of epinephrine also resulted in increased excretion of NM, ie, an increase of 25% in urine sample 1 and 33% in urine sample 2. The 24-hour excretion rates of E, M, and NM were significantly increased (Table 2). The increase in E plus M excretion compared with placebo infusion amounted to 0.64 μmoles ([0.17-0.04]+[1.13-0.62] μmol; Table 2), which is 36.1% of the 2.31 μmoles of epinephrine infused.

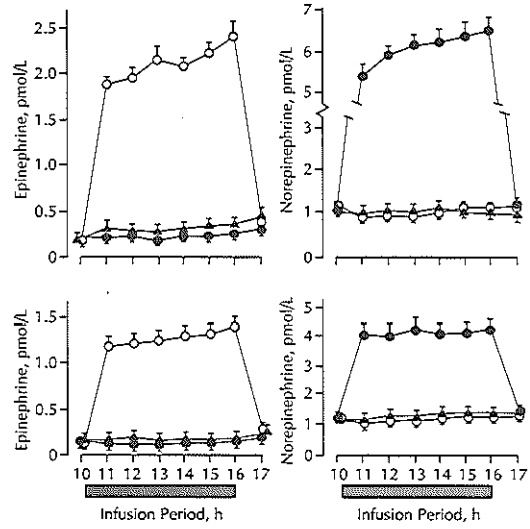

FIGURE
Mean and SEM values of arterial (top) and venous (bottom) plasma epinephrine and norepinephrine levels before, during, and after infusion of epinephrine (open circles), norepinephrine (closed circles), or placebo (closed triangles)

Unexpected was an increase of E excretion by 62% and 59% in urine sample 3, collected the day after the infusion, after norepinephrine and epinephrine infusion, respectively. The significance of this effect is as yet unclear. No significant effects of either infusion on excretion of MHPG, VMA, or creatinine were observed. With placebo infusion, excretion rates for E, M, NE, NM, MHPG and VMA were very similar to those reported by Koslow et al[10] for male, healthy controls and by Linnoila et al[17] and Davis et al[15] for healthy controls of both sexes (Table 2).

Comment

Infusion of norepinephrine resulted in increased urinary excretion of free NE and NM. Only 9.4% of the norepinephrine infused was recovered as NE and NM together in urine. Studies with infusion of tracer amounts of radiolabeled NE have indicated that a considerable portion of the norepinephrine infused is converted to VMA, the main metabolite of NE[22], but we did not find a significant effect on this metabolite. This may be due to the fact that the bulk of VMA excreted (18.3 μmol/d) originates from sources other than circulating NE, such as intraneuronal metabolism[20,22]. Changes in the small amount of VMA formed from circulating NE during the infusion may be obscured by this bulk of VMA. Urinary MHPG excretion was less during norepinephrine infusion, although not significantly so. This is in line with previous studies, which indicate that only small amounts of norepinephrine infused[22] or released[15] are excreted as MHPG. Thus, increased circulating NE resulting from infusion is most sensitively detected in urine by estimation of free NE and also of NM, but not of VMA or MHPG. This is similar to conclusions by other authors that urinary free NE[13,22,35] and total NM[20,21] are more sensitive indicators of sympathetic activity and resulting extraneuronal metabolism of NE than are VMA and MHPG[13,20-22,35].

Similar conclusions can be drawn with regard to infusion of epinephrine, which resulted in increased urinary excretion of E and M, but not of VMA or MHPG. Thus, increased circulating E is most sensitively detected in urine by estimation of E and also of M, but not of VMA or MHPG. These results lend support to the suggestion that proportionally strong increases in urinary E, NE, M and NM excretion are related to increased E and NE release in some depressed patients[13,15].

We also observed an increase in urinary excretion of NM due to infusion of epinephrine. Normetanephrine is formed from NE by the enzyme catechol-O-methyltransferase. We are not aware of studies indicating that NM can be formed from E, which would have to involve N-demethylation of M to form NM or of E to form NE. A more natural explanation would be an increase in

release of NE due to the epinephrine infusion, as has been reported previously[36,37]. It has been suggested that this increase in NE release is effected by E through stimulation of presynaptic ß2-receptors[36,37].

The subjects in the present study showed a sustained pressor response after the epinephrine infusion, which can be explained by enhanced release of NE[33]. The increase in NM excretion supports this hypothesis. Thus, we have physiological as well as biochemical indications of increased sympathetic activity due to epinephrine infused. In that case, one may have expected an increase also in urinary NE excretion and/or plasma or arterial levels of NE. Neither of these were observed in the present experiment. However, most of the NE released may not have reached the circulation[20]. It is conceivable that urinary NM is a more sensitive indicator of the increased sympathetic activity due to epinephrine infused than plasma or urinary NE.

These data may have implications for clinical monoamine research. For instance, significant correlations between urinary excretion rates of E, NE, M, and NM in depressed patients have been reported[10,16]. Our results can only be compared with those of studies with patients, if data on pure biological subgroups of patients are available. These are provided by Davis et al[15] on the basis of multivariate analyses. Those authors showed that a subgroup of depressed patients is characterized by increased urinary excretion of E, M, NE, and NM, jointly. Their results indicate that the hypersecretion of the adrenals (E) and peripheral sympathetic system (NE) are related and actually are based on the same underlying phenomenon[15].

On basis of our results, we hypothesize that hypersecretion of E may be the primary mechanism that leads to increased excretion of E and M and, secondarily, to amplified sympathetic activity with increased excretion of NM. The bimodal distribution of E and M in depressed patients, not present in healthy controls, and the absence of such a clear distinction in distribution patterns between patients and controls for NE and NM[15] to some extent supports our hypothesis of the primacy of E hypersecretion.

The present experiment involved infusion and not endogenous release of NE and E. It cannot be excluded that both these conditions cause different patterns of metabolism. With this in mind, our results have the following implications for measurement of urinary excretion rates of catecholamines and their metabolites: (1) free E and free NE are the most sensitive indicators of changes in circulating E and NE, respectively; (2) changes in circulating E or NE levels are not readily reflected in changes in urinary VMA or MHPG levels, probably because these changes are quantitavely unimportant compared to other metabolic origins of VMA and MHPG; and (3) changes in NM excretion rate can be due not only to changes in circulating NE but also to those in circulating E.

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CHAPTER 3

DOSE-RESPONSE EFFECTS OF INTRAVENOUS CLONIDINE

Chapter 3.1

Cardiovascular, Neuroendocrine, and Sedative Responses to Four Graded Doses of Clonidine in a Placebo-Controlled Study

Summary

Effects of four doses of the α_2-receptor agonist clonidine (CLO) (0.25, 0.5, 1 and 2 μg/kg IV) and placebo were studied in seven healthy men who volunteered in a double-blind randomized design in order to delineate possible presynaptic and postsynaptic components in the mechanism of action of CLO. Blood pressure, heart rate, plasma noradrenaline (NOR), plasma 3-methoxy-4-hydroxyphenylglycol (MHPG), plasma growth hormone (GH) and subjective sedation were monitored for a period of 1 hr following infusion of CLO. NOR and MHPG were analyzed also in urine, collected at 1 and 4 hr after the infusions. Dose-dependent decrements were observed in systolic and diastolic blood pressure and plasma NOR levels, and dose-dependent increases in subjective sedation and plasma GH. CLO did not influence plasma MHPG levels, whereas only urinary MHPG excretion was reduced 4 hr after infusion of 2 μg/kg CLO. Because no obvious differences between dose-response relations of plasma NOR (believed to be a presynaptic and peripheral effect), blood pressure (believed to be mainly a central presynaptic and postsynaptic effect) and subjective sedation (believed to be a central and probably postsynaptic effect) were observed, our results do not provide simple parameters to discern the multiple mechanisms of action of CLO. However, at a dose of 0.5 μg/kg CLO (a dose lower than that generally used) clear effects on plasma NOR, blood pressure, and sedation, but not on plasma GH (a central postsynaptic effect) or urinary MHPG (a presynaptic effect), were observed. When using CLO as a challenge test in psychiatric disorders, a design with 0.5 μg/kg CLO, in addition to the traditional 2 μg/kg CLO, may provide more information to characterize discrete abnormalities in the noradrenergic system at the level of the brainstem, the pituitary or the peripheral sympathetic nervous system.

Introduction

Administration of the α_2-receptor agonist clonidine is frequently used as a challenge test in psychiatric disorders such as panic disorder or depression in order to search for specific abnormalities in the noradrenergic system (Charney and Heninger 1986; Nutt 1989; Uhde et al 1989; Checkley et al 1981; Dolan and Calloway 1986). After a single intravenous dose of 1.5 or 2 μg/kg, clonidine induces hypotension, bradycardia, sedation, a reduction in plasma 3-methoxy-4-hydroxyphenylglycol (MHPG) levels, and an increase in growth hormone (GH) secretion (e.g., Nutt 1989). When compared with normal individuals, both patients with panic disorders or depression show altered responses following clonidine administration, which have been taken to signify an abnormal regulation of noradrenergic receptor function in subgroups of patients (Charney and Heninger 1986; Checkley et al 1981). The mechanisms underlying these effects are, however, unclear. Presumably, low doses of clonidine induce selective presynaptic α_2-receptor activation, whereas higher doses of clonidine activate both presynaptic and postsynaptic α_2-receptors, and still higher doses may affect α_1-receptors as well (Anden et al 1976). Furthermore, it is important (but difficult) to separate the central and peripheral actions of clonidine, as the drug rapidly penetrates the central nervous system due to its lipophilicity. Both plasma noradrenaline (derived from peripheral sympathetic nervous system activity) and plasma free MHPG (derived from both central and peripheral sources) (Kopin et al 1983) decrease after clonidine administration; probably this reflects stimulation of central brainstem inhibitory presynaptic α_2-adrenergic receptors (Nutt 1989). The sedation, bradycardia, and GH response probably reflect postsynaptic central α_2-receptor activation (Checkley et al 1981; Nassif et al 1983; McWilliam and Meldrum 1983), whereas clonidine reduces blood pressure by an effect on both presynaptic and postsynaptic α_2-adrenergic receptors in the brain and spinal cord (Nutt 1989; Brown and Harland 1984).

These complex α_2-adrenergic peripheral and central as well as presynaptic and postsynaptic effects of doses of clonidine in the 1.5-2 μg/kg range (Aghajanian 1984) may partially explain the lack of correlation between the different effects observed (Charney and Heninger 1986). However, the response patterns of the different parameters are not only time-dependent (Brown et al 1984), they also may vary with different doses of clonidine. We therefore studied the dose-response relationships of clonidine, employing doses lower than previously reported and explored whether it is possible to discriminate dose-dependent clusters or patterns in responsivity. In this study we covered a dose range of 0.25-2 μg/kg clonidine, administered intravenously, in a placebo-controlled randomized design in healthy volunteers with monitoring of

cardiovascular, neuroendocrine, and psychological parameters.

Methods

Subjects

Seven men with a mean age of 23.4 years (range 21-27 years) and a mean body weight of 76.4 kg (range 69-83 kg) participated in five experimental sessions each, after having given written informed consent. The subjects were paid volunteers, recruited from a student population by means of advertisement. The selection procedure included a comprehensive medical (medical history, hematologic and biochemistry panels, electrocardiogram) and psychological (personality questionnaires) screening to exclude subjects with medical or psychiatric illnesses, including alcohol or drug (ab)use. Two days before the first session and the following days until their last session, the subjects were requested to keep a regular sleep-wake schedule and to avoid abnormal physical or psychic exertions. The experimental procedures were approved of by the Medical Ethical Committee of the University Hospital Rotterdam Dijkzigt.

Design and Procedure

Each subject participated in five experimental sessions during which he received, in a double-blind randomized design, the following doses of clonidine (CLO) intravenously: 0 μg/kg, 0.25 μg/kg, 0.5 μg/kg, 1 μg/kg, or 2 μg/kg. CLO was diluted in 10cc saline and injected slowly over a period of 10 min through an indwelling catheter in the antecubital vein of the forearm.

The five sessions were held on separate days, at least 2 days apart. To avoid hypoglycemic effects on the catecholaminergic system, the subjects were asked to take their normal breakfast on each study day, without coffee or tea. Before the start of each session, the subjects were requested to void urine and drink a glass of mineral water. Coffee, tea and smoking were not allowed before or during the recordings. Each experimental session was performed between 10 and 12 AM. During this period hemodynamic, biochemical, and psychological measurements were obtained while the subjects rested comfortably in a semi-recumbent position in bed. At the end of each session, urine was collected for assay of catecholamines and MHPG. Following a standard light lunch, the subjects remained the afternoon in the hospital in a room where they could relax. Urine was again collected at 3 PM. At 3:30 PM the subjects were allowed to leave, after approval by the physician.

Measurements

Biochemical. Thirty min prior to the start of each session a catheter (Venflon, 18G, Viggo AB, Helsingborg, Sweden) was inserted in the antecubital vein of

the forearm, through which blood samples were drawn and CLO was administered. Blood samples were obtained after a baseline period of 20 min and at 15, 30, 45 and 60 min following the end of the infusion. Blood for assay of catecholamines (8 ml) was collected in chilled heparinized tubes containing 12 mg gluthatione. Blood samples were immediately centrifuged at 4°C and plasma was stored at -70°C. Catecholamines were assayed by means of high-performance liquid chromatography with fluorimetric detection (van der Hoorn et al 1989). Venous blood (4 ml per sample) was obtained in heparinized tubes, centrifuged, and frozen at -20°C for assay of growth hormone by means of an immunoradiometric assay (Euro-diagnostics BV, Apeldoorn, The Netherlands). Blood for assay of plasma MHPG (4 ml) was collected in siliconized tubes containing ethylenedinitrilo tetraacetic acid. MHPG was extracted using a slight modification of the procedure described by Moleman and Borstrok (1982) with iso-MHPG as internal standard. Quantification was done by means of high-performance liquid chromatography with electrochemical detection. Urine was collected in polyethylene containers with 0.5 g Na_2EDTA and 0.5 g $Na_2S_2O_5$ and kept at 4°C in the dark. After the session was completed, aliquots were transferred to a freezer at -70°C. Urinary catecholamines and MHPG were assayed by means of high-performance liquid chromatographic techniques with fluorescence detection and precolumn derivatization (van der Hoorn et al 1991; Moleman and Borstrok 1982).

Haemodynamic. During the session EKG and blood pressure were monitored continuously and recorded analogue on a Racal instrumentation recorder for off-line computer analyses. The EKG was derived using a precordial lead, amplified by a Nihon Kohden polygraph. Blood pressure was monitored and recorded by means of a servo-plethysmo-manometer for continuous, noninvasive measurement of finger arterial blood pressure, employing the volume clamp technique of Penaz (Penaz et al 1976; Wesseling et al 1982; Settels and Wesseling 1985) (Finapres 2300 NIBP monitor, Ohmeda, Englewood, Colorado, USA). Finger arterial blood pressure was measured via a finger cuff containing a photoplethysmographic volume transducer and inflatable air bladder. With this device a good estimation can be obtained of the arterial blood pressure measured invasively in the brachial artery. Details of measurement and analyses of the cardiovascular signals have been described before (Tulen et al 1991). Mean heart rate (HR) and mean systolic and diastolic blood pressure (SBP, DBP) were calculated over five periods: a 10-min period prior to the infusion of CLO (baseline) and 4 consecutive 10-min periods between the collection of the blood samples at 15, 30, 45 and 60 min after the infusion of CLO.

Psychological. Subjective mood was assessed directly before and one hour after the administration of CLO by means of two self rating questionnaires: a

shortened version of the Profile Of Mood States (POMS) (McNair et al 1971), which has been validated for the Dutch population (Wald and Mellenbergh 1990) and a Dutch translation of the Stanford Sleepiness Scale (SSS) (Hoddes et al 1973). In addition, every 15 min the subjects filled in Visual Analogue Scales (VAS) (based on Bond and Lader 1974) in order to assess time-dependent subjective changes before and after administration of CLO. The subjects rated their feelings on four 100 mm lines with the following dimensions: alert-drowsy, calm-excited, relaxed-tense and happy-sad. The VAS were scored in mm from the lefthand side of the 100 mm line to the mark made by the subject, corresponding to his state of feeling at that time. Only the results of the fatigue and vigor subscales of the POMS, the Stanford Sleepiness Scale and the visual analogue scale 'alert-drowsy' are presented, as indices of subjective sedation.

Statistical analyses

Data are presented as mean ($\pm$SD) for N=7, based on the absolute values per period. The growth-hormone data are presented for N=6 because of irregular baselines and responses in one subject during 4 of the 5 sessions. For the cardiovascular and plasma noradrenaline (NOR) data, response percentages relative to baseline values were calculated in order to correct for differences in baseline values. Difference scores versus baseline were calculated for the growth-hormone data and the VAS 'alert-drowsy'. Multivariate analyses of variance (MANOVA) for repeated measurements were used to evaluate the effect of the five doses of clonidine (within-subject factor: DOSE), the time dependency of the response profiles within a postinfusion period of 1 hr (within-subject factor: TIME), interaction effects between factors DOSE and TIME and the presence of between-subject effects. Duncan's multiple range test was used in order to analyze specific dose or time effects when a significant interaction was observed. A p-value of <0.05 was used to indicate a significant effect. For the SSS and the vigor and fatigue subscales of the POMS, Friedman analyses of variance (Siegel 1956) were used.

Results

Cardiovascular effects

Table 1 presents the absolute values of HR, SBP, and DBP as observed for a period of 1 hr after infusion of CLO. The baseline values of HR, SBP, and DBP were similar for the five sessions. Figures 1-3 show the percentage change relative to baseline to each of the infusions, for HR, SBP, and DBP, respectively.

Heart rate. Based on the absolute data, no significant DOSE effect on HR was

observed, but significant TIME and interaction effects (DOSExTIME) were present (Table 2). A significant DOSE effect was present for the HR responses (table 2, Figure 1). HR first showed a small (3%) increase 15 min after infusion

Table 1. Mean (SD) values of heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), plasma noradrenaline (NOR) levels, plasma growth hormone (GH) levels and drowsiness, before and after the infusion of clonidine

	CLO		Infusion			
	μg/kg	baseline	15 min	30 min	45 min	60 min
HR	0.00	66(11)	64(9)	64(8)	62(8)	61(7)
(bpm)	0.25	63(8)	63(6)	62(5)	61(5)	59(6)
	0.50	64(9)	66(10)	64(8)	62(8)	60(8)
	1.00	63(11)	64(10)	64(9)	63(10)	62(8)
	2.00	67(12)	69(12)	66(12)	63(11)	62(11)
SBP	0.00	124(19)	123(18)	123(17)	125(19)	126(20)
(mmHg)	0.25	119(16)	115(15)	113(12)	113(14)	116(16)
	0.50	125(22)	117(18)	113(20)	115(20)	119(11)
	1.00	116(20)	106(11)	106(11)	104(12)	108(11)
	2.00	124(24)	106(15)	98(12)	98(10)	98(14)
DBP	0.00	61(13)	62(11)	62(9)	64(11)	65(10)
(mmHg)	0.25	58(7)	58(5)	57(3)	57(4)	60(5)
	0.50	65(19)	63(16)	60(16)	62(16)	65(18)
	1.00	57(12)	54(6)	53(6)	52(5)	54(5)
	2.00	64(18)	58(14)	53(11)	53(10)	55(12)
plasma NOR	0.00	249(102)	209(58)	208(54)	212(57)	226(63)
(pg/ml)	0.25	169(40)	161(47)	164(36)	166(43)	184(60)
	0.50	187(39)	158(29)	153(38)	151(31)	191(74)
	1.00	175(23)	136(28)	127(28)	132(26)	135(21)
	2.00	176(39)	110(24)	103(27)	100(20)	100(23)
plasma GH	0.00	1.1(2)	1.1(2)	0.7(1)	0.5(1)	0.6(1)
(ng/ml)	0.25	0.5(1)	1.1(2)	1.1(2)	1.0(2)	1.0(2)
	0.50	0.5(1)	0.7(1)	1.9(3)	2.9(5)	2.4(4)
	1.00	1.4(3)	3.2(6)	5.8(7)	6.8(8)	6.1(8)
	2.00	0.7(1)	3.7(4)	6.6(6)	7.3(8)	6.1(8)
Drowsy	0.00	32(6)	33(19)	31(17)	31(19)	26(15)
(mm)	0.25	27(4)	26(9)	29(10)	29(14)	26(13)
	0.50	39(8)	48(21)	48(22)	50(20)	46(20)
	1.00	34(5)	50(18)	51(21)	49(22)	50(21)
	2.00	38(10)	71(23)	74(23)	76(22)	76(19)

Table 2. F-values and level of significance of MANOVA on HR, SBP, DBP, plasma NOR, plasma GH and subjective drowsiness, concerning the factors Dose, Time, their interaction and the between-subjects effects

	MANOVA F-values			
Parameter	Dose	Time	Dose x Time	Subject
HR	1.0	24.3***	2.3**	429.2***
HR response	4.4**	23.1***	2.1*	1.3
SBP	4.6**	3.9*	2.6**	649.3***
SBP response	17.1***	3.5*	2.3*	27.3**
DBP	2.2	3.2*	2.5**	424.0***
DBP response	11.8***	2.5	2.0*	0.9
NOR	15.3***	2.9	1.9*	189.2***
NOR response	31.9***	3.1*	2.1*	57.8***
GH	3.6*	1.2	1.8	4.7
GH response	4.2**	1.2	1.8	3.6
Drowsy	15.8***	0.4	0.4	81.7***
Drowsy response	9.6***	0.4	0.4	10.3*

The analyses were performed both on the absolute data and on the responses versus baseline. *:$p<0.05$; **:$p<0.01$; ***:$p<0.001$

of 0.5, 1 or 2 μg/kg CLO and then declined (Figure 1). After placebo (0 μg/kg) or 0.25 μg/kg we observed a gradual decrease in HR during the whole session.

Blood pressure.

SBP: A significant DOSE effect, as well as significant TIME and interaction effects were observed for SBP (Table 2). A dose-dependent SBP response was observed 15 min after infusion of CLO (Figure 2). A significantly larger decrease (20%) in SBP was observed with 2 μg/kg compared to 1 or 0.5 μg/kg CLO (8-9% decrease). The decreases observed after a dose of 1 or 0.5 μg/kg were significantly different from 0 μg/kg.

DBP: No significant DOSE effect was observed on the absolute DBP data, but significant TIME and interaction effects were present (Table 2). However, a significant DOSE effect on the DBP response was present at 15 min after

infusion of CLO (Figure 3; Table 2). The decrease in DBP 30 min after infusion of 2 μg/kg was significantly larger than the decrease observed after 1 or 0.5 μg/kg CLO. There was a gradual return to baseline values within 60 min after infusion of 1 or 0.5 μg/kg CLO (Figure 3). The lowest dose of 0.25 μg/kg CLO was not significantly different from placebo (0 μg/kg).

Table 3. Urinary excretion of NOR and MHPG, 1 and 4 hr after the infusion of CLO

Urinary excretion (metabolite/creat)		Collection periods (1) 10-12 AM	(2) 12-3 PM
	CLO μg/kg		
NOR	0.00	2.5 (0.8)	3.0 (1.0)
	0.25	2.4 (0.7)	2.7 (0.8)
	0.50	2.2 (0.6)	2.3 (0.7)
	1.00	2.0 (0.4)	2.0 (0.6)
	2.00	1.9 (0.6)	1.7 (0.7)
F-values	Dose	3.5*	19.0***
	Subject	94.3***	58.8***
	CLO μg/kg		
MHPG	0.00	0.86 (0.2)	0.95 (0.3)
	0.25	0.81 (0.3)	0.90 (0.3)
	0.50	0.92 (0.2)	0.92 (0.2)
	1.00	0.84 (0.3)	0.83 (0.3)
	2.00	0.85 (0.2)	0.71 (0.2)
F-values	Dose	0.9	3.7*
	Subject	102.2***	92.7***

Data are presented as mean (SD). F-values and level of significance of the factor Dose and the between-subject effects of the analyses of variance are also indicated.
*:$p<0.05$; **:$p<0.01$; ***:$p<0.001$

Plasma and urinary noradrenaline and MHPG

Plasma. Baseline values of the plasma NOR levels were not different for the five sessions (Table 2). Significant DOSE and interaction effects were observed (Table 2): plasma NOR levels showed a dose-dependent decrease 15 min after the infusions (Figure 4). All doses but 0.25 μg/kg induced significant reductions in plasma NOR levels compared to 0 μg/kg or baseline. All differences in responses to 2, 1, 0.5 and 0 μg/kg were significant. Both absolute MHPG levels and MHPG responses failed to show effects of CLO (data not presented).

Urine. NOR excretion decreased with increasing doses of CLO during both collection periods. The effect was larger during collection period 2 (Table 3). During collection period 1, only the differences between 0 and 1.0 μg/kg CLO or more were significant. During collection period 2, graded dose-response effects were apparent: the differences between the responses to 2, 1, 0.5 and 0 μg/kg CLO were significant. During collection period 2 infusion of 2 μg/kg CLO caused a significant decrease in MHPG excretion versus placebo (0 μg/kg) (Table 3).

Table 4. Mean (SD) preinfusion and postinfusion values of the SSS and fatigue and vigor subscales of the POMS

CLO	SSS		Fatigue		Vigor	
μg/kg	Pre	Post	Pre	Post	Pre	Post
0.00	1.6(1.0)	1.9(1.1)	0.7(1.1)	0.9(0.9)	13.6(3.1)	13.0(4.5)
0.25	1.7(0.8)	2.3(1.1)	0.3(0.5)	0.6(1.0)	13.4(4.0)	12.4(4.0)
0.50	1.7(1.0)	3.1(0.7)	1.3(1.8)	2.7(2.1)	13.0(4.3)	9.9(2.3)
1.00	2.0(1.2)	3.7(1.3)	1.6(2.2)	2.3(1.8)	11.3(4.5)	8.6(4.5)
2.00	2.1(1.1)	5.4(1.1)	1.4(1.9)	5.7(3.9)	12.1(4.0)	5.6(4.1)
Friedman						
X^2	2.2	20.1***	4.9	15.0**	4.5	15.7**

Friedman analyses of variance were performed on the preinfusion and postinfusion values separately. Chi-square values and level of significance are indicated.
**:$p<0.01$; ***:$p<0.001$

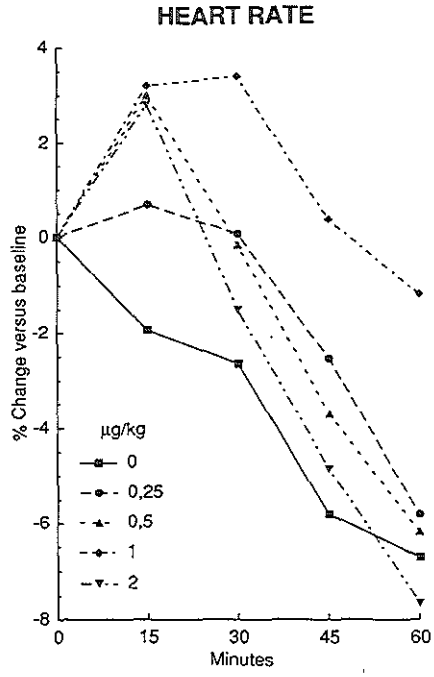

FIGURE 1
Mean percentage of changes in heart rate versus baseline during a period of 1 hour following the infusion of 0, 0.25, 0.5, 1, or 2 μg/kg clonidine.

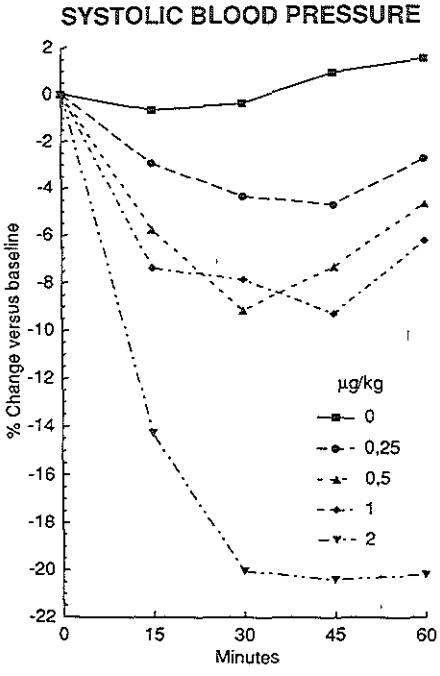

FIGURE 2
Mean percentage of changes in systolic blood pressure versus baseline during a period of 1 hour following the infusion of 0, 0.25, 0.5, 1, or 2 μg/kg clonidine.

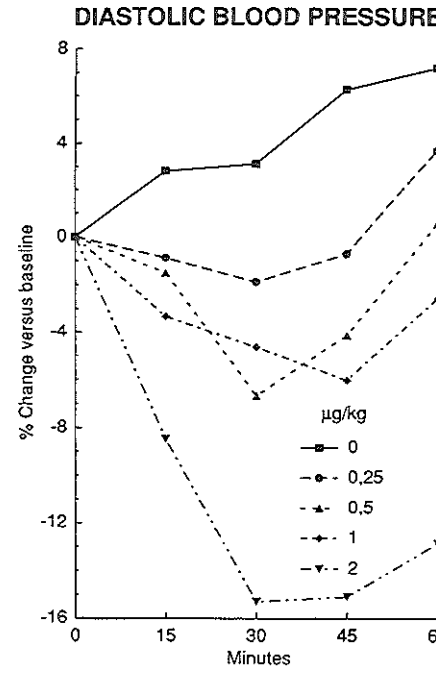

FIGURE 3
Mean percentage of changes in diastolic blood pressure versus baseline during a period of 1 hour following the infusion of 0, 0.25, 0.5, 1, or 2 μg/kg clonidine.

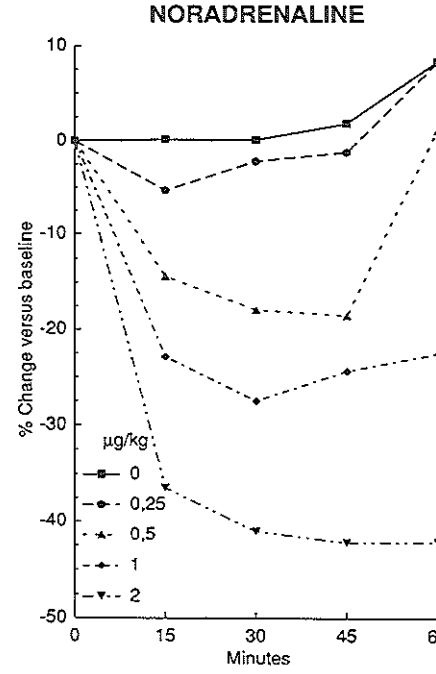

FIGURE 4
Mean percentage of changes in plasma noradrenaline levels versus baseline during a period of 1 hour following the infusion of 0, 0.25, 0.5, 1, or 2 μg/kg clonidine.

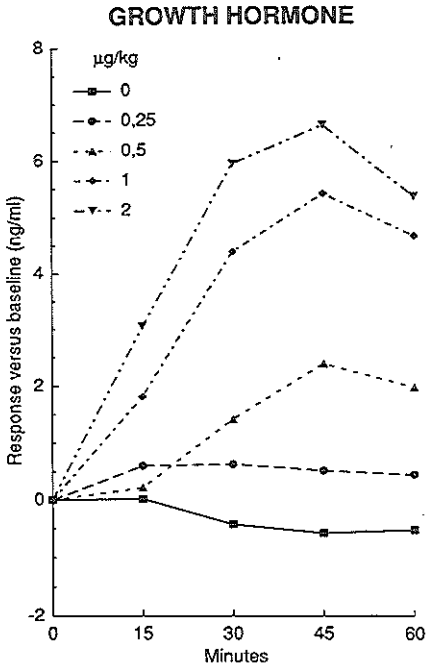

FIGURE 5
Mean responses (in ng/ml) versus baseline of plasma growth hormone levels during a period of 1 hour following the infusion of 0, 0.25, 0.5, 1, or 2 µg/kg clonidine.

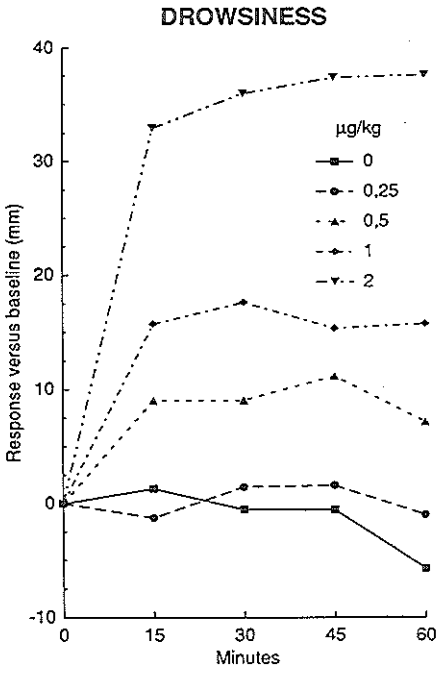

FIGURE 6
Mean responses (in mm) versus baseline of the self-ratings for sedation on the visual analogue dimension "alert-drowsy" during a period of 1 hour following the infusion of 0, 0.25, 0.5, 1, or 2 µg/kg clonidine.

Plasma Growth Hormone

Preinfusion levels of growth hormone were similar for the five sessions. A significant DOSE effect was observed for both the absolute growth-hormone data and the data relative to baseline (Tables 1,2; Figure 5), but no TIME or interaction effects: 15 min after infusion of CLO dose-response effects were observed. After infusion of 1 or 2 μg/kg CLO there was a significant increase in plasma growth hormone as compared to placebo (0 μg/kg). Responses to 1 and 2 μg/kg CLO were not different from each other. The lower doses of 0.25 and 0.5 μg/kg CLO did not induce a significant increase in plasma growth hormone levels.

Subjective sedation

Preinfusion values of the SSS and the fatigue and vigor scales of the POMS were similar for the five sessions. Significant DOSE effects were observed for all parameters (Table 4). There was an increase in sedation and a decrease in vigor with increasing dose of CLO. Within-session analyses (prevalues versus postvalues) revealed a significant increase on the SSS after 0.5 μg/kg CLO or more (Wilcoxon tests; $p < .05$), whereas fatigue showed a significant increase only after 2 μg/kg CLO and vigor decreased significantly after 1 μg/kg CLO or more. Between-session comparisons of the postinfusion values indicated a significant increase from 0 to 0.5 μg/kg CLO or more on the SSS, a significant increase from 0.5 to 2 and from 1 to 2 μg/kg CLO. Between-session comparisons of the postinfusion values on the fatigue scale showed a significant increase from 0 to 1 or 2 μg/kg CLO, whereas vigor decreased significantly from 0 to 2 μg/kg CLO.

The effects of CLO on drowsiness as assessed with the VAS 'alert-drowsy' are presented in Table 1 and the responses are presented in Figure 6. Baseline values were similar for the five sessions. A significant DOSE effect was present, but no TIME and interaction effects (Table 2). Fifteen min after infusion of CLO stable dose-dependent effects were observed (Figure 6): the response to 2 μg/kg was significantly larger than that to 0.5 or 1 μg/kg, both of which were significantly larger than that to 0.25 or 0 μg/kg CLO.

In summary, 15 min after infusion of CLO, significant dose effects were observed, indicating a significant increase in VAS drowsiness after a dose of 0.5 μg/kg CLO or more. This was confirmed by the results of the SSS. Subjective fatigue increased and vigor decreased significantly after a dose of 1 μg/kg or more. The dose of 0.25 μg/kg CLO had no significant sedative properties.

Discussion

Cardiovascular activity

Blood pressure. The dose-dependent effects of CLO on SBP and DBP confirm to some extent the findings of Brown et al (1990). These authors observed no differences in SBP response to a dose of 1.4 or 2.1 μg/kg CLO, but responses to both doses were significantly larger than the SBP response to 0.7 μg/kg CLO. We observed significant SBP and DBP responses to 0.5 μg/kg and higher doses. There was no difference in SBP response to a dose of 0.5 or 1 μg/kg CLO, but there was a significant difference in response between these doses and 2 μg/kg CLO. The data relative to baseline indicated a similar dose-response pattern for SBP and DBP; the response magnitudes were larger for SBP than for DBP, as observed in various other studies for doses in the 1.5-2 μg/kg range, whereas the maximal effect was delayed for DBP in comparison with SBP.

Heart rate. The small increase in HR 15 min after the infusion of 0.5, 1 or 2 μg/kg CLO may be compensatory to the fall in blood pressure, via activation of the baroreflex. This compensatory rise in heart rate has not been observed in other studies (Harron et al 1985; Wing et al 1977; Nutt 1986). The bradycardia as observed in several studies 1-1.5 hr after a dose of 1.5-2 μg/kg CLO (Charney and Heninger 1986; Siever and Uhde 1984) was not confirmed in this study. We observed a gradual decrease in HR to all doses, including placebo. This decrease could be interpreted erroneously as a clonidine-induced bradycardia in studies without a placebo control.

Noradrenergic function

Plasma and urinary noradrenaline. With the exception of 0.25 μg/kg CLO, the responses to the consecutive doses of CLO were all significantly different from each other (2 μg/kg < 1 μg/kg < 0.5 μg/kg < 0 μg/kg) for plasma NOR levels and urinary NOR excretion 4 hr after infusion. Plasma NOR levels already showed this response pattern 15 min after administration of CLO. The response magnitudes in urinary NOR excretion observed 4 hr after administration of CLO showed a pattern similar to the plasma NOR levels during the 1st hr postinfusion. The 40% decrease in plasma NOR levels after a dose of 2 μg/kg CLO is quantitatively similar to findings of studies using comparable doses (Murphy et al 1984; Veith et al 1984; Siever et al 1984).

Plasma and urinary MHPG. We observed no effects on plasma free MHPG levels within a period of 1 hr after CLO. Other studies have found a 10%-20% reduction of plasma free MHPG levels following a dose of 1.5-2.5 μg/kg CLO in normal controls (Siever et al 1984; Nutt and Molyneux 1986; Charney and Heninger 1986). Some have found these effects within 15 min after administra-

tion of CLO (Nutt and Molyneux 1986), others after 1 hr (Siever et al 1984; Charney and Heninger 1986), whereas effects were still present 3-4 hr after infusion of CLO, in comparison with placebo (Charney and Heninger 1986). These responses were small and were highly dependent upon baseline levels. Plasma MHPG may not be a sensitive indicator of α_2-adrenoceptor mediated NOR release (Scheinin et al 1991). However, 4 hr after administration of 2 μg/kg CLO, we found a significant 25% reduction in urinary MHPG excretion versus 0 μg/kg, which may indicate that urinary MHPG is more sensitive than plasma MHPG for this purpose.

Growth hormone

Our growth-hormone data necessitate a cautious interpretation. A light breakfast was allowed in this study, whereas reliable assessment of growth hormone levels normally requires an overnight fast. Nevertheless, our results are similar to those obtained by Brown et al (1990), who observed increases in growth hormone after 0.7 μg/kg CLO and higher doses.

Sedation

We observed sustained sedative effects 15 min after administration of CLO. Other studies reported gradual increases in sedation within a period of 30 min (Brown et al 1990) and/or maximal responses after 1 hr (Checkley et al 1981). The response magnitudes as observed in this study to 0.5, 1, or 2 μg/kg CLO are somewhat higher than those observed by Brown et al (1990) to 0.7, 1.4, or 2.1 μg/kg CLO. Differences in baseline values may be reponsible for this effect.

Presynaptic versus postsynaptic adrenergic effects

We attempted to delineate the presynaptic and postsynaptic components in the mechanisms of action of CLO by evaluating time-dependent response profiles to graded doses in a placebo-controlled design. For that purpose different parameters were analyzed, which are suggested to be regulated by different central and peripheral or presynaptic and postsynaptic noradrenergic mechanisms in which α_2-adrenoceptors are involved.

The hypotensive effect of CLO is thought to be regulated for the major part by presynaptic and postsynaptic α_2-adrenergic receptors at the level of the brain and the spinal cord (Kobinger and Pichler 1976; Zandberg et al 1979; Brown and Harland 1984). Our data indicate that these mechanisms already become activated at very low doses of CLO (0.5 μg/kg) within a short time interval (15 min) after administration.

Plasma and urinary NOR concentrations were used as an index of peripheral sympathetic nervous activity. The reduction in peripheral sympathetic

nervous activity after administration of CLO is due to a suppression of plasma NOR appearance rate (and not NOR clearance rate) (Veith et al 1984) and can be influenced by stimulation of central brainstem presynaptic α_2-adrenergic receptors (Schmidt et al 1967; Svensson and Usdin 1978) and by stimulation of presynaptic α_2-adrenergic receptors present on peripheral postganglionic sympathetic nervous system nerves (Langer 1981). Several studies underline the presence of a peripheral component in the CLO-induced fall in NOR levels (Brown and Harland 1984; Murphy et al 1984; Scheinin et al 1991). These effects of CLO to decrease central noradrenergic firing (Svensson and Usdin 1978) and sympathetic outflow (Schmidt et al 1967) resulted in effects on plasma and urinary NOR excretion that were clearly dose-dependent. Plasma NOR levels already respond within 15 min after infusion of 0.5 μg/kg CLO.

The growth-hormone response to clonidine represents an established central postsynaptic adrenoceptor effect (Charney and Heninger 1986): clonidine acts on postsynaptic α_2-adrenergic receptors in the hypothalamus to stimulate growth-hormone secretion. This mechanism is activated only at doses above 0.5 μg/kg CLO.

Sedation is mediated by central α-adrenergic receptors. Clonidine acts on the locus coeruleus to affect sleep and arousal mechanisms (De Sarro et al 1987), probably by affecting postsynaptic α_2-adrenoceptors (Nassif et al 1983). Our data suggest that these central postsynaptic α_2-adrenoceptor effects of CLO can be measured within 15 min after administration of a dose of 0.5 μg/kg CLO and higher, although changes in subjective fatigue and vigor only were apparent after a dose of 1 μg/kg CLO or more.

The major noradrenaline metabolite MHPG may reflect both central and peripheral noradrenergic activity (Kopin et al 1983; Maas et al 1968). The decrease in MHPG levels after administration of CLO is presumed to be a function of the action of CLO on central brainstem α_2-adrenergic receptors (Cederbaum and Aghajanian 1977). In this study effects on MHPG excretion were observed only 4 hr after infusion of 2 μg/kg CLO. The response pattern of this parameter clearly distinguished itself from the other parameters, which already responded to a minimal dose of 0.5 μg/kg CLO. This indicates that after a dose of 2 μg/kg CLO effects can be measured that are not apparent after the lower doses of CLO.

In conclusion, because the dose-response patterns of plasma and urinary NOR, SBP, and subjective sedation were all similar, our results do not provide simple parameters to discern presynaptic and postsynaptic effects, either peripherally or centrally, that represent the different components in the mechanism of action of clonidine. However, at a dose of 0.5 μg/kg significant changes were induced in plasma NOR levels, BP and sedation, but not in plasma or urinary MHPG or plasma growth hormone levels. Plasma NOR

levels proved to be the most sensitive and most clearly dose-dependent parameter. When using clonidine as a challenge test in psychiatric disorders, a design with 0.5 μg/kg, in addition to the traditional 2 μg/kg, may provide more information to characterize abnormalities in the noradrenergic system at the level of the brainstem, the pituitary or the peripheral sympathetic nervous system.

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Chapter 3.2

Cardiovascular Variability after Clonidine Challenge: Assessment of Dose-Dependent Temporal Effects by means of Spectral Analysis

Summary

Effects of four i.v. doses (0.25, 0.5, 1 and 2 μg/kg) of the α_2-adrenoceptor agonist clonidine (CLO) were studied in 7 normotensive male volunteers in a placebo-controlled double blind randomized design, in order to evaluate the role of α_2-adrenoceptors in spontaneous short-term cardiovascular fluctuations. Heart rate (HR), systolic and diastolic blood pressure (SBP, DBP; Finapres device), stroke volume (SV) and total peripheral resistance (TPR) were monitored for a period of 1 hour following infusion of CLO, while the subjects rested in a semi-recumbent position. For HR, SBP and DBP, power spectra and variation coefficients were calculated for consecutive time-segments of 2.5 min. Power density was assessed for three frequency bands: low (LFB: 0.02-0.06 Hz), mid (MFB: 0.07-0.14 Hz) and high (HFB: 0.15-0.40 Hz). Per time-segment, baroreflex sensitivity (BRS) was estimated as the gain (or modulus) in the MFB between the systolic pressure values and the R-R interval times.

Decreases in mean levels of SBP and DBP were observed within 15 min after infusion of 0.5 μg/kg CLO or more. HR first showed a small increase 15 min after infusion of 0.5, 1 and 2 μg/kg CLO, but declined subsequently as in all doses, including placebo. SV and TPR decreased after a dose of 2 μg/kg CLO. LFB and MFB power of HR were reduced after 2 μg/kg CLO, but only during the first 30 min following infusion; during this period respiratory depth was also diminished, indicating that these effects may reflect a reduction in sympathetic outflow as well as a reduction in vagal outflow. Respiratory frequency did not change after CLO, nor did BRS. DBP MFB power was reduced after 2 μg/kg CLO during the entire post-infusion period, probably as a reflection of reduced sympathetic outflow. SBP HFB power was significantly increased after 0.5 μg/kg CLO or more, but only after 30 min of infusion, which could be a consequence of alterations in both vagal outflow and mechanical respiratory properties.

Our data show that, within a dose-range of 0.25-2 μg/kg CLO, significant effects could be detected for SBP, DBP, and HR after 0.5 μg/kg or more,

whereas spontaneous short-term fluctuations in HR and DBP were influenced only after a dose of 2 μg/kg. The effects were small, but could be detected within a post-infusion period of 1 hour. Our data underline that sequential spectral analysis of spontaneous haemodynamic fluctuations can be employed to unravel time-dependent dynamics of sympathetic and vagal components within short-term cardiovascular control.

Introduction

The hypotensive effect of the α_2-receptor agonist clonidine is thought to be the net result of a reduction of sympathetic outflow, due to complex central actions of clonidine on noradrenergic neurons within the hypothalamus, medulla oblongata and spinal cord (1,2). Inhibitory activity from the nucleus tractus solitarius to the medullary vasomotor centre and parallel enhancement of vagal cardiac activity are part of the mechanisms involved in the hypotensive and bradycardial effects of clonidine (3,4). An increase in baroreflex sensitivity after clonidine administration has been reported (5,6), but data are not unequivocal (7). Primarily, the effects of clonidine are assumed to be the result of stimulation of central postsynaptic α_2-adrenoceptors, although presynaptic α_2-effects cannot be excluded (8). The influence of clonidine on the regulation of blood pressure and heart rate may also be the result of a reduction of noradrenaline release by means of action on peripheral presynaptic α_2-adrenoceptors (9,10). Thus, complex central and peripheral as well as pre- and postsynaptic α_2-adrenergic mechanisms are responsible for the alterations in sympathetic and vagal outflow after clonidine administration. In human cardiovascular research, it is difficult to delineate these components in cardiovascular control and to obtain simple and reliable indices of sympathetic and parasympathetic outflow.

Spectral analysis of short-term fluctuations in heart rate and blood pressure may reflect changes in sympathetic and vagal outflow, which can be used as quantitative indices of cardiovascular control systems (11-13). Three spectral peaks are usually defined: 1) a low frequency peak with variations around 0.04 Hz; for heart rate this peak is associated with both parasympathetic and sympathetic activity, while these low-frequency blood pressure fluctuations are linked with variations in peripheral vasomotor activity due to thermoregulatory influences and renin-angiotensin system activity (11,14,15), 2) a mid frequency peak with variations around 0.1 Hz (Mayer waves), which has been associated with sympathetic outflow (16,17) or with a resonance in the baroreflex control of peripheral resistance (18), and 3) a high frequency peak around the respiratory frequency, usually between 0.20-0.35 Hz (19,20), representing vagal activity for heart rate and a combination of vagal and

mechanical effects of respiration for blood pressure (21). Therefore, spectral analysis of haemodynamic parameters may be a useful method to describe changes in sympathetic and vagal outflow after clonidine administration. In addition, computing transfer functions between spectra of heart rate and systolic blood pressure offers the possibility to estimate the sensitivity of the cardiac component of the baroreflex (22) without application of a pressor agent or the neck suction method.

Clonidine markedly reduced midfrequency fluctuations in heart rate and blood pressure after an oral dose of 150 μg in hypertensive patients (23). Whether these responses are similar in normotensive subjects is sofar not known. The cardiovascular effects to clonidine are time-dependent and may vary with different doses (24,25). In a previous study (25), we have found significant decreases in plasma noradrenaline concentrations and blood pressure after a dose of 0.5 μg/kg clonidine i.v. in a placebo-controlled randomized design in healthy volunteers. In the present study we analyzed whether clonidine, along with the average changes in heart rate and blood pressure, has an effect on spontaneous short-term fluctuations in heart rate and blood pressure. We covered a dose-range from 0-2 μg/kg clonidine. Time-dependent effects were monitored in order to establish whether alterations in short-term haemodynamic adjustments occurred within a post-infusion period of 1 hour. In addition, stroke volume, total peripheral resistance, respiration, and baroreflex sensitivity (spectral method) (22) were analyzed in order to be able to interpret our findings in relation to cardiovascular control systems.

Methods

Subjects

Seven males with a mean age of 23.4 years (range 21-27 years) and a mean body weight of 76.4 kg (range 69-83 kg) participated each in five experimental sessions, after having given written informed consent. The study protocol was approved of by the Medical Ethical Committee of the University Hospital Rotterdam Dijkzigt. The selection procedure of the volunteers was based on a medical (medical history, hematologic and chemistry panels, ECG) and psychological (personality questionnaires) screening to exclude subjects with medical or psychiatric illnesses, including alcohol or drug abuse.

Design and Procedure

Details of the design and procedures of this study have been presented before (25). Each subject participated in five sessions, during which he received, in a randomized and double blind manner, the following doses of clonidine (CLO) intravenously: 0 μg/kg, 0.25 μg/kg, 0.5 μg/kg, 1 μg/kg or 2 μg/kg. CLO was

diluted in 10 ml saline and injected slowly over a period of 10 min through an indwelling catheter in the antecubital vein of the forearm.

The five sessions were recorded on separate days, at least 2 days apart. Each session was performed between 10:00-12:00 hrs. During this period, the subjects rested comfortably in a semi-recumbent position in bed. After a baseline period of 20 min, CLO was administered and the responses were monitored for a period of 1 hour.

Measurements. During each session spontaneous fluctuations in ECG and blood pressure were recorded on a multichannel FM-type analogue recorder (Racal Store 14 DS, Sarasota, Florida, USA) for off-line computer analyses. The ECG was derived using a precordial lead, amplified by a polygraph (Nihon Kohden, Tokyo, Japan). Blood pressure was monitored and recorded by means of a servo-plethysmo-manometer for continuous, non-invasive measurement of finger arterial blood pressure, employing the volume clamp technique of Penaz (26-29) (Finapres 2300 NIBP monitor, Ohmeda, Englewood, Colorado, USA). To control for respiratory linked variations during spontaneous cardiovascular fluctuations, thoracic and abdominal respiration were measured by means of Nihon Kohden impedance plethysmographs.

Analysis

The ECG and blood pressure signal were digitized at a sample frequency of 1024 Hz on a Personal Computer (Commodore PC 60-III) connected to an Analogue/Digital converter (Advantech PC-LabCard model PCL-718). R-R intervals in the ECG were detected with an accuracy of 1 msec and transposed to heart rate (HR) series. Systolic and diastolic blood pressure (SBP, DBP) were defined per R-R interval of the ECG, with an accuracy of 0.1 mmHg. For prolonged blood pressure recordings, the Finapres device has the disposal of a built-in "lock-adjust" procedure for automatic adjustment of the finger cuff pressure by means of a servosystem, which is activated in parallel with blood flow changes. This procedure occurs every 40 to 70 beats under stationary conditions. As a result, every 40 to 70 beats, 2-4 pulses were missing from the blood pressure recording. These values were estimated by means of a linear interpolation procedure (30). In addition, time-series of HR, SBP and DBP were scrutinized for stationarity and artifacts by means of a software program and visual inspection. A total of 4% of the time-segments were deleted from the final analyses, due to movement artifacts and non-stationarities. The respiratory signal was sampled with a frequency of 102.4 Hz.

Per recording, 5 periods were selected for analyses: a 10 min period prior to the infusion of CLO (baseline) and 4 consecutive 10 min periods between the collection of the blood samples at 15, 30, 45 and 60 min after the infusion of CLO.

Spectral analysis of heart rate and blood pressure. Within each 10 min period, 4 consecutive time segments of 150 sec (2.5 min) of HR, SBP and DBP time series were subjected to a discrete Fourier transform, based on non-equidistant sampling of the R-wave incidences (CARSPAN program) (30,31). With this method power spectral densities of rhythmic oscillations over a frequency range of 0.02-0.50 Hz were obtained, with a resolution of 0.01 Hz. For each time segment, the power was calculated for the total band (0.02-0.50 Hz), low frequency band (LFB:0.02-0.06 Hz), mid frequency band (MFB: 0.07-0.14 Hz) and high frequency band (HFB: 0.15-0.40 Hz), in addition to mean HR, SBP and DBP, variation coefficients (VC) of HR, SBP and DBP, stroke volume index (SV) and total peripheral resistance (TPR). Changes in SV were described by means of the pulse contour method as modified by Wesseling et al. (32). This method proved to be accurate, both when using the non-invasively obtained finger arterial pulse wave or the intrabrachial pulse wave, under varying hemodynamic circumstances, after corrections are made for the pressure dependent compliance of the aorta and for HR to compensate for reflections of pressure waves (33). TPR was computed as mean blood pressure divided by cardiac output.
Spectral energy was expressed in relative terms, i.e. in normalized values relative to the mean value of the considered signal (squared modulation index, MI^2, to be compared with squared variation coefficient) (34). Because the total power equals the squared variation coefficient minus the power of the DC component, total power data are not presented, but variation coefficients are. For the spectral data a logarithmic transformation was performed because of skewness of the distributions. As an index of baroreflex sensitivity (BRS), we computed per time segment the gain (or modulus) in the MFB between the systolic pressure values and the R-R interval times, based on those frequency points within the 0.07-0.14 Hz range with a coherence between the two signals of greater than or equal to 0.5 (22).

The results of the analysis of the 4 time segments per 10 min period were averaged; this procedure reduced a noise factor due to spontaneous segment to segment fluctuations (35) and allowed a statistical analysis of the time-dependent changes (i.e. over 4 consecutive 10 min periods) within a period of 1 hour.
Respiration. Mean (SEM) respiratory cycle duration (in sec) and inspiratory depth (in percentage of change versus baseline times 100) were calculated per 10 min period, on the basis of analysis of the thoracic respiratory signal. Since respiration was analyzed as a control for the respiratory related effects in the cardiovascular variations, we only analyzed these data after infusion of placebo (0 μg/kg) and the highest dose of CLO (2 μg/kg).

Statistical analyses

Data will be presented as mean (±SD) for N=7, based on the absolute values per period. Responses relative to baseline values were calculated also in order to correct for intra-individual differences in baseline values. Similarity of the baseline values of the 5 sessions was evaluated by means of a multivariate analysis of variance (MANOVA) for repeated measurements. MANOVA's for repeated measurements were also used to establish the effect of the 5 doses of clonidine (within-subject Factor: DOSE), the time-dependency of the response-profiles within a post-infusion period of 1 h (within-subject Factor: TIME), and the interaction-effects between factors DOSE and TIME. If a significant DOSE-effect was observed, additional MANOVA's were performed using pairwise comparisons to search for specific dose effects, including factor TIME in all analysis. Duncan's multiple range tests were used in order to search for specific dose effects related to specific time periods. A p-value of $<.05$ was used to indicate a significant effect.

Table 1. F-values of the MANOVAs for repeated measurements with factors Dose (0, 0.25, 0.5, 1, 2 μg/kg clonidine), Time (4 x 15 min), and their interaction Dose x Time

	Dose	Time	Dose x Time	Dose (response)
HR	0.30	26.55***	2.24*	4.01**
VC HR	2.58	5.91**	1.71	0.69
LFB HR	2.75*	4.83**	0.04	0.64
MFB HR	2.85*	2.95	1.46	0.99
HFB HR	2.21	1.58	1.84	1.58
SBP	5.68**	3.54*	2.96**	14.47***
VC SBP	0.20	2.00	1.04	0.53
LFB SBP	0.17	0.81	0.86	0.34
MFB SBP	2.03	5.54**	1.09	1.53
HFB SBP	4.91**	4.71*	1.28	4.13**
DBP	2.41	3.37*	2.72**	13.24***
VC DBP	0.75	8.80***	1.06	0.29
LFB DBP	0.76	11.26***	0.99	0.30
MFB DBP	6.10**	5.53**	1.39	2.91*
HFB DBP	2.28	1.08	1.06	0.77
BRS	0.62	0.78	1.39	0.21
SV	4.85**	25.91***	1.10	
TPR	3.12*	19.66***	0.94	

*:$p<0.05$; **: $p<0.01$; ***: $p<0.001$

Results

Per parameter, the baseline values of the 5 sessions were similar (for all parameters: $p > .05$, non-significant effects).

Heart rate (HR). CLO had no significant effect on the absolute values for HR data, but for HR responses versus baseline a significant DOSE-effect was apparent (Table 1,2; Figure 1). The significant interaction effect between factors DOSE and TIME (Table 1) was explained by a small, but significant, increase in HR of 3%, 15 min after infusion of 0.5, 1 or 2 μg/kg CLO (versus placebo, $p < .05$). This small increase in HR was followed by a gradual decline (Table 2), which was no longer significantly different from placebo. After placebo (0 μg/kg) and 0.25 μg/kg, we observed a gradual decrease in HR during the whole session. CLO had no effect on the HR variation coefficient or HFB power, although a trend towards a decrease was present for both parameters (respectively: $p=0.06$ and $p=0.09$, NS; Table 1). After infusion of 2 μg/kg CLO, LFB and MFB power were significantly reduced versus 0 μg/kg CLO (Table 1,3; Figure 1), but only during the first 30 min after infusion. When these data were corrected for differences in baseline, significant effects were no longer observed, although the averaged response pattern remained the same (Figure 1): the highest dose showing the largest effect.

Systolic blood pressure (SBP). A dose-dependent SBP response was observed within 15 min after infusion of CLO (Table 1,2; Figure 2). A significantly larger decrease (22%) in SBP was observed after 2 μg/kg, compared to 1 or 0.5 μg/kg CLO (9-11% decrease). The decreases observed after a dose of 1 or 0.5 μg/kg CLO were significantly different from 0 μg/kg. The SBP variation coefficient, the LFB and MFB power of SBP were not significantly affected by the different doses of CLO. However, CLO influenced SBP HFB power: 30 min after infusion, the increase versus placebo after a dose of 2 μg/kg CLO became significant for the absolute data, while the baseline corrected responses showed a significant increase after 0.5, 1 and 2 μg/kg CLO (Table 1, Figure 2).

Diastolic blood pressure (DBP). CLO only induced a significant dose-dependent effect on the DBP responses versus baseline (Table 1,2), and not on the absolute DBP data. A significant DOSE-effect on the DBP response was present at 15 min after infusion of CLO (Table 1,2; Figure 3). The decrease in DBP 30 min after infusion of 2 μg/kg was significantly larger than the decrease observed after 1 or 0.5 μg/kg CLO. The lowest dose of 0.25 μg/kg CLO had no significant effect on SBP or DBP. DBP variation coefficients and LFB and

HFB power were not influenced significantly by infusion of CLO. Fluctuations in the MFB power were significantly reduced by CLO (Table 1,3). Although this effect on MFB power appeared strongest when the effect on DBP was not yet maximal (during the first 15 min after the infusion), for the whole post-infusion period the highest dose of 2 μg/kg CLO induced a significant decrease versus 0 μg/kg ($p < .01$).

Table 2. Mean (VC) values of HR, SBP, DBP, and mean (SEM) response percentages versus baseline of SV and TPR, before and after 5 doses of clonidine (CLO)

	CLO (μg/kg)	baseline	15 min	30 min	45 min	60 min
HR (bpm)	0.00	66 (8.3)	64 (8.4)	63 (9.4)	61 (9.0)	61 (9.2)
	0.25	63 (8.5)	63 (7.9)	62 (8.0)	61 (9.2)	59 (8.2)
	0.50	64 (7.6)	66 (7.1)	64 (7.6)	61 (7.3)	60 (7.8)
	1.00	63 (8.8)	64 (7.3)	64 (8.2)	62 (8.1)	61 (9.0)
	2.00	67 (7.5)	69 (5.7)	65 (6.2)	63 (7.2)	61 (7.7)
SBP (mmHg)	0.00	127 (5.3)	125 (5.2)	125 (5.6)	128 (5.4)	129 (5.1)
	0.25	119 (5.9)	115 (5.3)	113 (5.7)	113 (5.9)	116 (5.3)
	0.50	128 (5.2)	120 (5.9)	116 (5.9)	118 (5.7)	122 (5.0)
	1.00	122 (5.5)	111 (5.1)	111 (5.6)	109 (5.4)	112 (5.8)
	2.00	129 (5.3)	110 (5.1)	102 (5.4)	101 (5.5)	102 (5.3)
DBP (mmHg)	0.00	62 (5.3)	63 (5.2)	63 (6.1)	65 (5.6)	66 (5.7)
	0.25	57 (6.0)	56 (5.4)	55 (6.3)	56 (6.6)	59 (5.8)
	0.50	67 (4.8)	65 (5.1)	61 (5.8)	63 (5.4)	66 (4.9)
	1.00	59 (5.5)	56 (4.9)	55 (5.7)	54 (5.9)	56 (6.4)
	2.00	67 (5.1)	60 (5.0)	55 (5.7)	54 (5.6)	56 (5.4)
SV response	0.00	0	-2.9 (1)	-4.3 (1)	-5.5 (1)	-6.4 (2)
(%)	0.25	0	-4.7 (2)	-6.3 (2)	-10.2 (3)	-11.4 (4)
	0.50	0	-7.5 (2)	-9.6 (2)	-10.2 (2)	-12.0 (2)
	1.00	0	-3.8 (4)	-5.4 (3)	-7.2 (3)	-8.6 (3)
	2.00	0	-9.2 (3)	-13.7 (2)	-15.8 (2)	-18.7 (3)
TPR response	0.00	0	6.5 (2)	8.5 (4)	16.9 (4)	21.1 (6)
(%)	0.25	0	2.0 (4)	3.0 (4)	11.8 (6)	22.0 (8)
	0.50	0	0.1 (4)	1.1 (3)	7.8 (2)	14.9 (3)
	1.00	0	-3.9 (4)	-2.3 (6)	-0.1 (6)	4.8 (6)
	2.00	0	-4.3 (3)	-1.9 (4)	3.4 (4)	12.3 (5)

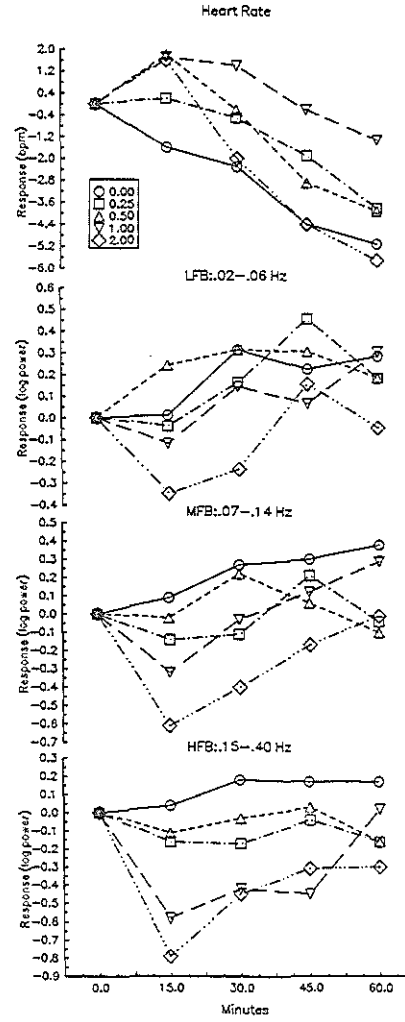

FIGURE 1
Mean responses versus baseline of heart rate (in bpm), and the logarithmic power values of the low (LFB), mid (MFB), and high frequency band (HFB) during a period of one hour following the infusion of 0, 0.25, 0.5, 1, or 2 μg/kg clonidine.

Stroke volume (SV). CLO significantly and dose-dependently reduced SV. A time dependent decrease in SV was observed during all sessions (Table 1,2). After a dose of 2 μg/kg CLO, SV decreased 9% within 15 min after infusion, to 19% after 60 min. This effect was significantly different from 0 μg/kg CLO. The decreases as observed after a dose of 0.25, 0.5 or 1 μg/kg CLO were too small or too variable to be significant versus 0 μg/kg.

Table 3. Mean (SEM) logarithmic power values of the LFB, MFB, and HFB of HR, SBP, and DBP, during baseline (bsl) and 15 and 60 min after infusion of 5 doses of clonidine (CLO)

	CLO	LFB			MFB			HFB		
	(μg/kg)	bsl	15 min	60 min	bsl	15 min	60 min	bsl	15 min	60 min
HR	0.00	7.4(.1)	7.4(.2)	7.6(.2)	7.2(.2)	7.3(.2)	7.6(.2)	7.3(.5)	7.3(.5)	7.5(.4)
	0.25	7.3(.2)	7.3(.2)	7.5(.2)	7.2(.2)	7.1(.2)	7.2(.1)	7.3(.5)	7.1(.5)	7.1(.4)
	0.50	6.9(.3)	7.2(.1)	7.1(.3)	6.9(.3)	6.9(.2)	6.8(.5)	7.0(.5)	6.9(.4)	6.8(.6)
	1.00	7.3(.3)	7.2(.2)	7.6(.2)	7.2(.3)	6.8(.3)	7.5(.2)	7.5(.5)	6.9(.5)	7.5(.4)
	2.00	7.1(.2)	6.8(.3)	7.1(.3)	6.9(.2)	6.3(.4)	6.9(.4)	7.0(.4)	6.2(.5)	6.7(.6)
SBP	0.00	7.0(.1)	7.0(.1)	6.9(.2)	6.0(.2)	6.1(.1)	6.2(.1)	5.3(.1)	5.3(.1)	5.3(.2)
	0.25	7.3(.2)	7.0(.2)	7.0(.3)	6.3(.1)	6.1(.2)	6.2(.2)	5.3(.1)	5.4(.1)	5.2(.2)
	0.50	7.0(.2)	7.2(.2)	6.9(.2)	5.9(.2)	6.0(.1)	5.9(.2)	5.0(.2)	5.3(.1)	5.3(.2)
	1.00	7.1(.3)	6.9(.3)	7.2(.3)	5.9(.3)	5.7(.2)	6.2(.2)	5.2(.2)	5.5(.2)	5.7(.2)
	2.00	7.1(.2)	7.0(.2)	7.2(.2)	6.0(.2)	5.7(.2)	5.9(.2)	5.1(.2)	5.6(.2)	5.7(.2)
DBP	0.00	6.9(.2)	6.8(.2)	7.2(.2)	6.4(.3)	6.5(.2)	6.5(.2)	5.3(.3)	5.3(.3)	5.2(.2)
	0.25	7.2(.2)	7.0(.1)	7.3(.2)	6.7(.1)	6.6(.1)	6.5(.1)	5.7(.3)	5.4(.3)	5.3(.2)
	0.50	6.7(.2)	6.8(.2)	7.0(.2)	6.2(.2)	6.1(.1)	6.0(.1)	5.1(.3)	4.9(.3)	4.9(.2)
	1.00	7.0(.4)	6.9(.3)	7.5(.3)	6.3(.3)	6.1(.2)	6.6(.2)	5.4(.4)	4.9(.3)	5.3(.3)
	2.00	7.0(.2)	7.1(.1)	7.1(.2)	6.3(.3)	5.8(.2)	5.9(.3)	5.1(.2)	4.8(.2)	5.0(.2)

Table 4. Mean (SEM) values of the baroreflex sensitivity (BRS), during baseline and 15, 30, 45, and 60 min after infusion of 5 doses of clonidine (CLO)

	CLO (μg/kg)	baseline	15 min	30 min	45 min	60 min
BRS	0.00	12.4 (1.9)	12.9 (2.5)	13.6 (1.9)	13.6 (1.7)	14.4 (1.6)
(ms/mmHg)	0.25	12.7 (2.5)	13.6 (3.6)	13.0 (3.0)	14.6 (2.6)	13.8 (2.9)
	0.50	11.5 (1.2)	12.3 (1.9)	13.3 (2.0)	11.2 (1.7)	13.3 (1.6)
	1.00	16.7 (6.0)	17.6 (7.3)	16.1 (5.3)	16.6 (5.9)	17.9 (5.8)
	2.00	11.8 (1.5)	12.1 (4.0)	14.0 (4.4)	12.7 (2.9)	16.4 (4.1)

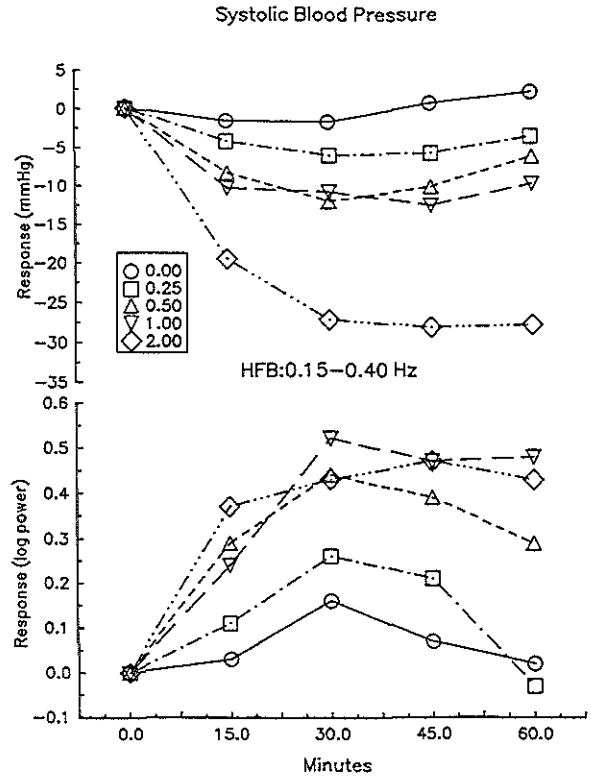

FIGURE 2
Mean responses versus baseline of systolic blood pressure (SBP; in mmHg) and the logarithmic power values of the high frequency band (HFB) during a period of one hour following the infusion of 0, 0.25, 0.5, 1, or 2 μg/kg clonidine.

Total peripheral resistance (TPR). TPR showed a time dependent increase during all sessions (Table 1,2). CLO induced a dose-dependent significant decrease on the TPR response percentages versus baseline (Table 1,2), which was already present 15 min after infusion of 1 or 2 μg/kg.

Baroreflex sensitivity (BRS). CLO did not induce an overall significant effect on BRS (Table 1), due to large between-session variability (Table 4).

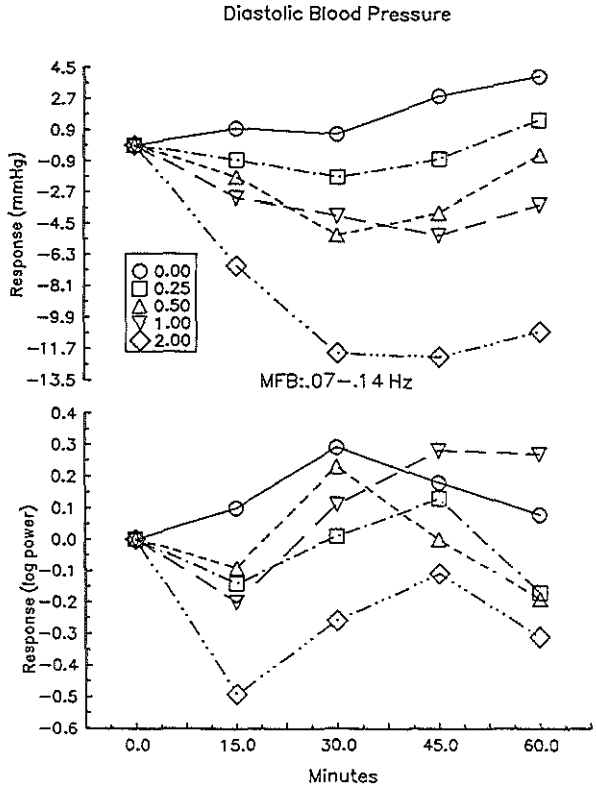

FIGURE 3
Mean responses versus baseline of diastolic blood pressure (DBP; in mmHg) and the logarithmic power values of the mid frequency band (MFB) during a period of one hour following the infusion of 0, 0.25, 0.5, 1, or 2 μg/kg clonidine.

Respiration. Respiratory cycle duration after infusion of 0 μg/kg was stable: 4.05(±0.24) sec during baseline, 3.99(±0.23) sec 15 min after infusion and 4.01(±0.26) sec after 60 min. After 2 μg/kg CLO, respiratory cycle duration also did not change: 4.17(±0.24) sec during baseline, 4.16(±0.28) sec after 15 min and 4.32(±0.27) sec 60 min after infusion. The percentage of change in inspiratory depth did show a significant effect of CLO ($p < .05$): 15 min after infusion of 2 μg/kg inspiratory depth decreased by 7.1%, while placebo induced a 4.5% increase. This effect was still present 30 min after infusion, but 45 min after infusion both placebo and 2 μg/kg CLO showed a small increase in respiratory depth versus baseline (2.7 and 2.9%, respectively). Thus, 2 μg/kg CLO induced a significant decrease in respiratory depth, but only during the first 30 min after infusion.

Discussion

Within a post-infusion period of 1 hour, CLO induced clear dose-dependent effects on SBP and DBP. There was a small increase in HR 15 min after the infusion of 0.5, 1 or 2 μg/kg CLO, indicating that the induced fall in blood pressure caused a compensatory rise in heart rate. This compensatory rise in heart rate was not observed in other studies (36,37). During the later part of the recording a gradual declining trend in HR was found after all doses, including placebo (0 μg/kg). In the dose-range of 0.25-2 μg/kg, we did not observe a transient increase in blood pressure shortly after infusion of CLO. SV declined significantly after 2 μg/kg, an effect which was strongest during the later part of the recording. TPR showed a small decrease after 1 and 2 μg/kg CLO; this effect was present within 15 min after infusion.

Clonidine did not significantly influence overall cardiovascular variability as measured by means of variation coefficients. Spectral analysis did indicate a clear effect, but only after the highest dose of 2 μg/kg. At this dose, LFB and MFB power of HR and MFB power of DBP were decreased. The effects on HR were significant only during the first 30 min post-infusion for the absolute data, while the DBP effects were present during the entire post-infusion period for both the absolute and relative data. This reduction in MFB fluctuations of DBP after 2 μg/kg CLO may be due to a reduction of oscillations or resonances in the baroreflex control of peripheral resistance at frequencies around 0.1 Hz, which may be reflected via the baroreflex in the HR fluctuations at these frequencies. However, this effect was not present for SBP MFB power, and BRS was not affected by CLO. Our findings correspond with the assumption that MFB fluctuations reflect changes in sympathetic outflow (17), which in this study coincided with a significant reduction in plasma noradrenaline concentrations (25). This effect was accompanied by significant reductions in absolute

levels of SBP and DBP. However, the effects on LFB and MFB power of HR may result partly from alterations in respiratory depth, which were reduced significantly during the first 30 min post-infusion. This effect of clonidine on HR fluctuations may reflect a parasympathetic change due to a central vagal-mediated action. That a central vagal-mediated action can indeed be reflected in changes in LFB and MFB fluctuations of HR is illustrated by studies with autonomic blocking agents such as atropine and detailed analysis of effects of spontaneous respiratory activity on HR variations (11,12,21,38-40). It should be noted that these effects on LFB and MFB fluctuations of HR occurred when the BP reduction was least.

Fluctuations in the HFB power of SBP appeared to increase above a dose of 0.5 μg/kg CLO, but only during the later part of the recording. This effect was not present in DBP. Both changes in central vagal outflow and respiratory induced 'mechanical' alterations of intrathoracic pressure may have contributed to this effect (41). On the basis of the present data, it is not possible to differentiate between these two possibilities.

CLO had no significant effect on BRS. Mancia et al. (7) suggested that clonidine can exert a hypotensive effect without a potentiating effect on the baroreceptor reflex. Our data underline this assumption, at least for the haemodynamic effects observed during a post-infusion period of 1 hour.

Our data confirm and extend the findings of Elghozi et al. (23) in hypertensive subjects. With our study in normotensive subjects we now have shown that within a dose-range of 0.25-2 μg/kg CLO, clear dose-response effects could be detected for SBP, DBP and HR, wherease spontaneous short-term fluctuations in HR and DBP were only significantly influenced after a dose of 2 μg/kg. These effects were small, but could be detected within a post-infusion period of 1 hour. Our data underline that sequential spectral analysis of spontaneous haemodynamic fluctuations can be employed to unravel time-dependent dynamics of sympathetic and vagal components within short-term cardiovascular control.

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CHAPTER 4

THE STROOP COLOR WORD TEST

Chapter 4.1

Characterization of Stress Reactions to the Stroop Color Word Test

Summary

Sympatho-adrenal activation induced by stress contributes to the development of pathological states such as hypertension and anxiety disorders. The Stroop Color Word Test (CWT) is evaluated as a test for the study of stress-induced sympathetic effects, on the basis of psychological, physiological and biochemical responses. The CWT induced increases in plasma and urinary adrenaline, heart rate, respiration rate, electrodermal activity, electromyography, feelings of anxiety, and decreased finger pulse amplitude.

Introduction

Many normal and abnormal psychic and somatic human behavioral states are associated with altered patterns of sympatho-adrenal activity. Altered sympatho-adrenal activity, especially abnormal catecholamine release, is believed to be of importance in the pathogenesis of hypertension, orthostatic hypotension and anxiety states (3,4,9,13).

Environmental events, which are subjectively perceived as stressors, evoke substantial increases in sympathetic nervous system activity and have been hypothesized to contribute to the development of the pathological states mentioned above. To characterize these sympathetic responses, i.e., how they may lead to pathological states and how they could be antagonized, tests are required that are capable of inducing: 1) psychological changes that indicate increased distress: the individual's perception and interpretation of the situation is of great importance for the observed physiological activation and variation, 2) physiological changes that indicate sympatho-adrenal activation as reflected in parameters such as heart rate, respiration rate, electrodermal activity, and peripheral blood flow, 3) muscular exertion as part of the fight-flight defence reaction (11,19), and 4) hormonal (and neuronal) changes as reflected in plasma and urinary catecholamines, and plasma cortisol and prolactin. In order to study these reactions, a controlled situation is needed and therefore it is necessary to induce anxiety or stress experimentally.

The present study was undertaken to investigate whether the Stroop Color Word Conflict Test meets all of the above-mentioned requirements. The Stroop Color Word Test, as presented in the version of Frankenhaeuser and Johansson (8), produces mental overstimulation as a result of cognitive conflict combined with time-pressure effects. This test has repeatedly been proven to increase plasma and urinary adrenaline concentrations (1,12). Although sympatho-adrenal activation during the Stroop test has been described before on the basis of psychological, physiological and biochemical parameters separately, it is necessary to combine these parameters in one study in order to evaluate the usefulness of this test for the above-mentioned purpose. The response patterns to the Stroop test were analyzed in a group of young healthy male subjects and compared with measurements obtained during rest.

Methods

Subjects and Procedure

Nine paid male volunteers (age range: 22-25 years; mean: 23.8) participated in two experimental sessions (a stress-session and a control-session) after giving informed consent. The experimental procedures were approved by the Medical Ethical Committee of the Erasmus University and Dijkzigt Hospital. All subjects were in good health; none reported excessive drinking or smoking habits. They were requested not to undertake unusual activities (exams, heavy training, abnormal drinking) for three days before the first recording session and the week between the two sessions. Of the two sessions, one was a stress-session and the other a control-session. The stress- and control-sessions were recorded on separate days, one week apart. The stress-session was the first session for six of the nine subjects.

During the stress-session, the volunteers were subjected twice to a 20-min version of the Stroop Color Word Test (CWT), with 20-min periods of rest before and after each CWT. The control-session consisted of five successive periods of rest (20 min each). The subjects were asked to remain seated in the same position during the rest periods as during the presentation of the CWT, with their eyes kept open.

The CWT was presented on videotape and was constructed to conform to a previously reported description (8). In short, four words (red, green, blue and yellow) were presented at random in one of four colors (red, green, blue and yellow) with random intervals of 0.8-1.7 sec, while the duration of the stimuli varied at random between 0.4-1.0 sec. The subjects had to indicate the color of the words on an answer sheet. During the second CWT a double-conflict version was used: auditory stimuli were added by presen-

ting the color word on sound track. Beforehand, a two-minute practice session was administered so that the subjects would be familiar with the test requirements.

Before the start of each experimental session, the subjects were requested to void urine and to drink a glass of mineral water. Coffee, tea and smoking were not allowed before or during the recordings. Both recording sessions were performed between 9:15-12:00 a.m.; physiological, biochemical and psychological measurements were obtained during these periods. After the sessions, urine was collected and a standard light lunch was served. During the afternoons the subjects spent their time reading and/or studying in a quiet room; urine was collected twice during this period.

Measurements and Analyses

Psychological. A shortened version of the POMS (Profile Of Mood States) (16) and the ZBV (Dutch version of Spielberger's State Trait Anxiety Inventory) (21) self-rating scales were administered before and after the sessions to assess changes in mood and anxiety. The shortened version of the POMS has been validated for the Dutch population (25).

Biochemical. Forty-five minutes before the start of the recordings a catheter (Venflon) was inserted in a vein of the nondominant forearm. Immediately after each 20-minute period, 15 ml of venous blood was drawn and collected into two tubes, one containing 19 mg of EGTA and 12 mg of gluthathione for assay of catecholamines and one containing heparin for assay of cortisol and prolactin. The tubes were immediately placed on ice and centrifuged within 15 minutes. Plasma for assay of adrenaline and noradrenaline was subsequently frozen at -70°C. Catecholamines from 1 ml of plasma were concentrated by a liquid-liquid extraction method (24) and then measured by a radioenzymatic procedure (20). Plasma for assay of cortisol and prolactin was frozen at -20°C. Cortisol was estimated by radioimmunoassay, using kits supplied by Diagnostic Product Corporation (DPC, Los Angeles, CA), and prolactin was assayed using a modified version of the method published by Miles et al. (17). Urine for determination of catecholamines and their metabolites was collected immediately after the sessions, 3 and 6 hours later. Urinary catecholamines were extracted (24) and assayed by a HPLC procedure after fluorescence derivatization (18).

Physiological. A Siemens-Elema (EEG-mingograf) electroencephalograph was used to amplify, calibrate and monitor the physiological signals. The ECG was derived by placing one electrode on the sternum, one below the left breast and one (reference) electrode below the right breast. Thoracic and abdominal respiration were measured by means of two mercury strain gauges

placed around the chest at the level of the nipples and the abdomen respectively. Skin resistance (Siemens Elema EMT 67) was recorded by two active Ag/AgCl electrodes with an effective area of 0.5 cm^2. The electrodes were attached to the medial phalanx of the index and ring finger of the nondominant hand. Peripheral vascular activity was monitored by means of a photoplethysmographic transducer, attached to the distal phalanx of the middle finger of the nondominant hand. EMG activity was derived from 2 Ag/AgCl electrodes, placed 3 cm apart, on the extensor muscles of the nondominant forearm. Before electrode placement the skin was cleaned with alcohol, and rubbed lightly with sandpaper; the interelectrode resistances had to be below 10 kOhm. All signals were recorded continuously during both sessions on a Racal instrumentation recorder.

Physiological analyses. The polygraphic recordings were checked visually for presence of artifacts. Because of technical shortcomings only six of the nine available GSR recordings of the stress-session were analyzed and seven of the control-session. All other recordings were of sufficient quality for further processing. Since the act of blood collection introduced movement artifacts and a short-lasting physiological arousal, only data collected after two minutes were used for analysis. All signals were sampled at 51.2 Hz on a MINC PDP-11/23 computer. The A/D conversion was performed at 4x real time speed. The data were further processed on a PDP-11/34 computer. Before A/D conversion, a level detector was used to trigger the incidence of the R-wave in the ECG. The output pulses of the trigger were fed into the computer for sampling. The time between the output pulses (the interbeat interval) was measured by a programmable clock and expressed in beats per minute. The EMG was full-wave rectified before processing; the digitized signal was integrated per minute for quantification. These integrated values were summed for the whole 20-minute period. The pulse amplitude of the plethysmogram was corrected for the amplification factor used for recording. Per R-R interval of the ECG, the amplitude of the pulse (max-min) was computed. GSR values (kOhms) were transformed to skin conductance values (micromho's). Mean SCL (Skin Conductance Level) was calculated per 20 minutes on basis of one sample per 10 sec. A logarithmic transformation was applied because of positive skewness of the distribution. Respiratory analyses were performed by means of a software program capable of computing different time and amplitude parameters per respiratory cycle. Only mean respiration rate per minute for the stress-session is presented here. Details of the respiratory changes during the Stroop test will be presented elsewhere.

Statistical Analyses

The data were averaged, per physiological parameter, to mean and SE (standard error of the mean) values per 20-minute period of rest or CWT. Results are presented for n=9, if not otherwise specified. Mean, median and range were defined for the nonparametric psychological data. Two-factor analyses of variance (ANOVA) for repeated measures (10) were used to assess the effects of the Stroop test (factor1: CWT/rest x factor2: subjects) with factor time as covariate. Two-tailed Student's t-tests were used for pairwise comparisons. For the psychological data Wilcoxon tests were applied (23). Pearson correlation coefficients were used to analyze the relation between initial value during rest and response-magnitude during stress. Linear regression analyses for related measurements were performed in order to assess the presence of trends in the physiological and biochemical data of the control-sessions.

Table 1. Psychological state, before and after the stress- and control-session

		Stress		Control	
		Pre	Post	Pre	Post
POMS					
Depression	mean	8.1	8.3	8.2	8.6
	median	8.0	8.0	8.0	8.0
	range	1.0	2.0	1.0	2.0
Anger	mean	8.0	7.9	7.7	7.9
	median	7.0	7.0	7.0	7.0
	range	6.0	4.0	4.0	5.0
Fatigue	mean	6.9	8.3	7.6	8.6
	median	7.0	8.0	6.0	8.0
	range	3.0	5.0	7.0	11.0
Vigor	mean	17.7	16.6	18.0	15.1
	median	20.0	18.0	19.0	15.0
	range	12.0	10.0	14.0	9.0
Tension	mean	8.0	8.8	8.6	6.6
	median	8.0	8.0	8.0	6.0
	range	4.0	5.0	6.0	2.0
ZBV					
Anxiety	mean	32.7	34.7	32.8	28.4
	median	32.0	33.0	33.0	28.0
	range	16.0	25.0	27.0	18.0

Mean, median and range per subscale of the POMS and the ZBV, of the pre- and post-session measures of the stress- and the control-session

Results

Psychological

Pre-and post-session values of the subscales of the POMS and the ZBV are presented in Table 1. The pre-session values of the stress- and control-session did not differ significantly on any factor. There was a significant decrease in tension after the control-session (Wilcoxon test: $Z=-2.02$; $p<0.05$), as well as a significant difference between post-session values of the stress- and control-session ($Z=-2.37$; $p<0.05$). The post-session values for state anxiety, as assessed by the ZBV, also differed significantly ($Z=2.07$; $p<0.05$).

Biochemical

The stress-session. The CWT produced a significant rise in plasma adrenaline, as compared to the rest periods (Fig. 1, Table 2). Though the subjects clearly differed in baseline adrenaline level, all showed an increase as a result of mental stress. With regard to noradrenaline, the ANOVA indicated a significant interaction effect between the stress and subject factor. Two subjects showed a decrease in noradrenaline level after both CWT periods, while there was one nonresponder. The other six subjects showed noradrenaline increases after both CWT periods. No changes in cortisol or prolactin levels were observed. Over the whole stress-session a gradual declining trend was found for both hormones as indicated by the highly significant F-values of the covariate 'time' (Table 2). A significant difference between subjects was found for both cortisol and prolactin, indicating that the differences between subjects were consistent between the different stress and rest periods.

The control-session. The significant results on the ANOVA covariate 'time' of the stress-session suggest trends possibly due to habituation or circadian fluctuations. Regression analyses on the data of the control-session must show these trends. No significant regression was found in the adrenaline and prolactin data (Table 2); a significant increase with time was observed in the noradrenaline data and a significant decrease in plasma cortisol concentration. These effects were not the same for all subjects, since between subjects differences in regression were significant in noradrenaline, cortisol and prolactin concentration. For adrenaline and cortisol, these results are in agreement with the findings on the ANOVA covariate 'time' of the stress-session. Noradrenaline, however, showed a positive trend in the control-session, not present in the stress-session. For prolactin, no trend was observed in the control-session, although a clear time-related effect was present in the stress-session (Table 2, Fig. 1). There were no significant

Table 2. ANOVA and regression analyses on the biochemical and physiological data

	Stress				Control		
	ANOVA (F-values)				Regression		
	CWT	Subject	Inter-action	Cov. Time	Mean Slope/period	F(1)	F(2)
Plasma							
Adrenaline	26.00***	11.12***	0.60	1.35	-1.39	1.73	0.36
Noradrenaline	8.07**	39.13***	2.64*	0.35	16.09	32.01***	7.46***
Cortisol	0.58	5.06***	1.29	19.07***	-24.26	53.91***	3.99**
Prolactin	0.39	56.92***	1.25	31.09***	-0.10	4.20	3.31*
Physiological							
Heart rate	153.71***	90.42***	8.43***	34.65***	-1.35	78.54***	1.85
EMG	118.78***	22.66***	29.36***	10.79**	-0.15	5.62*	0.76
Finger Pulse amplitude	27.66***	10.36***	1.09	11.77**	-0.11	62.97***	6.22***
SCL	33.85***	23.87***	4.24**	13.90**	-0.01	1.87	1.71

Stress-session: ANOVA F-values for the covariate 'time', the main factors (CWT/rest and subjects) and the interaction effect between the two factors are indicated per biochemical and physiological parameter. Control-session: Regression analyses per biochemical and physiological parameter: F(1): F-values of the regression analyses indicating the presence of increasing or declining regression in the data; F(2): F-values indicating differences in regression between the subjects. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$

Table 3. Urinary catecholamines during and after the stress- and control-session

Urinary excretion		Collection Periods 9-12 hr	12-15 hr	15-18 hr	Total 9-18 hr
Adrenaline					
Stress-session	mean	6.42	1.98	2.92	3.92
	s.e.	1.55	0.50	0.88	0.97
Control-session	mean	3.98	2.07	2.36	2.91
	s.e.	0.76	0.49	0.68	0.65
Noradrenaline					
Stress-session	mean	17.30	14.60	9.20	14.14
	s.e.	1.86	2.13	0.88	1.53
Control-session	mean	14.61	13.43	8.81	12.72
	s.e.	2.34	1.57	1.64	1.67

Mean and SE values of the urinary catecholamines, expressed in metabolite-/creatine x 10 (n=8)

differences between the first rest periods of the stress- and control-session with regard to adrenaline (t-test: t=-0.99; p=0.35), noradrenaline (t=1.24; p=0.25), cortisol (t=0.82; p=0.44) and prolactin (t=0.03; p=0.98), indicating comparable initial baselines in both situations.

Urinary catecholamines. In order to analyze the effect of the CWT on the urinary catecholamines, data of the stress-session were compared with data of the control-session (Table 3). A significant increase in urinary adrenaline concentration during the stress-session was found for the first collection period (9-12 hours) (paired t-test: t=2.64; $p<0.05$) and the total collection period (9-18 hours) (t=2.39; $p<0.05$). With regard to urinary noradrenaline concentration, we found no significant differences between stress- and control-session.

Physiological

The stress-session. The CWT caused a significant increase in heart rate, EMG activity, skin conductance and a significant decrease in pulse amplitude (Fig. 2; Table 2). Respiration rate per minute showed an increase during the CWT (Rest 1: mean=14.53, SE=0.76; CWT1: mean=17.56, SE=0.77; Rest 2: mean=14.64, SE=0.71; CWT2: mean=17.41, SE=0.69; Rest3: mean=14.59, SE=0.72). With regard to respiration rate the ANOVA showed a significant CWT effect (F=353.24; $p<0.001$), a significant subject effect (F=80.56; $p<0.001$), as well as a significant interaction effect

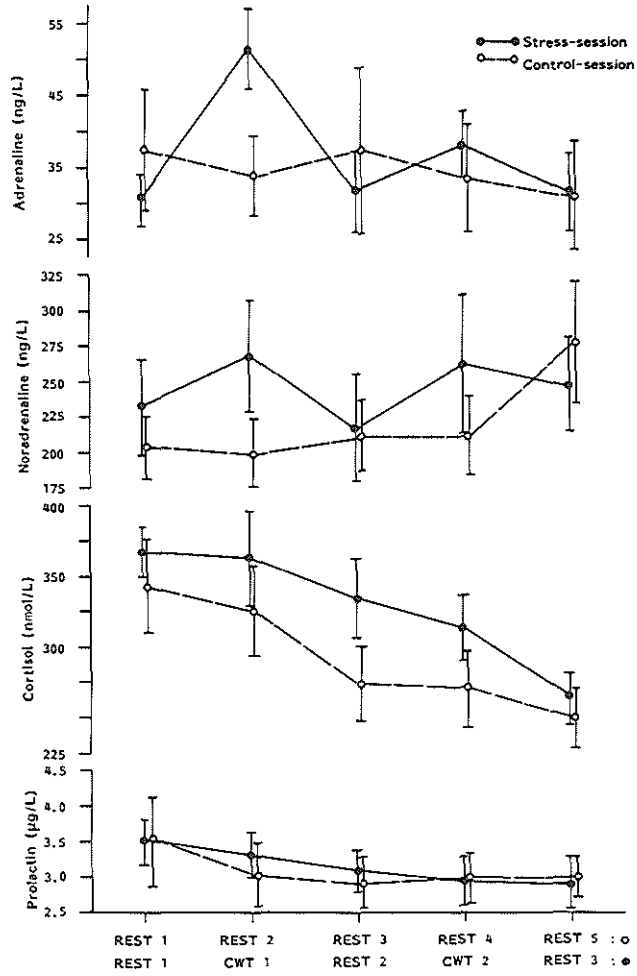

FIGURE 1
Mean±SE values per rest or CWT periods of the endocrine plasma parameters during the stress- and control-session.

(F=12.76; p<0.001), but the covariate time was not significant (F=0.07). The two-factor ANOVA also indicated interaction effects for heart rate, EMG and SCL (Table 2). Since these interactions might be due to a relation between magnitude of response and pre-stimulus level (26), we calculated product-moment correlation coefficients between the pre-stimulus values and the response-magnitudes to the CWT for all physiological parameters. We did this both for each rest and CWT period of the stress-session separately as well as for the averaged rest and CWT periods of the stress-session. Significant negative correlations were found for skin conductance level (r=-.916;

p=0.01, n=6) and respiratory frequency (r=-.736; p=0.04) for the averaged data. The interaction effects found for SCL and respiratory frequency can thus be explained by the initial values during rest. A high prestimulus level results in a small response to stress. For heart rate and EMG activity the correlations were -.337 (p=0.34) and .004 (p=0.99) respectively. Therefore, the interaction could not be explained on basis of the initial values.

Reproducibility and individual differences. Response-magnitudes to the first and second CWT were compared, based on mean values per period and minute-to-minute changes within periods. The second CWT consisted of a double-conflict task, introducing visual and auditory conflict. All parameters showed reduced response-magnitudes to the second CWT (Fig. 2). With regard to the biochemical data, plasma adrenaline also showed a reduced response-magnitude during the second CWT. Plasma noradrenaline showed an increased average response-magnitude to the second CWT, which was, however, accompanied by an increased variability, due to an absence of response or negative response in three of the subjects. Double-conflict, therefore, did not appear to lead to more sympathetic activation, compared to the first CWT. As a matter of fact, the subjects reported not to be distracted by the auditory stimulation.

Differences between individuals in stress response were observed for nearly all variables, as can be concluded from the interaction effects of the ANOVAs: the degree of inter-subject variability was highly significant for most variables, with the exception of adrenaline and peripheral blood flow. Noradrenaline was the only parameter which showed both positive and negative responses to the CWT.

The control-session. No significant differences were found between the first rest periods of the stress- and control-session with regard to heart rate (t=0.27; p=0.79), EMG (t=0.55; p=0.60), finger pulse amplitude (t=0.40; p=0.70) or SCL (t=0.36; p=0.74, n=6), indicating comparability of initial baselines in both situations. The physiological data of the control-session were also analyzed for trends, attributable to habituation or circadian fluctuations. A significant decline was found for heart rate, EMG activity and pulse amplitude, and a significant difference in regression between subjects for pulse amplitude only (Table 2). With regard to heart rate, EMG, and pulse amplitude, these findings are in agreement with the ANOVA results of the stress-session: the same trend is present during both sessions. SCL showed no significant regression during the control-session, while for the stress-session the covariate 'time' was significant. Recovery to baseline values after the CWT was apparently much slower for SCL than for the other parameters.

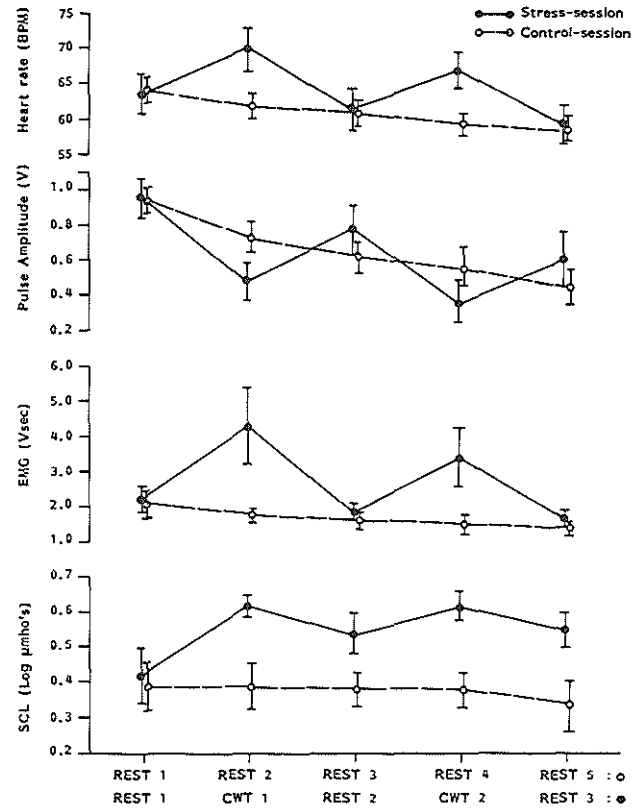

FIGURE 2
Mean±SE values per rest or CWT periods of the physiological parameters during the stress- and control-session.

Discussion

The aim of the present study was to test whether the CWT induces simultaneously four types of reactions required for a suitable stress test.
1) With regard to psychological effects, significant differences in subjective feelings of anxiety and tension were observed between the stress- and control-session. Increased feelings of discomfort and distress have been observed previously in response to the CWT, measured by ratio estimations comparing situational aspects with a 'standard' situation, or analogue ratings on alertness, distress and irritation (6-8). A sharp decrease in subjective arousal was observed when the test was presented five times with intervals of one week, indicating that increased subjective arousal was primarily observed during the first CWT session (6).

2) For all physiological parameters significant changes were observed. Although we observed a clear increase in heart rate during the CWT (6-7 beats/min), a much larger response was reported before (12) (28 beats/min). One explanation for this difference might be the fact that we used a relatively homogenous group of young subjects, all of about the same age, most of whom reported to be trained sportsmen. The response to the double-conflict CWT did not show the increased activation as observed by Frankenhaeuser et al. (8). However, they used a between-group design, while we used a within-group design.
3) EMG activity in the extensor muscles of the forearm was increased during the CWT. This may prove a valuable parameter for a suitable stress-test.
4) The CWT induced a significant increase in adrenaline concentration and variable results with regard to noradrenaline concentration for both plasma and urinary data. This is in agreement with previous findings (1,12). A significant increase in urinary cortisol secretion during the Stroop CWT has been reported in a group of male engineering students (5), while no significant responses of plasma cortisol and prolactin secretion were found in a group of women with oligomenorrhea (15). These last findings were confirmed by our study with healthy male volunteers. The CWT has, apparently, specific effects on the sympathetic-adreno-medullary system and not on the pituitary-adreno-cortical system (2).

The results, therefore, show that the CWT induces all four types of responses that are necessary for a test suitable to study stress-responses. However, it is not clear at this moment by what mechanism these responses are evoked. It could be argued that the CWT mainly increases arousal. Increases, specifically in adrenaline, in relation to the CWT [(14); this study] have been interpreted as indicating increased arousal. On the other hand, arousal during an ongoing task most often lasts for 10 minutes or less (22) and increased adrenaline after 20 minutes of CWT could be interpreted as an increase in effort, especially since effort may be closely connected to cognitive conflict presented with the CWT. Whether the CWT induces stress in the sense of a seriously overloaded effort mechanism (22) remains to be established. One way of exploring such mechanisms would be by administering anxiolytic drugs such as benzodiazepines and evaluating their effect on all reactions to the CWT. In the design of such an experiment habituation effects, response-magnitude in relation to initial values and spontaneous changes of basal values over time, as reported here, must be accounted for.

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CHAPTER 5

DOSE-RESPONSE EFFECTS OF INTRAVENOUS LORAZEPAM

Chapter 5.1

Dose-Dependent Effects of Intravenous Lorazepam on Cardiovascular Activity, Plasma Catecholamines and Psychological Function during Rest and Mental Stress

Summary

Dose-dependent effects of intravenously administered lorazepam on psychophysiological activity during rest and mental stress were studied in order to examine differential responses to doses which may induce anxiolysis or sedation. In a double-blind randomized cross-over study, nine male volunteers participated in a placebo and a lorazepam session, during which the subjects repeatedly performed a 10-min version of the Stroop Color Word Test (CWT), with 10 min of rest between the CWTs. Lorazepam was administered before each rest period in increasing doses of 0.0, 0.06, 0.13, 0.25 and 0.5 mg (total cumulative dose: 0.94 mg). Heart rate showed a dose-dependent decrease during rest with an ED_{50} of 0.13 mg lorazepam, while lorazepam had no effect on the cardiovascular and plasma catecholamine response magnitudes to the CWT. Subjective fatigue and reaction time increased significantly after 0.94 mg lorazepam, while at the same dose vigor decreased; state anxiety after the CWT was not influenced by lorazepam. These data show differential effects of lorazepam on cardiovascular, biochemical and psychological function. While heart rate was suppressed at low doses during rest and reaction time and subjective fatigue increased at doses which induced sedation, state anxiety and physiological response patterns to the CWT were not influenced by lorazepam.

Introduction

Anxiety is an emotional state in which psychic distress is associated with widespread physiological and biochemical changes, induced primarily by activation of the sympathetic adrenal-medullary system (Lader and Bruce 1986). In order to study the specificity of the relation between sympathetic adrenal-medullary activity and psychological function during anxiety or stress, controlled situations are needed in which anxiety or stress are induced

experimentally. The usefulness of the Stroop Color Word Test (CWT) as a stress-inducing performance task has been shown previously (Frankenhaeuser and Johansson 1976; Hjemdahl et al. 1984; Tulen et al. 1989). The CWT induces sympathetic adrenal-medullary activation as shown by increased cardiovascular activity and catecholamine secretion, in addition to psychic distress (Tulen et al. 1989). However, whether specific mechanisms related to anxiety, a non-specific increase in arousal or activation of effort mechanisms necessary to cope with the task (Sanders 1983) are responsible for the observed reactions is not clear. In order to analyze the mechanisms responsible for the responses to the CWT, administration of anxiolytic drugs may prove to be helpful. The benzodiazepines are the preferred anxiolytic drugs used today and induce different pharmacological effects at different dose levels: anxiolysis at low doses and sedation or sleep at higher doses (Geller and Seifter 1960). Suppression of sympathetic nervous system activity by benzodiazepines has been observed in man at doses which induce sedation (Duka et al. 1986; Roy-Byrne et al. 1988). These effects might, however, be different at doses which induce anxiolysis. It is therefore of interest to study specific dose-dependent sympathetic adrenal-medullary effects in relation to rest levels and stress-inducing performance tests.

In this study we investigated the cumulative dose-response effects of intravenously administered lorazepam during rest and during presentation of the CWT in a placebo-controlled design in healthy male volunteers. Lorazepam was chosen because of its short distribution half-life (intravenous: ± 4 min; Greenblatt et al. 1977) which allowed us to create a cumulative dose-response curve with five different doses within one experimental session. In addition, lorazepam has no active metabolites (Kyriakopoulos et al. 1978) which could complicate the interpretation of data. Five doses were administered, resulting in a total cumulative dose of ca. 1 mg lorazepam. The oral dose-equivalent of 1 mg lorazepam IV is circa 2 mg: a dose which has sedative effects (Patat et al. 1987; Preston et al. 1989). By studying the dose-dependency of effects of lorazepam IV on sympathetic adrenal-medullary activity and psychological function during rest and CWT, differential responses related to anxiolysis and sedation during rest and mental stress were explored.

Methods

Subjects, design and procedure

Nine male volunteers (mean: 23.9 years, range: 21-29) participated in a randomized double-blind cross-over study in two experimental sessions each: a placebo (PLA) and a lorazepam (LOR) session. The study was approved by

the Medical Ethical Committee of the University Hospital Dijkzigt. All subjects gave written informed consent and were paid for their participation in the study. The subjects underwent a medical screening: all were in good physical condition. Six of the nine subjects were non-smokers, three subjects smoked moderately (<5 cigarettes per day); heavy drinking was not reported.

During both sessions the subjects performed on five consecutive occasions a 10-min version of the Stroop Color Word Test (CWT), with 10 min of rest between the CWTs. Each CWT was followed by a Simple Reaction Time task (SRT) (duration task: 5 min). A schematic time-bar of the experimental procedures during each session is shown in Fig. 1. During the PLA session, a PLA injection (2.5 ml saline, slowly injected over 1 min) was administered five times intravenously, each time before the rest periods. During the LOR session, LOR was administered intravenously (in 2.5 ml saline solution, slowly injected over 1 min) before each rest period in increasing doses of 0.0 mg, 0.0625 mg, 0.125 mg, 0.25 mg and 0.5 mg (total cumulative dose: 0.9375 mg). Lorazepam has a distribution half-life (intravenous) of about 4 min; within 15 min a distribution of >95% is reached (Greenblatt et al. 1977) and its elimination half-life is about 12-13 h (Kyriakopoulos et al. 1978; Boulenger et al. 1984). The consecutive doses of LOR used in this study can therefore be supposed to induce a cumulative effect.

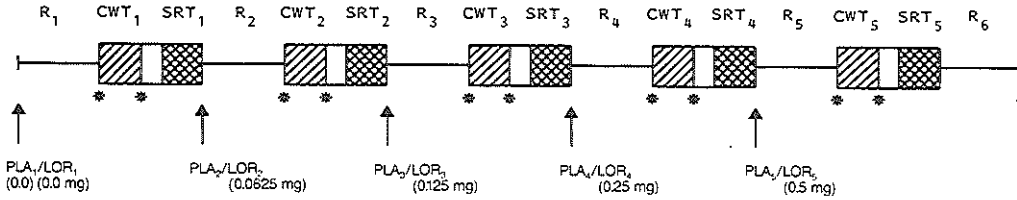

FIGURE 1
Schematic time-bar of the experimental procedures during the PLA and the LOR session. Injection moment (↑); CWT (hatched): Color Word Test; POMS: Profile Of Mood States, STAI: State-Trait Anxiety Inventory (blank); SRT (cross-hatched): Simple Reaction Time Task; PLA: placebo; LOR: lorazepam; R (-): rest period; blood sample (*).

The two experimental sessions per subject were recorded on separate days, 1 week apart. The subjects were requested to abstain from coffee, tea and smoking on both days, from the moment they got out of bed in the morning until they arrived in the laboratory. During the experimental sessions coffee, tea and smoking were not allowed. Before the start of each session, the subjects were requested to void urine and drink a glass of mineral water. Each session lasted from 09:00 to 12:30 hours: physiological, biochemical and

psychological measurements were performed during this period. The subjects were sitting in a comfortable chair during the entire session. After the session, a standard light lunch was served. Because sedative effects are observed after an intravenous cumulative dose of 1 mg LOR, in the afternoon the subjects remained in the hospital in a room where they could read or study. At 18:00 hours they were allowed to go home, after approval by the MD.

Tests

The Stroop Color Word Test (CWT). The CWT consists of four words (red, green, blue, yellow) which are presented on videotape, one word at a time, in four different colors (red, green, blue, yellow). The stimulus presentation and the interstimulus interval last about 1.5-2.0 s and conform to previously described versions of this test (Frankenhaeuser and Johansson 1976; Tulen et al. 1989). The subject has to indicate the color of the word on an answer sheet, with the specific request to do his very best and make as few errors as possible. The test induces a cognitive conflict (Stroop 1935), while time-pressure effects are added due to the rapid presentation of the stimuli. Within each session, five different 10-min versions of the CWT were used to avoid learning effects concerning word sequence. The number of errors made during each CWT was counted; the percentage of errors per CWT was calculated relative to the total number of stimuli presented. In order to become familiar with the specific requirements of the task, a two-min practice tape was presented at the beginning of the first session for instruction purposes.

Simple Reaction Time Task (SRT). A 5-min version of a visual reaction-time task was presented on a Personal Computer (Olivetti). The subject was requested to press a button as soon as possible after a small red square appeared on the monitor. The interstimulus-interval varied randomly between 1 and 5 s in order to avoid stimulus anticipation effects. Reaction time was calculated with an accuracy of 1 ms.

Recordings and analysis

Psychological. Before and after the whole session and immediately after each CWT, changes in subjective mood were assessed by means of a shortened version of the vigor and fatigue subscales of the Profile Of Mood States (POMS) (McNair et al. 1971) which was validated for the Dutch population (Wald and Mellenbergh 1990) and a shortened state version of the State-Trait Anxiety Inventory (STAI) (van der Ploeg et al. 1980; Knippenberg et al. 1990).

Physiological. A Nihon Kohden polygraph was used to amplify and calibrate the physiological signals. All signals were monitored continuously on paper by

means of a Siemens-Elema EEG mingograf, and at the same time recorded analogue on a Racal instrumentation recorder. The ECG was derived using a precordial lead. Blood pressure was recorded using a servo-plethysmo-manometer for continuous, non-invasive measurement of finger arterial blood pressure, employing the volume clamp technique of Penaz (Penaz et al. 1976; Wesseling et al. 1982; Settels and Wesseling 1985) (Finapres 2300 NIBP monitor, Ohmeda). ECG and blood pressure recordings were digitized with a sample frequency of 102.4 Hz on a Personal Computer (Olivetti), connected to a Labmaster Analogue/Digital converter. Before A/D conversion a Schmitt trigger was used to trigger the incidence of the R-waves in the ECG; the output pulses of the trigger were fed into the converter for sampling. The time between the output pulses (interbeat interval) was measured with a resolution of 10 ms. Systolic, diastolic and mean blood pressure (SBP, DBP, MBP) were defined per R-R interval of the ECG with a resolution of 0.2 mmHg. Obtaining blood samples caused momentary increases in heart rate and blood pressure and induced some movement artifacts: these periods were excluded from the analyses. One blood pressure recording showed technical shortcomings and was not analysed. Mean heart rate (in BPM) and mean SBP, DBP and MBP were calculated per rest period and for the first 5 min of each CWT. Only heart rate and MBP data will be presented because SBP and DBP data showed the same effects as MBP.

Biochemical. Forty-five min before the start of the recordings, a catheter (Venflon, 18G, Viggo AB, Helsingborg, Sweden) was inserted in an antecubital vein of the non-dominant forearm, through which blood samples were drawn and infusion of LOR/PLA took place. Blood samples (8 ml per sample) were drawn at the end of each rest period and during the second half of each CWT (the last 5 min) and collected in heparinized tubes containing 12 mg gluthathione for assay of catecholamines. The tubes were immediately placed on ice and centrifuged within 15 min. Plasma was subsequently frozen at -70°C. The catecholamines were assayed by means of high-performance liquid chromatography with fluorimetric detection (Van der Hoorn et al. 1989). The limits of detection for adrenaline and noradrenaline were 0.3 pg, with a low coefficient of variation (3-7%).

Statistical analyses

All analyses are presented for N=9 with the exception of the blood pressure analyses (N=8). For the physiological and biochemical data, two-tailed Student's t-tests were used for pairwise comparisons of the first rest periods of the two sessions to establish similarity of baseline values. Effects of LOR or PLA on rest values and CWT response magnitudes were analysed separately.

CWT response magnitude was defined as the difference in heart rate, blood pressure or catecholamines during the CWT and the preceding rest period. One-factor analyses of variance for repeated measurements were performed within each condition (LOR or PLA), to analyse specific dose effects (LOR condition: factor DOSE) or learning/habituation effects (PLA condition: factor TIME). In order to assess the effect of the cumulative doses of LOR the factor of increase in doses was taken into account to contrast between the successive rest periods or CWT responses (factor for contrast between the five dosages: 0, 1, 2, 4, 8). Dose-related changes in subjective fatigue, vigor or anxiety were analysed per condition (LOR or PLA) with a nonparametric one-factor analysis of variance (Friedman) (Siegel 1956), while Wilcoxon tests (Siegel 1956) were applied for pairwise comparisons between the different dose levels within each condition.

Where appropriate, dose-response curves were constructed and presented graphically, using the mean response of all individuals for each dose. ED_{50}s were calculated for each individual per variable, using linear interpolation. Paired Student's t-tests were used to compare ED_{50}s of the different responses.

Results

Cardiovascular activity and plasma catecholamines

Table 1 and Figs. 2 and 3 present the dose-related effects of lorazepam on cardiovascular activity and plasma catecholamines during rest and CWT, in relation to the placebo administrations.

Rest periods. Baseline values of the PLA and LOR sessions were similar for heart rate ($t=-1.61$; $p=.15$), MBP ($t=0.69$; $p=.51$), plasma adrenaline ($t=1.25$; $p=.24$) and plasma noradrenaline ($t=-2.11$; $p=.07$). The analyses of variance indicated a significant DOSE effect for heart rate in the LOR session (Table 2). After a cumulative dose of 0.19 mg LOR (LOR3) heart rate during rest decreased significantly in comparison with the first rest period (R1) ($t=4.95$; $p=.001$) (Fig. 2). This effect appears to be a specific effect of LOR, since no TIME effect was observed in the PLA session (Table 2). With regard to MBP, a significant TIME effect was observed in the PLA session (Table 2), indicating an increase in MBP in time. Increased values were observed mainly in the last two rest periods (R5 and R6, Fig. 2), the difference between R1 and R5 being significant ($t=-7.22$; $p<.001$). This increasing trend was not present in the LOR session. LOR had no effect on the plasma adrenaline levels; trends during the PLA session were not observed (Table 2). Figure 3 indicates a gradual increase in plasma noradrenaline levels during rest in the PLA session (especially during R5), which was not present in the LOR session. The TIME

Table 1. Mean (SD) values of the cardiovascular data and the plasma catecholamine levels during the consecutive rest periods (REST1 to REST6) and the CWT response magnitudes (CWT1 to CWT5) of the LOR and the PLA session

		REST1	REST2	REST3	REST4	REST5	REST6	CWT1	CWT2	CWT3	CWT4	CWT5
HR	PLA	64.1	65.4	63.9	64.0	61.8	61.7	8.6	6.5	6.3	5.9	5.3
(bpm)		(7.3)	(7.4)	(9.1)	(7.9)	(9.8)	(8.4)	(4.9)	(6.7)	(7.4)	(5.2)	(6.2)
	LOR	70.4	68.7	64.7	64.0	63.6	64.6	8.6	4.5	6.2	7.4	7.7
		(9.8)	(8.0)	(7.8)	(6.6)	(7.5)	(9.0)	(8.5)	(4.1)	(3.7)	(5.6)	(7.3)
MBP	PLA	76.6	79.3	81.1	83.2	88.8	87.6	14.5	8.4	8.3	4.6	4.3
(mmHg)		(13.9)	(13.8)	(12.4)	(12.5)	(14.6)	(14.1)	(5.6)	(3.9)	(5.8)	(3.8)	(9.1)
	LOR	79.7	82.3	84.7	85.5	88.3	88.9	17.7	9.2	7.9	8.2	8.0
		(15.3)	(12.5)	(13.6)	(10.8)	(11.2)	(11.3)	(11.1)	(6.7)	(4.2)	(6.9)	(4.5)
ADR	PLA	41.3	37.0	33.0	30.1	33.1	38.3	18.3	16.8	20.0	23.8	13.8
(pg/ml)		(25.5)	(17.3)	(16.1)	(16.0)	(18.1)	(24.5)	(19.2)	(27.7)	(19.2)	(12.0)	(12.4)
	LOR	31.4	30.1	27.8	29.2	29.8	30.8	22.6	19.8	20.2	15.6	15.3
		(18.7)	(11.5)	(10.9)	(11.0)	(10.2)	(14.3)	(14.5)	(12.8)	(9.7)	(8.4)	(12.6)
NOR	PLA	228.7	229.9	240.1	258.9	299.0	278.0	17.4	24.8	13.2	-3.0	-19.3
(pg/ml)		(69.8)	(71.1)	(77.7)	(93.9)	(111)	(84.8)	(40.0)	(27.4)	(36.3)	(31.1)	(50.6)
	LOR	196.6	200.7	206.7	225.2	214.7	230.1	15.1	28.7	9.7	-7.3	-6.8
		(52.2)	(58.0)	(71.6)	(97.9)	(66.7)	(94.7)	(30.0)	(75.6)	(18.6)	(30.3)	(35.8)

HR: heart rate, MBP: mean blood pressure, ADR: adrenaline, NOR: noradrenaline

Table 2. Analyses of variance for repeated measures on the cardiovascular and biochemical data: F-values and level of significance (p) of the within-subject effects of the main factor Dose (injection 1-5) during the lorazepam session and the main factor Time during the placebo session are presented. Level of significance and F-values of the between-subjects effects are also indicated. The analyses were performed on the rest periods and the CWT response magnitudes separately

		Rest periods				CWT			
		LOR		PLA		LOR		PLA	
		Dose	Subject	Time	Subject	Dose	Subject	Time	Subject
HR	F	7.62	716.78	0.54	773.69	1.11	19.76	1.13	13.77
	p≤	0.001	0.001	0.710	0.001	0.370	0.002	0.358	0.006
MBP	F	2.28	424.21	17.78	308.55	6.00	26.62	6.36	27.61
	p≤	0.085	0.001	0.001	0.001	0.001	0.001	0.001	0.001
ADR	F	0.33	62.55	2.23	36.93	0.91	35.74	0.50	19.15
	p≤	0.855	0.001	0.088	0.001	0.474	0.001	0.737	0.002
NOR	F	1.08	93.88	4.93	92.30	0.88	4.73	2.39	0.74
	p≤	0.382	0.001	0.003	0.001	0.490	0.066	0.074	0.418

HR: heart rate; MBP: mean blood pressure; ADR: adrenaline; NOR: noradrenaline

effect for plasma noradrenaline levels during the PLA session was significant (Table 2).

CWT responses. The response magnitudes to CWT1 of the LOR and PLA session were similar for heart rate (t=-0.02; p=.98), MBP (t=-0.76; p=.47), plasma adrenaline (t=0.81; p=.45) and plasma noradrenaline (t=-0.06; p=.95). There were no differences in heart rate responses to the consecutive CWTs in the PLA session (factor TIME: ns., Table 2), nor did LOR affect the response to the CWT (factor DOSE: ns., Table 2). A decrease in response magnitude was observed for MBP, both in the PLA session (a significant TIME effect, Table 2) and in the LOR session (a significant DOSE effect, Table 2), indicating learning or habituation effects to the CWT within each condition, but no drug-specific effects (Fig. 2). LOR had no effect on the plasma catecholamine response magnitudes to the CWTs (Table 2). Figure 3 shows an increase in plasma noradrenaline secretion during CWT1, CWT2 and CWT3 in relation to the preceding rest periods. However, thereafter a negative noradrenaline response is observed during CWT4 and CWT5.

In summary, during the rest periods the only effect of LOR was a dose-dependent decrease in heart rate; cardiovascular and plasma catecholamine response magnitudes to the CWT were not influenced by LOR. Nearly all variables showed significant (between) subject effects, indicating large interindividual variability (Tables 1 and 2).

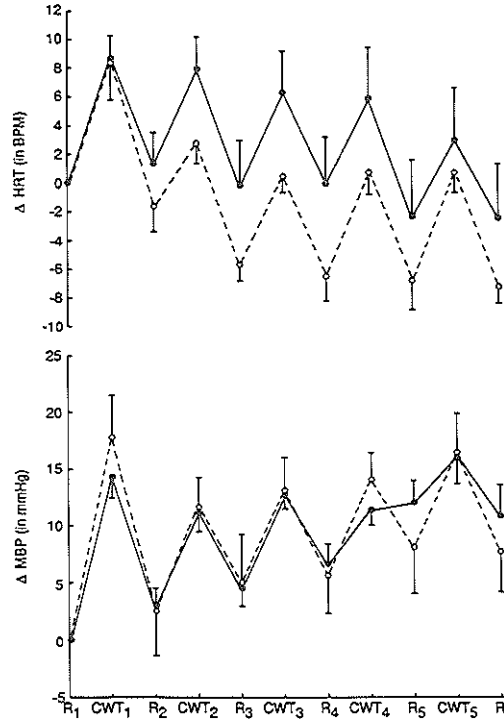

FIGURE 2
Dose-related effects of lorazepam IV (open circles) on cardiovascular activity during rest and CWT, in comparison with cardiovascular activity during placebo (closed circles) administrations. The upper curve presents mean (and SEM) heart rate of the consecutive rest and CWT periods, calculated relative to R1 (i.e. baseline: first rest period). The lower curve presents mean MBP, relative to R1 (see also FIGURE 1).

Performance on tasks and subjective mood

SRT. In Fig. 4 the dose-dependent effects of LOR on reaction time to the five consecutive SRTs are presented. Reaction time to SRT1 of the LOR and PLA session was similar ($t=0.95$; $p=.37$). A gradual increase in reaction time was observed in the LOR session (factor DOSE: $F=4.99$, $p=.003$; Subject: $F=392.64$, $p<.001$), but not in the PLA session (factor TIME: $F=.49$, $p=.74$; Subject: $F=408.38$, $p<.001$), which indicates a specific drug effect. Reaction time was significantly increased after a cumulative dose of 0.94 mg LOR (SRT1-LOR versus SRT5-LOR: $t=2.58$; $p=.03$; SRT5-LOR versus SRT5-PLA: $t=4.60$; $p=.001$) (Fig. 4).

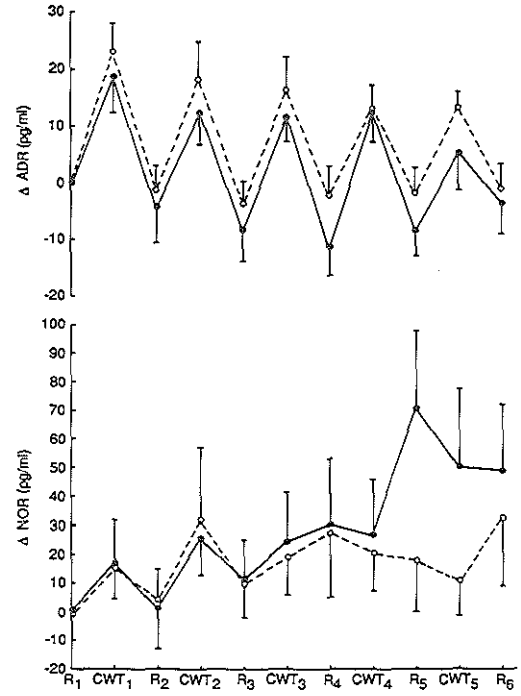

FIGURE 3
Dose-related effects of lorazepam IV on plasma catecholamines during rest and CWT, in comparison with plasma catecholamines during placebo administrations. (see legend to FIGURE 2 for explanation). ADR=adrenaline. NOR=noradrenaline.

CWT performance. The percentage of errors to the CWTs of the PLA session decreased from 6.7% during CWT1 to 4.8% during CWT5 (non-significant difference). During the LOR session the percentage of errors was similar from CWT1 (6.6%) to CWT4 (5.2%), but increased significantly during CWT5 (17.1%; t=-5.02, p=.001) in comparison with CWT1.

Subjective mood. Table 3 indicates the changes in fatigue, vigor and anxiety, as assessed immediately after each CWT, during the LOR and the PLA session. During the LOR session, we observed a gradual increase in fatigue: after a cumulative dose of 0.94 mg LOR (LOR5), this increase was significant in comparison with the baseline values (z=-2.20; p=.02). Similarly, a significant decrease in vigor was observed after a cumulative dose of 0.94 mg LOR (LOR5) in comparison with baseline values (z=-2.49; p=.01). Within-session analyses revealed a significant DOSE effect during the LOR session for both fatigue and vigor, but no effect at all on state anxiety.

Table 3. Mean and range values of subjective mood (fatigue, vigor, anxiety) for the five consecutive assessments after each CWT during the PLA and the LOR session. Friedman analysis of variance were performed for the PLA and LOR session separately; Chi-square and level of significance (p-values) are indicated per session

	Fatigue		Vigor		Anxiety	
	PLA session	LOR session	PLA session	LOR session	PLA session	LOR session
1	7.2(3)	7.2(4)	17.6(11)	18.2(15)	13.0(6)	12.7(5)
2	7.0(2)	7.1(3)	17.9(11)	17.1(10)	12.8(3)	13.0(4)
3	7.2(3)	7.8(7)	17.4(10)	16.3(9)	12.8(6)	13.0(8)
4	6.9(3)	8.6(8)	17.2(10)	15.9(9)	12.9(5)	13.8(9)
5	7.9(8)	10.2(13)	15.6(10)	14.3(13)	13.6(4)	12.9(7)
Chi^2	0.7	12.2	4.5	10.6	1.8	3.5
$p \leq$	0.95	0.01	0.34	0.03	0.78	0.48

Dose-response curves

Figure 5 shows the dose-response curves for heart rate, SRT, fatigue and vigor. The ED_{50}s calculated on basis of the individual dose-response curves were 0.13 mg $\pm$ 0.06 (mean$\pm$SD, N=9) for heart rate, 0.32$\pm$0.23 (N=8) for SRT, 0.24$\pm$0.27 (N=7) for vigor and 0.47$\pm$0.17 (N=7) for fatigue. Two individuals did not show a response on the vigor and fatigue scales and therefore were excluded from the analysis. Paired Student's t-tests revealed a significant difference between the ED_{50}s for heart rate versus fatigue (t=-5.27, p=0.002, N=7). From Fig. 5 it is apparent that the heart rate response has indeed reached a maximum, but this is not the case for the other three variables. Therefore, the ED_{50}s for SRT, fatigue and vigor are probably underestimated, as is also illustrated by the fact that two individuals did not yet show a response on the fatigue and vigor scale after a cumulative dose of 0.94 mg LOR.

To summarize the results:

1. Heart rate during rest was reduced dose-dependently with an ED_{50} of 0.13 mg LOR.
2. LOR induced an increase in SRT (ED_{50}>0.32) and fatigue (ED_{50}>0.47) and a decrease in vigor (ED_{50}>0.24). The ED_{50} of subjective fatigue was significantly higher than the ED_{50} of heart rate during rest.
3. The CWT induced consistent increases in heart rate and plasma adrena-

line levels, but LOR had no effect on the heart rate, MBP or catecholamine response magnitudes to the CWT.

4. LOR had no effect on state anxiety, nor on MBP or plasma catecholamines, although an increase in MBP and plasma noradrenaline was observed during the rest periods of the PLA session.

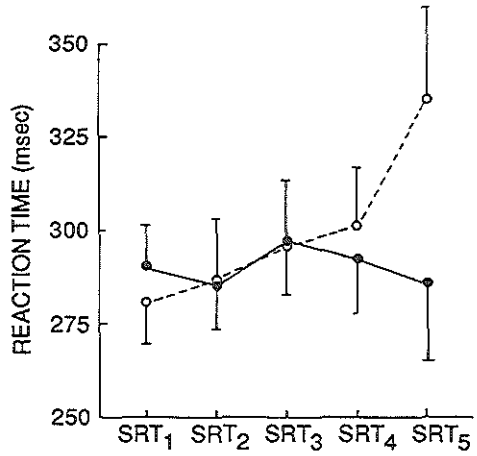

FIGURE 4
Dose-related effects of lorazepam (open circles) on simple reaction time, in comparison with simple reaction time to placebo (closed circles) administrations. Mean (and SEM) reaction time in ms as measured during the five consecutive SRTs of the LOR and PLA session.

Discussion

The aim of the present study was to separate sedative responses to lorazepam from anxiolytic responses which are supposed to occur at lower doses than sedative responses and to link either of these responses to effects on sympathetic adrenal-medullary activity.

Our results show a clear sedative effect of lorazepam as expressed by increased reaction time, increased number of errors on the CWT, increased fatigue and decreased vigor. Performance impairment, reduced attention or sedation have been described before after an oral dose of 2 mg LOR (Patat et al. 1987; Preston et al. 1989). In our study most of these responses occurred with an ED_{50} of 0.24 mg intravenously or higher, which is probably equivalent to an oral dose of 0.5 mg or higher.

Anxiolytic responses, however, were not observed as no effect of LOR on state anxiety or on any of the responses to the CWT could be detected, irrespective of the fact that the CWT reproducibly induced an increase in heart rate and plasma adrenaline levels. One might argue that the CWT as a stress-

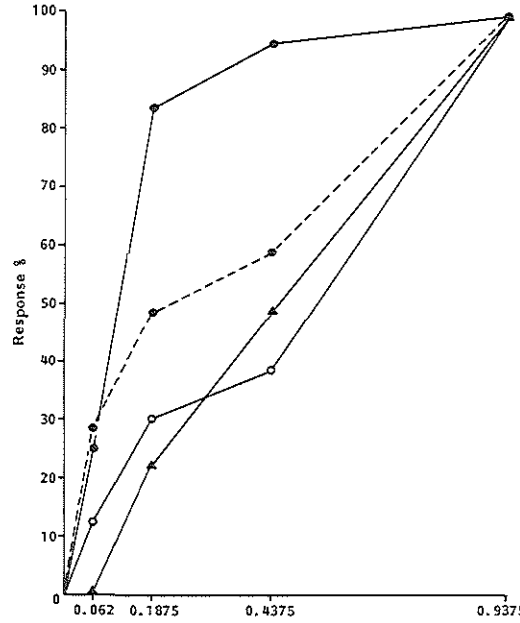

FIGURE 5
Mean (N=9) cumulative dose-response curves of LOR on heart rate (closed circles) during rest, fatigue (closed triangles), vigor (closed circles; dashed line) and reaction time (SRT) (open circles). Y-axis: heart rate (HRT) and vigor: % reduction; SRT and fatigue: % increase.

inducing performance task did not induce a relevant increase in anxiety in these healthy (non-anxious) volunteers and that therefore an anxiolytic effect of LOR could not be demonstrated. This is in line with the findings of File and Lister (1985), who report a failure of LOR to reduce experimentally-induced anxiety after oral doses of 1 and 2.5 mg.

Since no subjective anxiety, nor any effects of LOR on responses to the CWT were observed, the heart rate and adrenaline responses to each CWT may reflect an increase in effort mechanisms (Sanders 1983), which are necessary for the subject to cope with the task, rather than stress- or anxiety-related responses. Compensatory mechanisms as a result of subjective sedation may have partly masked a suppressing effect of LOR on the response magnitudes to the CWT, since motivation to perform a task is not negatively influenced by doses of LOR which induce sedation (File and Lister 1982). In order to establish whether the heart rate or adrenaline responses to the CWT are part of effort mechanisms necessary to perform a task, compensation

mechanisms to overcome sedation, or stress-response mechanisms on which LOR has no influence, application of anxiolytica without sedative properties (such as Buspirone; Cohn and Wilcox 1986) or anxiolytics with a specific influence on plasma catecholamines (such as Alprazolam; Grant et al. 1984) should be studied in a placebo-controlled design.

Evidence of direct or indirect involvement of the central benzodiazepine pathways in the regulation of sympathetic nervous system activity is scarce. Some studies report normal or increased heart rate after an oral administration of 1-2.5 mg LOR (Elliott et al. 1970; File & Lister, 1985), but other studies have shown a suppression of plasma catecholamine levels and blood pressure by benzodiazepines at doses which induce sedation (Roy-Birne et al. 1988, 1989; Duka et al. 1986). Our data are in agreement with these last observations since a cumulative dose of 0.94 mg LOR, which significantly induced subjective sedation, also suppressed plasma noradrenaline and MBP levels during rest. However, we found that heart rate already was reduced at lower doses with an ED_{50} of 0.13 mg intravenously. Since plasma catecholamines and blood pressure were unaffected at these doses of LOR, this effect may reflect an increase in vagal stimulation of the heart, rather than a suppression of sympathetic activity. This effect occurred at dosages expected to exert an anxiolytic effect and not sedative effects. Whether it is indeed related to vagal stimulation and clinical anxiolysis, however, could not be established in the present experiment and awaits further studies.

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Chapter 5.2

Effects of Lorazepam on Cardiac Vagal Tone during Rest and Mental Stress: Assessment by means of Spectral Analysis

Summary

Dose-dependent effects of intravenously administered lorazepam on haemodynamic fluctuations during rest and mental stress were studied by means of spectral analysis, in order to unravel sympathetic and parasympathetic components in cardiovascular control after benzodiazepine administration. In a double-blind randomized study, nine male volunteers participated in placebo and lorazepam sessions, during which the subjects repeatedly performed a 10-min version of the Stroop Color Word Test (CWT), with 10 min of rest between the CWTs. Lorazepam was administered before each rest period in increasing doses of 0.0, 0.06, 0.13, 0.25 and 0.5 mg (total cumulative dose: 0.94 mg). During the placebo session the subjects received 5 placebo injections. Heart rate (HR), blood pressure (BP; Finapres device) and respiration were recorded continuously. Power spectra were calculated per 2.5 min periods for HR, systolic (SBP) and diastolic BP (DBP). Spectral density was assessed for 3 frequency bands: low (LFB:0.02-0.06 Hz), mid (MFB:0.07-0.14 Hz) and high (HFB:0.15-0.40 Hz). Per time-segment baroreflex sensitivity (BRS) was computed as the gain (or modulus) in the MFB between the systolic pressure values and the R-R interval times.
During the consecutive periods of rest, lorazepam induced a dose-dependent decrease in HR, and an increase in BRS and LFB, MFB and HFB power of HR. These effects were significant after 0.19 mg lorazepam for HR, BRS and HFB power and after 0.44 mg lorazepam for the HR fluctuations in the LFB and MFB. Subjective fatigue increased significantly after 0.94 mg lorazepam. Lorazepam did not significantly influence BP variability or respiration rate during rest. During both sessions, the CWT significantly increased HR, SBP, DBP, and respiratory frequency and decreased BRS and LFB, MFB, and HFB power of HR and BP. Lorazepam did not induce dose-dependent effects on these responses to the CWT, although performance to the CWT deteriorated significantly after a dose of 0.94 mg.

Our data underline that benzodiazepines can exert an influence on cardiac vagal tone. In this experiment lorazepam induced dose-dependent

increases in cardiac vagal tone, resulting in decreased HR and increased HR variability during periods of rest. This effect was overruled during a mental stress task. The increase in vagal tone observed after low doses of lorazepam was not related to diminished sympathetic activity, altered respiration, or increased sedation.

Introduction

Benzodiazepines are the preferred drugs in the treatment of the anxiety symptoms in patients with cardiovascular dysfunctions. The effects of benzodiazepines on autonomic cardiovascular control mechanisms in man have been studied and interpreted primarily in relation to sympathetic nervous system activity (Duka et al., 1986; Marty et al., 1986; Roy-Byrne et al., 1988; Tulen et al., 1991) and not to parasympathetic mechanisms.

Analysis of variation patterns in cardiovascular parameters such as heart rate (HR) or blood pressure (BP) offers one approach to obtain non-invasive indices of parasympathetic activity. Previous research has shown that breathing linked variations in HR reflect a parasympathetic influence on the heart by means of alterations in cardiac vagal inhibition (Higgins et al., 1973; Katona et al., 1975; Eckberg, 1983). Beat to beat fluctuations in HR and BP are now widely studied to quantify parasympathetic and sympathetic influences within the complex neural control of short-term homeostatic cardiovascular processes (i.e., Akselrod et al., 1981, 1985; Pomeranz et al., 1985; Yongue et al., 1982; Malliani et al., 1991).

A normal vagal cardiac control has been associated with good health (Eckberg, 1980). Since Epstein et al. (1973) have observed that in patients with an acute myocardial infarction the vagal tone to the heart exerts a significant protective effect against lethal ventricular arrhythmias, a careful evaluation of the vagolytic or vagomimetic properties of anxiolytic drugs seems warranted. Animal research has suggested that benzodiazepines can affect cardiac vagal tone by means of GABA-ergic inhibitory mechanisms (DiMicco, 1987). A recent study of Adinoff et al. (1992) in human subjects supports this assumption.

Previously, we have reported the dose-response effects (dose range: 0.0-0.94 mg) of cumulative intravenous doses of lorazepam on sympathetic nervous system activity and psychological parameters (Tulen et al., 1991). We observed a significant decrease in heart rate at low doses of lorazepam (ED_{50}: 0.13 mg) during periods of rest, whereas sedation and suppression of sympathetic parameters occurred at a significantly higher cumulative dose of 0.94 mg. We suggested that this may reflect an increase in vagal stimulation of the heart, but were unable to substantiate this. We now have analyzed the

data of the above mentioned experiment by means of spectral analysis of beat to beat fluctuations in HR and BP. With this method three spectral peaks are usually defined within a time segment of several minutes (Sayers, 1973; Akselrod et al., 1981; 1985): 1) a low frequency peak with variations around 0.04 Hz; for HR this peak is associated with both parasympathetic and sympathetic activity (Akselrod et al., 1985), while these low-frequency BP fluctuations are linked with variations in peripheral vasomotor activity due to thermoregulatory influences (Kitney, 1975) or renin-angiotensin system activity (Akselrod et al., 1985), 2) a mid frequency peak with variations around 0.1 Hz (Mayer waves) which has been associated with changes in sympathetic tone (Pagani et al., 1986), or with a resonance in the baroreflex control of peripheral resistance (Madwed et al., 1989; Wesseling & Settels, 1985), and 3) a high frequency peak around the respiratory frequency, usually between 0.20-0.35 Hz, which for HR represents centrally mediated vagal activity (Angelone & Coulter, 1964, Davies & Nielson, 1967). For BP these fluctuations may result from centrally mediated HR fluctuations (Akselrod et al., 1985), although the mechanical effects of respiration may also contribute substantially to these fluctuations (Saul et al., 1991). In addition, we also analyzed baroreflex sensitivity according to the method described by Robbe et al. (1987).

The Stroop Color Word Test (CWT) was used in order to compare the effects of lorazepam during periods of rest and during periods of increased HR and BP due to mental stress as induced by the CWT (Frankenhaeuser and Johansson 1976; Hjemdahl et al., 1984; Tulen et al., 1989,1991).

Methods

Subjects

Nine male volunteers (mean age: 23.9 years; range:21-29) participated each in two sessions in a randomized double-blind study, after they had given written informed consent. The study procedures and protocol were approved by the Medical Ethical Committee of the University Hospital Rotterdam Dijkzigt. The screening procedure included a medical examination to exclude subjects with cardiorespiratory abnormalities. All subjects were in good physical condition. Subjects with a history of alcohol or drug abuse were excluded from the study.

Design, procedure and measurements

Details of the procedures have been presented before (Tulen et al., 1991). During both sessions, the subjects performed on 5 consecutive occasions a 10-min version of the Stroop Color Word Test (CWT), with 10 min of rest

between the CWTs. The CWT consists of four words (red, green, blue, yellow) which are presented on videotape, one word at a time, in four different colors (red, green, blue, yellow). The subject has to indicate the color of the word on an answer sheet, with the specific request to do his utmost best and make as few errors as possible. The test induces cognitive conflict (Stroop, 1935), while time-pressure effects are added due to the rapid presentation of the stimuli (on average one word per 1.5-2 sec). In order to become familiar with the requirements of the task, a 2-min practice tape was presented at the beginning of the first session for instruction purposes.

During one session (the placebo- or PLA-session), an intravenous PLA injection (2.5 ml saline, slowly injected over 1 min) was administered five times, each time before the rest periods. During the other session (the lorazepam- or LOR-session), LOR was administered intravenously (in 2.5 ml saline, slowly injected over 1 min) before each rest period in increasing doses of 0.0, 0.0625, 0.125, 0.25 and 0.5 mg (total cumulative dose: 0.9375 mg). The two sessions per subject were recorded on separate days, 1 week apart. Each session lasted from 09:00 to 12:30 hours. Physiological, biochemical and psychological measurements were obtained while the subjects were seated in a comfortable armchair during the entire recording.

Forty-five minutes before the start of the recordings, a catheter (Venflon, 18G, Viggo AB, Helsingborg, Sweden) was inserted into an antecubital vein of the non-dominant forearm, through which blood samples were drawn and infusion of LOR/PLA were given. Blood samples for assay of lorazepam concentrations were obtained 15 min after the injections (i.e. during the second half of each CWT: the last 5 min). Blood was collected in heparinized tubes; the tubes were immediately placed on ice and centrifuged within 15 min after collection. Plasma was subsequently frozen at -70° C. Lorazepam was assayed with a high-performance liquid chromatographic method according to Brodie et al. (1978), with the following modifications: extraction with dichloromethane and a methanol/-ammoniumphosphate-buffer (50/50) was used for elution of the column.

ECG, blood pressure and respiration were recorded continuously during the sessions on an FM-type analogue recorder (Racal Store 14 DS, Sarasota, Florida, USA) for off-line analyses per computer. The ECG was derived using a precordial lead, amplified by means of a polygraph (Nihon Kohden, Tokyo, Japan). Blood pressure was recorded using a servo-plethysmo-manometer for continuous non-invasive measurement of finger arterial blood pressure, employing the volume clamp technique of Penaz (Penaz et al., 1976; Settels and Wesseling, 1985) (Finapres 2300 NIBP monitor, Ohmeda, Englewood, CO, USA). The cuffed middle finger of the non-

dominant hand was kept at the level of the heart by means of a supportive arm-rest, in order to optimize the correspondence with intrabrachial pressure changes (Parati et al., 1989). Thoracic and abdominal respiration were measured separately by means of impedance plethysmographs (Nihon Kohden, Tokyo, Japan). Adhesive disposable Ag/AgCl electrodes (Red Dot, Medical Products Devision, 3M, St.Paul, USA) were used for the thoracic and abdominal respiration recordings, placed at the level of the nipples and the abdomen, respectively.

Analyses

The ECG and blood pressure signals were digitized at a sample frequency of 1024 Hz on a Personal Computer (Commodore PC 60-III) connected to an Analogue/Digital converter (Advantech PC-LabCard model PCL-718). R-R intervals in the ECG were detected with an accuracy of 1 ms and transposed to heart rate (HR) series. Systolic and diastolic blood pressure (SBP, DBP) were defined per R-R interval of the ECG, with an accuracy of 0.1 mmHg. For prolonged blood pressure recordings, the Finapres device has the disposal of a built-in "lock-adjust" procedure for automatic adjustment of the finger cuff pressure by means of a servosystem, which is activated in parallel with blood flow changes. This procedure takes place every 40 to 70 beats under stationary conditions. Since a total session in this study could last upto 3 hours, we employed this procedure to prevent slow drifts and unreliable recordings. As a result, every 40 to 70 beats, 2-4 pulses were missing from the blood pressure recording. By means of a linear interpolation between 2 preceding and 2 succeeding pulses the missing values were estimated, while a small amount of additional noise (0.25 times Standard Deviation) was added in order to prevent a temporary excessive reduction in variability due to the correction procedure itself (Mulder, 1988). In addition, time-series of HR, SBP and DBP were scrutinized for stationarity and artifacts by means of visual inspection. One blood pressure recording showed technical shortcomings and was not analyzed. The thoracic respiratory signal was sampled with a frequency of 102.4 Hz.

Per recording, the consecutive 10 min periods of rest were analyzed, in addition to the first 5 min of each CWT.

Spectral analysis of heart rate and blood pressure. Within each period of rest or CWT, consecutive time segments of 2.5 min of HR, SBP and DBP time series were subjected to a discrete Fourier transform, based on non-equidistant sampling of the R-wave incidences (CARSPAN program, Mulder et al., 1988). With this method power spectral densities of rhythmic oscillations over a frequency range of 0.02-0.50 Hz were obtained, with a frequency resolution of 0.01 Hz. For each time segment, power density was calcula-

ted for the total band (0.02-0.50 Hz), low frequency band (LFB:0.02-0.06 Hz), mid frequency band (MFB: 0.07-0.14 Hz) and high frequency band (HFB: 0.15-0.40 Hz), in addition to mean HR, SBP and DBP, and variation coefficients (VC) of HR, SBP and DBP. Spectral energy was expressed in relative terms, i.e. in normalized values relative to the mean value of the considered signal (squared modulation index, to be compared with squared variation coefficient; van Dellen et al., 1985). Because the total power equals the squared variation coefficient minus the low frequency DC component, total power data are not presented, but variation coefficients are. For the spectral data a logarithmic transformation was performed because of skewness of the distributions. As an index of baroreflex sensitivity (BRS), we computed per time segment the gain (or modulus) in the MFB between the systolic pressure values and the R-R interval times, based on those frequency points within the 0.07-0.14 Hz range with a coherence between the two signals of greater than or equal to 0.5 (Robbe et al., 1987). The results of the analysis of the time segments were averaged per consecutive rest or CWT period; this procedure reduced a noise factor due to spontaneous segment to segment fluctuations and allowed a statistical analysis of the dose- or time-dependent changes within the sessions.

Respiration. Mean (SEM) respiratory cycle duration (in sec) and inspiratory depth (in percentage of change versus baseline times 100) were calculated per period of rest or CWT, on basis of analysis of the thoracic respiratory signal. In addition, respiratory irregularities were quantified by computing the number of sighs (amplitude increase by a factor 2, versus the mean amplitude of the previous 30 respiratory cycles) or hypopnoeas (amplitude decrease by a factor 0.5) per period of rest or CWT.

Statistical analyses

Data will be presented as mean (SEM) for N=9 with the exception of the blood pressure analyses (N=8). Similarity of the baseline values of the 2 sessions was evaluated by means of t-tests for pairwise comparisons. MANOVA's for repeated measurements were performed for each condition separately: for the LOR-condition, MANOVA's were used to establish the effect of the CWT versus the rest periods (within-subject Factor STRESS: rest/CWT), the 5 doses of LOR (within-subject Factor DOSE: 5 consecutive rest and CWT periods) and the interaction between factors STRESS and DOSE (reflecting the effect of increasing doses of lorazepam on the consecutive CWT responses, i.e. the CWT response magnitudes versus the preceding rest periods). For the PLA-condition, the MANOVA's were employed to establish the effect of the CWT versus the rest periods (within-subject Factor STRESS: rest/CWT), the time-dependency or habituation effects (within-

subject Factor TIME: 5 consecutive rest and CWT periods) and the interaction effect between factors STRESS and TIME (reflecting habituation effects in the consecutive CWT response magnitudes). If a significant main effect or interaction effect was observed, t-tests for pairwise comparisons were used in order to search for specific dose- or time-related effects. A p-value of $<.05$ was used to indicate a significant effect.

Results

Plasma concentrations of lorazepam

The plasma LOR concentrations were proportional to the cumulative dose administered (Table 1).

Table 1. Plasma concentrations of lorazepam

dosis (mg)	cumulative dosis (mg)	plasma lorazepam (ng/ml)
0.0000	0.0000	0.00 (0.00)
0.0625	0.0625	3.56 (0.44)
0.1250	0.1875	6.22 (0.52)
0.2500	0.4375	14.56 (3.54)
0.5000	0.9375	20.00 (0.91)

Cardiovascular variability

Baseline values of the PLA- and LOR-session were similar for all cardiovascular parameters ($p>.05$; NS).

Heart rate (HR). The CWT significantly increased HR and significantly decreased HR VC and fluctuations in the LFB, MFB and HFB during both the PLA- and the LOR-session (Table 2,4, Figure 1). During the PLA-session, the CWT response magnitudes showed no habituation during the 5 consecutive CWT presentations (no significant interaction effects, Table 2); significant time-dependent trends were only observed for the LFB fluctuations, showing a small gradual increase in power during the entire session (Table 2, Figure 1). LOR induced a significant dose-dependent decrease in HR, while HR VC and LFB, MFB and HFB fluctuations increased dose-dependently (Table 2, Figure 1,3). Since no significant interaction effects between factors STRESS and DOSE for HR and LFB, MFB and HFB fluctuations were observed, it can be concluded that the resting values were

Table 2. MANOVA F-values and level of significancies for the PLA- and LOR-session separately

	PLACEBO			LORAZEPAM		
	Stress	Time	Stress x Time	Stress	Time	Stress x Time
HR	12.82**	1.40	0.37	16.67**	12.04***	0.96
VC HR	29.54***	1.69	1.72	59.57***	15.23***	3.82**
LFB HR	25.87***	2.90*	0.45	37.83***	13.45***	0.58
MFB HR	42.52***	1.61	2.43	24.83***	8.68***	1.36
HFB HR	11.81**	0.74	1.10	34.04***	10.85***	0.67
SBP	12.39**	4.60**	3.15*	11.95**	0.53	7.13***
VC SBP	7.77*	5.12**	0.11	22.86**	1.70	0.44
LFB SBP	21.35**	4.98**	1.05	98.70***	0.38	0.04
MFB SBP	15.53**	0.96	0.50	17.48**	0.34	0.40
HFB SBP	3.78	1.89	1.24	39.26***	0.99	1.32
DBP	16.92**	5.68**	5.04**	32.06***	0.94	4.34**
VC DBP	13.42**	1.08	0.13	18.00**	2.03	0.27
LFB DBP	17.03**	0.62	0.60	54.66***	3.35*	0.18
MFB DBP	58.54***	2.36	0.15	27.53***	0.15	0.36
HFB DBP	5.87*	0.33	2.50	15.30**	0.88	0.48
BRS	10.89**	0.57	1.05	12.88**	7.14**	0.78
Resp cycle	15.75**	0.68	0.95	28.38***	1.49	0.93
Resp depth	6.60*	1.48	1.08	4.39	0.21	0.35
Sighs	2.97	2.60*	1.09	0.75	2.00	1.01
Hypopnoeas	0.04	2.00	0.99	0.45	0.83	0.55

*: $p<0.05$; **: $p<0.01$; ***: $p<0.001$

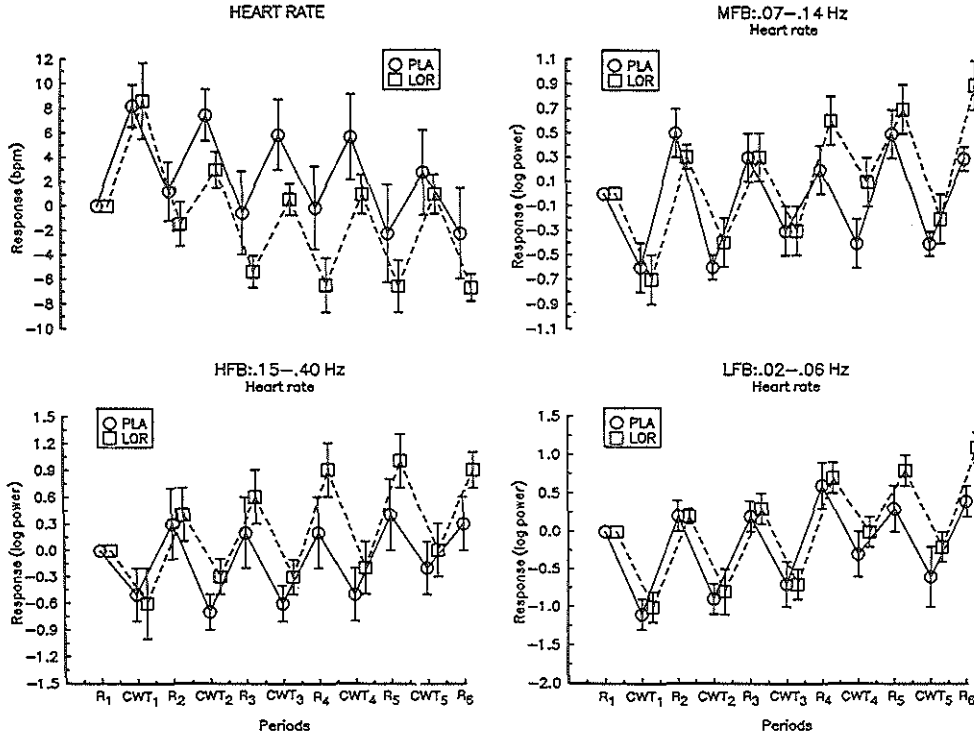

FIGURE 1
Mean (±SEM) values of HR and HFB, MFB, and LFB log power of HR during the consecutive periods of rest and CWT.

affected by the increasing doses of LOR, while the response magnitudes to the consecutive CWTs remained the same. After a cumulative dose of 0.19 mg LOR, HR decreased and HFB fluctuations increased significantly versus the first rest period (REST1 versus REST3: t-tests: $p=.003$ and $p=.03$, respectively). After a cumulative dose of 0.44 mg LOR, HR VC and fluctuations in LFB and MFB were significantly increased versus REST1 (REST1 versus REST4: $p<.01$).

Systolic blood pressure (SBP). The CWT significantly increased SBP, and significantly decreased SBP VC and LFB and MFB power during both the PLA- and the LOR-session (Table 2,4, Figure 2). During the PLA-session, the fluctuations in the HFB were not significantly affected by the CWT ($p=.09$, NS), but LOR significantly decreased HFB fluctuations during the CWT. During both sessions a significant decrease in the consecutive SBP CWT response magnitudes was observed (interaction effects significant),

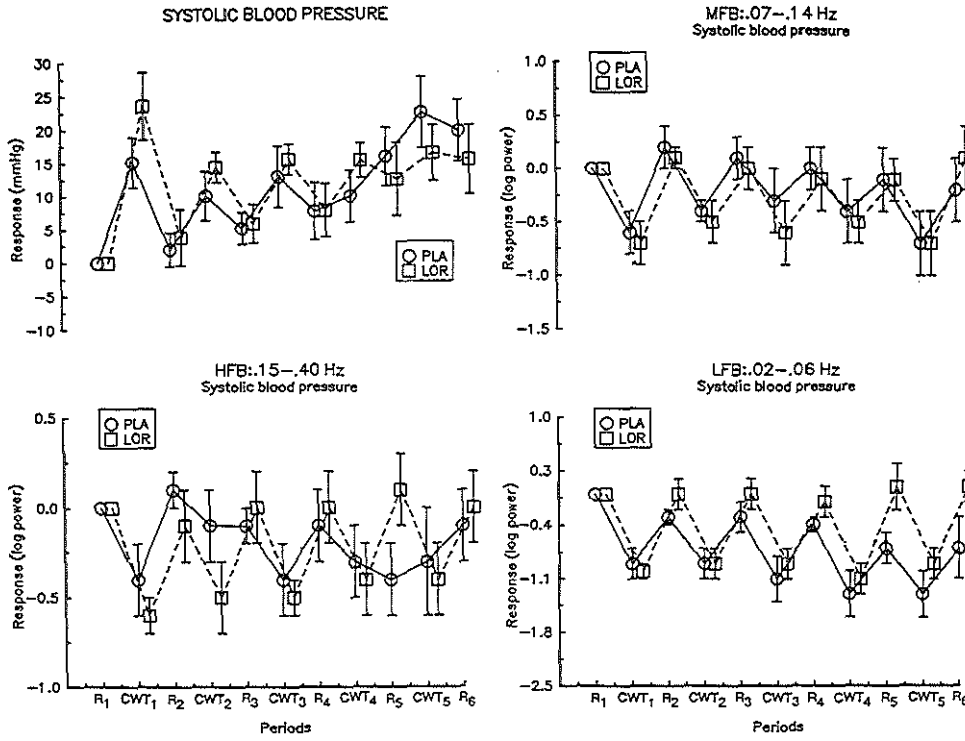

FIGURE 2
Mean (±SEM) values of SBP and HFB, MFB, and LFB log power of SBP during the consecutive periods of rest and CWT.

indicating learning or habituation effects to the CWT, but no drug-specific effects. During the PLA-session we observed a time-dependent increase in SBP, which was not present during the LOR-session (Table 2, Figure 2). This increase in SBP during the PLA-session was accompanied by a significant time-dependent decrease in VC and LFB fluctuations. LOR did not influence the CWT response magnitudes of SBP VC, and LFB, MFB and HFB fluctuations; these CWT responses also showed no habituation effects during the consecutive presentations (Table 2).

Diastolic blood pressure (DBP). The CWT significantly increased DBP and significantly decreased DBP VC and LFB, MFB and HFB fluctuations during both the PLA- and the LOR-session (Table 2,3,4). As with SBP, DBP increased time-dependently during the PLA-session, while the consecutive CWT response magnitudes showed a habituation effect during both the PLA- and the LOR-session. The LFB fluctuations increased dose-dependent-

Table 3. Mean (SEM) data of DBP (mmHg), and LFB, MFB, and HFB logarithmic power of DBP, and BRS (ms/mmHg) during the consecutive rest and CWT periods, for the placebo (PLA) and lorazepam (LOR) session separately

		REST1	CWT1	REST2	CWT2	REST3	CWT3	REST4	CWT4	REST5	CWT5	REST6
DBP	PLA	59(4)	72(5)	61(4)	70(4)	63(4)	70(4)	65(4)	70(4)	70(5)	72(5)	67(4)
	LOR	60(6)	75(7)	62(4)	70(4)	64(5)	71(5)	65(4)	73(4)	68(4)	76(4)	69(4)
LFB DBP	PLA	7.3(.2)	6.4(.2)	7.2(.3)	6.6(.2)	7.4(.3)	6.6(.3)	7.5(.3)	6.6(.4)	7.4(.3)	6.8(.2)	7.7(.3)
	LOR	7.3(.3)	6.4(.2)	7.3(.2)	6.6(.1)	7.4(.2)	6.6(.2)	7.6(.2)	6.7(.2)	7.8(.1)	6.9(.2)	7.9(.2)
MFB DBP	PLA	6.5(.3)	5.9(.3)	6.7(.3)	6.1(.2)	6.8(.3)	6.3(.3)	6.6(.3)	6.2(.3)	6.6(.4)	6.1(.2)	6.7(.2)
	LOR	6.7(.3)	6.2(.2)	6.8(.2)	6.2(.2)	6.7(.3)	6.2(.2)	6.7(.3)	6.3(.3)	6.8(.3)	6.2(.2)	6.9(.2)
HFB DBP	PLA	5.2(.1)	5.1(.3)	5.7(.3)	5.0(.2)	5.6(.3)	5.0(.3)	5.5(.3)	5.1(.4)	5.3(.3)	5.3(.3)	5.7(.3)
	LOR	5.5(.4)	5.0(.1)	5.5(.3)	5.1(.2)	5.6(.3)	4.9(.2)	5.7(.2)	5.1(.2)	5.8(.3)	5.2(.2)	5.9(.2)
BRS	PLA	15(2)	12(3)	16(2)	10(2)	15(1)	12(1)	15(1)	12(2)	19(3)	13(2)	16(2)
	LOR	11(2)	8(1)	13(2)	11(1)	13(2)	11(1)	16(2)	12(1)	17(2)	13(2)	19(3)

Table 4. Mean (SEM) values of variation coefficients (VC) of HR, SBP, and DBP, and mean (SEM) values of respiratory cycle duration (in sec), respiratory depth (% change vs baseline), and the number of sighs and hypopnoeas, during the consecutive periods of rest and CWT, for the placebo (PLA) and the lorazepam (LOR) session separately

		REST1	CWT1	REST2	CWT2	REST3	CWT3	REST4	CWT4	REST5	CWT5	REST6
VC HR	PLA	8.3(0.6)	6.3(1.0)	9.8(0.7)	5.9(0.6)	9.6(0.7)	6.6(0.7)	10.7(1.3)	7.2(0.9)	10.8(1.1)	7.0(0.8)	10.2(0.9)
	LOR	7.7(0.9)	5.3(0.4)	8.7(0.9)	5.5(0.6)	9.4(0.9)	5.6(0.5)	10.5(0.8)	7.2(0.8)	12.2(1.1)	6.8(0.7)	12.7(1.1)
VC SBP	PLA	6.6(0.8)	5.4(0.6)	6.0(0.6)	5.0(0.5)	5.6(0.5)	4.6(0.5)	5.7(0.6)	4.2(0.5)	5.3(0.5)	4.4(0.6)	5.2(0.6)
	LOR	6.3(0.5)	4.1(0.4)	6.3(0.7)	4.7(0.5)	5.9(0.6)	4.2(0.4)	5.6(0.5)	3.9(0.4)	6.0(0.5)	4.6(0.4)	5.8(0.6)
VC DBP	PLA	6.3(0.7)	4.9(0.6)	6.6(0.7)	4.7(0.4)	6.7(0.9)	5.0(0.7)	7.1(0.9)	5.5(0.9)	7.1(1.0)	5.2(0.6)	7.5(1.1)
	LOR	6.5(0.7)	4.8(0.3)	6.6(0.7)	5.0(0.3)	6.8(0.7)	4.7(0.3)	6.9(0.5)	4.9(0.4)	7.3(0.6)	5.9(0.6)	7.7(0.8)
R. cycle	PLA	4.1(0.4)	3.1(0.2)	4.3(0.4)	3.2(0.2)	4.0(0.2)	3.2(0.2)	4.0(0.2)	3.3(0.2)	4.3(0.4)	3.2(0.2)	4.2(0.4)
	LOR	3.9(0.2)	2.9(0.1)	3.9(0.3)	3.2(0.2)	3.7(0.2)	3.1(0.2)	3.8(0.2)	3.1(0.2)	3.9(0.2)	3.1(0.2)	3.6(0.2)
R. depth	PLA	100(0)	91(4)	104(3)	92(6)	100(4)	89(4)	97(3)	94(7)	94(7)	87(4)	96(4)
	LOR	100(0)	88(5)	103(6)	90(5)	99(5)	89(6)	99(4)	90(6)	98(4)	91(7)	95(6)
Sighs	PLA	3.4(1.0)	3.0(1.0)	5.7(1.3)	4.0(0.6)	5.9(1.6)	3.3(0.7)	5.1(1.1)	4.2(0.8)	5.2(1.2)	1.9(0.3)	4.8(1.1)
	LOR	3.7(0.7)	2.8(0.7)	3.9(0.9)	3.3(0.6)	4.3(1.3)	3.6(0.7)	4.7(1.6)	5.1(0.9)	6.8(1.8)	4.3(0.5)	5.9(1.9)
Hypo's	PLA	5.3(2.6)	3.7(0.9)	2.6(0.9)	4.7(1.8)	4.4(1.3)	4.8(1.3)	4.0(1.7)	3.2(1.0)	2.6(0.9)	4.9(1.4)	3.2(1.5)
	LOR	3.7(1.5)	4.3(1.4)	3.4(0.5)	3.6(0.6)	3.0(0.9)	3.2(1.2)	3.0(1.3)	5.3(0.9)	4.8(1.2)	4.6(1.0)	7.6(4.1)

ly during the LOR-session (Table 2,3) due to a gradual increase in LFB power during the rest periods.

Baroreflex sensitivity

BRS decreased significantly during the CWTs of both the PLA- and the LOR-session (Table 2,3). The response magnitudes to the consecutive CWTs showed no time- or dose-dependent effects. However, during the LOR-session the BRS levels during the rest-periods increased dose-dependently; after a cumulative dose of 0.19 mg LOR, BRS was significantly increased versus REST1 (REST1 versus REST3: p=.03).

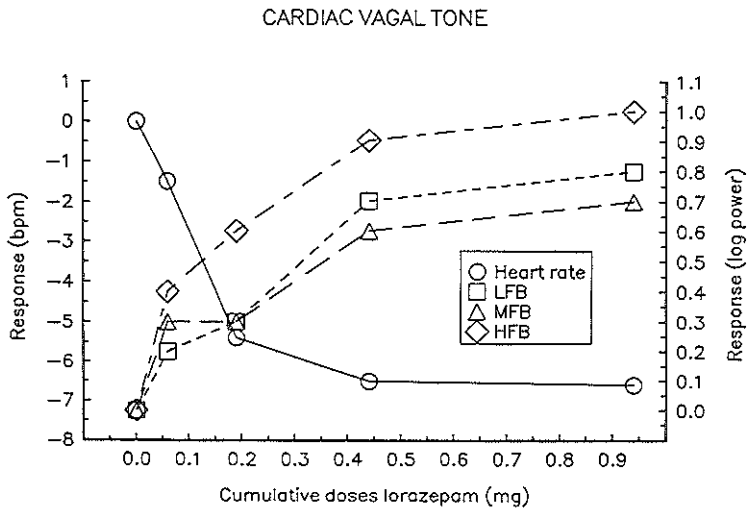

FIGURE 3
Dose-response effects of changes in HR and changes in LFB, MFB and HFB log power of HR, after intravenous administration of lorazepam.

Respiration

Respiratory cycle duration decreased significantly during the CWTs of the PLA- and the LOR-session (Table 2,4). During the PLA-session this was accompanied by a significant reduction in respiratory depth; for the LOR-session there was a trend towards significance (Table 2, p=.07, NS). The number of sighs or hypopnoeas showed no systematic changes during the PLA- or the LOR-session due to highly variable data. Response magnitudes of the consecutive CWTs were not affected by LOR or PLA, for all the respiratory parameters measured.

Discussion

Cardiovascular variability during rest

Previously, we suggested that the observed decrease in HR might indicate a stimulating effect of LOR on cardiac vagal tone during rest (Tulen et al., 1991). The present results of dose-dependent increases in BRS, and LFB, MFB, and HFB power of HR and the absence of changes in BP variability lend direct support for this hypothesis. It has been shown that high, mid and low frequency fluctuations in HR can be influenced by parasympathetic mechanisms during situations of rest (Akselrod et al., 1985; Pomeranz et al., 1985) and the increase in BRS may also be the result of parasympathetic stimulation (Eckberg et al., 1971).

Adinoff et al. (1992) reported an opposite effect in a similar, although not placebo-controlled, research protocol in healthy subjects after intravenous administration of diazepam: a dose-dependent increase in HR and a dose-dependent attenuation of cardiac vagal tone. Although their, as well as our, data underline an effect of benzodiazepines on cardiac vagal tone, the direction of the effect clearly is not similar for all benzodiazepines. In our design, we additionally used repeated presentations of the CWT between the rest periods: it can not be excluded that rebound phenomena due to strain of the tasks influenced the responses during periods of rest. However, pharmacokinetic differences between diazepam and lorazepam may be more relevant for the interpretation of these experiments. We have chosen lorazepam because it has a short distribution half-life and no active metabolites, which in combination with an elimination half-life of 12-13 h makes a cumulative dose administration possible. This assumption is supported by our pharmacokinetic data (Table 1). Diazepam, on the other hand, is much more lipophilic, which will result in high brain concentrations immediately after intravenous administration and a swift decrease within minutes. In mice, e.g., it has been shown that diazepam concentration in the brain is reduced tenfold between 10 and 30 min after intravenous administration, whereas lorazepam concentrations were rather constant during the first hour after administration (Greenblatt and Sethy, 1990). Adinoff et al. (1992) do not present plasma data of diazepam and its active metabolites, nor do they present data on respiratory or sedative effects of diazepam, which could explain the effects on vagal tone. However, the opposite results obtained with diazepam and lorazepam can only be clarified when both drugs are compared within the same experiment. Opposite effects on cardiac vagal activity of two different GABA-ergic systems have been described (Wible and DiMicco, 1986) and, although benzodiazepines may not necessarily be involved, similar opposite mechanisms can not be excluded for benzodiazepines.

The observed increase in vagal tone induced by LOR could be caused by respiratory effects. Benzodiazepines are known to cause respiratory depression, especially after intravenous administration of high doses (Danneberg, 1986; Berggren et al., 1987). Regarding LOR, either a lack of respiratory effects (Gasser et al., 1975; Elliott et al., 1971), respiratory stimulation (Paulson et al., 1983; Dodson et al., 1976), as well as respiratory inhibition (Wettstein et al., 1990) have been reported. In this study with low doses, LOR did not induce clear respiratory effects on the parameters we measured, indicating that the cardiac effects cannot be explained by changes in respiration.

Sedative effects of lorazepam, mediated by suppression of locus coeruleus firing (Grant et al., 1980), might also explain the observed effects on HR, BRS and HR fluctuations. Previously, we have shown that after the highest cumulative dose (0.94 mg), LOR induced significant sedative effects as indicated by performance impairment, increased fatigue and decreased vigor (Tulen et al., 1991). After the highest dose plasma noradrenaline concentrations were also lowered in comparison with the PLA-session, reflecting a suppression of sympathetic nervous system activity, but only after the highest dose of LOR. However, HR, HFB fluctuations and BRS already changed significantly after 0.19 mg LOR, whereas MFB and LFB fluctuations changed significantly after 0.44 mg LOR. These parasympathetic effects of LOR at lower doses do not appear to be related to sedation.

Overall, the present data indicate a stimulating effect of LOR on cardiac vagal cardiac tone during periods of rest.

Cardiovascular variability to the Stroop Color Word Test (CWT)

The CWT significantly increased HR, SBP, DBP and respiration rate and significantly decreased BRS, respiratory depth and LFB and MFB fluctuations in HR, SBP and DBP as well as HFB fluctuations in HR and DBP. LOR did not induce clear effects on the variability responses to the CWT. The SBP and DBP response magnitudes to the CWTs showed a habituation effect during both sessions; the other parameters showed similar response magnitudes to the 5 consecutive CWTs. Our results of decreased HR and BP variability in the three frequency bands during a mental task correspond with findings of others (Mulder & Mulder, 1981; Langewitz & Rüddel, 1989; Veldman et al., 1985). Similarly, a reduction in BRS during psychological stress has been observed in a number of studies, in which BRS during mental stress was computed either after application of a pressor agent (Conway et al., 1983; Sleight et al., 1978), the neck suction method (Ditto & France, 1990), or by means of non-invasive methods (Pagani et al., 1991; Robbe et al., 1987; Steptoe & Sawada, 1989). Overall, the reduced variability in HR

and BP during the CWT resembles a pattern of a parasympathetic withdrawal (Akselrod et al., 1985; Pomeranz et al., 1985). However, previously we have established that the CWT induces a defence-like reaction by means of sympatho-adrenomedullary activation; the CWT increased HR, plasma and urinary adrenaline, electrodermal activity and muscular tension, whereas it decreased finger pulse amplitude (Tulen et al., 1989). The present study does not permit a differentiation between parasympathetic withdrawal, sympathetic activation, or both, as a possible cause for the changes in cardiovascular variability observed during the CWT. It is clear, however, that LOR had no effect on these cardiovascular responses to the CWT, although performance to the task was severely deteriorated after the highest dose (0.94 mg LOR), apparently due to the sedative effects of LOR (Tulen et al., 1991).

In conclusion, our study shows that spectral analysis of beat to beat fluctuations in HR and BP represents a useful tool to unravel the mechanisms of cardiovascular control after benzodiazepine administration. LOR induced dose-dependent increases in cardiac vagal tone. This effect of LOR was apparent only during periods of rest and was overruled during a mental stress task. The increase in vagal tone observed at low doses of LOR was not related to diminished sympathetic activity, altered respiration, or increased sedation.

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Chapter 6

Concluding Remarks

Catecholamines and mood

In this thesis, two of the four experiments were aimed at directly manipulating concentrations of plasma catecholamines by means of infusions of adrenaline, noradrenaline, or clonidine. In these studies, subjective mood was assessed in a passive laboratory situation, with no requirements of mental or physical exertions. No changes were observed in subjective mood during or after a sustained infusion of adrenaline or noradrenaline, whereas a dose-dependent sedative type of response was observed after infusion of clonidine. Both results are in line with previous research on the psychological effects of catecholamine-infusions or clonidine-challenges in healthy subjects (discussion chapters 2.1 and 3.1).

During performance of the CWT, plasma catecholamine concentrations increased, specifically plasma adrenaline concentrations. A small, but significant, increase in state-anxiety was observed after the CWT, in comparison with a passive control situation (chapter 4). We observed no anxiolytic responses to the CWT after administrations of lorazepam (chapter 5). Lorazepam induced a dose-dependent increase in subjective fatigue and a deterioration of task performance. One might argue that the CWT did not induce a relevant increase in anxiety in the healthy (non-anxious) volunteers, and that therefore an anxiolytic effect of lorazepam could not be demonstrated. In that respect, responses of heart rate and plasma adrenaline to the CWT may reflect an increase in effort mechanisms (Sanders, 1983), which are necessary for the subject to cope with the task, rather than anxiety-related responses. In order to establish whether the heart rate or adrenaline responses to the CWT are part of effort-mechanisms necessary to perform a task, compensatory mechanisms to overcome sedation, or stress-related mechanisms on which lorazepam has no influence, application of anxiolytics without sedative properties (such as buspirone; Cohn & Wilcox, 1986), or anxiolytics with a specific influence on plasma catecholamines (such as alprazolam; Grant et al., 1984) should be studied in a placebo-controlled design.

Catecholamines and cardiovascular variability

Neural homeostatic cardiovascular regulation is for a large part effected

through the interplay of sympathetic and vagal activity. The balance between sympathetic and vagal outflows is tonically and phasically modulated by the interaction of at least three major factors: central neural integration, peripheral inhibitory reflex mechanisms with negative feedback characteristics (baroreceptors and vagal afferents), and peripheral excitatory reflex mechanisms with positive feedback characteristics (sympathetic afferents) (Malliani et al., 1991). In this thesis, homeostatic cardiovascular mechanisms, in relation to changes in circulating catecholamine concentrations, were evaluated by means of spectral analysis of fluctuations in heart rate and blood pressure. Specific effects could be detected on the spectral power of the different frequency components of heart rate and blood pressure during infusion of adrenaline, noradrenaline, clonidine, lorazepam, as well as during the CWT.

Infusion of adrenaline, noradrenaline and clonidine underlined that low- and/or mid-frequency fluctuations of heart rate, but specifically blood pressure, can reflect changes in sympathetic modulation as a result of peripheral or central (in the case of clonidine) stimulation of adrenergic receptors. However, as indices of sympathetic activity, plasma concentrations of catecholamines clearly have no unequivocal relationship with the spectral power of the low- or mid-frequency bands of heart rate or blood pressure. The dynamics of sympathetic modulation (in interaction with parasympathetic modulation) within the neural cardiovascular regulation are just too complex to be accurately reflected in concentrations of plasma catecholamines.

Administration of lorazepam confirmed the vagal modulation of low-, mid-, but especially high-frequency fluctuations of heart rate during situations of rest. During the CWT, the reduced fluctuations in heart rate and blood pressure could be attributed to both sympathetic stimulation and vagal inhibition; on the basis of the present experiments, no distinction could be made between the relative contribution of each component to the reduction of cardiovascular variability.

Our results confirmed that spectral analysis of heart rate and blood pressure is a valuable tool for the assessment of the dynamics of neural cardiovascular regulation. However, our results also showed that mid- and high-frequency fluctuations of heart rate or blood pressure can not be used as simple indices of a reciprocal sympatho-vagal balance, as suggested by Pagani et al. (1986). Also, changes in heart rate and blood pressure variability clearly are not similar under different experimental conditions. Moreover, our studies indicated the necessity to differentiate low- from mid-frequency fluctuations, because these bands may represent different components of homeostatic cardiovascular regulation. These aspects will have to be taken into consideration in both clinical and experimental studies. Further studies

are required on the interrelationships between indices of cardiovascular variability in order to establish the relevance of both inter- and intra-individual differences.
At this moment there are only a few studies available on a detailed analysis of heart rate variability in depression and panic disorders (i.e., Yeragani et al., 1991,1992). More studies are necessary, especially on spectral analysis of both heart rate and blood pressure variations, in order to substantiate the usefulness of this method to assess alterations in sympathetic and parasympathetic cardiovascular modulation in psychiatric disorders. This also contributes to the evaluation of its diagnostic value.

Plasma and urinary catecholamines

In three of the four experiments, we measured concentrations of catecholamines in both plasma and urine. The data presented in chapters 2 and 3 showed that the urinary concentration of noradrenaline proved a sensitive indicator of noradrenaline concentration in plasma. However, during performance of a task which involves local muscle activity at the site of blood sampling (such as the CWT, chapter 4), some caution regarding interpretations is required; in that situation, measurements of urinary noradrenaline may reflect global sympathetic nervous activity more accurately than venous plasma noradrenaline concentrations (which may be affected by local skeletal muscle activity: Brown et al., 1981; Floras et al., 1986). Increased plasma adrenaline concentration, due to infusion of adrenaline or due to task performance, is sensitively detected in urine by estimation of adrenaline concentration. Since only small amounts of circulating adrenaline or noradrenaline are recovered as adrenaline or noradrenaline concentrations in urine (chapter 2.3), stimuli will need to have a sufficient intensity and length in order to observe effects in urine. Our CWT-study (chapter 4) showed that two 20-min versions of the CWT, during a urinary collection period of three hours, were sufficient to still reflect significant increases in urinary adrenaline concentrations. However, our clonidine-study (chapter 3.1) showed that it may be relevant to take into account a time-delay of several hours to detect peak effects in urine.

Our data also have clinical implications for catecholamine research (as discussed in chapter 2.3). Urinary catecholamines and their metabolites have been most extensively researched in depressed patients. Subgroups of depressed patients have been found to be characterized by increased urinary excretion of adrenaline, metanephrine, noradrenaline, and normetanephrine, jointly (Davis et al., 1988). These findings suggested that the hypersecretion of the adrenal medulla and peripheral sympathetic nervous system are interrelated and may be based on the same underlying phenomenon. On the basis of our results we may hypothesize that hypersecretion of adrenaline

may be the primary mechanism that leads to increased excretion of adrenaline and metanephrine and, secondarily, to amplification of sympathetic activity with consequently increased excretion of normetanephrine. The bimodal distribution of adrenaline and metanephrine in depressed patients, not present in healthy controls, and the absence of such a clear distinction in distribution patterns between patients and controls for noradrenaline and normetanephrine, to some extent supports this hypothesis. Further clinical studies are required, in which comparisons are made between plasma concentrations of catecholamines and urinary excretion patterns as reflected in catecholamines and their metabolites, in order to substantiate this hypothesis.

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Summary

Activation of the sympatho-adrenomedullary system, resulting in increased plasma concentrations of adrenaline and noradrenaline, can occur in response to a variety of stressful or demanding situations, and is believed to be of importance in the pathogenesis of affective disorders, anxiety disorders and hypertension. However, the manner in which these effects come about is often obscured by the net result of the complex interplay between central, autonomic (sympathetic and parasympathetic), and metabolic mechanisms. The research presented in this thesis aimed to contribute to the clarification of this issue based on four psychophysiological experiments. These experiments were designed to separately manipulate concentrations of circulating adrenaline and noradrenaline by means of infusions of catecholamines, pharmacological interventions, or psychological (mental stress) challenges in healthy male subjects. Special attention was paid to the indices of sympathetic activity, such as plasma or urinary catecholamines and their metabolites, and specific frequency fluctuations in cardiovascular variations as analyzed by means of spectral analysis.

Chapter 1 starts with a synopsis of the modes of action of the catecholamines in the central and autonomic nervous system. Spectral analysis as a potential non-invasive technique to assess the sympathetic and parasympathetic mechanisms in homeostatic cardiovascular processes is subsequently described. Different approaches for catecholamine research are postulated, after which the aims of our studies are presented.

Chapter 2 presents the results of a 6-hour infusion study of adrenaline, noradrenaline, or placebo. This study reflected a situation of a sustained peripheral increase in concentrations of circulating catecholamines. During infusion of adrenaline, arterial plasma adrenaline concentrations increased 10-fold, which induced significant increases in heart rate, stroke volume, low-frequency band (0.02-0.06 Hz) power of systolic and diastolic blood pressure, plasma insulin, renin, aldosterone and glucose concentrations; adrenaline significantly decreased mean blood pressure, diastolic blood pressure, and total peripheral resistance. Noradrenaline infusion caused a 5-fold increase in arterial plasma noradrenaline concentrations and induced a significant decrease in heart rate and mid-frequency band (0.07-0.14 Hz) power of systolic and diastolic blood pressure. Noradrenaline significantly increased mean blood pressure, diastolic blood pressure, plasma renin, and glucose concentrations. Changes in subjective mood were not observed during the infusions, nor after the infusions had been stopped. Infusion of adrenaline or noradrenaline also had no significant effect on systolic blood

pressure, baroreflex sensitivity, respiration rate, high-frequency band (0.15-0.40 Hz) fluctuations in heart rate or blood pressure, plasma prolactin, growth hormone, cortisol, and triglycerides. It is concluded that: 1) Moderate cardiovascular and metabolic effects can be caused by sustained increases in circulating catecholamines within the physiological range. The effects were present shortly after initiation of the infusions, remained fairly constant during the 6-hour infusion period and disappeared within one hour after the infusions had been stopped. This indicated that no regulatory mechanisms counteracted the perturbations induced by the sustained infusions of adrenaline or noradrenaline. 2) Subjective mood is not affected by a prolonged increase in circulating catecholamines in the high physiological range. This extends previous observations concerning the absence of mood changes to short-lasting catecholamine-infusions in healthy volunteers. 3) Low-frequency fluctuations in heart rate and blood pressure need to be separated from mid-frequency fluctuations, because these bands may represent different components of baroreflex mediated changes during situations of increased sympatho-adrenomedullary activation. Our results also imply that, under the conditions of the present experiment (supine rest, 6 hours), changes in plasma catecholamines can not be unequivocally labelled as indices of altered sympatho-adrenal control of cardiovascular homeostasis. 4) Urinary adrenaline and noradrenaline concentrations are sensitive indicators of increased circulating adrenaline and noradrenaline concentrations, respectively. Changes in circulating adrenaline and noradrenaline concentrations are not readily reflected in changes in urinary VMA or MHPG concentrations, whereas increased normetanephrine excretion may not only be induced by infusion of noradrenaline, but also by adrenaline.

In chapter 3, dose-response effects of intravenous clonidine are evaluated on the basis of cardiovascular, neuroendocrine, and psychological parameters, in order to delineate the mechanisms of action of clonidine at the level of the brainstem, the pituitary, or the peripheral sympathetic nervous system. Dose-dependent decrements were observed in systolic and diastolic blood pressure and plasma noradrenaline concentrations, and dose-dependent increases in subjective sedation and plasma growth hormone. Clonidine reduced low- and mid-frequency band power of heart rate after 2 μg/kg, but only during the first 30 min following infusion. Mid-frequency band power of diastolic blood pressure was reduced after 2 μg/kg clonidine during the entire post-infusion period, whereas high-frequency band power of systolic blood pressure was significantly increased after 0.5 μg/kg clonidine or more. Clonidine did not influence plasma MHPG concentrations, whereas only urinary MHPG excretion was reduced 4 hours after infusion of 2 μg/kg clonidine. It is concluded that: 1) Our results do not provide simple parame-

ters to discern the multiple mechanisms of action of clonidine, because no obvious differences were observed between dose-response relations of plasma noradrenaline (believed to be a presynaptic and peripheral effect), blood pressure (believed to be mainly a centrally pre- and postsynaptic effect) and subjective sedation (believed to be a central and probably postsynaptic effect). However, at a dose of 0.5 μg/kg clonidine (a dose lower than that generally used), clear effects on plasma noradrenaline, blood pressure, and sedation, but not on plasma growth hormone (a central postsynaptic effect) or urinary MHPG (a presynaptic effect), were observed. These data, therefore, indicated that when using clonidine as a challenge test in psychiatric disorders, a design with 0.5 μg/kg, in addition to the traditional 2 μg/kg clonidine, may provide more information to characterize discrete abnormalities in the noradrenergic system at the level of the brainstem, the pituitary or the peripheral sympathetic nervous system. 2) Furthermore, clonidine suppresses low- and mid-frequency band fluctuations in heart rate and especially mid-frequency band fluctuations in diastolic blood pressure. Our data underline that sequential spectral analysis of spontaneous haemodynamic fluctuations can be employed to unravel time-dependent dynamics of sympathetic and vagal components within short-term cardiovascular control.

In chapter 4, the Stroop Color Word Test (CWT) is evaluated as a test for the study of stress-induced sympatho-adrenomedullary effects, on the basis of psychological, physiological and biochemical responses. It was found that: 1) The CWT induces significant increases in plasma adrenaline concentrations, heart rate, respiration rate, electrodermal activity, electromyography, and feelings of anxiety, and significantly decreased finger pulse amplitude. The CWT has variable effects on plasma noradrenaline concentrations, and no significant effect on plasma cortisol and prolactin. 2) The CWT significantly increases urinary adrenaline concentrations, but has no significant effect on urinary noradrenaline concentrations. Habituation effects, response-magnitudes in relation to baseline values, and spontaneous changes of basal values over time, must be accounted for in order to evaluate responses to the task reliably.

Chapter 5 presents the dose-response effects of intravenously administered lorazepam on cardiovascular activity, plasma catecholamines and psychological function during rest and mental stress. Heart rate decreased dose-dependently, whereas baroreflex sensitivity, and low-, mid-, and high-frequency band fluctuations in heart rate increased dose-dependently during periods of rest. These effects were significant after 0.19 mg lorazepam for heart rate, baroreflex sensitivity, and high-frequency band power, and after 0.44 mg lorazepam for the heart rate fluctuations in the low- and mid-frequency band. Lorazepam did not significantly influence blood pressure

variability or respiration rate during rest. During placebo, as well as during lorazepam administrations, the CWT significantly increased heart rate, systolic blood pressure, diastolic blood pressure, respiratory frequency, plasma adrenaline concentrations, and significantly decreased baroreflex sensitivity and low-, mid-, and high-frequency fluctuations of both heart rate and blood pressure. Lorazepam had no effect on the cardiovascular and plasma catecholamine response magnitudes to the CWT. Subjective fatigue and reaction time increased significantly after 0.94 mg lorazepam, while at the same dose vigor decreased; state anxiety was not influenced by lorazepam. It is concluded that: 1) Lorazepam has differential effects on cardiovascular, biochemical, and psychological function. While heart rate is suppressed and heart rate variability is increased at low doses during rest and reaction time and subjective fatigue increase at doses which induced sedation, state anxiety and physiological response patterns to the CWT are not influenced by lorazepam. More specific pharmacological interventions are required to analyze the response mechanisms to the CWT. 2) Lorazepam induces dose-dependent increases in cardiac vagal tone, resulting in decreased heart rate and increased heart rate variability during periods of rest. This effect is overruled during a mental stress task. The increase in vagal tone, as observed after low doses of lorazepam, appears unrelated to diminished sympathetic activity, altered respiration, or increased sedation.

Chapter 6 contains a brief evaluation of our major findings. Methodological and clinical implications of the studies are briefly discussed, and suggestions for further research are provided.

Samenvatting

Activatie van het sympaticus-bijniermerg-systeem (SBS) resulteert vaak in een toename van plasma-concentraties van adrenaline en noradrenaline. SBS-activatie vindt plaats gedurende stressvolle en veeleisende situaties en wordt tevens verondersteld te zijn betrokken bij de pathogenese van affectieve stoornissen, angststoornissen en hypertensie. Op welke manier deze betrokkenheid tot stand komt is echter vaak onduidelijk als gevolg van de complexe wisselwerking tussen centrale, autonome (sympatische en parasympatische) en metabole mechanismen. Het onderzoek dat in dit proefschrift is beschreven tracht bij te dragen aan het inzicht in deze processen. Tijdens vier psychofysiologische studies werden concentraties van circulerend adrenaline en noradrenaline gemanipuleerd door middel van infusies van catecholaminen, farmacologische interventies of een mentale stresstaak bij gezonde mannelijke vrijwilligers. Er werd uitgebreid aandacht besteed aan indices van sympatische activiteit, zoals concentraties van catecholaminen en hun metabolieten in plasma of urine, en bepaalde cardiovasculaire fluctuaties geanalyseerd met behulp van spectraalanalyse.

Hoofdstuk 1 geeft een beknopt overzicht van de werkingsmechanismen van adrenaline en noradrenaline in het centrale en het perifere autonome zenuwstelsel. Vervolgens wordt spectraalanalyse besproken als noninvasieve methode voor het bepalen van sympatische en parasympatische componenten in de variabiliteit van de bloeddruk en de hartslag. Verschillende invalshoeken voor catecholamine-onderzoek worden beschreven, waarna de doelstellingen van de studies worden gepresenteerd.

In hoofdstuk 2 worden de resultaten van een infuus-studie van adrenaline, noradrenaline, en placebo weergegeven. In dit experiment werd een situatie gecreëerd van een aanhoudende (zes uur durende) verhoging van circulerende catecholamine-concentraties. Gedurende het adrenaline-infuus nam de arteriële plasma-concentratie van adrenaline toe met een factor 10. Deze stijging induceerde een significante toename in de hartslag, het slagvolume, de lage-band (0.02-0.06 Hz) fluctuaties in systolische en diastolische bloeddruk, en plasma-concentraties van insuline, renine, aldosteron en glucose. De gemiddelde bloeddruk, diastolische bloeddruk, en perifere weerstand namen significant af gedurende het adrenaline-infuus. Het noradrenaline-infuus zorgde voor een 5-voudige stijging van de arteriële plasma-concentraties van noradrenaline. Dit resulteerde in een significante daling van de hartslag en de midden-band (0.07-0.14 Hz) fluctuaties in de systolische en diastolische bloeddruk. Daarnaast nam de gemiddelde bloeddruk significant toe, alsmede de diastolische bloeddruk en de plasma renine- en

glucose-concentraties. Er werden geen veranderingen gemeten in subjectieve stemming tijdens of na de infusies. Infunderen van adrenaline of noradrenaline had ook geen significant effect op de systolische bloeddruk, de baroreflexgevoeligheid, de ademfrequentie, de hoge-band (0.15-0.4 Hz) fluctuaties in hartslag of bloeddruk, en de plasma-concentraties van prolactine, groeihormoon, cortisol en triglyceriden. Op basis hiervan werden de volgende conclusies getrokken: 1) Matige cardiovasculaire en metabole veranderingen kunnen veroorzaakt worden door aanhoudende verhogingen van circulerende catecholaminen met concentraties binnen het fysiologische bereik. De effecten waren kort na de start van de infusies meetbaar, bleven redelijk constant gedurende de infuusperiode van zes uur en verdwenen binnen één uur na het stoppen van de infusies. Dit geeft aan dat de responsen op de aanhoudende infusies van adrenaline of noradrenaline niet werden gecompenseerd door tegengestelde regelmechanismen. 2) Subjectieve stemming wordt niet beïnvloed door een aanhoudende verhoging van circulerende catecholaminen in het hoge fysiologische bereik. Deze bevinding komt overeen met eerdere observaties betreffende het uitblijven van veranderingen in de stemming na kortdurende catecholamine-infusies in gezonde volwassenen. 3) Lage-band fluctuaties in hartslag en bloeddruk dienen gescheiden te worden bestudeerd van midden-band fluctuaties. Deze banden kunnen verschillende componenten van baroreflex-gemedieerde veranderingen weergeven gedurende situaties van toegenomen SBS-activiteit. Deze resultaten geven ook aan dat, onder de condities van het huidige experiment (liggend op bed, zes uur infuus), veranderingen in plasma-concentraties van catecholaminen niet eenduidig geïnterpreteerd kunnen worden als indices van veranderde SBS-controle binnen het cardiovasculaire regelsysteem. 4) Urine-concentraties van adrenaline en noradrenaline zijn gevoelige indicatoren van concentraties van, respectievelijk, adrenaline en noradrenaline in de circulatie. Veranderingen in circulerende concentraties van adrenaline en noradrenaline worden niet gereflecteerd in veranderingen in urine-concentraties van VMA of MHPG. Een toegenomen normetanefrine-uitscheiding kan niet alleen door een infuus van noradrenaline, maar ook door een infuus van adrenaline worden veroorzaakt.

In hoofdstuk 3 worden de dosis-respons effecten van intraveneus toegediend clonidine geëvalueerd aan de hand van cardiovasculaire, neuroendocrine, en psychologische parameters. Deze studie werd opgezet om de werkingsmechanismen van clonidine op de hersenstam, de hypofyse en het perifere sympatische zenuwstelsel te ontrafelen. Een dosis-afhankelijke daling werd vastgesteld voor de systolische en diastolische bloeddruk en de plasma-concentraties van noradrenaline. Een dosis-afhankelijke toename werd gevonden voor subjectieve sedatie en de plasma-concentraties van groeihor-

moon. Clonidine reduceerde lage- en midden-band fluctuaties in de hartslag na een dosis van 2 μg/kg, maar alleen gedurende de eerste dertig minuten na het infuus. De midden-band fluctuaties in de diastolische bloeddruk waren afgenomen gedurende de gehele postinfuus-periode na een dosis van 2 μg/kg clonidine, terwijl de hoge-band fluctuaties in de systolische bloeddruk significant toenamen na een dosis van 0.5 μg/kg of hoger. Op basis hiervan werd het volgende geconcludeerd: 1) Het is niet mogelijk om simpele parameters te verschaffen om de multiple werkingsmechanismen van clonidine te onderscheiden. Er werden geen duidelijke verschillen geobserveerd tussen de dosis-respons relaties van plasma-concentraties van noradrenaline (een presynaptisch en perifeer effect), bloeddruk (vooral een centraal pre- en postsynaptisch effect), en subjectieve sedatie (een centraal en waarschijnlijk postsynaptisch effect). Echter, na een dosis van 0.5 μg/kg clonidine (een lagere dosis dan gebruikelijk) werden duidelijke effecten gevonden in plasma-concentraties van noradrenaline, bloeddruk en sedatie, maar niet in plasma-concentraties van groeihormoon (een centraal postsynaptisch effect) en urine-concentraties van MHPG (een presynaptisch effect). Dit geeft aan dat, indien clonidine als belastingstest voor het noradrenerge systeem wordt gebruikt, een studie-protocol met 0.5 μg/kg, in toevoeging op de traditionele 2 μg/kg clonidine, meer informatie zou kunnen verschaffen met betrekking tot het karakteriseren van discrete abnormaliteiten in het noradrenerge systeem op het niveau van de hersenstam, de hypofyse of het perifere sympatische zenuwstelsel. 2) Clonidine onderdrukt lage- en midden-band fluctuaties in de hartslag en vooral midden-band fluctuaties in de diastolische bloeddruk, binnen een postinfuus-periode van één uur. Deze gegevens onderstrepen dat spectraalanalyse van spontane cardiovasculaire fluctuaties gebruikt kan worden om tijdsafhankelijke sympatische en parasympatische processen binnen het cardiovasculaire regelsysteem te analyseren.

In hoofdstuk 4 wordt de Stroop Kleur Woord Test (CWT) geëvalueerd als test voor het bestuderen van stress-geïnduceerde SBS activiteit, op basis van psychologische, fysiologische en biochemische responsen. Er werd het volgende gevonden: 1) De CWT induceerde een significante toename in plasma-adrenaline-concentratie, hartslag, ademfrequentie, electrodermale activiteit, spieractiviteit, en gevoelens van angst. Tijdens de CWT nam de puls-amplitude, gemeten aan de middelvinger, significant af. De CWT had wisselende effecten op plasma-concentraties van noradrenaline, en geen effect op plasma-concentraties van cortisol of prolactine. 2) Na de CWT nam de concentratie van adrenaline in de urine significant toe, terwijl de CWT geen effect had op de noradrenaline-concentraties in de urine. Om de responsen op de taak betrouwbaar te kunnen evalueren dient er rekening gehouden te worden met habituatie effecten, responsgrootte op de taak in

relatie tot uitgangswaarden, en spontane veranderingen in basale waarden over de tijd.

In hoofdstuk 5 staan de resultaten beschreven van een studie naar de dosis-afhankelijke effecten van lorazepam op cardiovasculaire activiteit, plasma catecholaminen, en psychologische parameters gedurende rust en het uitvoeren van de CWT. De hartslag nam dosis-afhankelijk af, terwijl de baroreflexgevoeligheid, en de lage-, midden- en hoge-band fluctuaties in de hartslag dosis-afhankelijk toenamen gedurende de rustperioden. Deze effecten waren significant na 0.19 mg lorazepam voor de hartslag, de baroreflexgevoeligheid en de hoge-band fluctuaties in de hartslag, en na 0.44 mg lorazepam voor de hartslag-fluctuaties in de lage- en de midden-band. Lorazepam had geen invloed op de bloeddruk-fluctuaties of de ademfrequentie tijdens de rustperioden. De CWT induceerde, zowel na de placebo- als na de lorazepam-toedieningen, een significante toename in de hartslag, de systolische en diastolische bloeddruk, de ademfrequentie en de plasma adrenaline-concentratie, en een significante afname in de baroreflexgevoeligheid en de lage-, midden- en hoge-band fluctuaties van zowel de hartslag als de bloeddruk. Lorazepam had geen effect op de responsgrootte van de cardiovasculaire en biochemische parameters gedurende de CWT. Subjectieve vermoeidheid en reactietijd namen significant toe na 0.94 mg lorazepam, terwijl na dezelfde dosis alertheid afnam. Subjectieve angst werd niet beïnvloed door lorazepam. Op basis hiervan werden de volgende conclusies getrokken: 1) Lorazepam induceert verschillende effecten op cardiovasculaire, biochemische en psychologische functies. Terwijl hartslag afnam en hartslag-variabiliteit toenam bij lage doseringen gedurende rustperioden, en reactietijd en subjectieve vermoeidheid toenamen bij sederende doseringen, werden toestandsangst en fysiologische responspatronen op de CWT niet beïnvloed door lorazepam. Meer specifieke farmacologische interventies zijn noodzakelijk om de responsmechanismen op de CWT te analyseren. 2) Lorazepam induceert een dosis-afhankelijke toename in vagus-activiteit op het hart. Dit resulteert in een afname van de hartslag en een toename van hartslag-variabiliteit tijdens perioden van rust. Dit effect wordt teniet gedaan door de CWT. De toename in vagus-activiteit zoals geobserveerd na lage doseringen lorazepam, blijkt niet gerelateerd aan verminderde sympaticus-activiteit, veranderde ademhaling of toegenomen sedatie.

Hoofdstuk 6 bevat een korte evaluatie van de belangrijkste bevindingen. Methodologische en klinische implicaties van de studies worden kort besproken en suggesties voor vervolgonderzoek gepresenteerd.

Verantwoording

De in dit proefschrift beschreven studies werden verricht op de afdeling Psychiatrie van het Academisch Ziekenhuis Rotterdam Dijkzigt (hoofd: Joost Schudel) en de werkgroep Pathofysiologie van Gedrag van de Erasmus Universiteit Rotterdam (hoofd: Lolke Pepplinkhuizen). Op deze plaats wil ik graag die personen bedanken die betrokken waren bij de tot standkoming en uitvoering van de verschillende experimenten.

In de allereerste plaats gaat mijn dank uit naar Peter Moleman (afdeling Psychiatrie, AZR-Dijkzigt; Moleman Research BV), onder wiens inspirerende leiding de eerste psychofysiologische experimenten met betrekking tot de werking van het catecholamine-metabolisme werden opgezet. Zijn concrete en nauwgezette manier van werken vormde een belangrijke steun bij de vervaardiging van de protocollen en het schrijven van de eerste artikelen.

Psychofysiologisch onderzoek, zoals beschreven in dit proefschrift, doet een beroep op een specifieke infrastructuur. Ik ben zowel mijn promotor Lolke Pepplinkhuizen, als het hoofd van de afdeling Psychiatrie, Joost Schudel, bijzonder erkentelijk voor het feit dat zij de voorwaarden hebben geschapen om dit type onderzoek te kunnen verrichten en mij in de gelegenheid hebben gesteld om een infrastructuur op te bouwen. Lolke Pepplinkhuizen dank ik tevens voor zijn commentaar op en bijdragen aan de uiteindelijke versie van dit proefschrift.

De samenwerking met de afdeling Interne I was eigenlijk vanaf het eerste psychofysiologische experiment op het gebied van het catecholamine-metabolisme een feit, hetgeen een bijzonder gunstige invloed had op de productiviteit van de onderzoekslijn. Ik ben mijn promotor Arie Man in 't Veld dan ook dankbaar voor de interesse die hij altijd heeft getoond voor dit onderzoek, maar daarnaast ook voor de vele constructieve opmerkingen bij het schrijven van de artikelen en het vervaardigen van dit proefschrift. Frans Boomsma ben ik zeer erkentelijk voor zijn medewerking aan de biochemische invalshoek van het onderzoek, maar tevens voor het feit dat hij het gehele proefschrift nog eens kritisch heeft willen nalezen op taalkundige onjuistheden.

Voor de signaalanalyse van variatie-patronen in hartfrequentie en bloeddruk werd dankbaar gebruik gemaakt van de expertise van de vakgroep Experimentele en Arbeids Psychologie van de Rijksuniversiteit Groningen (hoofd: Bert Mulder). Ben Mulder en Arie van Roon zorgden ervoor dat implementatie van de benodigde spectraal-technieken mogelijk werd. Naast hen wil ik graag Bert Mulder bedanken voor de vele adviezen betreffende de analyse en interpretatie van onze data.

De praktische uitvoering van de experimenten vond plaats in één van de onderzoekruimten van de afdeling Klinische Neurofysiologie. De gastvrijheid die ik de afgelopen jaren op deze afdeling heb genoten vormde een belangrijke basis voor het opbouwen van een infrastructuur, alsmede voor de uitvoering van de projecten. Ik ben Frans van der Meché (hoofd afdeling Neurologie), Robert-Jan Schimsheimer en Jan Meulstee (Klinische Neurofysiologie) dan ook zeer erkentelijk voor de

collegiale samenwerking en hun ondersteuning van het psychofysiologisch onderzoek. De software voor het analyseren van de fysiologische signalen werd vervaardigd door Hugo van Steenis, wiens altijd aanwezige behulpzame inzet ervoor zorgde dat de vele analyses voor mij en voor de stagiaires haalbaar werden. Ton Mus en Hans van der Sluis stonden steeds garant voor de technische ondersteuning. Naast hen wil ik op deze plaats ook de laboranten van de afdeling Klinische Neurofysiologie bedanken voor de prettige samenwerking.

De farmacologische en biochemische invalshoek van de experimenten vereiste de aanwezigheid van een medische expertise, die een psychofysioloog niet vanuit de academische opleiding meekrijgt. Voor de medische begeleiding van de protocollen, alsmede voor de ondersteunig met betrekking tot de praktische handelingen die nodig waren voor de vele bloedafnamen, wil ik een aantal personen dan ook bijzonder danken. Het 'catecholamine-infuus'-experiment was het resultaat van een samenwerkingsproject met Interne I en kon dankzij de enthousiaste inzet van Peter Blankestijn worden uitgevoerd. In overleg met Ben van de Wetering (afdeling Psychiatrie) werd de 'clonidine'-studie opgezet, als voorbereiding op een onderzoek naar de werking van clonidine bij patiënten met het syndroom van Gilles de la Tourette; Michel Kruijk en Renée von Saher waren nauw betrokken bij de uitvoering van dit experiment. Robert Vermaat en Aad de Lijster zorgden ervoor dat het 'lorazepam'-experiment uitvoerbaar werd.

De analyse van de fysiologische signalen werd soms gekenmerkt door een uitputtingslag met de computer. Ik ben zowel Finance van den Heuij als Frank Smeets dank verschuldigd voor het feit dat zij, als post-doctoraal stagiaires, een deel van deze analyses voor hun rekening hebben willen nemen.

De biochemische variabelen vormden een belangrijk aspect van het onderzoek. De personen van de verschillende laboratoria die hun medewerking hebben verleend aan deze experimenten wil ik dan ook hartelijk bedanken. Frans Boomsma (Interne I) was steeds bereid de vele catecholamine bepalingen in plasma en urine te verzorgen, Durk Fekkes (Werkgroep Pathofysiologie van Gedrag) verrichtte de metingen met betrekking tot plasma MHPG, Rens Zwang (CKCL) verzorgde de urine MHPG bepalingen en Frans Derkx (Interne I) was verantwoordelijk voor de plasma renine, aldosteron en insuline metingen. Het laboratorium van Interne III (hoofd: Steven Lamberts) verrichtte de bepalingen met betrekking tot plasma cortisol, groeihormoon en prolactine, terwijl in het CKCL (hoofd: Jan Lindemans) de creatinine, glucose en triglyceride spiegels werden bepaald.

De aanlevering van de dubbelblinde ampullen voor de 'lorazepam'- en de 'clonidine'-studies werd verzorgd door de apotheek van het AZR-Dijkzigt. Graag dank ik Peter Roos en Hayo Graatsma voor hun nauwgezette medewerking aan deze projecten.

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Curriculum Vitae

J.H.M. Tulen werd op 11 december 1956 geboren te Lisse. Van 1969 tot 1975 bezocht zij het Fioretti College te Lisse, alwaar in juni 1975 het Atheneum-B diploma werd behaald. In datzelfde jaar werd een aanvang gemaakt met de studie psychologie aan de Universiteit van Amsterdam. De doctoraalfase van deze studie omvatte een specialisatie in de psychofysiologie en vanaf oktober 1980 tot januari 1984 was zij werkzaam als kandidaatsassistent bij de vakgroep Psychofysiologie (hoofd: Prof. Dr. P. Visser). Bijvakken gedurende de doctoraalfase betroffen het gebied van de klinische neurofysiologie (supervisie: Prof. Dr. P.E. Voorhoeve) en de theoretische psychosomatiek (supervisie: Prof. Dr. J. Bastiaans). In mei 1984 werd het doctoraalexamen in de psychologie behaald (cum laude). Van december 1984 tot oktober 1986 volgde een part-time aanstelling als wetenschappelijk ambtenaar aan de vakgroep Psychofysiologie van de Universiteit van Amsterdam. Sinds juni 1984 is zij als universitair docent werkzaam bij het instituut Psychiatrie van de Erasmus Universiteit Rotterdam (hoofd: Prof. Dr. W.J. Schudel), terwijl zij vanaf september 1988 tevens verbonden is aan de Werkgroep Pathofysiologie van Gedrag (hoofd: Prof. Dr. L. Pepplinkhuizen) van dezelfde universiteit.

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