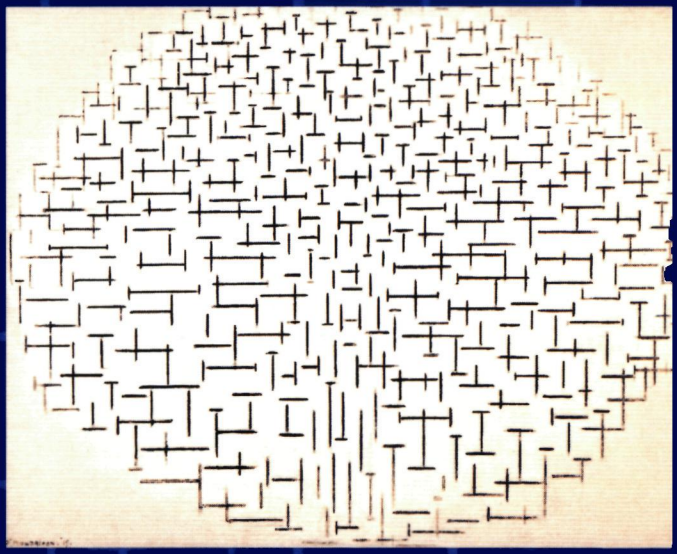
CATECHOLAMINE KINETICS IN PRIMARY HYPERTENSION



Marie-Cécile Jacobs

Catecholamine Kinetics in Primary Hypertension

ISBN 90 9010140 3

Grafische vormgeving en druk: Datawyse | Universitaire Pers Maastricht
Omslagillustratie: P. Mondriaan. Compositie nr. 10. Interpretatie: "Positieve en negatieve signalen die een beweeglijke structuur vormen, analoog aan het sympatisch zenuwstelsel".

Catecholamine Kinetics in Primary Hypertension

een wetenschappelijke proeve op het gebied
van de Medische Wetenschappen

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Katholieke Universiteit Nijmegen
volgens besluit van het College van Decanen
in het openbaar te verdedigen
op dinsdag 10 december 1996
des namiddags om 1.30 uur precies

door

Marie-Cécile Gertrude Sophie Jacobs
geboren op 5 augustus 1963 te Mechelen

Promotores

Prof. dr Th. Thien

Prof. dr P. Smits

Co-promotor

Dr J.W.M. Lenders

Manuscriptcommissie

Dr F. Russel

Prof. dr A.G.H. Smals

Prof. dr F.W.A. Verheugt

The studies described in this thesis were performed in the Department of Medicine, Division of General Internal Medicine, University Hospital Nijmegen, The Netherlands and were supported by a grant (no 89.212) from the Dutch Heart Association.

William Harvey (1578 - 1657), de ontdekker van de bloedsomloop, leidde zijn meesterwerk over de werking van het hart (Exercitatio anatomica de motu cordis et sanguinis in animalibus) in, door het orgaan te vergelijken met zijn koning Karel I en de zon, het centrum van leven en souverein van het lichaam. Tegenwoordig huldigen we echter de opvatting dat het hart geen meester maar knecht is, onderworpen aan de eisen van andere organen, gestuurd door het autonome zenuwstelsel.

Naar Dr AJ Dunning. Uitersten. Beschouwingen over menselijk gedrag.

Contents

Chapter 1

Introduction

The sympathetic nervous system plays a key role in the regulation of cardiovascular homeostasis. Reflex-induced adjustments in sympathetic activity are required to maintain arterial blood pressure in several physiological conditions like change of body position or exercise [1]. Without rapid constriction of resistance vessels by increments in sympathetic nerve activity, upright posture would not be possible for the human being. Since the hallmark of primary hypertension is an increased total peripheral vascular resistance, it is presumed that increased sympathetic activity contributes to the pathogenesis of hypertension. Numerous studies have tried to define the pathogenetic role of the sympathetic nervous system in primary hypertension. The activity and reactivity of the sympathetic nervous system are very difficult to assess quantitatively because of its high variability and its non-uniform outflow to the different organs.

The sympathetic nervous system

The sympathetic nervous system consists of nerve networks that entwine blood vessels, especially arterioles, throughout the body and that invade organs like heart, viscera and endocrine glands. The sympathetic outflow to these effector organs is controlled by the central nervous system, whose activity is continuously modulated by information received from an array of peripheral sensors. The sensors in the carotid sinus and aortic arch are referred to as arterial baroreceptors, whereas the sensors in the atria, ventricles and lungs are called cardiopulmonary baroreceptors. Nerve pulses from baroreceptors inhibit tonically sympathetic outflow to restrain the blood pressure and heart rate between certain values. When the arterial pressure in the carotid sinus or in the aortic arch increases, the resulting increase in afferent nerve activity leads to a decrease of sympathetic outflow to the heart and blood vessels and an increase in vagal outflow to the heart. A decrease in heart rate and cardiac contractility ensue together with dilation of the resistance vessels in muscles, kidneys, and splanchnic vascular bed. The decrease in cardiac output and peripheral vascular resistance restores the arterial blood pressure level. In contrast, a reduction of blood pressure at the site of the arterial baroreceptors deactivates the baroreceptors with a subsequent increase of blood pressure to its original

level. The cardiopulmonary baroreceptors are activated or deactivated by an increase or decrease in intrathoracic blood volume that is sufficient to alter the cardiac filling pressures but insufficient to change mean arterial or pulse pressure. This results in decreased or increased sympathetic outflow respectively, with ensuing reflex vasodilatation or vasoconstriction of the resistance vessels, thus restoring the cardiac filling pressures. Experimental studies concerning the baroreflex commonly employ head-up tilt and lower body negative pressure (LBNP). Selective unloading of cardiopulmonary baroreceptors can be achieved by application of LBNP at less then -20 mmHg, which decreases cardiac filling pressure without detectable changes in arterial blood pressure [2]. LBNP exceeding the -20 mmHg decreases arterial blood pressure, resulting in combined arterial and cardiopulmonary baroreceptor unloading. Efferent sympathetic nerve impulses cause exocytotic release of the neurotransmitter norepinephrine (NE) from the prejunctional sympathetic nerve terminals into the neuroeffector junctions of arteriolar walls and heart, where it exerts its effect through postjunctional adrenoceptors on the effector cells. A small proportion of the released NE spills over into the circulation. The adrenal medulla, being specialized sympathetic nervous tissue where NE is metabolized to epinephrine (EPI), releases EPI and NE directly into the circulation [3]

Measurement of sympathetic activity: plasma catecholamine concentrations

The plasma concentration of NE is dependent on the rate of its spillover into the circulation and on the rate of its clearance from the circulation. When sympathetic nerve pulses arrive at the nerve terminal, NE is released from intraneuronal vesicles into the synaptic cleft. A substantial proportion of the released NE is inactivated intrasynaptically, mainly by neuronal reuptake. Only a small fraction spills over from the synaptic cleft into the venous drainage of the organ. Since in most vascular beds NE spillover rate exceeds the clearance rate, there is usually an arteriovenous increment in the plasma NE concentration of an organ. The venous plasma NE concentration in a particular organ is not only determined by the NE spillover rate in that organ but also by the NE concentration of the inflowing arterial blood and by the extraction of NE during passage of blood through the organ. Therefore it is not surprising that the antecubital forearm venous plasma NE concentration is only a moderately reliable measure of sympathetic activity. Actually it merely reflects regional forearm sympathetic activity than global sympathetic activity of the entire body. For the latter purpose, arterial sampling is preferred over venous sampling [4,5].

EPI is directly released into the circulation from the adrenal medulla. The EPI concentration in mixed venous blood reflects sympathetic outflow to the adrenal medulla. The antecubital venous plasma EPI concentration is unreliable for the determination of sympathetic activity to the adrenal medulla because EPI is subject to extraction in the forearm [6]. Changes in the arterial plasma EPI level can be due to alterations in the

release of EPI from the adrenal medulla but also from alterations in clearance in several vascular beds.

Measurement of sympathetic activity: catecholamine kinetic studies

In order to unravel the contributions of spillover and clearance to the resulting plasma catecholamine concentration, kinetic techniques using radiotracers have been developed. The catecholamine tracer dilution method permits measurement of spillover and clearance rates of NE and EPI [7]. Total body as well as regional kinetics of NE and EPI in particular vascular beds can be measured. For the assessment of catecholamine kinetics, tritiated NE or EPI are infused at a constant rate to achieve steady state radiotracer concentrations in plasma. Under steady state conditions, the total body catecholamine spillover rate of a catecholamine can be calculated as the quotient of the radiotracer infusion rate and specific radioactivity of the catecholamine in plasma. Catecholamine clearance rate of a catecholamine from the body can be calculated from the quotient of radiotracer infusion rate and the plasma catecholamine radiotracer concentration. An important underlying assumption is that a well-mixed central plasma pool exists for the endogenous catecholamines and the radiotracers. Another relevant assumption is that any rerelease of the radiotracer from sympathetic nerves is negligible in comparison to the radiotracer infusion rate.

Total body spillover of EPI provides an estimation of adrenal medullary sympathetic activity or more specifically of the adrenomedullary EPI secretion. Total body NE spillover rate provides a measure of overall sympathetic tone but does not indicate the source of the released NE. Because organ-specific increases in sympathetic outflow can occur, methods for estimation of regionalized increments in NE release have been developed. The assessment of sympathetic activity in specific organs is complicated by the fact that all organs release NE into and remove NE from the circulation in a variable manner, depending on sympathetic activity in the organ and the proportion of cardiac output to that organ. NE spillover in an organ can be estimated by the following equation: organ NE spillover = $[(C_v - C_a) + C_a(NE_E)] \times$ plasma flow, where C_v is the venous plasma NE concentration, C_a is the arterial plasma NE concentration and NE_E is the fractional extraction of tritiated NE. It should be emphasized that the spillover of NE into the circulation is not identical with the rate of release of NE from the sympathetic nerve terminals into the synaps. Thus, with the tracer dilution method, the NE spillover rate but not the neuronal NE release rate can be measured [8,9]. The rate of release and the rate of spillover of NE can differ for several reasons. The neuronal NE release can be modulated by several endogenous compounds, including NE and EPI themselves or other cotransmitters like peptides, purines and dopamine. All these compounds may act at specific presynaptic receptors at the nerve terminal, affecting intrasynaptic neuronal release of NE [10,11]. The neuronal NE spillover rate can also be influenced by intrinsic factors like the width of the neuronal cleft, the efficacy of

intrasynaptic inactivation mechanisms such as neuronal or extraneuronal NE clearance rates and local blood flow [10-12].

Measurement of sympathetic activity: microneurography

Prejunctional sympathetic nervous activity can be recorded intraneuronally by microneurography of peripheral nerves [13]. This technique permits direct observation of the exact timing of sympathetic nerve traffic to skeletal muscles. Moreover, nerve activity can be separated from effects of hormonal or local actions at the receptor site. However, small changes in intraneuronal discharge frequency are difficult to quantify whereas they may have large effects at the target site. Finally, sympathetic outflow, measured in one nerve of the skeletal muscles of one arm or leg, does not automatically represent sympathetic nerve activity to other organs in the body, especially during sympathetic stimulation.

Measurement of sympathetic activity: plethysmography and heart rate variability

The effects of the sympathetic nervous system on the cardiovascular system at the postjunctional level can be assessed by measuring forearm blood flow to skeletal muscles with venous occlusion mercury-in-strain gauge plethysmography [14] or by measuring heart rate variability by spectral analysis. Heart rate variability measurement is a method to estimate indirectly cardiac sympathetic and parasympathetic activity [15]. The responsiveness of a target organ for a given nerve impulse rate is influenced by neural, local or circulating vasoactive factors, such as the type and density of the adrenoceptors and their affinity for NE, the locally operating uptake mechanisms, the rate of enzymatic degradation of NE, the synthesis and release of interfering metabolites, the vasomotor effects of locally synthesised substances like prostaglandins, histamine, adenine nucleotides, 5-hydroxytryptamine and effects of circulating substances like EPI and angiotensin II. Intrinsic factors like vascular size and structure also play a role.

The sympathetic nervous system in primary hypertension

No consensus exists on the role of the sympathetic nervous system in the pathophysiology of primary hypertension. Increased sympathetic nerve activity is suggested by the increased cardiac output and increased heart rate in the early phase of hypertension and by the increased vascular resistance in the later phase of established hypertension. When hypertension has reached this later phase, structural cardiovascular abnormalities have developed and predominate in the maintenance of high blood pressure. They can possibly mask an eventual sympathetic dysfunction. The exact nature of a sympathetic defect in primary hypertension remains unknown. The methods nowadays available for the assessment of sympathetic activity can only measure a part of the system. It seems impossible to measure overall sympathetic activity of this extremely differentiated and

highly variable system. Because of the capacity of the sympathetic nervous system to adapt very rapidly, it still remains difficult to unravel causes and consequences.
Several abnormalities in the sympathetic nervous system at different levels have been described. Shortly after the discovery of the carotid sinus baroreflex, it was suggested that dysfunction of the baroreflex might play a role in the pathogenesis of primary hypertension in humans. Hypertension in humans appears to be related to a depression of the arterial baroreceptor reflex sensitivity for heart rate control [16], and to a lesser degree for peripheral resistance control [17]. On the other hand, the responses of forearm vascular resistance and sympathetic nerve traffic (microneurography) to cardiopulmonary baroreceptor deactivation in borderline hypertension, were shown to be augmented [18,19]. The reflexive responses in vascular resistance in established hypertensives were similar to the responses in normotensives [20]. The cardiac responses to arterial baroreflex stimulation with head-up tilt seem also to be impaired, whereas sympathetic nerve traffic to skeletal muscles is preserved in early hypertension [21]. It is however still unknown, whether impaired baroreflexes cause hypertension, or vice versa. Irrespective of what is cause or consequence, it was shown that baroreceptor endings are reset to a higher blood pressure level in the hypertensive patient. After resetting, the receptor endings have lower peak firing frequencies and also lower increases in firing rate for stepwise increments in distending pressure. This means that although the receptors are reset to the higher arterial pressure, they are less sensitive to changes of blood pressure and presumably less efficient in buffering further rises in pressure [22]. Resetting of the baroreflex may occur at the local baroreceptor level or at the central nervous system level. It may be related to physical changes at the baroreceptor site, such as reduced elasticity in the arterial wall of the carotid sinus and aorta. Other mechanisms may be an altered central processing of afferent baroreflex information.
Many efforts have been spent to the measurement of the sympathetic neurotransmitter NE. Venous plasma NE concentrations however, were not found to be increased in the majority of studies. Younger patient groups, with probably more recent onset hypertension than older patients, seem to have higher plasma NE concentrations, though controversial data have been published [23]. After development of the radiotracer dilution method, more precise measurements of sympathetic activity in human hypertension were possible. Assessment of organ-specific sympathetic outflow suggests that the increased sympathetic outflow is regionalized mainly to the heart and kidneys in young hypertensive patients [24,25], and not to the lungs or hepatosplanchnic complex [24]. The demonstrated increased muscle sympathetic nerve activity (MSNA) in skeletal muscles of the forearm of young borderline hypertensives [26] would imply an increased regional forearm NE spillover but this has never been demonstrated convincingly. The important difference between NE spillover and neuronal NE release is underlined by the finding that neuronal reuptake (uptake-1) may be impaired in primary hypertension. When uptake-1 is indeed decreased, this would result in increased spillover of NE into the circulation despite the absence of increased sympathetic nerve traffic and neuronal

NE release. Additional important factors to be taken into account are the duration of the hypertension and the gender of the subjects. The increased MSNA in borderline or mild hypertension could not be demonstrated anymore in established hypertension. Previous reports on gender-related effects on plasma catecholamine levels have not been followed by studies on gender-dependent differences in NE and EPI kinetics [27].
Several lines of evidence suggest that increased release of adrenomedullary EPI may be the primary abnormality in primary hypertension. Small repetitive increases in circulating EPI by an increased release of adrenomedullary EPI might stimulate presynaptic β_2-adrenoceptors. This would facilitate NE release from sympathetic nerve terminals with a subsequent increase in peripheral vasculature resistance and blood pressure. EPI can possibly also act as a cotransmitter after neuronal uptake. In addition to these actions at the neuroeffector junction, stimulation of β_2-adrenoceptors by EPI also facilitates hypothalamic and adrenal catecholamine release. This might even cause a positive feedback loop, through which even a small increase in circulating EPI can sustain amplification of neurotransmission at the central and peripheral level [28]. Plasma EPI concentrations are slightly elevated in many but not all young subjects with borderline or mild hypertension [23] but are mostly measured in antecubital venous blood. Since venous plasma EPI concentrations do not reflect adrenomedullary EPI secretion because of the major extraction of EPI through the forearm, it would be more interesting to know whether patients with hypertension have increased arterial plasma EPI levels.
Mechanisms of postsynaptic hyperresponsiveness include altered adrenoceptor number and function or altered cardiovascular structure. The increased heart rate, cardiac output and forearm blood flow, as described in many young patients with borderline hypertension, could be a consequence of increased postjunctional β_1- and β_2-adrenoceptor responsiveness. In some studies an increase in mononuclear leucocyte β_2-adrenoceptor number has been described, but the majority of studies in older patients and those with established hypertension have demonstrated a decreased number of postjunctional β_2-adrenoceptors [29]. Besides alterations in receptor density, an attenuation of the coupling between β_2-adrenoceptors and G_S-proteins was observed in primary hypertension, suggesting a defect in β_2-adrenergic signal transducton in hypertension. A difficulty in the interpretation of these studies is that the lymphocyte β-adrenoceptor numbers and β-adrenoceptor-stimulated adenylate cyclase activity are themselves subject to modification by catecholamines.

Sympathetic activity during antihypertensive treatment

Many effective antihypertensive agents exert their action by interfering with sympathetic neurotransmission, blocking cardiac or vascular adrenoceptors or stimulating central neural adrenoceptors.
Alpha$_1$-adrenoceptor antagonists like doxazosin lower blood pressure by blocking postjunctional α_1-adrenoceptors, causing vasodilation without tachycardia [30,31].

During chronic α_1-blockade the arterial plasma NE level is increased, which could be due to vasodilation-induced baroreflex stimulation [32] or to decreased clearance of NE due to decreased extraction by the vasodilation. The absence of tachycardia suggests that no sympathetic counterregulation occurs and the unchanged heart rate has been attributed to absence of antagonism of prejunctional α_2-adrenoceptors [33].
Beta$_1$-adrenoceptor antagonists like atenolol are effective antihypertensive drugs. The antihypertensive action has been ascribed to blockade of sympathetic activity, either in the central nervous system or through presynaptic modulation of NE release. Resetting of baroreflex control of blood pressure has also been considered [34]. Previous reports have described variable effects of different β-adrenoceptor antagonists on plasma NE and EPI levels [34] in hypertension, despite similar antihypertensive effects. No NE and EPI kinetic data are available during longterm treatment with β_1-adrenoceptor antagonists but inconsistent results were found in acute [12,35,36] and chronic [37] studies with β_1-selective [36] and non-selective [12,35-37] β-adrenoceptor antagonists. These results can not be generalized, since β_1-selective and non-selective β-antagonists can affect NE release differently through presynaptic β_2-adrenoceptors [34], whereas short and longterm treatment have different effects on organ blood flow and may differently affect NE clearance [34].

Outline of this thesis

This thesis focuses on the assessment of sympathetic nervous activity in primary hypertension by the isotope dilution technique. We measured sympathoneural and adrenomedullary activities by using steady state infusions of tritiated catecholamines in a large group of patients with mild primary hypertension. For this purpose we needed a sensitive assay for the simultaneous quantification of endogenous plasma catecholamine concentrations and its radiotracers. Catecholamine kinetics were determined at rest and during sympathetic stimulation by lower body negative pressure (LBNP). In chapter 2 is described how the HPLC method with fluorometric detection allows simultaneous sensitive measurement of the radiolabeled and endogenous catecholamines in the same eluted HPLC fraction. In chapter 3, the effects of prolonged low-intensity and high-intensity LBNP on NE and EPI kinetics in normotensives are described. Since some subjects are prone to a vasodepressor syncope during prolonged high-intensity LBNP, we studied the neurohumoral regulation during low-intensity LBNP in those subjects who developed a syncope during high-intensity LBNP. This is described in chapter 4. Chapter 5 contains a study on NE and EPI kinetics in a large group of mildly hypertensive patients, both at rest and during sympathetic stimulation. Chapter 6 and 7 deal with catecholamine kinetics during longterm treatment with the selective α_1-ad-

renoceptor antagonist doxazosin or with the β_1-adrenoceptor antagonist atenolol in subjects with primary hypertension.

References

1. Abboud FM, Heistad DD, Mark AL, Schmid PG. Reflex control of the peripheral circulation. Progr Cardiovasc Dis 1976;13;371-403
2. Mark AL, Mancia G. Cardiopulmonary baroreflexes in humans. In: Handbook of Physiology. The cardiovascular system. Eds Shepherd JT, Abboud FM. Bethesda: American Physiological Society 1983;3(part 2):795-813
3. Weiner N. Control of the biosynthesis of adrenal catecholamines by the adrenal medulla. In: Geiger SR (Ed). Handbook of physiology. A critical, comprehensive presentation of physiological knowledge and concepts. Section 7 Endocrinology. Volume 6 Adrenal gland. 1975, 357-366
4. Esler M, Jennings G, Korner P, Blombery P, Sacharias N, Leonard P. Measurement of total and organ-specific norepinphrine kinetics in humans. Am J Physiol 247 (Endocrinol Metab 10):E21-E28, 1984
5. Hjemdahl P. Physiological aspects on catecholamine sampling. Life Sci 1987;41:841-844
6. Kjeldsen SE, Westheim A, Aakesson I, Eide I, Leren P. Plasma adrenaline and noradrenaline during orthostasis in man: The importance of arterial sampling. Scand J Clin Lab Invest 1986;46:397-401
7. Esler M, Leonard P, O'Dea K, Jackman G, Jennings G, Korner P. Biochemical quantification of sympathetic nervous activity in humans using radiotracer methodology: Fallibility of plasma noradrenaline measurements. J Cardiovasc Pharmacol 1982;4:S152-S157
8. Eisenhofer G, Esler MD, Meredith IT, Dart A, Cannon RO, Quyyumi AA, Lambert G, Chin J, Jennings GL, Goldstein DS. Sympathetic nervous function in human heart as assessed by cardiac spillovers of dihydroxyphenylglycol and norepinephrine. Circulation 1992;85:1775-1785
9. Chang PC, Kriek E, van der Krogt JA, van Brummelen P. Does regional norepinephrine spillover represent local sympathetic activity? Hypertension 1991;18:56-66
10. Langer SZ. Presynaptic regulation of the release of catecholamines. Pharmacol Rev 1981;32;337-362
11. Langer SZ, Cavero I, Massingham R. Recent developments in noradrenergic neurotransmission and its relevance to the mechanism of action of certain antihypertensive agents. Hypertension 1980;2:372-382
12. Rosen SG, Supiano MA, Perry TJ, Linares OA, Hogikyan RV, Smith MJ, Halter JB. β-Adrenergic blockade decreases norepinephrine release. Am J Physiol 1990;258 (Endocrinol Metab 21):E999-E1005
13. Hagbarth KE, Vallbo AB. Pulse and respiratory grouping of sympathetic impulses in human muscle nerves. Acta Physiol Scand 1968;74:96-108
14. Brakkee AJM, Vendrik AJH. Strain gauge plethysmography, theoretical and practical notes on a new design. J Appl Physiol 1966;21:701-704
15. Pagani M, Lombardi F, Guzetti S, Rimoldi O, Furlan R, Pizzinelli P, Sandrone G, Malfatto G, Dell'Orto S, Piccaluga S, Turil M, Baselli G, Cerutti S, Malliani A. Power spectral analysis of heart rate and arterial blood pressure variabilities as a marker of sympathovagal interaction in man and conscious dog. Circ Res 1986;59:178-193
16. Gribbin B, Pickering TG, Sleight P, Peto R. Effect of age and high blood pressure on baroreflex sensitivity in man. Circ Res 1971;29:424-431
17. Duprez D, de Pue N, Clement DL. Peripheral vascular responses during carotid baroreceptor stimulation in normotensive and hypertensive subjects. Clin Sci 1987;73:635-640

18 Mark AL, Kerber RE Augmentation of cardiopulmonary baroreflex control of forearm vascular resistance in borderline hypertension Hypertension 1982,4 39-46
19. Rea RF, Hamdan M Baroreflex control of muscle sympathetic nerve activity in borderline hypertension Circulation 1990,82 856-862
20 Grassi G, Giannattasio C, Cleroux J, Cuspidi C, Sampieri L, Bolla GB, Mancia G Cardiopulmonary reflex before and after regression of left ventricular hypertrophy in primary hypertension Hypertension 1988,12 227-237
21 London GM, Weiss YA, Pannier BP, Laurent SL, Safar ME Tilt test in primary hypertension Differential responses in heart rate and vascular resistance Hypertension 1987,10 29-34
22 Sleight P, Robinson JL, Brooks D, Rees PM Characteristics of single carotid sinus baroreceptor fibers and whole nerve activity in the normotensive and renal hypertensive dog Circ Res 1977,14 750-758
23 Goldstein DS Plasma catecholamines and primary hypertension An analytical review Hypertension 1983,5 86-99
24 Esler M, Jennings G, Biviano B, Lambert G, Hasking G Mechanism of elevated plasma noradrenaline in the course of primary hypertension J Cardiovasc Pharmacol 1986,8(suppl 5) S39-S43
25 Goldstein DS, Eisenhofer G, Garty M, Sax FL, Keiser HR, Kopin IJ Pharmacologic and tracer methods to study sympathetic function in primary hypertension Clin Exper Theory and Practice 1989,A11(suppl 1) 173-189
26 Anderson EA, Sinkey CA, Lawton WJ, Mark AL Elevated sympathetic nerve activity in borderline hypertensive humans evidence from intraneural recordings Hypertension 1989,14 177 183
27 Lenders JWM, de Boo Th, Lemmens WAJ, Reijenga J, Willemsen JJ, Thien Th Comparison of blood pressure response to exogenous epinephrine in hypertensive men and women Am J Cardiol 1988,61 1288-1291
28 Floras JS Epinephrine and the genesis of hypertension Hypertension 1992,19 1 18
29 Michel MC, Brodde O-E, Insel PA Peripheral adrenergic receptors in hypertension Hypertension 1990,16 107-120
30 Manos J A long term study of doxazosin in the treatment of mild or moderate primary hypertension in general medical practice Am Heart J 1991,121 346-51
31 Lund-Johansen P, Omvik P, Haugland H Acute and chronic haemodynamic effects of doxazosin in hypertension at rest and during exercise Br J Clin Pharmac 1986,21,45S-54S
32 Eklund B, Hjemdahl P, Seideman P, Atterhog JH Effects of prazosin on hemodynamics and sympathoadrenal activity in hypertensive patients J Cardiovasc Pharmacol 1983,5 384-91
33 Reid JL, Rubin PC Catecholamines and blood pressure In Catecholamines II ed U Trendelenburg, Weiner N, 1989, Ch 17, p 319-356
34 Man in t Veld AJ, Schalekamp MADH Effects of 10 different β-adrenoceptor antagonists on hemodynamics, plasma renin activity, and plasma norepinephrine in hypertension The key role of vascular resistance changes in relation to partial agonist activity J Cardiovasc Pharmacol 1983,5(suppl 1) S30-S45
35 Best JD, Halter JR Blood pressure and norepinephrine spillover during propranolol infusion in man Am J Physiol 1988,248 R40-406
36 Chang PC, Grossman E, Kopin IJ, Goldstein DS On the existence of functional beta-adrenoceptors on vascular sympathetic nerve endings in the human forearm J Hypertension 1994,12 681-690
37 Esler M, Jackman G, Leonard P, Skews H, Bobik A, Jennings G Effect of propranolol on noradrenaline kinetics in patients with primary hypertension Br J Clin Pharmac 1981,12 375-380

Chapter 2

Highly sensitive and specific HPLC with fluorometric detection for determination of plasma epinephrine and norepinephrine applied to kinetic studies in humans.

Clinical Chemistry 1995;41:1455-1460

J.J. Willemsen
H.A. Ross
M.C. Jacobs
J.W.M. Lenders
Th. Thien
L.M.J.W. Swinkels
T.J. Benraad

Highly sensitive and specific HPLC with fluorometric detection for determination of plasma epinephrine and norepinephrine applied to kinetic studies in humans

Abstract

An HPLC separation method combined with fluorometric detection was extended to enable simultaneous assessment of plasma ^{3}H-labeled and endogenous epinephrine and norepinephrine. Forearm fractional extraction of ^{3}H-labeled epinephrine and norepinephrine and of endogenous epinephrine was measured in 40 healthy volunteers who were receiving a continuous infusion of ^{3}H-labeled epinephrine and norepinephrine. Concentrations of arterial and venous epinephrine were 26.8 ± 1.95 (mean ± SE) and 6.8 ± 0.75 ng/L, respectively. Arterial and venous norepinephrine and dopamine were also measured, with respective values of 140.7 ± 8.5 and 192.1 ± 15.1 for norepinephrine, and 13.1 ± 0.78 and 11.3 ± 0.70 ng/L for dopamine. The forearm fractional extraction of ^{3}H-labeled epinephrine was slightly but significantly higher (0.790 ± 0.016) than the forearm fractional extraction of either ^{3}H-labeled norepinephrine or endogenous epinephrine (0.748 ± 0.0146 and 0.745 ± 0.0185 respectively; $P<0.001$), the correlations being highly significant ($r = 0.80$, $P<0.001$) in both cases. The small difference between the forearm fractional extractions of epinephrine and of ^{3}H-labeled epinephrine allows calculation of the apparent spillover of epinephrine. However, this spillover was negligible compared with forearm norepinephrine spillover (0.0112 ± 0.0031 vs. 1.369 ± 0.128 ng/L per minute. The high sensitivity of this measurement of venous epinephrine widens the possibilities for studying epinephrine kinetics under physiological conditions.

Introduction

Several methods have been introduced for measurement of plasma catecholamines (CA). The early fluorometric method [1] was followed by the more sensitive and specific radioenzymatic assay [2,3]. The latter, in turn, has largely been replaced by less cumbersome and faster procedures such as HPLC with electrochemical detection [4-8]. However, for measurement of physiological concentrations of epinephrine (EPI), particularly in venous plasma, sensitivity is restrictive and, as a consequence, will make kinetic studies of EPI inappropiate. Measurements of the tritiated EPI (^{3}H-EPI), however, using HPLC with electrochemical detection to perform kinetic studies, are

completely different from those of the concentrations of EPI and are relatively easy to obtain. For this reason, measurement of ^{3}H-EPI is not the limiting factor in kinetic studies of EPI [5,9]. One method described for CA measurement uses HPLC separation of CA fluorescent derivatives with subsequent fluorometric detection [10,11]. This method reportedly has higher sensitivity for measurement of EPI. One of these studies [10] reported that a processed EPI calibrator of 2 ng/L could be detected. In the present study, we concentrated plasma extracts to assess the sensitivity and accuracy of the proposed assay. The rationale of the present study, however, was to investigate whether the high sensitivity and accuracy for assessment of endogenous EPI achieved with this new method, combined with simultaneous separation and measurement of ^{3}H-EPI, supports the possibility of performing kinetic studies of EPI under physiological conditions. Therefore, we compared the forearm fractional extraction (FFE) of ^{3}H-EPI vs endogenous EPI, on the assumption that FFE of endogenous EPI would be equal, or at least be close to, that of infused ^{3}H-EPI.

Materials and methods

Subjects and sampling

Forty volunteers (ages 37 ± 6 years, mean ± SD) participated in the study. All subjects gave their written informed consent. The study protocol was approved by the Hospital Ethics Committee. All participants underwent a physical examination before entry into the study. None of the subjects had cardiovascular or other major diseases, and none was under pharmacological treatment of any kind. The participants were in supine position during the procedure, which was carried out in a temperature-controlled room at 21°C. Forearm blood flow was measured according to Brakkee and Vendrik [12]. A brachial artery was cannulated for drawing arterial blood samples, and an intravenous catheter was inserted into a brachial vein in the same arm for collecting venous blood samples. A forearm venous catheter in the contralateral arm was used for intravenous infusion of ^{3}H-EPI and ^{3}H-labeled NE (^{3}H-NE). Thirty minutes after installation of the catheters, a 15 μCi/m^{2} bolus of ^{3}H-EPI and ^{3}H-NE was injected. Thereafter ^{3}H-EPI and ^{3}H-NE were infused simultaneously at a continuous rate of 0.35 μCi/m^{2} per minute. Then, 30 min after the start of the infusion, forearm arterial and venous blood samples were collected simultaneously. The blood samples were collected in precooled tubes on melting ice, containing 0.25 mol/L EGTA and 0.2 mol/L glutathione in distilled water (pH 7.4). The blood was centrifuged (10 min at 1500g, 4°C) immediately after collection, and plasma was stored at -20°C. At the end of the experiment, portions of the ^{3}H-EPI and ^{3}H-NE infusates were stored at -80°C. Plasma samples and ^{3}H-EPI and ^{3}H-NE infusates were analysed together within 2 months after collection.

Materials and instrumentation

For the liquid-liquid extraction and derivatization, we used the same materials as described by van der Hoorn et al. [10]. L-[N-methyl-^{3}H]-EPI (specific activity 55-85 KCi/mol) and L-[ring 2,5,6-^{3}H]-NE (specific activity 30-60 KCi/mol) were obtained from Du Pont, New England Nuclear ('s Hertogenbosch, The Netherlands). The purity of each batch of radiolabeled compound was established by HPLC with direct injection of the underivatized tritiated compounds onto a 250 × 4.6mm C_8 column, after which fractions were collected and their radioactivity counted. The samples containing ^{3}H-EPI and ^{3}H-NE were sterilized with the use of a micropore filter (0.22 μm) and diluted in isotone saline (9 g/L NaCl), containing acetic acid (0.2 mol/L) and ascorbic acid (1 g/L), to prevent oxidation. The samples were stored until use at a concentration of approximately 70 mCi/L at -80°C for ≤ 3 months. Sterilization, dilution and partition into aliquots took place under nitrogen. The aliquots were capped and, just before the study, the tracer was diluted in isotone saline.

The system configuration for HPLC consisted of a Model 610 pump, a Model 600E system controller, a Model 470 scanning fluorescence detector (16 μL flow cell), and an automated sample processor WISP 710B (all from Waters Associates, Millford, MA). Chromatography data were edited with Baseline 815 Chromatography Workstation Version 3.30 software. The separations were performed on a silica analytical Nova-Pak™ (Waters Associates) C_{18} (150 × 3.9mm) column fitted with a Nova-Pak C_{18} guard column. The ^{3}H-EPI and ^{3}H-NE activities were collected for 1 minute with the use of a Gilson fraction collector, model 201-202 (Gilson Medical Electronics, Villiers le Bel, France), which was connected to the WISP 710B and programmed to start collecting the ^{3}H-EPI and ^{3}H-NE fractions at the start of the EPI and NE peak positions, respectively, in the chromatogram. To these fractions was added 8 mL of scintillation fluid (Aqualuma; Lumac-LSC BV, Groningen, The Netherlands). The ^{3}H-EPI and ^{3}H-NE radioactivities were counted for 20 minutes in a Packard Tri-Carb 460 C liquid scintillation counter (background count rate, 11.7 ± 0.4 counts/min; efficiency, 40%).

Sample preparation and chromatography

The present laboratory procedure, an extension of the procedure described by van der Hoorn et al. [10], includes specific measurement of infused tracers in the same eluate. Each assay run included a standard mixture of NE (250 ng/L), EPI (250 ng/L) and dopamine (DA, 500 ng/L); a reagent blank; a control plasma; the venous and arterial plasma samples; and the ^{3}H-EPI and the ^{3}H-NE infusates from an experiment in one subject. To different glass tubes were added 1 mL of the standard mixture in 0.01 mol/L HCl (A), 1 mL of 0.01 mol/L HCl (B), or 1 mL of control plasma or 1 mL venous or arterial plasma (C). We than added 50-μL aliquots of ^{3}H-EPI or ^{3}H-NE infusate to 1 mL of venous baseline plasma sample that had been collected before ^{3}H-EPI and ^{3}H-NE were infused (D), or to 1 mL of 0.01 mol/L HCl (D'). To each of these tubes

we added 250 µL (500 pg) of isoproterenol (IP) solution as internal standard. Another 50 µL of ^{3}H-EPI or ^{3}H-NE infusate was pipetted into a counting vial for direct measurement of the ^{3}H-EPI and ^{3}H-NE (R_T). Using these solutions, we determined the procedural recoveries of ^{3}H-EPI and ^{3}H-NE, and investigated the effect of the plasma matrix on the measurement of ^{3}H-EPI and ^{3}H-NE.
Extraction of catecholamines was performed as described previously [10], but with the following modifications. We used only one-half of the previous volumes of reagents for the liquid-liquid extraction and made the final extraction of the CA with 37.5 µl of 0.08 mol/L acetic acid. To convert the CA into their diphenyl-quinoxalin derivatives, we successively added 50 µL of acetonitrile, 12.5 µL of 1.75 mol/L Bicine (pH 7.05), and 25 µL of 1,2-diphenylethylenediamine solution (0.1 mol/L in 0.1 mol/L HCL), started the reaction by adding 5 µL of potassium ferricyanide (20 mmol/L in distilled water), and incubated the mixture for 60 min in a closed oven at 37°C. After derivatization, we injected 120 µL of the solution into the chromatographic system.

Assay performance

To obtain "zero" plasma, we heated pooled plasma from healthy volunteers at 37°C until NE, EPI and DA detector responses had become equal to the response of reagent blank. For assessment of lower detection limits, we added to this zero plasma (a) 1 ng/L NE and EPI and 2 ng/L DA and (b) 2 ng/L NE and EPI and 4 ng/L DA. To these two samples as well as to the zero sample we then added 250 µl (500pg) of the IP solution and assayed each sample five times.
Within-run variation was assessed by means of five determinations each of five samples, ranging from low to moderate CA concentrations. Sample 1 was obtained from a patient who had undergone bilateral adrenalectomy. Samples 2 and 3 originated from two different pooled plasmas, and samples 2a and 3a were prepared by CA supplementation of samples 2 and 3.
Between-run variation was assessed by assaying a control (pooled) plasma over a period of 1,5 years in 52 different runs.
Assay accuracy was assessed by measuring CA in supplemented samples and calculating the proportion of the added CA that was thus refound. To the zero plasma mentioned, as well as to sample 3 (which had normal CA concentrations), we added 250 ng/L NE and EPI and 250 or 500 ng/L DA. To sample 2 we added smaller quantities of CA: 25 ng/L NE and 2.5 ng/L EPI and DA.
The precision and accuracy of measurements of radiolabeled CA were determined as described in the Calculations section.

Calculations

Measurement of ^{3}H-Catecholamines. Identical amounts of IP internal standard were added to 1 mL of unknown plasma (C) and to the radioactivity calibration solution (D)

(50 µL of the ^{3}H-EPI or ^{3}H-NE infusate mixed with 1 mL of venous baseline plasma). From the amounts of ^{3}H activity (in counts/min or dpm) of the calibrator solution (D) and plasma samples (C) collected after derivatization and HPLC ($R_{CA,D}$ and $R_{CA,C}$ respectively) and the ^{3}H-activity of 50-µL infusate (R_T), we can calculate the original activities of ^{3}H-labeled CA (^{3}H-CA) in 1 mL of plasma sample by means of the following formula:

$$^3\text{H-CA}_C = \frac{R_{CA,C}}{R_{CA,D}} \times \frac{P_{IP,D}}{P_{IP,C}} \times R_T \qquad (1)$$

where $P_{IP,C}$ and $P_{IP,D}$ denote the µV readings corresponding to IP internal standard peak heights of sample C and radioactivity calibrator D respectively.

After correction for the volume actually injected relative to the total volume after derivatization, the ratio $R_{CA,D}$ / R_T yields the true procedural recoveries of CA from sample D.

The recovery for the unknown plasma samples (C) can be estimated by multiplying the ratio $R_{CA,D}$ / R_T by $P_{IP,C}$ / $P_{IP,D}$. Division of $R_{CA,C}$ by this recovery gives the original activity in 1 mL unknown plasma sample C.

Validation of ^{3}H-CA measurement. Although the true procedural recoveries of the ^{3}H-CA can be measured as outlined in the previous paragraph, this value cannot be used to determine the ^{3}H-CA concentration in an unknown plasma sample because the loss of CA during the procedures of extraction and derivatization may differ between samples. Instead, the internal standard method, which also is used to estimate the endogenous CA, is applied here. Therefore, we assume a direct proportionality between the recoveries of ^{3}H-CA and IP in different matrices. The validity of this assumption was verified by the following experiment. To different plasma samples D(i), known amounts of tracer $R_{T(i)}$ and known amounts of internal standard IP,D(i) were added. In parallel with these samples, a sample D' (without plasma), to which the same amount of tracer and IP was added, was analyzed. The ratios $R_{CA,D(i)}$ / $P_{IP,D(i)}$ and $R_{CA,D'}$ / $P_{IP,D'}$ were then compared for 14 different plasma samples, each analyzed on different days; the quotient Q(i) was calculated according to:

$$Q(i) = \frac{R_{CA,D(i)}}{R_{CA,D'}} \times \frac{P_{IP,D'}}{P_{IP,D(i)}} \qquad (2)$$

where $R_{CA,D'}$ denotes the amount of ^{3}H activity in the fraction collected after derivatization and HPLC of the 50 µL of infusate added to 1 mL of 0.01 mol/L HCl, and $P_{IP,D'}$ denotes the µV readings of the corresponding internal standard peak height in the chromatogram. If this quotient Q(i) approximates unity, it means that the assumption is valid and is independent of different matrices.

Fractional extraction. FFE was calculated as 1 – [V]/[A], where V and A represent venous and arterial concentrations of ^{3}H-EPI or ^{3}H-NE. Assuming unidirectional clearance of

endogenous EPI and no local EPI release, its fractional extraction was calculated in the same way.

Forearm spillover. Forearm spillover (ng/L per minute) was calculated from FFE of ^{3}H-EPI or ^{3}H-NE and forearm plasma flow, where forearm plasma flow = forearm blood flow × (1 – hematocrit), in mL/L per minute, as follows:

$$\text{Forearm spillover} = \text{forearm plasma flow} \times [V - A \times (1 - \text{FFE})]$$

where V and A denote venous and arterial endogenous CA concentrations, respectively.

Statistics. Differences were tested by a paired t-test. Correlation coefficients were determined according to Pearson.

Results

Analytical evaluation

The present HPLC method allows good separation of the CA. The sequence of elution was NE-EPI-DA-IP with retention times of 2.2, 3.75, 5.5, and 7.5 min, respectively (Fig 1). Even with these very short retention times, the separation of the tritiated compounds was complete. No radioactivity originating from ^{3}H-NE could be detected in the ^{3}H-EPI zone of the chromatogram, and vice versa. The procedural recoveries of the different ^{3}H-NE and ^{3}H-EPI infusates of the same batch, added to different 1-mL venous baseline plasma samples, were 37.1% ± 2.5% (mean ± SD, n=14) for ^{3}H-NE and 52.0% ± 2.2% for ^{3}H-EPI. The quotient Q(i), calculated with Eq.2, was 0.968 ± 0.025 (mean ± SD, n=14) for ^{3}H-NE and 0.983 ± 0.028 for ^{3}H-EPI, respectively. The CVs of these latter ratios (2.6% and 2.8%, respectively) reflect the interassay CVs for the radiolabeled compounds with high count rates. The purity of each batch of (underivatized) radiotracers was >99%.

In all instances where CA had been added, peaks corresponding to the fluorescence signal of derivatized NE, EPI and DA could be readily identified. Retention times varied, with CVs of 0.4%, 0.8% and 0.7% for NE, EPI and DA, respectively. CVs for EPI and DA could be further reduced to 0.2% and 0.3%, respectively, by normalization, with the retention times of IP in the corresponding chromatograms. The 99% confidence intervals of these retention times were calculated to allow us to classify the very small apparent peaks that were discernible in the chromatographs of the zero plasmas as representing either residual CA or baseline noise. In only one of five observations on zero plasmas was the "peak" retention time within those confidence limits. Thus we concluded that these apparent peaks must be classified as baseline noise. In all instances, responses for plasmas containing 1, 2 and 4 ng/L CA were much higher than the zero response and were close to expected values (Fig 2). In addition, the differences between 1 and 2 ng/L and between 2 and 4 ng/L were highly significant ($P < 0.001$) (Table 1).

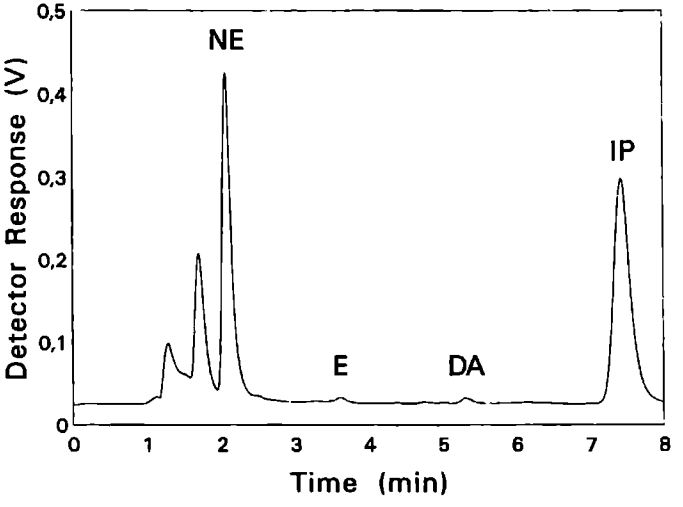


Figure 1. Chromatogram of a plasma sample containing 378 ng/L norepinephrine (NE), 6.1 ng/L epinephrine (EPI), 11.7 ng/L dopamine (DA) and 500 ng/L of added isoproterenol (IP).

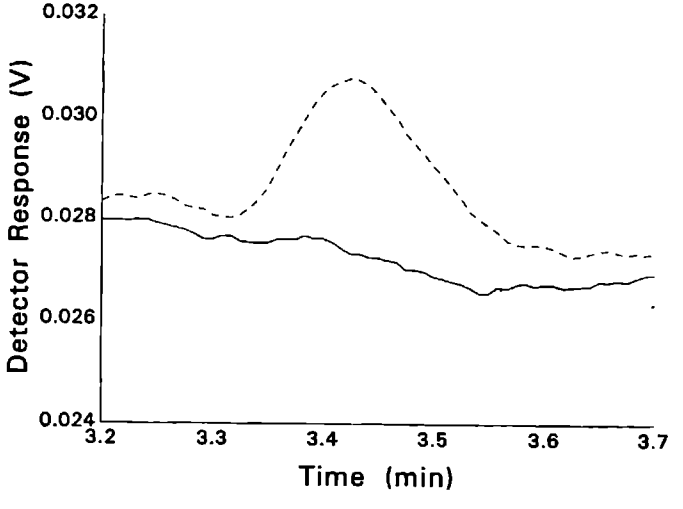


Figure 2. Example of chromatographic recording of detector response to 0, 1 and 2 ng/L epinephrine (EPI), expressed in volts.
0 ng/L (solid line), 1 ng/L (dotted line), 2 ng/L (dashed line) were added to zero plasma.

Table 1. Assay sensitivity: detector response in the lower concentration range (additions to zero plasma).

Addition ng/L	Detector response [a]		
	NE	EPI	DA
1	1.03 ± 0.145	1.15 ± 0.200	
2	1.84 ± 0.095	1.84 ± 0.103	1.99 ± 0.188
4			3.14 ± 0.163

[a] expressed in ng/L (mean ± SD, n=5)

Defining the detection limit as three times the (pooled) SD in the lowest measurement range (1 and 2 ng/L) resulted in detection limits of 0.37, 0.58 and 0.47 ng/L for NE, EPI and DA, respectively.
CA additions to two of three samples resulted in normal to moderately elevated values. Quintuplicate measurements of the five samples thus obtained yielded estimates of within-run precision (Table 2).
Between-run precision, assessed over 1.5 years in 52 different assays of a control pooled plasma, was as follows (mean ± SD, CV %): NE, 173.3 ± 14.7 (8.5%); EPI, 28.0 ± 2.03 (7.2%); and DA, 18.1 ± 2.15 (11.9%) ng/L. For assessment of accuracy, 250 ng/L NE and EPI and 500 ng/L DA were added to sample 3 (Table 2). Of these additions 99.9% ± 2.6%, 104.6% ± 3.2% and 101.7% ± 1.7%, respectively, were found. Additions of 250 ng/L NE, EPI and DA to the zero plasma were made in 10 replicates each. We found (mean ± SD) 94.9% ± 5.0%, 98.6% ± 3.6%, and 94.0% ± 4.4%, respectively, of the added materials. To sample 2 (Table 2), much lower quantities were added: 25 ng/L NE and 2.5 ng/L for EPI and DA; 24.1 ± 11.8, 2.84 ± 0.76 and 1.85 ± 0.49 ng/L were refound. The data from samples 2 and 2a also demonstrate that the precision of this assay allows for small changes in plasma concentrations to be detected; i.e., beginning with 20 or 10 ng/L, 2.5 ng/L increments in EPI and DA led to significantly ($P < 0.001$) higher signals. Thus changes of 10% already will be easily detectable.

Plasma catecholamine values in subjects at rest

A total of 40 arterial and 40 venous plasma paired samples were obtained 60 min after installing the cannulae. From the concentrations of ^{3}H-EPI and ^{3}H-NE and endogenous EPI, we could calculate fractional extraction (see above). Mean and SE of these concentrations are shown in Table 3.

Table 2. Within-run variation of assay for plasma samples.

Sample	Mean ± SD, ng/L (and CV, %; n = 5)		
	NE	EPI	DA
1	162.9 ± 3.63 (2.2)	1.59 ± 0.350(21.9)	8.35 ± 0.383(4.6)
2	249.6 ± 11.58 (4.6)	20.7 ± 0.69 (3.4)	10.6 ± 0.34 (3.3)
2a [a]	273.7 ± 12.0 (4.3)	23.5 ± 0.82 (3.5)	12.4 ± 0.61 (4.9)
3	221.8 ± 5.03 (2.3)	35.9 ± 0.73 (2.0)	20.1 ± 0.40 (2.0)
3a [b]	471.5 ± 5.23 (2.6)	296.5 ± 11.48 (3.9)	528.6 ± 12.23 (2.3)

[a] Sample 2 + 25 ng/L NE + 2.5 ng/L EPI + 2.5 ng/L DA;
[b] Sample 3 + 250 ng/L NE + 250 ng/L EPI + 500 ng/L DA.

Table 3. Concentrations and activities of EPI and NE measured in 40 plasma samples taken at rest.

	Mean ± SE	
	Venous	Arterial
[EPI], ng/L	6.8 ± 0.75	26.9 ± 1.9
[NE], ng/L	192.1 ± 15.1	140.7 ± 8.5
^{3}H-EPI, dpm/L × 10^{-3}	126.0 ± 8.5	622.0 ± 22.3
^{3}H-NE, dpm/L × 10^{-3}	180.0 ± 9.8	729.0 ± 23.5

Forearm fractional extraction and forearm spillover

Average fractional extractions (mean ± SE) of ^{3}H-NE, ^{3}H-EPI and EPI were rather close: 0.748 ± 0.0146, 0.790 ± 0.0160 and 0.754 ± 0.0185. The difference between FFE of labeled and unlabeled EPI, albeit small, was statistically significant ($P<0.001$) and led to calculation of an apparant spillover of 0.0112 ± 0.0031 ng/L per minute. This, however, is two orders of magnitude lower than the forearm spillover of NE: 1.369 ± 0.128 ng/L per minute. The FFE correlations of tritiated NE and EPI with endogenous EPI were highly significant, $r = 0.80$ ($P<0.001$) in both cases. As expected, FFE of unlabeled NE was negative (-0.615 ± 0.0624) because of NE release in the forearm.

Discussion

High sensitivity, especially for plasma EPI, has been attained in the present approach. This was accomplished by concentrating the plasma extracts and keeping the background noise very low compared with the peak heights. High sensitivity was obtained

not only with the use of pure standard but also for 1 and 2 pg of EPI and NE added to 1 mL of zero plasma, which gave peaks that were clearly higher than the zero response. Moreover, the calculated concentrations were close to expected values. At an EPI concentration of 20 ng/L, within-run differences of only 10% already will be significant. This is of particular importance in detecting trends in or effects on venous plasma concentrations of EPI in dynamic studies. The high sensitivity for assessment of endogenous EPI, combined with simultaneous separation and measurement of ^{3}H-EPI, yields the possibility of performing kinetic studies under physiological conditions.

The true losses during extraction and derivatization cannot be assessed, because no quantitative relation between the amount of quinoxalin derivative and fluorescence signal can be established. This is a drawback compared with methods that use electrochemical detection of unmodified CA. However, the assumption of equal ratios between CA and IP recovery implicit in the calculation of plasma CA levels appears to be justified, as demonstrated by the quantitative recovery of pure standard added to different plasmas. Assessment of ^{3}H-EPI and ^{3}H-NE likewise depends on the validity of this assumption. Because in this case we could measure the procedural recovery of tritiated CA that had been added to plasma (37.1 ± 2.5% for ^{3}H-NE and 52.0 ± 2.2% for ^{3}H-EPI) (mean ± SD, n=14), the existence of a direct proportionality between the true recovery and internal standard peak height could be demonstrated by experiments in which ^{3}H-EPI or ^{3}H-NE and IP are added to plasma as well as to 0.01 mol/L HCL. This was done for 14 different plasmas on 14 days. The average quotient Q (mean ± SD; 0.968 ± 0.025 for ^{3}H-NE and 0.983 ± 0.028 for ^{3}H-EPI, calculated with the use of Eq. 2) demonstrates that the validity of this key assumption is matrix independent.

The between-run CV of the quotient Q (2.6% for ^{3}H-NE and 2.8% for ^{3}H-EPI) is essentially the same as the CV that would have been obtained with still another sample to which high count rates of ^{3}H-CA had been added and assessed with sample D as a calibrant (Eq. 1). This is because, as inspection of Eqs. 1 and 2 shows, the quotient is subject to the same sources of variation, the only exception being the much lower count rates in the plasma samples (C) obtained after infusion of ^{3}H-CA. At these low count rates, counting error contributes to or even dominates the overall variation. It can be shown that, with a counting error of 6% or higher, the contribution of the other sources of variation, as reflected by the CV of quotient Q, becomes negligible (< 10%). Because this counting error of the activities in these plasma samples (C) with the lowest measured count rate is about 7% (achieved by counting the radioactivity of these samples for 20 min), the CV of the radioactivity measurement in these plasma samples is expected to be approximately 7%.

A high degree of purity of the tracers before processing is a necessary prerequisite for validity of the determination of ^{3}H-CA. Damage occurring during derivatization and separation, however, leads only to lower procedural recovery, which then is completely compensated for in Eq. 1, which follows the same internal standard principle as for determinations of unlabeled CA.

Additionally, the conversion of the CA into diphenyl-quinoxalin derivatives involves only the dihydroxy groups on the catechol nucleus and therefore will not displace the tritium label from the catechol nucleus or anywhere else on the molecule.

Optimal derivatization conditions differ significantly between the three CAs. Conditions leading to an optimal recovery of one CA may lead to lower recovery of another. Because the differences between the plasma concentrations are obvious and require higher recoveries for the lower-concentration substances, the conditions chosen for the present study are a compromise that produces acceptable, though not optimal, recoveries for all three CAs.

The bulk of plasma EPI is derived from adrenal medullary secretion. Synthesis of EPI in the central nervous system does not lead to release into plasma. It has been suggested that EPI is coreleased with neuronal NE from sympathetic nerves after uptake into sympathetic nerves from the circulation [13]. Such neuronal release of EPI, however, has been demonstrated only in the heart at very high rates of nerve stimulation, but not in unstimulated skeletal muscle [14]. Therefore, inasmuch as no difference in kinetics between exogenous ^{3}H-substituted and unmodified EPI could be demonstrated [9], we anticipated that the FFE of endogenous EPI would equal or at least be close to that of infused ^{3}H-EPI, and indeed, in this study only a small difference was observed. The origin of this small but significant difference between FFE of administered and endogenous EPI of about 5% is uncertain. First, the possibility of an assay artifact has to be considered. A systematic error averaging 1.7 ng/L in the estimations of venous EPI could account for this difference. However, even in the case of venous EPI estimation, such an error is unlikely because these amounts are well above the detection limit and in a concentration range with intraassay CV not expected to exceed 10%. Second, there might still be an isotope effect, too small to be detected by this approach, as reported by Eisenhofer et al. [9]. Third, if the rate of equilibration of the neuronal EPI pool lags somewhat behind that of the arterial pool of EPI, a smaller proportion of ^{3}H-EPI entering the neuronal pool will be returned into the venous compartment than for endogenous EPI. This would account for the observed difference in ^{3}H-EPI and endogenous EPI forearm fractional extraction. Finally, this difference could be explained by actual net release of endogenous EPI from the neuronal pool (spillover). The present technique is quite well suited for addressing this subject as well as for other studies on catecholamine kinetics.

Acknowledgments

We thank Harry van Hoof for his cooperation and helpful discussions.

References

1 Lund A Simultaneous fluorimetric determinations of adrenaline and noradrenaline in blood Acta Pharmacol Toxicol 1950,6 137-46

2 Peuler JD, Johnson GA Simultaneous single isotope radioenzymatic assay of plasma norepinephrine, epinephrine and dopamine Life Sci 1977,21 625-35

3 Hoffmann JJML, Willemsen JJ, Lenders JWM, Benraad TJ Reduced imprecision of the radioenzymatic assay of plasma catecholamines by improving the stability of the internal standards Clin Chim Acta 1986,156 221-26

4 Hjemdahl P Catecholamine measurements by high performance liquid chromatography Am J Physiol 1984,247 E13-20

5 Medvedev OS, Esler MD, Angus JA, Cox HS, Eisenhofer G Responses to nitroprusside-induced hypotension and 2-deoxyglucose-induced glucopenia in the rabbit Naunyn Schmiedebergs Arch Pharmacol 1990,341 192-99

6 Goldstein DS, Feuerstein G, Izzo JL, Kopin IJ, Keiser HR Validity and reliability of high pressure liquid chromatography with electrochemical detection for measuring plasma levels of norepinephrine and epinephrine in man Life Sci 1981,28 467-75

7 Eisenhofer G, Goldstein DS, Stull R, Keiser HR, Sunderland T, Murphy DL, Kopin IJ Simultaneous liquid-chromatographic determination of 3,4-dihydroxyphenylglycol, catecholamines, and 3,4 dihydroxyphenylalanine in plasma, and their responses to inhibition of monoamine oxidase Clin Chem 1986,32 2030-33

8 Hjemdahl P Inter-laboratory comparison of plasma catecholamine determinations using several different assays Acta Physiol Scand 1984,527 43-54

9 Eisenhofer G, Esler MD, Cox HS, Meredith IT, Jennings GL, Brush JE Jr, Goldstein DS Differences in the neuronal removal of circulating epinephrine and norepinephrine J Clin Endocrinol Metab 1990,70 1710-20

10 van der Hoorn FAJ, Boomsma F, Man in 't Veld AJ, Schalekamp MADH Determination of catecholamines in human plasma by high-performance liquid chromatography comparison between a new method with fluorescence detection and an established method with electrochemical detection J Chromatogr 1989,487 17-28

11 Mitsui A, Nohta H, Ohkura Y High-performance liquid chromatography of plasma catecholamines using 1,2-diphenylethylenediamine as precolumn fluorescence derivatization reagent J Chromatogr 1985,344 61-70

12 Brakkee AJM, Vendrik AJH Strain gauge plethysmography, theoretical and practical notes on a new design J Appl Physiol 1966,21 701-04

13 Peronnet F, Nadeau R, Boudreau G, Cardinal R, Lamontagne D, Yamaguchi N, De Champlain J Epinephrine release from the heart during left stellate ganglion stimulation in dogs Am J Physiol 1988,254 R659-62

14 Esler M, Jennings G, Lambert G, Meredith I, Horne M, Eisenhofer G Overflow of catecholamine neurotransmitters to the circulation source, fate, and functions Physiol Rev 1990,70 963-85

Chapter 3

Differential effects of low-and high-intensity lower body negative pressure on norepinephrine and epinephrine kinetics in humans

Clinical Science 1996;30:337-343

M.C. Jacobs
D.S. Goldstein
J.J. Willemsen
P. Smits,
Th. Thien
J.W.M. Lenders

Differential effects of low- and high-intensity lower body negative pressure on norepinephrine and epinephrine kinetics in humans

Abstract

Lower body negative pressure provides a means to examine neurocirculatory reflexive responses to decreases in venous return to the heart. We assessed whether the pattern of catecholaminergic responses to lower body negative pressure depends on the intensity of the stimulus (-15 vs -40 mmHg). In 14 healthy subjects, responses of forearm blood flow and norepinephrine spillover and of total body norepinephrine and epinephrine spillover were assessed during infusion of ^{3}H-norepinephrine and ^{3}H-epinephrine during -15 and -40 mmHg of lower body negative pressure. During lower body negative pressure at -15 mmHg, heart rate and pulse pressure did not change, but forearm vascular resistance increased by 25-50%. Forearm norepineph-rine spillover increased by about 50%, from 0.63 ± 0.16 to 0.94 ± 0.23 pmol · min^{-1} · 100 ml^{-1} ($P<0.05$). Total body norepinephrine spillover did not change, and total body epinephrine spillover increased significantly by about 30%. Clearances of norepinephrine and epinephrine were unchanged. During lower body negative pressure at -40 mmHg, heart rate increased and pulse pressure decreased. Forearm vascular resistance increased by about 100%, and forearm norepinephrine spillover increased by 80%, from 0.73 ± 0.19 to 1.32 ± 0.36 pmol · min^{-1} · 100 ml^{-1} ($P<0.05$). Total body norepinephrine spillover increased by 30%, and total body epinephrine spillover increased by about 50%. Clearances of both norepinephrine and epinephrine decreased. The results are consistent with the view that selective deactivation of cardiopulmonary baroreceptors during low-intensity lower body negative pressure increases sympathoneural traffic to forearm skeletal muscle and increases adrenomedullary secretion without a concomitant generalized increase in sympathoneural outflows. Concurrent deactivation of cardiopulmonary and arterial baroreceptors during high-intensity lower body negative pressure evokes a more generalized increase in sympathoneural activity, accompanied by further increased adrenomedullary secretion and decreased plasma clearances of norepinephrine and epinephrine. The findings support differential increases in skeletal sympathoneural and adrenomedullary outflows during orthostasis, with more generalized sympathoneural responses to systemic hypotension.

Introduction

The lower body negative pressure (LBNP) technique, introduced by Greenfield et al [1] in 1963, has been used frequently for examination of cardiopulmonary baroreflex function. By applying different levels of subatmospheric pressure to the lower half of the body, one can study reflexive neurocirculatory responses to decreases in venous return to the heart. Results of the studies of Zoller et al. [2] and Johnson et al. [3] have led to the view that LBNP at intensities less than -20 mmHg selectively deactivates cardiopulmonary baroreceptors and that, in humans, alterations in cardiopulmonary baroreceptor afferent traffic preferentially influence sympathetic outflows to skeletal muscle and skin. LBNP at intensities greater than -20 mmHg also deactivates arterial baroreceptors. Microneurographic studies have shown that both low- and high-intensity LBNPs increase efferent sympathoneural outflows to skeletal muscles in the arms and legs [4-6]. Because of differentiated autonomic response patterns during different forms of stress, microneurographic data about skeletal muscle and skin sympathetic nerve activity may not detect alterations in sympathetic nerve activity in other organs.
Using a tracer approach, Baily et al [7] reported increases in forearm norepinephrine (NE) spillover but not in total body NE spillover (NE entry into arterial plasma) during low-intensity LBNP. Total body NE spillover responses to high-intensity LBNP have not been reported. Little is known about baroreflex regulation of adrenomedullary secretion in humans during LBNP or orthostasis. One study reported an increase in venous plasma epinephrine (EPI) levels during low-intensity LBNP in humans [8]. The meaning of this finding is unclear, since venous plasma EPI levels depend not only on adrenomedullary secretion but also on clearance of EPI from arterial plasma and on local catecholamine removal in the forearm.
This study assessed effects of low- and high-intensity LBNP on total body and forearm kinetics of NE and EPI in healthy humans, using steady-state infusions of tracer amounts of ^{3}H-NE and ^{3}H-EPI [9].

Materials and methods

Subjects

Fourteen healthy male subjects (aged 25-38 years) participated in the study after giving their written informed consent. The study protocol was approved by the hospital ethics committee. Before entry into the study, all participants had a normal physical examination. None suffered from cardiovascular or other major diseases, and they took no medication.

Study protocol

All subjects abstained from nicotine, alcohol, and caffeinated foods and beverages for at least 24 h before the study. The subjects were allowed to eat a light breakfast 2 h before the study. All experiments were carried out in the morning in a temperature-controlled observation room.

During the study the subjects remained supine, with the lower body sealed at the iliac crests in an airtight Plexiglas^{TM} box. The LBNP applied was recorded by a manometer connected to the inside of the box.

After local anaesthesia using lidocaine, a cannula was inserted percutaneously into a brachial artery, for monitoring arterial blood pressure and heart rate (Hewlett Packard, Böblingen, Germany) and for drawing arterial blood samples. In the same arm, a cannula was inserted for collecting venous blood. Another venous cannula was inserted in the contralateral arm, for infusion of ^{3}H-NE or ^{3}H-EPI.

Forearm blood flow (FBF) was measured using venous occlusion strain-gauge plethysmography [10] on the forearm contralateral to that used for ^{3}H-NE infusion and it was positioned 10 cm above the mid-thoracic level. Measurements of FBF were obtained after exclusion of the hand circulation by inflating a wrist cuff to 100 mmHg above systolic blood pressure for at least 1 minute [11].

After instrumentation, the subjects rested for 30 min. During the last 3 min, baseline recordings of blood pressure, heart rate, and FBF (nine flow curves) were obtained. Blood pressure was recorded simultaneously with each FBF measurement. Thereafter, arterial and venous blood samples were drawn simultaneously for determinations of plasma catecholamines. Six subjects were infused with the radiotracer ^{3}H-NE (L-[ring-2,5,6-^{3}H]-NE (specific activity 30-60 Ci/mmol)) and another six were infused with ^{3}H-EPI (L-[N-methyl-^{3}H]-EPI (specific activity 55-85 Ci/mmol)). An intravenous bolus of 15 $\mu Ci \cdot m^{-2}$ (0.55 $MBq \cdot m^{-2}$) was administered, followed by a constant infusion of 0.35 $\mu Ci \cdot min^{-1} \cdot m^{-2}$ (0.013 $MBq \cdot min^{-1} \cdot m^{-2}$) for 120 min. In two subjects ^{3}H-NE and ^{3}H-EPI were infused simultaneously. The first LBNP application began 30 min after the start of infusion of the radiotracers.

LBNP at -15 mmHg was applied for 30 min. Blood pressure, heart rate, FBF and blood samples were taken in sequence, beginning after 7, 17 and 27 min of LBNP, so that the blood samples were drawn at 10, 20, and 30 min of LBNP. A 30-min rest period ensued, followed by another 30 minutes of LBNP at -40 mmHg. Blood pressure, pulse rate, FBF and blood samples were obtained at the same time points as during LBNP at -15 mmHg.

Materials

Tritiated NE and EPI were obtained from Du Pont New England Nuclear ('s-Hertogenbosch, The Netherlands). The radionuclides were sterilized using a 0.22 µm filter and diluted in 0.9% NaCl containing acetic (0.2 $mol \cdot L^{-1}$) and ascorbic (1 $mg \cdot ml^{-1}$) acids. Aliquots of 70 µCi ^{3}H-NE and ^{3}H-EPI were stored at -80°C until used.

Sterilization, dilution, and aliquoting were carried out under nitrogen. Just before use, an aliquot was diluted in 0.9% NaCl. The syringe containing the radiotracer was weighed just before and just after the infusion, in order to verify the infusion rate. Samples of the infusates were taken at the end of the infusion and stored at -80°C.

Analytical methods

Blood samples were collected in chilled tubes containing gluthatione (0.2 mol · L^{-1}) and EGTA (0.25 mol · L^{-1}). The tubes were centrifuged at 4°C and the plasma was separated and stored at -20°C. Assays of samples and infusates were carried out within 2 months of each study.
Plasma samples were assayed for concentrations of EPI and NE using HPLC with fluorometric detection after precolumn derivatization with the fluorescent agent 1,2-diphenylethylenediamine [12]. A Gilson fraction collector (Model 201-202), which was connected to an automatic sample injector (Wisp 710B), was used for collecting ^{3}H-NE and ^{3}H-EPI into scintillation vials, according to the retention times of NE and EPI standards.

Data analysis

Forearm vascular resistance (FVR) was calculated by dividing mean arterial blood pressure by FBF and was expressed in resistance units.
The clearance of NE from arterial plasma was calculated from the infusion rate of ^{3}H-NE and the steady-state arterial plasma concentration of the tracer, ^{3}H-NE_a:

$$\text{Total body NE clearance } (l \cdot min^{-1}) = \frac{\text{infusion rate } (dpm \cdot min^{-1})}{^3\text{H-NE}_a \ (dpm \cdot L^{-1})}$$

Total body NE spillover, the estimated rate of appearance of endogenous NE in arterial plasma, representing generalized sympathetic nerve activity, was calculated from the arterial plasma NE concentration (NE_a) and the arterial steady-state clearance of NE, according to the equation:

$$\text{Total body NE spillover } (nmol \cdot min^{-1}) = NE_a \ (nmol \cdot L^{-1}) \times \text{total body NE clearance } (L \cdot min^{-1})$$

Analogously, NE spillover in the forearm was estimated from:

$$\text{Forearm spillover } (pmol \cdot min^{-1} \cdot 100\ ml^{-1}) = (FPF \times NE_a \times f_{NE}) + FPF \times (NE_v - NE_a)$$

where $f_{NE} = (^3\text{H-NE}_a - ^3\text{H-NE}_v) / ^3\text{H-NE}_a$, the fractional extraction of the tracer in the forearm; FPF is the forearm plasma flow, in units of ml · min^{-1} · 100 ml^{-1}, calculated from the FBF and hematocrit; and ^{3}H-NE_v is the venous plasma concentration of ^{3}H-NE.

The removal of NE in the forearm was calculated from:

Forearm removal (pmol · min^{-1} · 100 ml^{-1}) = FPF × NE_a × f_{NE}

The clearance of EPI from arterial plasma and the estimated rate of appearance of endogenous EPI in arterial plasma were calculated according to the same formulas.
Results are expressed as means ± SEM. For each variable, the mean of the three observations at the three time points during each LBNP was calculated. Within each group, the effects of LBNP on each variable were tested by the Wilcoxon signed-ranks test. This test was also used for testing differences between LBNP at -15 and at -40 mmHg. A P-value below 0.05 defined statistical significance.

Results

Baseline measurements

At baseline, total body spillover of NE (2.81 ± 0.34 nmol · min^{-1}) was about seven to eight times higher than that of EPI (0.36 ± 0.05 nmol · min^{-1}). Clearances of NE and EPI from arterial plasma were similar (3.17 ± 0.34 and 2.84 ± 0.14 L · min^{-1}).

LBNP at -15 mmHg

Mean arterial blood pressure, pulse pressure, and heart rate did not change during LBNP at -15 mmHg (Table 1). FBF decreased significantly by about 30% and FVR increased by about 25-50%.
The increase in venous plasma NE was larger than that of arterial plasma NE during LBNP at -15 mmHg, while the increase in venous plasma EPI was smaller than that of arterial plasma EPI (Table 2). The total body spillover of NE did not increase during LBNP at -15 mmHg (Fig 1), whereas the forearm spillover of NE increased in seven out of eight subjects, from 0.63 ± 0.16 to 0.94 ± 0.23 pmol · min^{-1} · 100 ml^{-1} ($P<0.05$) (Fig 1). The clearance of NE from arterial plasma tended to decrease ($P< 0.10$). The removal of NE from the forearm did not change during LBNP at -15 mmHg (Table 2). The fractional extraction of NE increased slightly, from 0.64 ± 0.04 to 0.73 ± 0.03 ($P<0.05$). The total body spillover of EPI increased by about 30% during LBNP at -15 mmHg, from 0.36 ± 0.05 to 0.48 ± 0.07 nmol · min^{-1} (Table 2). The individual responses of total body spillover of EPI are shown in Fig 2. The clearance of EPI from arterial plasma and the removal of EPI from the forearm did not change during LBNP at -15 mmHg (Table 2).

Table 1. Hemodynamic variables before (basal) and during LBNP at -15 and at -40 mmHg.

	basal	LBNP -15	basal	LBNP -40
^{3}H-NE infusion (n=8)				
Mean arterial pressure (mmHg)	90 ± 3	89 ± 3	92 ± 2	93 ± 3
Pulse pressure (mmHg)	66 ± 3	63 ± 4	62 ± 3	55 ± 3*
Heart rate (beats/min)	58 ± 4	57 ± 4	56 ± 4	63 ± 4*
FBF (ml · min^{-1} · 100 ml^{-1})	1.81 ± 0.27	1.38 ± 0.22*	1.85 ± 0.24	1.00 ± 0.22*
FVR (arbitrary units)	64 ± 3	80 ± 12*	57 ± 8	122 ± 19*
^{3}H-EPI infusion (n=8)				
Mean arterial pressure (mmHg)	81 ± 2	83 ± 2	88 ± 2	90 ± 2
Pulse pressure (mmHg)	58 ± 3	57 ± 3	53 ± 5	47 ± 6*
Heart rate (beats/min)	54 ± 3	53 ± 2	53 ± 2	61 ± 3*
FBF (ml · min^{-1} · 100 ml^{-1})	1.59 ± 0.23*	1.11 ± 0.20*	1.49 ± 0.25	0.90 ± 0.23*
FVR (arbitrary units)	59 ± 8	90 ± 14*	74 ± 12	147 ± 32*

Values are expressed as means ± SEM. Statistical significance (Wilcoxon signed-rank test): * P<0.05.

Table 2. Plasma concentrations of NE and EPI, total body and forearm spillover and removal of NE before (basal) and during LBNP at -15 and at -40 mmHg.

	basal	LBNP -15	basal	LBNP -40
^{3}H-NE infusion (n=8)				
Venous NE (nmol · L^{-1})	0.90 ± 0.10	1.44 ± 0.20*	1.13 ± 0.19	2.60 ± 0.30*
Arterial NE (nmol · L^{-1})	0.90 ± 0.06	1.10 ± 0.10*	1.01 ± 0.12	1.88 ± 0.07*
Total body NE spillover (nmol · min^{-1})	2.81 ± 0.34	2.78 ± 0.30	3.00 ± 0.42	3.89 ± 0.30*
NE clearance (L · min^{-1})	3.17 ± 0.34	2.57 ± 0.19	3.12 ± 0.39	2.08 ± 0.15*
Forearm NE spillover (pmol · min^{-1} · 100 ml^{-1})	0.63 ± 0.16	0.94 ± 0.23*	0.73 ± 0.19	1.32 ± 0.36*
Forearm NE removal (pmol · min^{-1} · 100 ml^{-1})	0.62 ± 0.11	0.67 ± 0.13	0.70 ± 0.15	0.91 ± 0.24
^{3}H-EPI infusion (n=8)				
Venous EPI (nmol · L^{-1})	0.06 ± 0.01	0.08 ± 0.01*	0.07 ± 0.01	0.14 ± 0.02*
Arterial EPI (nmol · L^{-1})	0.13 ± 0.02	0.18 ± 0.02*	0.19 ± 0.02	0.34 ± 0.03*
Total body EPI spillover (nmol · min^{-1})	0.36 ± 0.05	0.48 ± 0.07*	0.50 ± 0.07	0.72 ± 0.08*
EPI clearance (L · min^{-1})	2.84 ± 0.14	2.63 ± 0.17	2.51 ± 0.18	2.07 ± 0.16*
Forearm EPI removal (pmol · min^{-1} · 100 ml^{-1})	0.07 ± 0.01	0.07 ± 0.01	0.10 ± 0.02	0.11 ± 0.03

Values are expressed as mean ± SEM. Statistical significance (Wilcoxon signed-rank test): * P < 0.05.

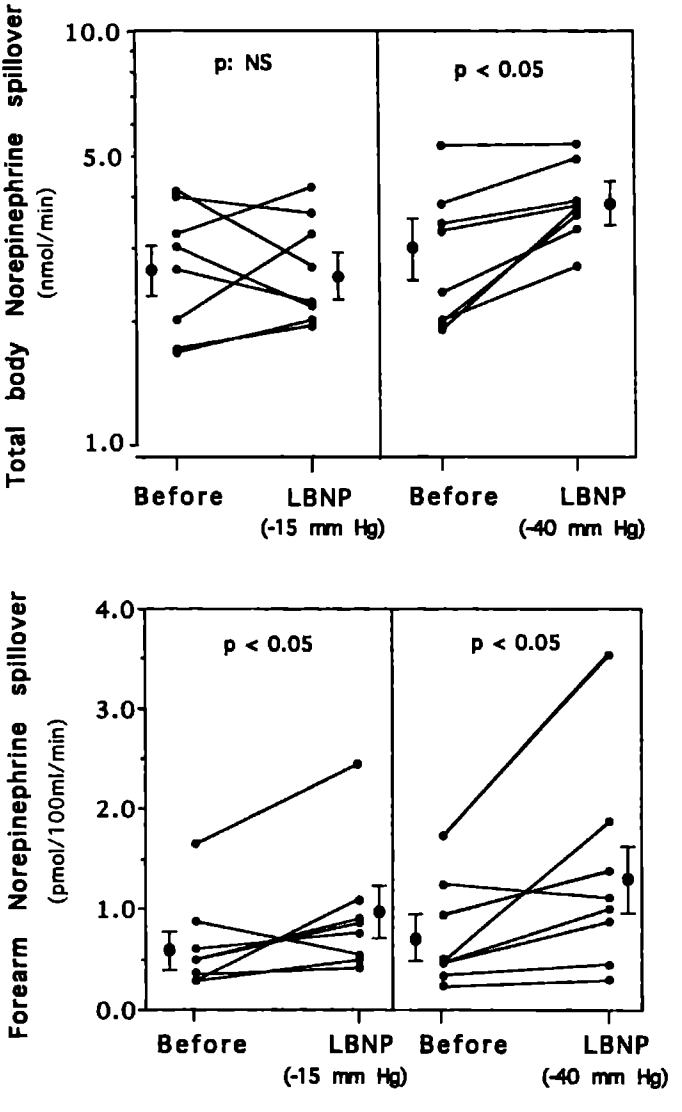


Figure 1. Total body NE spillover and forearm NE spillover before and in response to LBNP at -15 and at -40 mmHg. NS, not significant.

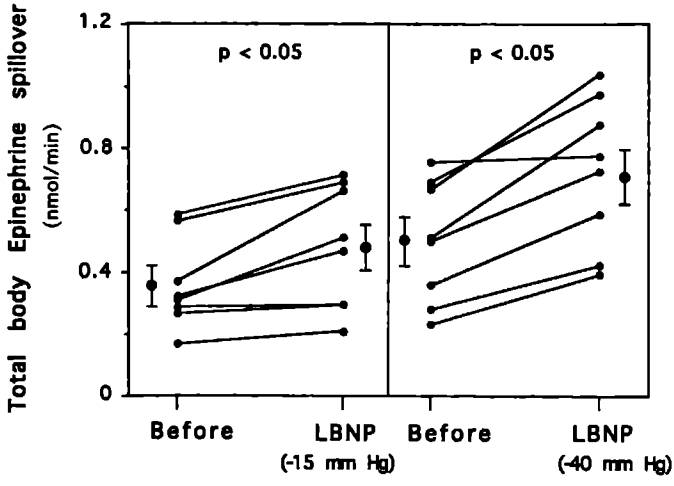


Figure 2. Total body EPI spillover before and in response to LBNP at -15 and -40 mmHg.

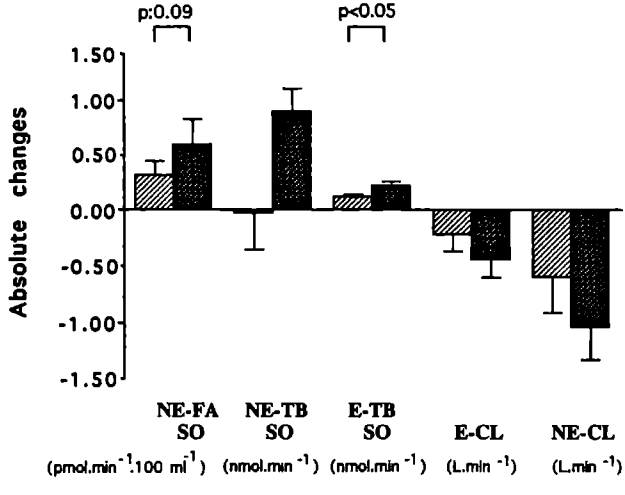


Figure 3. Absolute responses of forearm NE spillover (NE-FA SO), total body NE spillover (NE-TB SO) and NE clearance (NE-CL) in eight subjects, and of total body EPI spillover (E-TB SO) and EPI clearance (E-CL) in eight subjects, to LBNPs at -15 (light bar) and -40 mmHg (dark bar).

LBNP at -40 mmHg

Mean arterial blood pressure did not change significantly during LBNP at -40 mmHg but pulse pressure decreased and heart rate increased significantly in both groups (Table 1). FBF decreased significantly by about 40-50% and the FVR increased by about 100%. The increase of venous plasma NE was larger than that of arterial plasma NE during LBNP at -40 mmHg (Table 2). In contrast to LBNP at -15 mmHg, the total body spillover of NE increased by about 30% during LBNP -40 mmHg while the forearm spillover of NE increased by about 80% from 0.73 ± 0.19 to 1.32 ± 0.36 pmol · min^{-1} · 100 ml^{-1} (Fig 1). Fig 1 shows that the forearm spillover of NE increased in seven out of eight subjects. The clearance of NE from arterial plasma decreased significantly while the removal of NE from the forearm did not change significantly during LBNP at -40 mmHg (Table 2). The fractional extraction of NE increased slightly from 0.62 ± 0.05 to 0.76 ± 0.03 ($P<0.05$) during LBNP at -40 mmHg.
The total body spillover of EPI increased by about 50% in all subjects during LBNP at -40 mmHg (Table 2 and Fig 2). The clearance of EPI from arterial plasma decreased while removal of EPI from the forearm remained unchanged (Table 2).

Comparison of LBNP at -15 and at -40 mmHg

Comparison of the responses during LBNP at -15 and -40 mmHg was made for the variables whose values changed during both LBNP periods. The increase in FVR was larger during LBNP at -40 than at -15 mmHg ($P<0.05$) (Table 1). The increments in venous and arterial plasma NE and EPI were also significantly larger during LBNP at -40 than at -15 mmHg (Table 2). The increase in forearm spillover of NE tended to be larger at -40 mmHg (0.59 ± 0.24 pmol · min^{-1} ·100 ml^{-1}) than at -15 mmHg (0.31 ± 0.13 pmol · min^{-1} · 100 ml^{-1}, $P=0.09$, Fig 3). The increment in total body spillover of EPI was larger during LBNP at -40 mmHg (0.22 ± 0.04 nmol · min^{-1}) than at -15 mmHg (0.12 ± 0.03 nmol · min^{-1}, $P< 0.05$, Fig 3).

Discussion

The main new findings of this study were: (1) low-intensity LBNP increased forearm NE spillover and EPI spillover without affecting total body NE spillover; (2) high-intensity LBNP increased total body NE spillover; (3) LBNP elicited intensity-related increments in total body spillover of EPI and decreases in plasma clearances of NE and EPI.
Zoller et al. [2] and Johnson et al. [3] demonstrated that a LBNP of less than -20 mmHg selectively deactivates cardiopulmonary baroreceptors. At negative pressures exceeding -20 mmHg, there is additional deactivation of arterial baroreceptors. Our data are in

agreement with selective deactivation of cardiopulmonary baroreceptors during low-intensity LBNP (-15 mmHg), since we found no increase in heart rate or a decrease in pulse pressure in response to this stimulus. During LBNP at -40 mmHg, heart rate increased and pulse pressure decreased. At this stage, it is impossible to examine arterial baroreceptors separately from cardiopulmonary baroreceptors with LBNP since arterial baroreceptor deactivation cannot be attained without simultaneous pertubation of the cardiopulmonary baroreceptors. Two other factors impede an examination of the arterial baroreceptors separately. First, the stronger intensity of LBNP at -40 mmHg does not only cause a concurrent deactivation of arterial baroreceptors but probably also elicits a gradually stronger deactivation of cardiopulmonary baroreceptors. Second, there is a functional interaction between arterial and cardiopulmonary baroreceptors that does not allow assessment of effects of arterial baroreceptor deactivation by subtracting the effects of unloading of cardiopulmonary baroreceptors from that of simultaneous deactivation of arterial and cardiopulmonary baroreceptors.

During low-intensity LBNP, there was an increase in forearm NE spillover but not in total body NE spillover. This not only confirms that cardiopulmonary baroreceptors play an important role in reflexive regulation of FBF but also indicates the regionalization of sympathoneuronal responses to this stressor. In contrast, total body spillover of NE increased during concomitant deactivation of cardiopulmonary and arterial baroreceptors, suggesting more diffuse increases in sympathetic nerve outflows. Measurements of total body spillover of NE during orthostasis may therefore fail to detect regional sympathoneuronal responses. Differentiated sympathoneuronal response patterns have also been demonstrated for several other stressors.

The plasma clearance of NE tended to decrease during cardiopulmonary baroreceptor deactivation and decreased further during simultaneous deactivation of arterial and cardiopulmonary baroreceptors. These decreases in plasma NE clearance probably resulted from decreases in cardiac output and splanchnic blood flow, as has been demonstrated during cardiopulmonary and arterial baroreceptor deactivation [13, 14].

Apart from sympathoneural activation during LBNP, we also found small but significant elevations in venous and arterial EPI levels. The increments were larger during high-intensity LBNP than during low-intensity LBNP. Increments in plasma EPI levels during low-intensity LBNP are in agreement with a previous study [8]. Since we found no decrease in the clearance of EPI and an increase in total body spillover of EPI, the increments in plasma EPI levels during low-intensity LBNP probably result from increased adrenomedullary secretion. During combined deactivation of cardiopulmonary and arterial baroreceptors, there was a further increase in adrenomedullary secretion of EPI, but now the decreased clearance of EPI contributed to the larger increment in plasma EPI levels during high-intensity LBNP.

LBNP at −45 to −50 mmHg is equivalent to head-up tilt to 90°, with respect to the amount of blood translocated. As shown previously, plasma NE and EPI levels nearly double during orthostasis, and this is in agreement with the plasma catecholamine

responses during LBNP at -40 mmHg in the present study. These increments in plasma catecholamine levels result from both increased arterial NE spillover and decreased plasma NE clearance [15]. In the present study, using LBNP at -40 mmHg, responses of spillover and clearance were qualitatively similar to those reported previously for head-up tilt.

LBNP induced a vasoconstrictor response in the forearm that was largest when both types of baroreceptors were deactivated simultaneously during high-intensity LBNP. This is in agreement with the further increase in forearm NE spillover during high-intensity LBNP. The larger responses of FVR and forearm NE spillover during high-intensity LBNP can be explained by either a stronger deactivation of cardiopulmonary baroreceptors, or by the concurrent deactivation of arterial baroreceptors or both. Since there must be arterial baroreceptor deactivation during high-intensity LBNP [2,3], our data indicate that arterial baroreceptors are probably as important for baroreflex control of the forearm skeletal muscle circulation as cardiopulmonary baroreceptors. This conclusion is supported by the findings of microneurographic studies, showing that sympathetic nerve traffic responses were larger during deactivation of arterial baroreceptors than during deactivation of cardiopulmonary baroreceptors [6, 16]. Previous studies have suggested that cardiopulmonary baroreceptors play a major role in the reflex control of the skeletal muscle circulation [17].

The contribution of the skin to the FBF and thus to the forearm NE spillover should be considered. Some but not all previous studies showed that LBNP produced graded vasoconstriction of the skin vessels, thus contributing to the forearm vasoconstrictor response [7, 18]. Assuming that sympathetic nerve traffic to the skin is also increased during LBNP, the increase in forearm NE spillover may overestimate the increase in muscle sympathoneural activity; however, if the increments in NE spillover from the skin and the muscles were proportionally the same at -15 and at -40 mmHg, then the conclusion that arterial baroreceptors are important for reflex regulation of skeletal muscle circulation would be still valid.

In conclusion, the data of this study indicate that selective deactivation of cardiopulmonary baroreceptors during low-intensity LBNP increases NE release in the forearm and EPI release by the adrenals. Concurrent deactivation of arterial baroreceptors during high-intensity LBNP produces further increases in forearm vasoconstriction and NE spillover, with increases also in total body NE and EPI spillover and decreases in clearances of NE and EPI.

References

1. Greenfield ADM, Brown E, Goei JS, Plassaras GC. Circulatory responses to abrupt release of blood accumulated in the legs. Physiologist 1963; 6: 191.

2 Zoller RP, Mark AL, Abboud FM, Schmid PG, Heistad DD The role of low pressure baroreceptors in reflex vasoconstrictor responses in man J Clin Invest 1972, 51 2967-72
3 Johnson JM, Loring B, Niederberger M, Eisman MM Human splanchnic and forearm vasoconstrictor responses to reductions of right atrial aortic pressures Circ Res 1974, 34 515-24
4 Victor RG, Leimbach WN Effects of lower body negative pressure on sympathetic discharge to leg muscle in humans J Appl Physiol 1987, 63 2558-62
5 Rea RR, Wallin BG Sympathetic nerve activity in arm and leg muscles during lower body negative pressure in humans J Appl Physiol 1989, 66 2778-81
6 Sundlof G, Wallin BG Fffect of lower body negative pressure on human muscle nerve sympathetic activity J Physiol 1978, 278 525-32
7. Baily RG, Prophet SA, Shenberger JS, Zelis R, Sinoway LI Direct neurohumoral evidence for isolated sympathetic nervous system activation to skeletal muscle in response to cardiopulmonary baroreceptor unloading Circ Res 1990, 66 1720 8
8 Grassi G, Gavazzi C, Cesura AM, Picotti GB, Mancia G Changes in plasma catecholamines in response to reflex modulation of sympathetic vasoconstrictor tone by cardiopulmonary receptors Clin Sci 1985, 68 503-10
9 Esler M, Jennings G, Lambert G, Meredith I, Horne M, Eisenhofer G Overflow of catecholamine neurotransmitter to the circulation source, fate and functions Physiol Rev 1990, 70 963-85
10 Brakkee AJM, Vendrik AJH Strain gauge plethysmography, theoretical and practical notes on a new design J Appl Physiol 1966, 21 701-4
11 Lenders JWM, Janssen GJ, Smits P, Thien Th Role of the wrist cuff in forearm plethysmography Clin Sci 1991, 80 413-7
12 Willemsen JJ, Ross HA, Jacobs MC, Lenders JWM, Thien Th, Benraad TJ Highly sensitive and specific HPLC with fluorometric detection for determination of plasma epinephrine and norepinephrine applied to kinetic studies in humans Clin Chem 1995, 41 1455-60
13 Baily RG, Leuenberger U, Leaman G, Silber D, Sinoway LI Norepinephrine kinetics and cardiac output during nonhypotensive lower body negative pressure Am J Physiol 1991, 260 H1708-H1712
14 Hirsch AT, Levenson DJ, Cutler SS, Dzau VJ, Creager MA Regional vascular responses to prolonged lower body negative pressure in normal subjects Am J Physiol 1989, 257 H219-H225
15 Davis D, Sinoway LI, Robison J, Minotti JR, Day FP, Baily R, Zelis R Norepinephrine kinetics during orthostatic stress in congestive heart failure Circ Res 1987, 61 (suppl I) I-87-90
16 Jacobsen TN, Morgan BJ, Scherrer U, Vissing SF, Lange RA, Johnson N, Steves Ring W, Rahko PS, Hanson P, Victor RG Relative contributions of cardiopulmonary and sinoaortic baroreflexes in causing sympathetic activation in the human skeletal muscle circulation during orthostatic stress Circ Res 1993, 73 367-78
17 Abboud FM, Eckberg DL, Johannsen UJ, Mark AL Carotid and cardiopulmonary baroreceptor control of splanchnic and forearm vascular resistance during venous pooling in man J Physiol 1979, 286 173-84
18 Tripathi A, Nadel ER Forearm skin and muscle vasoconstriction during lower body negative pressure J Appl Physiol 1986, 60 1535-41

Chapter 4

Neurohumoral antecedents of vasodepressor reactions

Based on European Journal of Clinical Investigation 1995;25:754-761

M.C. Jacobs
D.S. Goldstein
J.J. Willemsen
P. Smits
Th. Thien
R.A. Dionne
J.W.M. Lenders

Neurohumoral antecedents of vasodepressor reactions

Abstract

Vasodepressor (vasovagal) syncope, the most common cause of acute loss of consciousness, can occur in otherwise vigorously healthy people during exposure to stimuli decreasing cardiac filling. Antecedent physiological or neuroendocrine conditions for this dramatic syndrome are poorly understood. This study compared neurocirculatory responses to non-hypotensive lower body negative pressure in subjects who subsequently developed vasodepressor reactions during hypotensive lower body negative pressure with responses in subjects who did not.

In 26 healthy subjects, lower body negative pressure -15 and -40 mmHg was applied to inhibit cardiopulmonary and arterial baroreceptors. All the subjects tolerated 30 min of lower body negative pressure at -15 mmHg, but during subsequent lower body negative pressure at -40 mmHg 11 subjects had vasodepressor reactions, with sudden hypotension, nausea, and dizziness. In these subjects, arterial plasma epinephrine responses to lower body negative pressure both at -15 and at -40 mmHg exceeded those in subjects who did not experience these reactions. In 16 of the 26 subjects, forearm norepinephrine spillover was measured; in the eight subjects with a vasodepressor reaction, mean forearm norepinephrine spillover failed to increase during lower body negative pressure at -15 mmHg (Δ = -0.06 ± (SEM) 0.04 pmol · min^{-1} · 100 ml^{-1}), whereas in the eight subjects without a vasodepressor reaction, mean forearm norepinephrine spillover increased significantly (Δ = 0.31 ± 0.13 pmol · min^{-1} · 100ml^{-1}). Plasma levels of β-endorphin during lower body negative pressure at -15 mmHg increased in some subjects who subsequently had a vasodepressor reaction during lower body negative pressure at -40 mmHg.

The findings suggest that a neuroendocrine pattern including adrenomedullary stimulation, skeletal sympathoinhibition, and release of endogenous opioids can precede vasodepressor syncope.

Introduction

Vasodepressor syncope, or fainting, is the most frequent cause of sudden loss of consciousness [1] and can occur in otherwise healthy people during exposure to emotional stress, heat, prolonged upright posture, or pain. The hemodynamic hallmark of vasodepressor reactions is systemic vasodilation [2], especially in skeletal muscle [3, 4], with or without vagally mediated bradycardia. Microneurographic studies have

shown that precipitously decreased skeletal sympathetic nervous outflow accompanies the vasodilation [5]. Plasma norepinephrine (NE) levels, measured immediately after or at the time of fainting, are decreased or inappropriately normal [6, 7] and release of NE into the venous drainage in the heart [8] and kidneys [9] decreases virtually to zero. In contrast, plasma epinephrine (EPI) levels often are increased [6]. Vagal bradycardia does not cause vasodepressor reactions, because parasympatholytic drugs may not reverse or prevent the hypotension [10].

Mechanisms responsible for the abrupt decreases in sympathetic outflows have been unclear. One possibility is sudden central resetting of baroreflexes, so that hypotension fails to release sympathoneural outflows from baroreceptor restraint. Since endogenous opioids augment the extent of sympathoinhibition during a hypotensive stimulus (e.g. haemorrhage) [11], sudden release of endogenous opioids in the brain could contribute to baroreflex resetting and thereby produce sympathoinhibition despite hypotension. Vasopressin also augments baroreflex restraint of sympathoneural outflows [12], and extremely high circulating vasopressin levels often accompany vasodepressor syncope [13]. Alternatively, stimulation of ventricular stretch receptors that increase activity of non-myelinated, inhibitory afferent C-fibers [14,15], or collapse-firing of low pressure baroreceptors in the right atrium and great veins [16], evoke vasodepressor reactions. The common feature of the central and peripheral hypothesized mechanisms is sympathoinhibition despite hypotension.

The suddenness and unexpectedness of vasodepressor reactions have hampered efforts to identify antecedent hemodynamic and neurohumoral events. Prolonged head-up tilt, with or without intravenous isoproterenol infusion [17], or relatively large amounts of lower body negative pressure (LBNP) [18] can provoke these episodes. In the current study, we examined circulatory, catecholaminergic, and β-endorphin responses to a small decrease in cardiac filling during mild LBNP (-15 mmHg) and to systemic hypotension during subsequent more intense LBNP (-40 mmHg) in healthy people. LBNP at less than -20 mmHg reduces central venous pressure without altering arterial blood pressure or pulse pressure, suggesting relatively selective inhibition of cardiac baroreceptors [19], whereas higher intensity LBNP decreases blood pressure and pulse pressure, inhibiting cardiac and arterial baroreceptors. We compared responses in groups of subjects who did, vs. those who did not, develop a vasodepressor reaction during high-intensity LBNP, in order to identify possible neurochemical antecedents of vasodepressor syncope.

To assess neurohumoral responses during inhibition of cardiopulmonary and arterial baroreceptors, plasma catecholamine concentrations were measured. Plasma NE levels, however, have some limitations as a measure of sympathetic activity since they are the net result of the rate by which NE spills over into the circulation (after neuronal release and neuronal uptake) and the rate by which NE is removed from the plasma. Therefore we used the radiotracer dilution technique [20] that enables calculation of the total body spillover of NE into the circulation. This is a better, although not ideal, measure of total

body neuronal NE release and thus over-all sympathetic activity. Since in many conditions sympathetic outflow is not uniformly distributed to different organs, calculation of the regional spillover of NE in an organ is preferred as a measure of regional sympathetic activity. Forearm spillover and removal of NE can be calculated by measuring forearm blood flow, venous and arterial plasma NE levels and the extraction of the tracer tritiated NE. Measurement of NE spillover and removal in the forearm enables to identify whether increments in venous forearm plasma NE levels are due to an increased neuronal release to or to a decreased removal of NE from the forearm circulation.

Patients and methods

Subjects

Twenty-six healthy male subjects participated in the study after giving their written informed consent. The study protocol was approved by the Hospital Ethics Committee. Before entry into the study, all participants had a normal physical examination. None suffered from cardiovascular or other major diseases, and they took no medication. None of the participants had a history of syncopal attacks.

Study protocol

All subjects abstained from nicotine, alcohol, and caffeinated foods and beverages for at least 24 h before the study. The subjects were allowed to eat a light breakfast 2 h before the study. All experiments were carried out in the morning in a temperature-controlled observation room.

During the study, the subjects remained supine, with the lower body sealed at the iliac crests in an airtight Plexiglas™ box. The applied LBNP pressure was recorded by a manometer connected to the inside of the box.

After local anesthesia using lidocaine, a cannula was inserted percutaneously into a brachial artery, for monitoring arterial blood pressure and heart rate (Hewlett Packard GmbH, Böblingen, Germany) and for drawing arterial blood samples. In the same arm, a cannula was inserted for collecting venous blood. In 16 of the 26 subjects, another venous cannula was inserted in the contralateral arm, for infusion of ^{3}H-norepinephrine (^{3}H-NE).

Forearm blood flow (FBF) was measured using venous occlusion strain-gauge plethysmography [21]. The forearm contralateral to that used for ^{3}H-NE infusion was positioned 10 cm above the mid-thoracic level. Measurements of FBF and blood samples were obtained after exclusion of the hand circulation by inflating a wrist cuff to 100 mmHg above systolic blood pressure for at least 1 min [22].

After instrumentation, the subjects rested for at least 30 min. During the last 3 min, baseline recordings of blood pressure, heart rate, and FBF (nine flow curves) were obtained. Blood pressure was recorded simultaneously with each FBF measurement. Thereafter, arterial and venous blood samples were drawn simultaneously for determinations of plasma catecholamines.
LBNP at -15 mmHg was then applied for 30 min. Blood pressure, heart rate, FBF recordings and blood samples were taken in sequence beginning after 7, 17 and 27 min of LBNP, so that the blood samples were drawn at 10, 20, and 30 min of LBNP. A 30 min rest period ensued, followed by another 30 min of LBNP -40 mmHg. Blood pressure, pulse rate, FBF, and blood samples were obtained at the same time points as for LBNP -15 mmHg.

^{3}H-NE infusion

^{3}H-NE (levo-[ring-2,5,6-^{3}H]-NE) was infused to assess plasma NE kinetics. Tritiated NE was obtained from Du Pont New England Nuclear ('s-Hertogenbosch, the Netherlands). The radionuclide was sterilized using a 0.22 µm filter and diluted in 0.9% NaCl containing acetic (0.2 mol·L^{-1}) and ascorbic (1 mg · mL^{-1}) acid. Aliquots of 50 µCi ^{3}H-NE were stored at -80°C until used. Sterilization, dilution, and aliquoting were carried out under nitrogen. Just before use, an aliquot was diluted in 0.9% NaCl . The radiotracer was administered intravenously as a bolus of 15 µCi · m^{-2} followed by a constant infusion of 0.35 µCi · min^{-1} · m^{-2} for 120 min. The first LBNP application began at 30 min of infusion of ^{3}H-NE. The weight of the syringe containing the radiotracer was measured just before and just after the infusion, in order to check the infusion rate. Samples of the infusate were taken at the end of the infusion.

Analytical methods

Blood samples were collected in chilled tubes containing gluthatione (0.2 mol·L^{-1}) and EGTA (0.25 mol · L^{-1}). The tubes were centrifuged at 4°C and the plasma separated and stored at -20°C. Assays of all samples and infusates occurred within 2 months of each study.
Plasma samples were assayed for concentrations of epinephrine (EPI), total NE, and ^{3}H-NE, using high performance liquid chromatography (HPLC) with fluorometric detection after precolumn derivatization with the fluorescent agent 1,2-diphenylethylenediamine [23]. A fraction collector connected to an automatic sample injector (WISP 710B, Waters-Millipore, Milford, MA, USA), was used for collecting ^{3}H-NE into scintillation vials, according to the retention time of NE standard.
Arterial plasma immunoreactive β-endorphin levels were measured by a radioimmunoassay [24] in the 16 subjects in whom plasma NE kinetics were studied.

Data analysis

Forearm vascular resistance (FVR) was calculated by dividing mean arterial blood pressure by FBF and was expressed in resistance units (RU).
The clearance of NE from arterial plasma was calculated from the infusion rate of ^{3}H-NE and the steady-state arterial plasma concentration of ^{3}H-NE, ^{3}H-NE$_a$:

$$\text{Total body NE clearance } (L \cdot min^{-1}) = \frac{\text{Infusion rate } (dpm \cdot min^{-1})}{^3\text{H-NE}_a\ [dpm \cdot L^{-1}]} \quad (1)$$

Total body NE spillover, the estimated rate of appearance of endogenous NE in arterial plasma, was calculated from the arterial plasma NE concentration (NE$_a$) and the arterial steady-state clearance of NE, according to the equation:

$$\text{Total body NE spillover } (nmol \cdot min^{-1}) = \text{NE}_a\ (nmol \cdot L^{-1}) \times \text{total body NE clearance } (L \cdot min^{-1}) \quad (2)$$

Analogously, NE spillover in the forearm was estimated from:

$$\text{Foream NE spillover } (pmol \cdot min^{-1} \cdot 100\ ml^{-1}) = (FPF \times NE_a \times f_{NE}) + (FPF \times (NE_v - NE_a)) \quad (3)$$

where $f_{NE} = (^3\text{H-NE}_a - ^3\text{H-NE}_v) / ^3\text{H-NE}_a$, the fractional extraction of the tracer in the forearm; FPF is the forearm plasma flow, in units of $mL \cdot min^{-1} \cdot 100\ mL^{-1}$, calculated from the forearm blood flow and haematocrit; and ^{3}H-NE$_v$ is the venous plasma concentration of ^{3}H-NE.
The removal of NE in the forearm was calculated from:

$$\text{Forearm NE removal } (pmol \cdot min^{-1} \cdot 100\ ml^{-1}) = FPF \times NE_a \times f_{NE} \quad (4)$$

Subjects were classified post hoc into two groups. Subjects in group A underwent both 30 min periods of LBNP without experiencing dizziness, nausea, weakness, or hypotension. Subjects in group B underwent LBNP at -15 mmHg without symptoms but developed a vasodepressor reaction during LBNP at -40 mmHg. The criteria for a vasodepressor reaction were hypotension (systolic blood pressure <100 mmHg or a rapid decrease of >20 mmHg) and symptoms (nausea, dizziness, or weakness).
Results are expressed as means ± SEM. For each LBNP period, values for all dependent variables were averaged across the three time points during LBNP. To assess the effects of LBNP, the Wilcoxon signed rank test was used to compare the baseline with the average value of a variable during LBNP. Responses at each time point were expressed as the absolute change at a time point from the baseline value, and two-way analyses of variance (ANOVA's) for repeated measures were used to compare the changes as a function of time in the two groups. A P-value less then 0.05 defined statistical significance.

Results

Of the 26 subjects, 15 (group A) underwent 30 min of LBNP at -15 mmHg as well as at -40 mmHg without any signs of a vasodepressor reaction. The remaining 11 subjects (group B) developed a vasodepressor reaction during LBNP at -40 mmHg. There were no differences in age, resting blood pressure, or heart rate between the two groups (Table 1). All but one subject in group B had both hypotension and bradycardia during LBNP at -40 mmHg, bradycardia being defined as a pulse rate less than 50 bpm or a rapid decrease of more than 10 bpm. In group B subjects, LBNP at -40 mmHg was terminated immediately when the first signs of a vasodepressor reaction emerged. All group B

Table 1. Clinical characteristics of group A (without syncope) and group B (with syncope)

Parameter	Group A	Group B
Number (n)	15	11
Age (years)	30 ± 5	29 ± 6
Height (cm)	184 ± 8	184 ± 9
Weight (kg)	82.0 ± 10.6	81.7 ± 10.3
Quetelet index ($kg \cdot m^{-2}$)	24.4 ± 3.2	24.2 ± 3.6
SBP (mmHg)	120 ± 9	125 ± 10
DBP (mmHg)	72 ± 8	73 ± 9
HR (bpm)	70 ± 10	67 ± 7

Mean ± SD are given. The data in this table show blood pressure and heart rate values obtained at the screening visit prior to entry in the study. SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate.

subjects complained of nausea, and some became pale; one lost consciousness. The vasodepressor response occurred in the first 10 min of LBNP at -40 mmHg in two subjects, between 10 and 20 min in seven subjects, and in the last 10 min in two subjects. Consequently, the data summarized below for LBNP at -40 mmHg are incomplete for group B.

LBNP at -15 mmHg

During LBNP at -15 mmHg, FBF decreased and FVR increased significantly in both groups (Table 2), and the groups did not differ in values for these variables. Blood pressure, pulse pressure, and heart rate did not change during LBNP at -15 mmHg in either group.

Baseline levels of venous plasma NE were similar in the two groups (0.92 ± 0.10 nmol $\cdot L^{-1}$ in group A and 1.00 ± 0.09 nmol $\cdot L^{-1}$ in group B). During LBNP, venous plasma NE levels increased significantly in both groups to 1.38 ± 0.13 nmol $\cdot L^{-1}$ in group A and 1.26 ± 0.11 nmol $\cdot L^{-1}$ in group B at 30 min of LBNP. There was a tendency for

Table 2. Haemodynamic data during LBNP at -15 mmHg in group A (without syncope, n=15) and group B (with syncope, n=11).

		Group A	Group B
FBF (mL · min^{-1} · 100 mL^{-1})	Baseline	1.61 ± 0.19	1.46 ± 0.19
	LBNP -15	1.16 ± 0.15**	1.06 ± 0.15**
FVR (resistance units)	Baseline	64 ± 8	71 ± 10
	LBNP -15	90 ± 10**	99 ± 13**
SBP (mmHg)	Baseline	126 ± 4	128 ± 4
	LBNP -15	127 ± 3	130 ± 4
DBP (mmHg)	Baseline	73 ± 3	67 ± 2
	LBNP -15	75 ± 3	69 ± 3
Pulse pressure (mmHg)	Baseline	61 ± 2	64 ± 2
	LBNP -15	59 ± 2	61 ± 2
Heart rate (bpm)	Baseline	55 ± 3	59 ± 2
	LBNP -15	54 ± 3	60 ± 2

Mean ± SEM are given. SBP, systolic blood pressure; DBP, diastolic blood pressure; FBF, forearm blood flow; FVR, forearm vasular resistance. ** $P < 0.01$ with respect to baseline.

smaller venous plasma NE responses in the group with subsequent syncope but the increments in venous NE levels were not significantly different between the two groups (Fig 1).

Baseline arterial plasma NE levels also did not differ between the groups. Arterial NE levels increased from 0.94 ± 0.08 to a maximal value of 1.10 ± 0.08 nmol · L^{-1} in group A and from 0.92 ± 0.11 nmol · L^{-1} to 1.03 ± 0.09 nmol · L^{-1} in group B.

Baseline arterial EPI levels were similar in the two groups (0.16 ± 0.03 nmol · L^{-1} in group A and 0.17 ± 0.03 nmol · L^{-1} in group B). In both groups, arterial EPI levels increased significantly during LBNP at -15 mmHg; however, in group A, arterial EPI levels plateaued at about 0.22 nmol · L^{-1} after 10 min, whereas in group B, arterial EPI levels progressively rose to a peak value of 0.34 ± 0.05 nmol · L^{-1}. Thus, the absolute increments in arterial EPI levels were larger in the group with than in the group without subsequent syncope (Fig 2). Venous plasma EPI responses did not differ between the two groups.

In the 16 subjects who received a ^{3}H-NE infusion, mean forearm NE spillover at baseline was not significantly different between group A (n=8) and group B (n=8) subjects (0.63 ± 0.16 vs 0.76 ± 0.16 pmol · min^{-1} · 100 mL^{-1}, Table 3). During LBNP at -15 mmHg, the average response of forearm NE spillover across all time points was larger in the

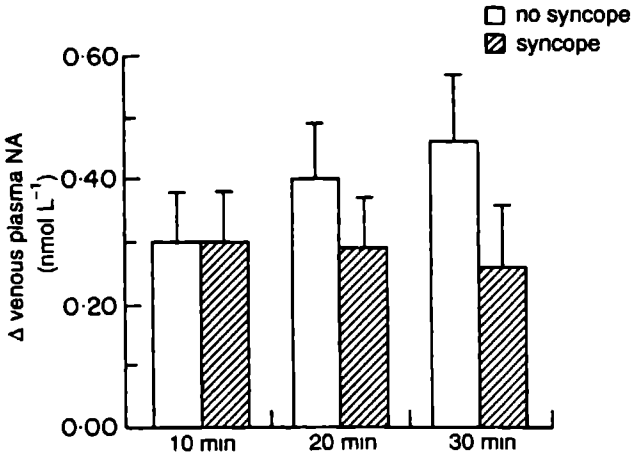


Figure 1. Increments in venous plasma norepinephrine (NA, means ± SEM) after 10, 20, and 30 min of lower body negative pressure for group A (n=15, no syncope) and group B (n=11, syncope).

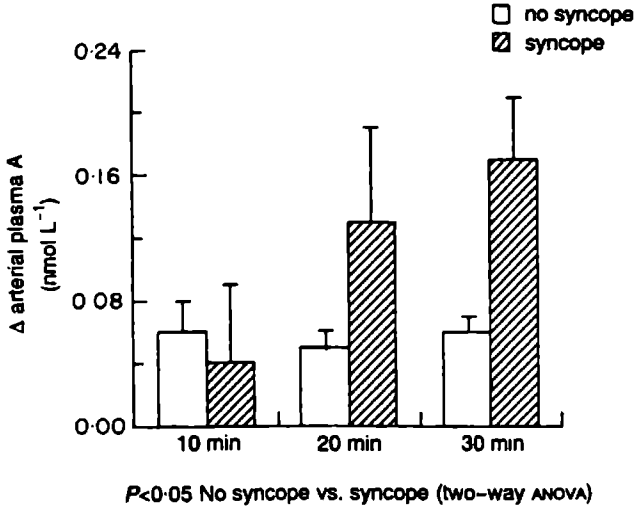


Figure 2. Increments in arterial plasma epinephrine (A, means ± SEM) after 10, 20, and 30 min of lower body negative pressure for group A (n=15, no syncope) and group B (n=11, syncope).

group A subjects (0.31 ± 0.13 pmol · min^{-1} · 100 mL^{-1}) than in the group B subjects (-0.06 ± 0.04 pmol · min^{-1} · 100 mL^{-1}, P< 0.05, Figure 3).

Values for total body NE spillover did not change significantly in either group during LBNP at -15 mmHg (Table 3). Values for total body NE clearance decreased by about 30% in group A and did not change in group B. In neither group did values for forearm NE removal change during LBNP at -15 mmHg.

Baseline plasma β-endorphin levels did not differ between the groups and did not change significantly during LBNP at -15 mmHg in group A (27 ± 3 pg · mL^{-1} at baseline to 30 ± 4 pg · mL^{-1} during LBNP). In group B, plasma β-endorphin increased in three of the eight subjects; the average increase, from 38 ± 11 pg · mL^{-1} at baseline to 62 ± 28 pg · mL^{-1} during LBNP at -15 mmHg, was not significant.

Table 3. Norepinephrine kinetics values during LBNP at -15 mmHg in group A (without syncope, n=8) and group B (with syncope, n=8).

		Group A	Group B
Arterial plasma NE ($nmol \cdot L^{-1}$)	Baseline	0.90 ± 0.06	0.88 ± 0.15
	LBNP -15	1.10 ± 0.12*	0.95 ± 0.10
Forearm NE spillover ($pmol \cdot min^{-1} \cdot 100\ mL^{-1}$)	Baseline	0.63 ± 0.16	0.76 ± 0.16
	LBNP -15	0.94 ± 0.23*	0.70 ± 0.13
Total body NE spillover ($nmol \cdot min^{-1} \cdot m^{-2}$)	Baseline	1.39 ± 0.19	0.99 ± 0.16
	LBNP -15	1.37 ± 0.15	1.26 ± 0.16
Total body NE clearance ($L \cdot min^{-1} \cdot m^{-2}$)	Baseline	1.56 ± 0.18	1.30 ± 0.14
	LBNP -15	1.26 ± 0.08*	1.32 ± 0.07

Means ± SEM are given. NE, norepinephrine. * $P < 0.05$ with respect to baseline.

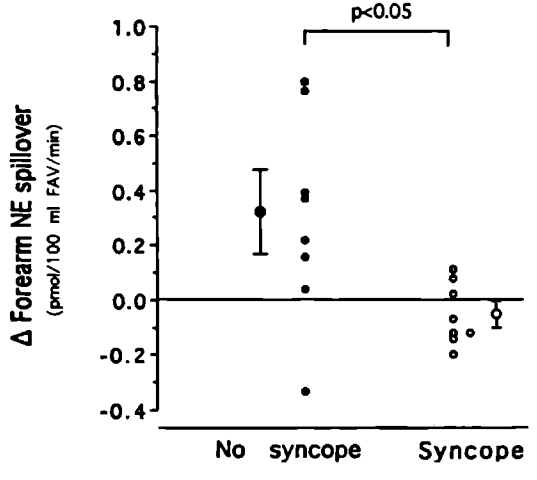


Figure 3. Individual responses of forearm norepinephrine (NE) spillover in eight subjects without and eight subjects with syncope. FAV= forearm volume.

LBNP at -40 mmHg

In both group A and group B, FBF decreased and FVR increased during LBNP at -40 mmHg, analogous to the changes during LBNP at -15 mmHg (Table 4); however, the changes in values for both variables were larger during LBNP at -40 mmHg than during LBNP at -15 mmHg. Systolic blood pressure and pulse pressure decreased and heart rate increased, both in the group A subjects and in the nine group B subjects.

Baseline levels of venous plasma NE were similar in the two groups (1.08 ± 0.13 nmol · L^{-1} in group A and 1.10 ± 0.12 nmol · L^{-1} in group B). During LBNP, venous plasma

Table 4. Haemodynamic data during LBNP at -40 mmHg in group A (without syncope, n=15) and group B (with syncope, n=9 #).

		Group A	Group B
FBF	Baseline	1.49 ± 0.17	1.16 ± 0.16
(mL · min^{-1} · 100 mL^{-1})	LBNP -40	0.88 ± 0.14***	0.65 ± 0.10**
FVR	Baseline	71 ± 8	94 ± 11
(resistance units)	LBNP -40	135 ± 18***	165 ± 21***
SBP	Baseline	129 ± 2	140 ± 5
(mmHg)	LBNP -40	125 ± 3***	131 ± 5***
DBP	Baseline	73 ± 3	75 ± 3
(mmHg)	LBNP -40	75 ± 3	75 ± 3
Pulse pressure	Baseline	57 ± 3	65 ± 3
(mmHg)	LBNP -40	50 ± 4***	56 ± 3***
Heart rate	Baseline	54 ± 2	57 ± 2
(bpm)	LBNP -40	62 ± 3***	68 ± 4**

Means ± SEM are given. SBP, systolic blood pressure; DBP, diastolic blood pressure; FBF, forearm blood flow; FVR, forearm vasular resistance. # Represents the data of nine subjects of group B (n=11) who tolerated the first 10 min of LBNP at -40 mmHg but who later developed a vasodepressor reaction. Two subjects had a vasodepressor reaction in the first 10 min of LBNP at -40 mmHg. ** $P < 0.01$, *** $P < 0.001$ with respect to baseline.

Table 5. Norepinephrine kinetics values during LBNP at -40 mmHg in group A (without syncope, n=8) and group B (with syncope, n=6 #).

		Group A	Group B
Arterial plasma NE	Baseline	1.01 ± 0.12	0.82 ± 0.11
(nmol · L^{-1})	LBNP -40	1.88 ± 0.08**	1.50 ± 0.21*
Forearm NE spillover	Baseline	0.73 ± 0.19	0.60 ± 0.17
(pmol · min^{-1} · 100 ml^{-1})	LBNP -40	1.32 ± 0.36*	0.73 ± 0.15
Total body NE spillover	Baseline	1.47 ± 0.20	1.05 ± 0.18
(nmol · min^{-1} · m^{-2})	LBNP -40	1.91 ± 0.15**	1.87 ± 0.24
Total body NE clearance	Baseline	1.51 ± 0.17	1.25 ± 0.10
(L · min^{-1} · m^{-2})	LBNP -40	1.02 ± 0.07**	1.31 ± 0.20

Means ± SEM are given. NE, norepinephrine. # Represents the data for six subjects of group B (n=8) who tolerated the first 10 min of LBNP at -40 mmHg but who developed a vasodepressor reaction later. Two subjects had a vasodepressor reaction in the first 10 min of LBNP at -40 mmHg. * $P<0.05$ and ** $P<0.01$ with respect to baseline.

NE levels increased significantly in both groups to 2.12 ± 0.22 nmol · L^{-1} in group A and 2.11 ± 0.22 nmol · L^{-1} in group B.
In the group A subjects, arterial plasma NE increased from 1.01 ± 0.10 to 1.76 ± 0.09 nmol · L^{-1} ($P<0.01$). In the nine group B subjects, the arterial plasma NE response (from 0.93 ± 0.10 to 1.50 ± 0.14 nmol · L^{-1}, $P<0.01$) after 10 min of LBNP at -40 mmHg did not differ significantly from that in the group A subjects.
Arterial plasma EPI levels increased in the group A subjects from 0.21 ± 0.02 nmol · L^{-1} to 0.39 ± 0.04 nmol · L^{-1} ($P<0.01$) during LBNP at -40 mmHg and increased markedly in the nine group B subjects from 0.30 ± 0.04 nmol · L^{-1} to 1.24 ± 0.44 nmol · L^{-1} ($P<0.01$) after 10 min of LBNP.
In group A total body NE spillover increased significantly by about 30% during LBNP at -40 mmHg, and NE clearance decreased significantly by about 40% (Table 5); in group B, total body NE spillover increased in five subjects, but this response was not significant (Table 5). Forearm NE spillover increased in the group A subjects, from 0.73 ± 0.19 to 1.32 ± 0.36 pmol · min^{-1} · 100 mL^{-1}, $P<0.05$, whereas in group B there was no significant change (Table 5).
Plasma β-endorphin levels did not change during LBNP at -40 mmHg in group A (34 ± 6 pg · mL^{-1} at baseline, 38 ± 5 pg · mL^{-1} during LBNP), whereas in group B plasma β-endorphin increased in six of the eight subjects; the average increase, from 59 ± 12 pg · mL^{-1} at baseline to 82 ± 22 pg · mL^{-1} during LBNP at -40 mmHg, was not significant.

Discussion

Few occurrences in clinical medicine match vasodepressor reactions for abruptness, unexpectedness, and drama. The neurocirculatory basis for vasodepressor reactions was long presumed to be diffusely increased vagal "tone", since people experiencing them often have nausea, sweating, and bradycardia; however, cholinergic blockade may not prevent or reverse the hypotension. More recently, microneurographic [5] and neurochemical [6, 7] evidence has convincingly demonstrated virtually complete shutdown of sympathoneural cardiovascular outflows, with generally preserved or augmented adrenomedullary secretion [25]. This combination could contribute to the skeletal vasodilation that virtually always accompanies vasodepressor reactions.
Lower body negative pressure at -15 mmHg inhibits cardiac "low pressure" baroreceptors. During LBNP at -15 mmHg, skeletal muscle sympathoneural outflow normally increases reflexively [7, 26, 27]. Exposure to LBNP at -40 mmHg inhibits cardiac and arterial baroreceptors, increasing sympathoneural outflow in several vascular beds and concurrently increasing adrenomedullary secretion. Diffuse reflexive sympathoadrenal stimulation in this setting probably supports systemic blood pressure and cerebral perfusion.

In the present study, sudden vasodepressor reactions occurred in 11 of 26 healthy subjects during prolonged exposure to LBNP at -40 mmHg. The study design enabled review of catecholaminergic function *before* the actual vasodepressor reactions, that is during LBNP at -15 mmHg. The findings indicate that a combination of attenuated noradrenergic and exaggerated adrenomedullary and possibly endogenous opioid responses to mildly decreased cardiac filling (LBNP at -15 mmHg) characterized subjects who subsequently developed vasodepressor reactions during exposure to more severely decreased cardiac filling (group B). These subjects failed to increase forearm NE spillover during exposure to LBNP at -15 mmHg and had enhanced increases in arterial EPI levels.

Forearm vasoconstriction occurred in the group B subjects during LBNP at -15 mmHg, despite the absence of increased forearm NE spillover. The basis for the forearm vasoconstriction without concurrently increased regional NE spillover cannot be determined from the present data. Several explanations are possible, including augmented extraneuronal removal of NE escaping the neuroeffector junctions, release of vasoconstrictor peptides such as NPY or ATP, blockade of formation of endothelium-derived relaxing factors, or concurrent inhibition of sympathetic cholinergic vasodilation.

It is a possibility that this pattern of sympathoinhibition and adrenomedullary stimulation reflects a central neural process and arises from sudden resetting of baroreflexes. Analogous resetting occurs during haemorrhage [28] and hypoglycaemia [29] in laboratory animals and produces a neurocirculatory positive feedback loop that leads rapidly to hypotension and circulatory shock. Moreover, electrical stimulation of specific hypothalamic regions can concurrently increase vagal and decrease sympathoneural outflows, evoking hypotension [30]. Disruption of vagal afferents or blockade of receptors for vasopressin or endogenous opioids can reverse the sympathoinhibition attending haemorrhage or hypoglycaemia, and it is therefore possible that central processes involving these compounds may also operate in some vasodepressor reactions.

Vasovagal syncope frequently occurs in emotionally distressing circumstances. Individuals who developed a vasodepressor reaction had enhanced plasma EPI responses. Exaggerated endogenous EPI responses may also be part of a centrally generated pattern that triggers or reinforces a neurocirculatory positive feedback loop. Consistent with this view, a recent study in a small group of subjects also reported increases in plasma EPI levels before the onset of fainting and suggested that increased plasma EPI contributed to the vasodilation [31]. This helps to explain why β-adrenoceptor blockade can be useful in treating patients with recurrent vasodepressor syncope [32,33]. Exogenous administration of EPI alone does not cause syncope in healthy people.

Several investigators have proposed that in volume-depleted subjects, cardiac contraction around a near-empty ventricle at end-systole can paradoxically stimulate inhibitory ventricular myocardial receptors, evoking a depressor reflex [14]. This mechanism, although widely accepted, has never actually been demonstrated as a cause of vasovagal syncope. On the contrary, infusion of a vasodilator has been reported to evoke vasode-

pressor syncope in a heart transplant recipient who lacked cardiac innervation [34], and heart transplant recipients do not differ from healthy control subjects in their susceptibility to LBNP-induced vasodepression [35]. In cats and rats, but not in dogs, interference with cardiac vagal C-fibre afferents prevents haemorrhage-induced vasodepression [15]. These findings suggest that the occurrence of vasodepressor syncope in humans may not require altered neuronal afferent input from ventricular baroreceptors and that the relative contributions of peripheral and central mechanisms vary among species.

The central aetiology hypothesis has direct therapeutic implications, since β-adrenoceptor blockade, administration of an α-adrenoceptor agonist or sympathomimetic amine, blockade of endogenous opiate effects, or blockade of vasopressin receptors would be expected to abort or prevent vasodepressor syncope. The beneficial effects of β-blockade were noted above [32, 33]. Treatment with dextroamphetamine [36], pseudoephedrine or phenylephrine [37] appears to prevent syncope in patients with positive tilt testing. Pre-treatment with naloxone, however, does not prevent vasodepressor responses during repeat exposure to LBNP in healthy subjects with previous LBNP-induced syncope [38]. We did not find reports about effects of vasopressin antagonists.

In summary, healthy subjects who developed vasodepressor reactions during high-intensity LBNP had antecedent exaggerated adrenomedullary and attenuated sympathoneural responses during mild LBNP; some subjects who developed a vasodepressor reaction during high-intensity LBNP had β-endorphin responses during mild LBNP. The findings are consistent with the view that sudden, central resetting of baroreflex function can evoke a pattern of sympathoneural inhibition combined with adrenomedullary and vagal activation, producing neurocirculatory positive feedback loops that lead precipitously to hypotension and syncope.

References

1. Wayne HH. Syncope. Physiologic considerations and an analysis of the clinical characteristics in 510 patients. Am J Med 1961;30:418-38.
2. Glick G, Yu PN. Hemodynamic changes during spontaneous vasovagal reactions. Am J Med 1963;34:42-5.
3. Barcroft H, Edholm OG. On the vasodilation in human skeletal muscle during posthaemorrhagic fainting. J Physiol 1945;104:161-75.
4. Greenfield ADM. An emotional faint. Lancet 1951;i:1302-3.
5. Wallin BG, Sundlof G: Sympathetic outflow to muscles during vasovagal syncope. J Auton Nerv Syst 1982;6:287-91.
6. Goldstein DS, Spanarkel M, Pitterman A, Toltzis R, Gratz E, Epstein S, Keiser H. Circulatory control mechanisms in vasodepressor syncope. Am Heart J 1982;104:1071-5.
7. Ziegler MG, Echon C, Wilner KD, Specho P, Lake CR, McCutchen JA. Sympathetic nervous withdrawal in the vasodepressor (vasovagal) reaction. J Auton Nerv Syst 1986;17: 273-8.

8 Esler M, Jennings G, Lambert G, Meredith I, Horne M, Eisenhofer G.Overflow of catecholamine neurotransmitters to the circulation: Source, fate, and functions. Physiol Rev 1990;70:963-85.
9. Tidgren B, Hjemdahl P, Theodorsson E, Nusberger J. Renal responses to lower body negative pressure in humans. Am J Physiol 1990;259:F573-9.
10. Lewis T. Vasovagal syncope and the carotid sinus mechanism. Br Med J 1932;2:873-6.
11. Morita H, Nishida Y, Motochigawa H, Uemura N, Hosomi H, Vatner SF. Opiate receptor-mediated decrease in renal nerve activity during hypotensive hemorrhage in conscious rabbits. Circ Res 1988; 63:165-72.
12. Floras JS, Aylward PE, Abboud FM, Mark AL. Inhibition of muscle sympathetic nerve activity in humans by arginine vasopressin. Hypertension 1987;10:409-16.
13. Riegger GA,Wagner A. Excessive secretion of vasopressin during vasovagal reaction. Am Heart J 1991;121:602-3.
14. Oberg B, Thoren P. Increased activity in left ventricular receptors during hemorrhage or occlusion of caval veins in the cat. A possible cause of the vaso-vagal reaction. Acta Physiol Scand 1972;85:164-73.
15. Rea RF, Thames MD. Neural control mechanisms and vasovagal syncope. Cardiovasc Electrophysiol 1993;4:587-95.
16. Dickinson CJ. Fainting precipitated by collapse-firing of venous baroreceptors. Lancet 1993;342:970-2.
17. Almquist A, Goldenberg IF, Milstein S, Chen MY, Chen WC, Hansen R, Gornick CC, Benditt DG. Provocation of bradycardia and hypotension by isoproterenol and upright posture in patients with unexplained syncope. N Eng J Med 1989;320:346-51.
18. Epstein SE, Stampfer M, Beiser GD. Role of the capacitance and resistance vessels in vasovagal syncope. Circ Res 1968:37:524-33.
19. Zoller RP, Mark AL, Abboud FM, Schmid PG, Heistad DD. The role of low pressure baroreceptors in reflex vasoconstrictor responses in man. J Clin Invest 1972;51:2967-72.
20. Esler M, Jackman G, Bobik A, Kelleher D, Jennings G, Leonard P, Skews H, Korner P. Determination of norepinephrine apparent release rate and clearance in humans. Life Sci 1979;25:1461-70
21. Brakkee AJM, Vendrik AJH. Strain gauge plethysmography, theoretical and practical notes on a new design. J Appl Physiol 1966;21:701-4.
22. Lenders JWM, Janssen GJ, Smits P, Thien Th. Role of the wrist cuff in forearm plethysmography. Clin Sci 1991;80:413-7.
23. Van der Hoorn FAJ, Boomsma F, Man in't Veld AJ, Schalekamp MADH. Determination of catecholamines in human plasma by high-performance liquid chromatography: Comparison between a new method with fluorescence detection and an established method with electrochemical detection. J Chrom 1989;487:17-28.
24. Troullos ES, Hargreaves KM, Goldstein DS, Stull R, Dionne RA. Epinephrine suppresses stress-induced increases in plasma immunoreactive β-endorphin in humans. J Clin Endocrinol Metab 1989;69:546-51.
25. Chosy JJ, Graham DT. Catecholamines in vasovagal fainting. J Psychosom Res 1965:9:189-94.
26. Baily RG, Prophet SA, Shenberger JS, Zelis R, Sinoway LI. Direct neurohumoral evidence for isolated sympathetic nervous system activation to skeletal muscle in response to cardiopulmonary baroreceptor unloading. Circ Res 1990;66:1720-8.
27. Johnson JM, Rowell LB, Niederberger M, Eisman MM. Human splanchnic and forearm vasoconstrictor responses to reductions of right atrial and aortic pressures. Circ Res 1974;34: 515-23.
28. Victor RG, Thoren P, Morgan DA, Mark AL. Differential control of adrenal and renal sympathetic nerve activity during hemorrhagic hypotension in rats. Circ Res 1989;64:686-94.
29. Nijima A. The effect of 2-deoxy-D-glucose and D-glucose on the efferent discharge rate of sympathetic nerves. J Physiol 1975; 251:231-43.
30. Koizumi K, Kollai M. Control of reciprocal and non-reciprocal action of vagal and sympathetic efferents: Study of centrally induced reactions. J Auton Nerv Syst 1981;3:483-501.

31. Robinson BJ, Johnson RH. Why does vasodilation occur during syncope? Clin Sci 1988;74:347-50.
32. Goldenberg IF, Almquist A, Dunbar DN, Milstein S, Pritzker MR, Benditt DG. Prevention of neurally-mediated syncope by selective beta-1-adrenoceptor blockade. Circulation 1987;76(suppl 4):133.
33. Muller G, Deal BJ, Strasburger JF, Benson DW Jr. Usefulness of metoprolol for unexplained syncope and positive response to tilt testing in young persons. Am J Cardiol 1993;71:592-5.
34. Scherrer U, Vissing S, Morgan BJ, Rollins JA, Tindall RSA, Ring S, Hanson P, Mohanty PK, Victor RG. Vasovagal syncope after infusion of a vasodilator in a heart-transplant recipient. N Engl J Med 1990;322:602-4.
35. Lightfoot JT, Rowe SA, Fortney SM. Occurrence of presyncope in subjects without ventricular innervation. Clin Sci 1993;85:695-700.
36. Susmano A, Volgman AS, Buckingham TA. Beneficial effects of dextroamphetamine in the treatment of vasodepressor syncope. PACE Pacing Clin Electrophysiol 1993;16:1235-9.
37. Strieper MJ, Campbell RM. Efficacy of alpha-adrenergic agonist therapy for prevention of pediatric neurocardiogenic syncope. J Am Coll Cardiol 1993;22:594-7.
38. Smith ML, Carlson MD, Thames MD. Naloxone does not prevent vasovagal syncope during simulated orthostasis in humans. J Auton Nerv Syst 1993;45:1-9.

Chapter 5

Adrenomedullary secretion of epinephrine is increased in mild primary hypertension

Hypertension (in press)

M.C. Jacobs
J.W.M. Lenders
J.J. Willemsen
Th. Thien

Adrenomedullary secretion of epinephrine is increased in mild primary hypertension

Abstract

To assess whether patients with mild primary hypertension have excessive activities of the sympathoneural and adrenomedullary systems, we examined total body and forearm spillovers and clearances of norepinephrine and epinephrine in 47 subjects with mild primary hypertension (25 males, 22 females, age 36.5 ± 5.9 years) and in 43 normotensive subjects (19 males, 24 females, age 38.1 ± 6.7 years). The isotope dilution method with infusions of tritiated norepinephrine and epinephrine was used at rest and during sympathetic stimulation by lower body negative pressure at -15 and at -40 mmHg. Hypertensive subjects had a higher arterial plasma epinephrine concentration (0.20 ± 0.01 nmol · L^{-1}; mean ± SEM) than the normotensives (0.15 ± 0.01 nmol · L^{-1}) ($p<0.01$). The increased arterial plasma epinephrine levels appeared to be due to a higher total body epinephrine spillover rate in the hypertensives (0.23 ± 0.02 nmol · min^{-1} · m^{-2}) then in the normotensives (0.18 ± 0.01 nmol · min^{-1} · m^{-2}) ($p<0.05$) and not to a decreased plasma clearance of epinephrine. The arterial plasma norepinephrine level, the total body and forearm norepinephrine spillover rates and the plasma norepinephrine clearance were not altered in the hypertensives. The responses of the catecholamine kinetic variables to lower body negative pressure were not consistently different between normotensives and hypertensives. These data indicate that patients with mild primary hypertension have: 1) elevated arterial plasma epinephrine concentrations that are due to an increased total body epinephrine spillover rate, indicating an increased adrenomedullary secretion of epinephrine, 2) have no increased generalized sympathoneural activity and no increased forearm norepinephrine spillover, and 3) have similar responses of both the sympathoneural and adrenomedullary systems to sympathetic stimulation by lower body negative pressure.

Introduction

The sympathetic nervous system plays not only an important role in the regulation of blood pressure but is probably also involved in the pathogenesis of human primary hypertension. Several lines of evidence indicate that hypertensive subjects have an elevated sympathetic neural outflow. Biochemical measurements like plasma catecholamine concentrations have been used extensively to document this. Several studies reported on increased plasma norepinephrine (NE) levels or increased plasma epineph-

rine (EPI) levels in hypertensive patients, in particular under the age of forty years [1]. Plasma catecholamine concentrations are however not a reliable index of sympathoneuronal and adrenomedullary activities for several reasons. First, plasma NE and EPI levels are determined not only by the rate at which these catecholamines enter plasma but also by their rates of removal [2]. Secondly, sympathetic outflow to different organs is not uniform and in particular during sympathetic stimulation, sympathoneuronal responses show a differentiated pattern across different organs, depending on the kind of stimulus [3]. Thus, venous and even arterial plasma NE and EPI concentrations have a limited value as measures of sympathetic and adrenomedullary activities.

The isotope dilution method can provide more detailed information since there is a proportional relationship between the sympathetic nerve firing rate to an organ and the rate of spillover of NE into the circulation [4]. This method enables calculating spillover and clearance rates of NE and EPI, both for the whole body and for specific vascular beds. Several groups investigated NE kinetics in hypertensive patients, showing normal total body NE spillover with decreased neuronal uptake of NE or increased total body NE spillover in young hypertensives [5,6,7]. Other investigators however found no significant difference in total body spillover of NE between normo-and hypertensives [8].

In contrast to NE kinetics, much less attention has been paid to EPI kinetics. Several studies reported on increased plasma EPI levels in hypertensives [1], but it is unclear whether these increased plasma EPI concentrations are due to an increased adrenomedullary secretion of EPI or to a diminished clearance of EPI from plasma. Up to now, no direct comparison between normo- and hypertensives has been carried out with regard to EPI kinetics.

The purpose of the present study was to assess simultaneously sympathoneural and adrenomedullary activities as measured by the isotope dilution technique, using simultaneous infusions of tritiated NE and tritiated EPI in untreated patients with mild primary hypertension. Since excessive activity of the sympathoneuronal and adrenomedullary systems may only be disclosed during sympathetic stimulation, the kinetics of NE and EPI were also assessed during low and high intensities of lower body negative pressure (LBNP) [9,10].

Methods

Subjects

Forty-seven patients with mild primary hypertension and 43 normotensive control subjects participated in the study. Before entry in the study all participants (ages 20-45 years) had a negative medical history and a normal physical examination. Thirty-one of the 47 hypertensive patients had discontinued their antihypertensive medication for at

least four weeks before the study while the remaining 16 subjects had not yet received any antihypertensive treatment. Blood pressure was measured at two occasions (in triplicate at each occasion after 10 minutes of supine rest) at two-weeks intervals using a mercury sphygmomanometer. Mild hypertension was defined as having a diastolic blood pressure between 90-105 mmHg four weeks after stopping the antihypertensive therapy. Secondary hypertension was excluded according to standard clinical criteria. Normotension was defined as having a blood pressure of less than 140/90 mmHg. Subjects with a body weight higher than the ideal body weight +10% were excluded from the study [11]. The normotensive control subjects were recruited by means of a newspaper announcement. The study protocol was approved by the Hospital Ethics Committee and all subjects gave their written informed consent.

Study protocol and procedures

All subjects were required to abstain from alcohol, nicotine, and caffeinated foods and beverages for at least 24 hours before each study. The subjects were allowed to take a light breakfast two hours before the study. All experiments were carried out in the morning in a temperature-controlled room (21-22 °C). During the study the subjects remained supine. The lower body of the subjects was sealed at the iliac crests in an airtight Plexiglass^{TM} lower body negative pressure box. The applied subatmospheric pressure was recorded by a manometer connected to the inside of the box.

After local anesthesia, a brachial artery was cannulated (Angiocath, 20G, Deseret Medical, Becton Dickinson, Sandy, UT, USA) for monitoring blood pressure and heart rate (Hewlett Packard GmbH, Böblingen, Germany) and for drawing arterial blood samples. An intravenous cannula was inserted into a deep brachial vein in the ipsilateral arm for drawing venous blood samples. A venous cannula in the contralateral arm was used for simultaneous infusion of the radiotracers. Forearm blood flow (FBF) was recorded in the same arm that was also used for collecting arterial and venous blood samples, using venous occlusion strain-gauge plethysmography (Hokanson EC4, D.E. Hokanson, Washington, USA) with air-filled cuffs [12]. During measurement of FBF and blood sampling, the hand circulation was excluded by inflation of a wrist cuff to 100 mmHg above systolic blood pressure [13]. After instrumentation, the subjects rested for 30 minutes. Thereafter both radiotracers were infused each as a bolus of 15 $\mu Ci \cdot m^{-2}$, followed by a constant infusion at a continuous rate of 0.35 $\mu Ci \cdot min^{-1} \cdot m^{-2}$ for a total duration of 90 minutes. During the last 3 minutes of the rest period of 30 minutes, baseline recordings of intra-arterial blood pressure, heart rate, and FBF were obtained and arterial and venous blood samples were drawn for measurement of labeled and unlabeled plasma catecholamines. Blood pressure was recorded simultaneously with the FBF measurement. FBF was measured three times per minute. Thereafter LBNP was applied at an intensity -15 mmHg for 15 minutes. Blood pressure, heart rate, and FBF recordings and blood samples were collected in this sequence in the last 3 minutes of

the LBNP period. After 30 minutes of rest, another LBNP period at -40 mmHg for 15 minutes followed with similar obtainment of blood pressure, heart rate, FBF recordings and arterial and venous blood samples. The weights of the syringes containing the radiotracers were measured before and after the infusion to verify the infusion rate. Samples of the infusates were taken at the end of each infusion.
^{3}H-NE (levo-[ring-2,5,6-^{3}H]-norepinephrine) (specific activity 30-60 Ci/mmol) and ^{3}H-EPI (levo-[N-methyl-^{3}H]-epinephrine) (specific activity 50-85 Ci/mmol) (Du Pont New England Nuclear, 's Hertogenbosch, the Netherlands) were sterilized using a micropore filter (0.22 µm) and diluted in NaCl 0.9%, containing acetic (0.2 mol/L) and ascorbic (5.7 mmol/L) acid. Sterilization, dilution and storage took place under nitrogen. The vials were stored until usage at -80 °C for maximal 3 months. Just prior to a study, an aliquot of each radiotracer was diluted in normal saline for intravenous infusion.

Analytical methods

Blood samples were collected in prechilled tubes containing 0.25 mol/L EGTA and 0.2 mol/L glutathione and immediately placed on melting ice. Plasma was separated by refrigerated centrifugation and frozen until assayed within two months from collection. The samples were analyzed for concentrations of unlabeled NE and EPI and ^{3}H-NE and ^{3}H-EPI, using high performance liquid chromatography (HPLC) with fluorometric detection after selective precolumn derivatization of the catecholamines with the fluorescent agent 1,2-diphenylethylenediamine [14].
The detection limits of unlabeled NE and EPI are 2.2 and 3.2 pmol/L respectively. The intra-assay coefficients of variation of unlabeled NE and EPI at plasma levels of 1.31 and 0.11 nmol/L are 2.3% and 3.4% respectively. At plasma levels of 1.02 and 0.15 nmol/L, inter-assay coefficients of variation are 8.5% and 7.2% respectively. The detection limit of ^{3}H-NE and ^{3}H-EPI is 6 dpm. The interassay coefficient of variation of ^{3}H-NE and ^{3}H-EPI is 7.0% in venous plasma samples.

Data analysis

Forearm vascular resistance (FVR) was calculated as the quotient of mean arterial blood pressure (MAP) and FBF and was expressed in Arbitrary Units (AU). The average of the hemodynamic data during the three minutes recording was taken.
The total body clearance rate of each catecholamine was calculated from the infusion rate of each tritiated catecholamine (^{3}H-CA) and the steady state arterial plasma concentration of each tritiated catecholamine (^{3}H-CA$_{art}$) according to the formula: Total body CA clearance (L · min^{-1} · m^{-2}) = ^{3}H-CA infusion rate (dpm · min^{-1} · m^{-2}) / ^{3}H-CA$_{art}$(dpm · L^{-1}). Total body spillover rate of each catecholamine was calculated from the arterial plasma catecholamine concentration (CA$_{art}$) and the total body clearance of a catecholamine according to the formula: Total body CA spillover (nmol

· min^{-1} · m^{-2}) = CA_{art} (nmol · L^{-1}) × total body CA clearance (L · min^{-1} · m^{-2}). The regional kinetic variables of each catecholamine in the forearm have been expressed per 100 ml forearm volume (FAV). Regional catecholamine spillover in the forearm was estimated from the following equation: Forearm spillover (pmol · 100ml^{-1} FAV · min^{-1})= ((CA_{ven} – CA_{art}) + (CA_{art} × fractional extraction)) × FPF, where fractional extraction = (^{3}H-CA_{art} – ^{3}H-CA_{ven}) / ^{3}H-CA_{art} and FPF is the forearm plasma flow in ml · 100 ml^{-1} FAV · min^{-1}, calculated from the FBF and hematocrit. The forearm clearance of each catecholamine was calculated according to the formula: Forearm clearance (ml · 100ml^{-1} FAV · min^{-1}) = FPF × fractional extraction of each catecholamine.

Data are expressed as mean ± SEM unless indicated otherwise. Differences between normo- and hypertensive subjects were tested by the Mann-Whitney U test. For each variable, the responses to LBNP were tested by the Wilcoxon Matched-Pairs Signed Ranks Tests. For calculating correlations between hemodynamic and catecholamine kinetic variables the Spearman correlation was used. A p-value of less than 0.05 was considered to be significant.

Results

Baseline values

The descriptive characteristics of all participants are summarized in Table 1. There was no significant difference between the ages of the normotensive and hypertensive groups but the hypertensives had a slightly but significantly higher Quetelet index than the normotensives. As expected, blood pressure was higher in the hypertensives than in the normotensives and this applied also for the heart rate. Forearm blood flow was similar

Table 1. Clinical characteristics of the hypertensive patients and the normotensive subjects.

Characteristic	NT	HT
Number	43	47
Males/females	19/24	25/22
Age, years	36.5 ± 5.9	38.1 ± 6.7
Quetelet Index, kg · m^{-2}	23.1 ± 3.0	24.8 ± 2.2**
Systolic BP, mmHg	119 ± 14	150 ± 15
Diastolic BP, mmHg	73 ± 9	98 ± 8
HR, beats per minute	67 ± 10	72 ± 11*
Urinary sodium, mmol/mmol creatinine	11.1 ± 4.0 (n=37)	10.4 ± 3.8 (n=43)

NT indicates normotensives; HT, hypertensives; BP, blood pressure; HR, heart rate;
Values are mean ± SD. * $p<0.05$ and ** $p<0.01$ versus normotensives.

Table 2. The baseline values and the responses (Δ) of the catecholamine kinetic variables to lower body negative pressure (LBNP) at -15 mmHg in the hypertensives and normotensives

	Baseline level		Δ at LBNP at -15 mmHg	
Catecholamine kinetic variable	NT	HT	NT	HT
Venous plasma NE, nmol · L^{-1}	1.13 ± 0.09	1.29 ± 0.09	0.34 ± 0.05 §	0.35 ± 0.05 §
Arterial plasma NE, nmol · L^{-1}	0.83 ± 0.05	0.95 ± 0.05	0.23 ± 0.03 §	0.18 ± 0.03 §
TB NE Spill, nmol · min^{-1} · m^{-2}	0.85 ± 0.06	0.87 ± 0.05	0.07 ± 0.03	0.06 ± 0.03 ˣ
TB NE CL, L · min^{-1} · m^{-2}	1.03 ± 0.03	0.95 ± 0.03	-0.15 ± 0.02 §	-0.10 ± 0.03 ˣˣ
FA NE Spill, pmol · $100ml^{-1}$ · min^{-1}	0.77 ± 0.07	0.90 ± 0.06	0.08 ± 0.05	0.10 ± 0.04 ˣ
FA NE CL, ml · $100ml^{-1}$ · min^{-1}	0.64 ± 0.04	0.69 ± 0.04	-0.08 ± 0.03 §	-0.10 ± 0.03 ˣˣ
Venous plasma EPI, nmol · L^{-1}	0.04 ± 0.01	0.06 ± 0.01**	0.00 ± 0.00	0.00 ± 0.01
Arterial plasma EPI, nmol · L^{-1}	0.15 ± 0.01	0.20 ± 0.01**	0.06 ± 0.01 §	0.06 ± 0.01 §
TB EPI Spill, nmol · min^{-1} · m^{-2}	0.18 ± 0.01	0.23 ± 0.02*	0.02 ± 0.01 ˣ	0.03 ± 0.01 ˣ
TB EPI CL, L · min^{-1} · m^{-2}	1.22 ± 0.04	1.14 ± 0.04	-0.22 ± 0.03 §	-0.16 ± 0.03 §
FA EPI Spill, pmol · $100ml^{-1}$ · min^{-1}	0.008 ± 0.002	0.009 ± 0.004	-0.001 ± 0.002	0.000 ± 0.006
FA EPI CL, ml · $100ml^{-1}$ · min^{-1}	0.69 ± 0.04	0.74 ± 0.05	-0.07 ± 0.04 ˣ	-0.12 ± 0.03 §

NE indicates norepinephrine; EPI indicates epinephrine; NT, normotensives; HT, hypertensives; TB, total body; FA, forearm; Spill, spillover; CL, clearance. Values are mean ± SEM; * $p<0.05$ ** $p<0.01$ versus normotensives; ˣ $p<.05$ ˣˣ $p<.001$ § $p<.0001$ versus baseline values

in both groups with 1.65 ± 0.12 and 1.51 ± 0.10 ml/100ml/minute respectively while forearm vascular resistance was slightly higher in the hypertensive (81 ± 4 AU) than in the normotensive subjects (70 ± 4 AU) ($p<0.05$).

Both arterial and venous plasma NE concentrations were not significantly different between hypertensives and normotensives (Table 2). No differences were found in total body NE spillover and clearance between hypertensives and normotensives. Regional forearm NE spillover and clearance were also similar in the two groups (Table 2, Figure 1). There was no correlation between systolic, diastolic, or mean arterial blood pressure and arterial plasma NE level or total body NE spillover.

The baseline arterial and venous plasma EPI concentrations were significantly higher in the hypertensive ($p<0.01$) than in the normotensive group (Table 2, Figure 2). Among all subjects, there were weak but significant correlations between arterial plasma EPI level and systolic blood pressure ($r = 0.29$; $p<0.01$), diastolic blood pressure ($r = 0.31$; $p<0.01$), and heart rate ($r = 0.25$; $p<0.05$). The total body spillover of EPI was significantly increased in the hypertensives (Figure 3), while total body and forearm clearances of EPI were similar in both the hypertensive and normotensive groups. In both groups there were extremely low forearm spillovers of EPI that were both significantly different from zero ($p<0.01$) but there was no difference between normo- and hypertensives (Table 2).

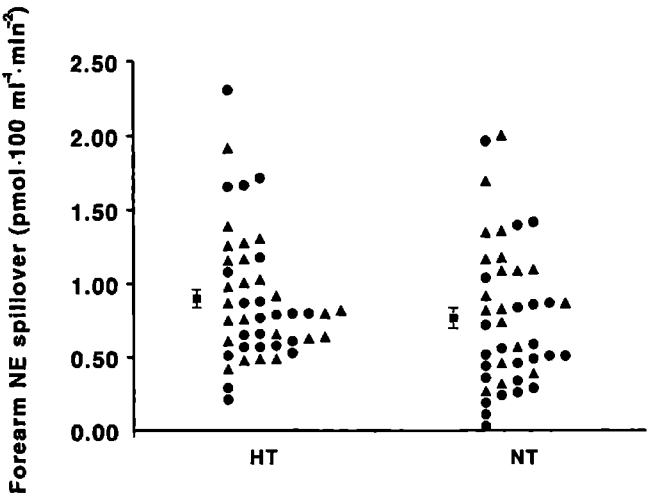


Figure 1. Individual values of norepinephrine (NE) spillover from the forearm in mild primary hypertensive patients (HT) and normotensive control subjects (NT). Triangles indicate male subjects and circles indicate female subjects.

Lower body negative pressure

During LBNP at -15 mmHg, both systolic and diastolic blood pressure did not change but the heart rate increased slightly but significantly in both groups by +1 ± 1 and +1 ± 1 beats/minute. Pulse pressure decreased in the normotensives by 2 ± 1 mmHg ($p<0.01$) and by 3 ± 1 mmHg in the hypertensives ($p<0.01$) but these decrements were not significantly different. Forearm vascular resistance increased similarly in both groups by +17 ± 3 and +22 ± 3 AU.

The venous and arterial plasma NE concentrations increased significantly by 31 ± 3% and 31 ± 4% in the normotensives, and by 34 ± 5% and 24 ± 4% in the hypertensives (Table 2). The increments of forearm and total body NE spillover reached only significance in the hypertensive group and not in the normotensive group but these increments were not significantly different between the groups (Table 2). Total body NE clearance decreased significantly in both groups.

The venous plasma EPI concentration did not change during LBNP at -15 mmHg but the arterial plasma EPI level increased by 36 ± 5% in the normotensives and 38 ± 7% in the hypertensives (NS) (Table 2). The increments in arterial plasma EPI levels during LBNP appeared to be due to both an increase in total body EPI spillover (+13 ± 5%, normotensives and +19 ± 6%, hypertensives) and to a reduction in total body EPI clearance (-17 ± 2% and -12 ± 3%). The forearm spillovers of EPI did not increase significantly during LBNP in both groups (Table 2).

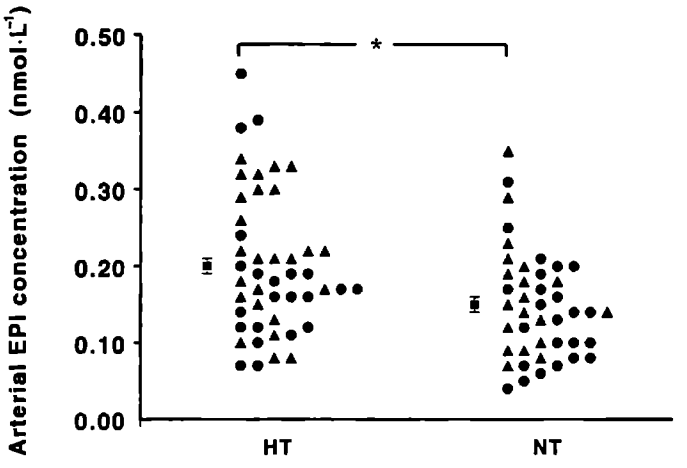


Figure 2. Individual values of arterial plasma epinephrine (EPI) concentration in mild primary hypertensive patients (HT) and normotensive control subjects (NT). Triangles indicate male subjects and circles indicate female subjects. * $p<0.01$ hypertension versus normotension.

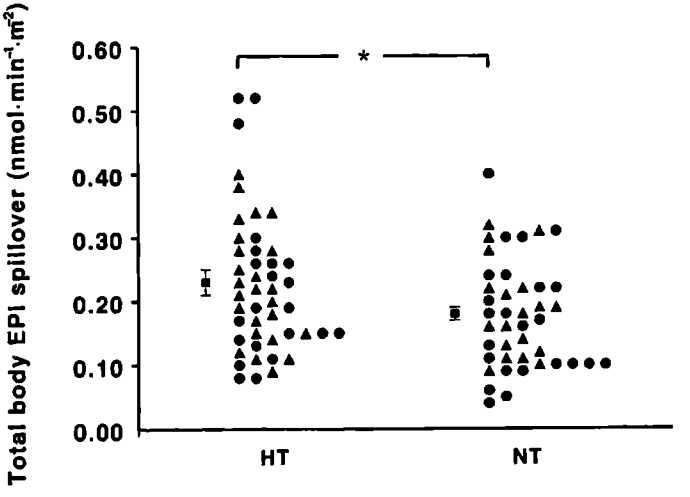


Figure 3. Individual values of total body epinephrine (EPI) spillover in mild primary hypertensive patients (HT) and normotensive control subjects (NT). Triangles indicate male subjects and circles indicate female subjects. * $p<0.05$ hypertension versus normotension.

Table 3. The baseline values and the responses (Δ) of the catecholamine kinetic variables to lower body negative pressure (LBNP) at -40 mmHg in the hypertensives and normotensives

	Baseline levels		Δ at LBNP at -40 mmHg	
Catecholamine kinetic variable	NT	HT	NT	HT
Venous plasma NE, nmol · L^{-1}	1.17 ± 0.10	1.26 ± 0.09	1.02 ± 0.10 §	1.18 ± 0.09 §
Arterial plasma NE, nmol · L^{-1}	0.86 ± 0.05	0.94 ± 0.05	0.64 ± 0.06 §	0.83 ± 0.06 *§
TB NE Spill, nmol · min^{-1} · m^{-2}	0.83 ± 0.06	0.86 ± 0.05	0.30 ± 0.04 §	0.46 ± 0.06 §
TB NE CL, L · min^{-1} · m^{-2}	0.96 ± 0.03	0.92 ± 0.03	-0.15 ± 0.02 §	-0.17 ± 0.02 §
FA NE Spill, pmol · $100ml^{-1}$ · min^{-1}	0.82 ± 0.08	0.82 ± 0.08	0.36 ± 0.11 §	0.36 ± 0.07 §
FA NE CL, ml · $100ml^{-1}$ · min^{-1}	0.64 ± 0.04	0.62 ± 0.04	-0.17 ± 0.04 §	-0.14 ± 0.05 §
Venous plasma EPI, nmol · L^{-1}	0.04 ± 0.01	0.06 ± 0.01**	0.02 ± 0.01 §	0.06 ± 0.01**§
Arterial plasma EPI, nmol · L^{-1}	0.19 ± 0.01	0.25 ± 0.02**	0.19 ± 0.02 §	0.34 ± 0.07 §
TB EPI Spill, nmol · min^{-1} · m^{-2}	0.21 ± 0.02	0.27 ± 0.02*	0.12 ± 0.02 §	0.23 ± 0.06 §
TB EPI CL, L · min^{-1} · m^{-2}	1.09 ± 0.04	1.06 ± 0.04	-0.21 ± 0.03 §	-0.24 ± 0.03 §
FA EPI Spill, pmol · $100ml^{-1}$ · min^{-1}	0.005 ± 0.001	-0.006 ± 0.005	-0.005 ± 0.002 ^{x}	-0.025 ± 0.019 ^{x}
FA EPI CL, ml · $100ml^{-1}$ · min^{-1}	0.71 ± 0.04	0.69 ± 0.05	-0.22 ± 0.04 §	-0.19 ± 0.04 §

NE indicates norepinephrine; EPI indicates epinephrine; NT, normotensives; HT, hypertensives; TB, total body; FA, forearm; Spill, spillover; CL, clearance. Values are mean ± SEM; * $p<0.05$ ** $p<0.01$ versus normotensives; ^{x} $p<0.05$ § $p<0.0001$ versus baseline values

During LBNP at -40 mmHg, pulse pressure decreased significantly more in the hypertensives (-13 ± 1 mmHg) than in the normotensives (-9 ± 1 mmHg) ($P<0.05$) while the heart rate increased similarly in both groups by 10 ± 1 and 9 ± 1 beats per minute respectively (NS).

The venous plasma NE concentrations increased similarly by 94 ± 6% and 111 ± 11% in the normotensives and hypertensives respectively. Arterial NE increased more in the hypertensive (96 ± 8%) than in the normotensive group (83 ± 8%) (Table 3) while there was no difference between the normotensive and hypertensive groups with regard to the responses of forearm and total NE spillovers, and total body and forearm NE clearances (Table 3).

The responses of the arterial plasma EPI concentrations increased by 108 ± 10% in the normotensives and 136 ± 18% in the hypertensives, which are not significantly different responses (Table 3). The total body spillover of EPI increased by 69 ± 12% and 81 ± 13%, whereas the total body clearance of EPI decreased by 16 ± 6% and 21 ± 3%. These differences between normo- and hypertensives were not significant. The forearm spillovers of EPI showed a slight but significant decrease in both groups.

Discussion

The present study not only confirms the previously reported elevated plasma EPI levels in patients with primary hypertension but also demonstrates that this is due to an increased adrenomedullary release of EPI into the bloodstream and not to a decreased clearance of EPI. This is indicated by the increased total body spillover of EPI in combination with an unaltered EPI clearance. Several previous studies have demonstrated elevated plasma EPI levels in patients with primary hypertension [1,15-18], suggesting increased adrenomedullary secretion of EPI in hypertensives. Although EPI is released from the adrenal medulla directly into the bloodstream, arterial plasma EPI levels can not be used as an index of adrenomedullary EPI secretion because most of the circulating EPI is removed rapidly from the circulation [19]. To take into account the removal of EPI from the circulation, adrenomedullary EPI secretion can be assessed by the isotope dilution technique [20]. This technique requires a high analytical sensitivity for measuring low venous EPI concentrations and venous ^{3}H-EPI activity [14]. Using this technique, the present study demonstrates for the first time that the elevated plasma EPI levels in primary hypertensives are due to an increased adrenomedullary secretion of EPI. Since the adrenal medulla serves as a sympathetic ganglion, the increased adrenal EPI release in the mild hypertensives reflects an increased sympathetic outflow selectively to the adrenal medulla in these patients.

An increased sympathetic nervous system activity has been implicated as an important pathophysiological mechanism in primary hypertension for a long time [21]. An increased activity of the sympathetic system may not only be manifest at different levels of the sympathoneuronal axis but has also been demonstrated as an increased local NE spillover in certain organs like the heart, kidneys, brain and skeletal muscles [6,7]. The present observation demonstrates that this increased sympathetic outflow extends also to the adrenal medulla. In contrast, global sympathoneuronal activity, estimated as total body NE spillover, was not elevated in the hypertensive subjects. This is at variance with some but not all previous studies, demonstrating an increased total body NE spillover in hypertensive subjects [5,6]. It should be noted that reports on NE kinetics or microneurographic data in hypertensive subjects have mainly been based on male subjects [22]. The present study comprises an approximately equal number of male and female subjects in both groups.

A particular additional finding in the present study is the forearm spillover of EPI in both normo- and hypertensives. These spillover rates, although extremely low, are significantly different from zero. Under normal conditions, EPI is mainly synthesized in the adrenal medulla and in certain brain nuclei. However, in patients with heart failure but also in healthy subjects during intensive aerobic exercise, EPI can be released from the heart. This released EPI is EPI that is predominantly derived from the circulation, from which it is taken up by sympathetic nerves [23]. Apparently, forearm EPI can recycle since it can be co-released along with NE from sympathetic nerves. Synthesis of

EPI in the human forearm is unlikely, since the key enzyme (phenylethanolamine N-methyltransferase), necessary for the synthesis of EPI from NE, has not been demonstrated in the forearm. Alternative possible explanations for the measured forearm EPI spillover include an assay artefact, an isotope effect of ^{3}H-EPI, or a delayed recycling of the ^{3}H-EPI as compared to unlabeled endogenous EPI because of a too short infusion time of the tracer [14]. Thus it is unclear whether the measured spillover of EPI in the forearm indicates a basal release of EPI. During sympathetic stimulation, as is the case during high-intensity LBNP, forearm EPI spillover did not increase but even decreased slightly. This could be related to an increased forearm extraction of EPI because of the sympathetic-induced decrease in forearm blood flow.

Sympathetic stimulation by low-intensity LBNP did not disclose any further differences in sympathoneuronal and adrenomedullary activities between normo- and hypertensive subjects. Low-intensity LBNP deactivates cardiopulmonary baroreceptors [9,10], and by using the microneurographic technique it has previously been shown that cardiopulmonary baroreceptor control of sympathetic nerve activity is enhanced in patients with mild primary hypertension [24]. In our study however, the response of foream NE spillover to cardiopulmonary baroreceptor deactivation in the hypertensive subjects was not enhanced. Although we did not measure muscle sympathetic nerve traffic by microneurography, it is unlikely that sympathetic nerve traffic response to LBNP was enhanced in the hypertensives because the forearm vascular resistance response in the hypertensives was also not increased. The apparent discrepancy between the results obtained by microneurography and by NE kinetics might be explained by a different study population (borderline hypertensives versus mild hypertensives) or by an interfering effect of peripheral sympathoneural mechanisms like neuronal re-uptake of NE. In addition, it should be noted that we did not measure central venous pressure in this study. If the hypertensives would have had a smaller decrease in central venous pressure in response to low-intensity LBNP than the normotensives, this might also be an explanation for the similar increments in forearm NE spillover in the hypertensives.

High-intensity LBNP deactivates both cardiopulmonary and arterial baroreceptors [9,10]. The arterial plasma levels of NE and EPI and total body spillovers of NE and EPI tended to increase more during high-intensity LBNP in the hypertensive than in the normotensive group. This is probably caused by the larger fall in pulse pressure in the hypertensive group during LBNP and does therefore not necessarily mean that the hypertensives have an enhanced arterial baroreceptor control of sympathoneural and adrenomedullary activities. Previous studies have also shown a normal arterial baroreceptor control of sympathetic activity in patients with borderline hypertension [24]. In view of the larger decrease in pulse pressure in the hypertensives, the apparent normal heart rate response to arterial baroreceptor deactivation in the hypertensives fits with the well-documented impaired arterial baroreceptor control of heart rate in mildly hypertensives [25].

It is still an unresolved issue whether an increased release of adrenal EPI is of pathophysiological significance in primary hypertension. Based on evidence obtained in isolated tissue preparations and animal experiments [26-28], Brown and Macquin hypothesized that intermittent increments in circulating EPI facilitate the neuronal release of NE by a stimulating effect of EPI on the presynaptic β_2-adrenergic receptors, thus contributing to the development of hypertension [29]. Support for this hypothesis came also from studies in humans, showing that EPI has a facilitatory effect on peripheral noradrenergic transmission and that this effect was enhanced in patients with primary hypertension [30,31]. Patients who develop hypertension would be more susceptible to stressful stimuli and exhibit elevated stress-related increments in circulating EPI. There is indeed an abundance of evidence that, in particular at a young age, patients with primary hypertension have increased sympathetic responses to psychological kinds of stress [15,32]. The slightly increased plasma EPI concentrations in the hypertensive subjects fit with the hypothesis of Brown and Macquin [29].
A question of particular concern is whether the increased plasma EPI levels may contribute to the deleterious cardiovascular sequelae of hypertension on the long term. The development of these complications may be mediated by the well-known adverse effects of catecholamines like induction of cardiac arrythmias, stimulation of vascular and ventricular hypertrophy, and platelet activation [33-35]. Although the arterial plasma EPI levels in the subjects with mild hypertension were only slightly higher than in the normotensives, it can not be excluded that chronic exposure of the heart and blood vessels to this circulating EPI may be harmful on the longterm.
In conclusion, this study demonstrates that patients with mild primary hypertension have increased plasma EPI levels and that this is due to an increased basal adrenomedullary secretion of EPI. This indicates that mildly hypertensive subjects have an increased sympathetic outflow to the adrenals. In contrast to some previous studies, we did not find an increased general sympathoneural activity, measured as total body NE spillover. No gross abnormalities were noted during sympathetic stimulation by different levels of LBNP. It is tempting to speculate that the increased plasma EPI levels in the hypertensives may be of pathophysiological significance for the development of the cardiovascular complications of hypertension on the longterm.

Acknowledgements

We thank Eugenie Olde Riekerink for her assistance during the study.

References

1. Goldstein DS. Plasma catecholamines in essential hypertension: An analytical review. Hypertension 1983;5:86-99.

2 Esler M, Jennings G, Lambert G, Meredith I, Horne M, Eisenhofer G Overflow of catecholamine neurotransmitters to the circulation Source, fate, and functions Physiol Rev 1990,70 963-985
3 Folkow B Physiological aspects of primary hypertension Physiol Rev 1982,62 347-504
4 Esler M, Jennings G, Korner P, Willett I, Dudley F, Hasking G, Anderson W, Lambert G Assessment of human sympathetic nervous system activity from measurements of norepinephrine turnover Hypertension 1988,11 3-20
5 Esler M, Jackman G, Bobik A, Leonard P, Kelleher D, Skews H, Jennings G, Korner P Norepinephrine kinetics in essential hypertension Defective neuronal uptake of norepinephrine in some patients Hypertension 1981,3 149-156
6 Esler M, Jennings G, Biviano B, Lambert G, Hasking G Mechanism of elevated plasma noradrenaline in the course of essential hypertension J Cardiovasc Pharmacol 1986,8(Suppl 5) S39-S43
7 Ferrier C, Esler MD, Eisenhofer G, Wallin G, Horne M, Cox HS, Lambert G, Jennings GL Increased norepinephrine spillover into the jugular veins in essential hypertension Hypertension 1992,19 62-69
8 Goldstein DS, Horwitz D, Keiser HR, Polinsky RJ, Kopin IJ Plasma l-[^{3}H]-norepinephrine, d-[^{14}C]-norepinephrine, and d,l-[^{3}H]-isoproterenol kinetics in essential hypertension J Clin Invest 1983,72 1748-1758
9 Zoller RP, Mark AL, Abboud FM, Schmid PG, Heistad DD The role of low pressure baroreceptors in reflex vasoconstrictor responses in man J Clin Invest 1972,51 2967-2972
10 Johnson JM, Rowell LB, Niederberger M, Eisman MM Human splanchnic and forearm vasoconstrictor responses to reductions of right atrial and aortic pressures Circ Res 1974,34 515-523
11 Statistical Bulletin of the Metropolitan Life Insurance Company 1959,40
12 Brakkee AJM, Vendrik AJH Strain gauge plethysmography, theoretical and practical notes on a new design J Appl Physiol 1966,21 710-714
13 Lenders JWM, Janssen GJ, Smits P, Thien Th Role of the wrist cuff in forearm plethysmography Clin Sci 1991,80 413-417
14 Willemsen JJ, Ross HA, Jacobs MC, Lenders JWM, Thien Th, Benraad TJ Highly sensitive and specific HPLC method with fluorometric detection for determination of plasma epinephrine and norepinephrine applied to kinetic studies in humans Clin Chem 1995,41 1455-1460
15 Eliasson K, Hjemdahl P, Kahan T Circulatory and sympathoadrenal responses to stress in borderline and established hypertension J Hypertens 1983,1 131 139
16 Bertel O, Buhler FR, Kiowski W, Lutold BE Decreased beta-adrenoceptor responsiveness as related to age, blood pressure, and plasma catecholamines in patients with essential hypertension Hypertension 1982,2 130-138
17 Franco-Morselli R, Elghozi JL, Joly E, di Giuilli S, Meyer P Increased plasma adrenaline concentrations in benign essential hypertension Br Med J 1977,2 1251 1254
18 Kjeldsen SE, Eide I, Aakesson I, Leren P Increased arterial catecholamine concentrations in 50 year-old men with essential hypertension Scand J Clin Lab Invest 1983,43 343-349
19 Ginn R, Vane JR Disappearance of catecholamines from the circulation Nature 1968,219 740 742
20 Rosen SG, Linares OA, Sanfield JA, Zech LA, Lizzio VP, Halter JB Epinephrine kinetics in humans radiotracer methodology J Clin Endocrinol Metab 1989,69 753-761
21 Julius S, Esler MD, Randall OS Role of the autonomic nervous system in mild essential hypertension Clin Sci Mol Med 1975,48 243s-252s
22 Anderson EA, Sinkey CA, Lawton WJ, Mark AL Elevated sympathetic nerve activity in borderline hypertensive humans Evidence from direct intraneural recordings Hypertension 1989,14 177-183
23 Esler M, Eisenhofer G, Jennings G, Meredith I, Lambert G, Thompson J, Dart A Is adrenaline released by sympathetic nerves in man? Clin Auton Res 1991,1 103-108
24 Rea RF, Hamdan M Baroreflex control of muscle sympathetic nerve activity in borderline hypertension Circulation 1990,82 856 862
25 Takeshita A, Tanaka S, Kuroiwa A, Nakamur M Reduced baroreceptor sensitivity in borderline hypertension Circulation 1975,51 738-742

26. Adler-Graschinsky E, Langer SZ. Possible role of a beta-adrenoceptor in the regulation of noradrenaline release by nervous stimulation through a positive feed-back mechanism. Br J Pharmacol 1975;53:43-50.
27. Majewski H, Tung LH, Rand MJ. Adrenaline activation of prejunctional beta-adrenoceptors and hypertension. J Cardiovasc Pharmacol 1982;4:99-106.
28. Quinn P, Borkowski KR, Collins MG. Epinephrine enhances neurogenic vasoconstriction in the rat perfused kidney. Hypertension 1984;7:47-52.
29. Brown MJ, Macquin I. Is adrenaline the cause of essential hypertension? Lancet 1981;2:1079-1082.
30. Floras JS, Aylward PE, Victor RG, Mark AL, Abboud FM. Epinephrine facilitates neurogenic vasoconstriction in humans. J Clin Invest 1988;81:1265-1274.
31. Floras JS, Aylward PE, Victor RG, Mark AL, Abboud FM. Epinephrine facilitates neurogenic vasoconstriction in borderline hypertensive subjects. J Hypertens 1990;8:443-448.
32. Lenders JWM, Willemsen JJ, de Boo T, Lemmens WAJ, Thien T. Disparate effects of mental stress on plasma noradrenaline in young normotensive and hypertensive subjects. J Hypertens 1989;7:317-323.
33. Meredith IT, Broughton A, Jennings GL, Esler MD. Evidence of a selective increase in cardiac sympathetic activity in patients with sustained ventricular arrhythmias. N Engl J Med 1991;325:618-624.
34. Ardlie NG, Glew G, Schwartz CJ. Influence of catecholamines on nucleotide-induced platelet aggregation. Nature 1966;212:415-417.
35. Egan B, Julius S. Vascular hypertrophy in borderline hypertension: relationship to blood pressure and sympathetic drive. Clin Exp Hypertens 1985;A7:243-255.

Chapter 6

Chronic α_1-adrenergic blockade increases sympathoneural but not adrenomedullary activity in patients with primary hypertension

Journal of Hypertension 1995;13:1837-1841

M.C. Jacobs
J.W.M. Lenders
J.J. Willemsen
Th. Thien

Chronic α_1-adrenergic blockade increases sympathoneural but not adrenomedullary activity in patients with primary hypertension

Abstract

Doxazosin, a selective α_1-adrenoceptor antagonist, lowers blood pressure by reducing peripheral vascular resistance without causing reflex tachycardia. To discover whether antihypertensive treatment of hypertension with an α_1-adrenoceptor blocker is accompanied by an increase in sympathoadrenomedullary activity, we studied plasma catecholamine kinetics before and during treatment with doxazosin.

Eleven patients with primary hypertension were studied before and after 3 months' treatment with doxazosin (4-8 mg daily). ^{3}H-norepinephrine and ^{3}H-epinephrine were infused simultaneously and blood samples were collected to calculate plasma catecholamine kinetics before and during sympathoadrenomedullary stimulation (lower body negative pressure).

Doxazosin decreased systolic and diastolic blood pressure and forearm vascular resistance, whereas heart rate did not change significantly. During doxazosin, baseline arterial plasma norepinephrine increased from 0.97 ± 0.07 to 1.21 ± 0.07 nmol/l, and this appeared to be due to an increase in total body norepinephrine spillover from 1.54 ± 0.15 to 1.84 ± 0.16 nmol/min; norepinephrine clearance did not change significantly. Forearm norepinephrine spillover also increased, from 0.89 ± 0.18 to 1.48 ± 0.23 pmol/100ml per min. In contrast, arterial plasma epinephrine, total body epinephrine spillover and epinephrine clearance were not significantly affected by doxazosin treatment. The response of plasma norepinephrine and total and forearm spillover of norepinephrine to lower body negative pressure (-40 mmHg) was significantly increased during doxazosin administration, whereas the responses of the epinephrine kinetic parameters were not altered.

The blood pressure reduction induced by a chronic administration of the α_1-adrenoceptor blocker doxazosin elicits a baroreceptor-mediated reflexive increase in sympathoneural but not in adrenomedullary activity. The latter finding might partly explain why the heart rate is not increased during chronic treatment with this α_1-adrenoceptor blocking drug.

Introduction

Doxazosin is a selective α_1-adrenoceptor blocking drug with a slow onset of action; it lowers blood pressure by decreasing systemic vascular resistance [1,2]. In contrast to the

acute administration of α_1-adrenoceptor blockers, chronic administration in hypertensive patients does not elicit a reflexive increase in the heart rate. This suggests that baroreflex-mediated sympathetic activity is not increased during chronic treatment with doxazosin. The lack of reflex tachycardia has also been attributed to the absence of an interruption of the presynaptic inhibitory effect by norepinephrine (NE) upon neuronal NE release [3]. Central α_1-adrenoceptor antagonism has been suggested as an alternative mechanism for the absence of reflex tachycardia [4]. However, treatment with prazosin, a similar α_1-adrenoceptor blocker to doxazosin but with a rapid onset of action, induces an increase in plasma levels of NE [5-7]. Although it has been assumed that the increased plasma NE levels are caused by an increase in baroreflex-mediated sympathetic activity, no definitive explanation is available for the increase in plasma NE levels. Elevated plasma NE levels might be due to a reflexive increase in sympathoneuronal release of NE or to a reduced clearance of NE.

The main objective of the present study was to assess the effects of a chronic blood pressure reduction by the α_1-adrenoceptor blocker doxazosin on the baroreflex-mediated activity of the sympathoadrenomedullary system. For this purpose, we used the isotope dilution method with steady-state infusion of tritiated catecholamines [8]. Before and after chronic doxazosin treatment (3 months), spillover and clearance of NE and epinephrine EPI were assessed in primary hypertensives and this was examined before and during stimulation of the sympathoadrenomedullary system by two different intensities of lower body negative pressure (LBNP).

Subjects and methods

Subjects

Eleven subjects with primary hypertension (mean ± SD age: 35.8 ± 2.3 years) participated in the study. Before entry all participants had a normal physical examination and none were found to suffer from cardiovascular or other diseases. Secondary hypertension was excluded according to standard clinical criteria and all subjects had a normal renal function. The mean ± SD Quetelet index was 23.6 ± 0.7 kg/m^2. Antihypertensive treatment was withdrawn at least 4 weeks before the study; after it was withdrawn, blood pressure was measured three times at 2-week intervals. The mean ± SD basal systolic/diastolic blood pressure before treatment with doxazosin was 147 ± 3/95 ± 2 mmHg and the heart rate was 77 ± 3 beats/minute. All subjects gave written informed consent and the study protocol was approved by the Hospital Ethics Committee.

Study protocol

All patients were studied twice: before and after treatment with doxazosin for 3 months. Each patient started with doxazosin 2 mg once a day and blood pressure was monitored every 3 weeks. The dose was increased every 3 weeks by 2 mg to a maximum of 8 mg a day unless diastolic blood pressure had fallen by at least 25% on the current dose. Patients with a sufficient blood pressure fall remained on the titrated dose until the end of the 3-month period. After 3 months, two patients were taking 4 mg a day, one was taking 6 mg a day, and nine were taking 8 mg doxazosin a day.

On each study day, the subjects were allowed a light breakfast. All were required to abstain from alcohol, nicotine, and caffeinated foods and beverages for at least 24 h before each study day. All studies were carried out in the morning in a room with constant temperature. During the study the subjects remained supine in a lower body negative pressure box that was used to stimulate sympathoadrenomedullary activity. After instrumentation, radiotracer infusions (see below) were started and the subjects rested for 30 minutes. During the last 3 min, baseline recordings of blood pressure, heart rate, and nine forearm blood flow curves were obtained. Then arterial and venous blood samples were drawn simultaneously to determine plasma concentrations of endogenous and tritiated catecholamines.

Thereafter, LBNP was applied at -15 mmHg for 15 min. Blood pressure, heart rate, and forearm blood flow recordings and blood samples were collected in sequence beginning after 12 min of LBNP. A rest period of 30 min ensued and then another 15 minutes of LBNP at -40 mmHg was applied, and blood pressure, heart rate and forearm blood flow recordings and blood samples were obtained as before.

Procedures

A brachial artery was cannulated to monitor blood pressure and the heart rate (Hewlett Packard GmbH, Böblingen, Germany) and to draw arterial blood samples. An intravenous catheter was inserted into a deep brachial vein in the ipsilateral arm for collecting venous blood samples. A forearm venous catheter in the contralateral arm was used for simultaneous infusion of ^{3}H-norepinephrine (^{3}H-NE) and ^{3}H-epinephrine (^{3}H-EPI). Forearm blood flow was recorded by venous occlusion strain-gauge plethysmography with air-filled cuffs [9]; during this measurement and while blood samples were drawn, the hand circulation was excluded by inflating a wrist cuff to 100 mmHg above systolic blood pressure [10].

To assess catecholamine kinetics, ^{3}H-NE (L-[O-2,5,6-^{3}H]-norepinephrine) and ^{3}H-EPI (L-[N-methyl-^{3}H]-epinephrine), with high specific activity, were infused intravenously. Tritiated catecholamines were obtained from Du Pont New England Nuclear ('s Hertogenbosch, the Netherlands), sterilized using a micropore filter (0.22 µm) and diluted in 0.9% NaCl , containing acetic (0.2 mol/l) and ascorbic (1 mg/ml) acid. The aliquots were stored until used at -80°C for a maximum of 3 months. Sterilization,

dilution and aliquoting were carried out under nitrogen. Just before use, an aliquot of each radiotracer was diluted in 0.9% NaCl.
After a bolus injection of each tracer at 15 μCi/m^2 of each tracer, both tracers were infused continuously for 90 min at a rate of 0.35 μCi/m^2 per min. The weights of the two syringes containing the radiotracers were measured before and after the infusion, to verify the infusion rate. Samples of the infusate were taken at the end of the infusion and stored at -80 °C until assayed.

Analytical methods

Blood samples were collected in prechilled tubes containing ethyleneglycol-bis-(β-aminoethylether)-N,N,N',N'-tetraacetic acid (0.25 mol/l) and glutathione (0.2 mol/l). The blood samples were placed on melting ice. Plasma was separated by refrigerated centrifugation and frozen until assayed within 2 months from collection. Plasma samples were analysed for concentrations of both NE, EPI, ^{3}H-NE and ^{3}H-EPI, using high-performance liquid chromatography with fluorometric detection after selective precolumn derivatization of the catecholamines with the fluorescent agent 1,2-diphenylethylenediamine [11]. Using a Gilson fraction collector, model 201-202, (Gilson Medical Electronics, Villiers le Bel, France) connected to an automatic sample injector, Wisp 710B, (Waters Associates, Milford, Massachusetts, USA), we collected ^{3}H-NE and ^{3}H-EPI into scintillation vials for 1 min, starting at the beginning of the peaks of NE and EPI in the standard mixture.

Data analysis

Forearm vascular resistance was calculated by dividing mean arterial blood pressure by forearm blood flow and was expressed in arbitrary units (AU). The average of the haemodynamic data during 3 min of recording was calculated.
The clearance of NE from arterial plasma was calculated by dividing the rate of ^{3}H-NE infusion by the steady-state arterial plasma concentration of ^{3}H-NE. Total body NE spillover, the estimated rate of appearance of endogenous NE in arterial plasma, was calculated by multiplying the steady-state arterial plasma NE concentration by the clearance. Analogously, NE spillover in the forearm (pmol/min per 100 ml), was estimated as:

$$\text{Forearm NE spillover} = FPF \times NE_a \times f_{NE} + (FPF \times (NE_v - NE_a))$$

where FPF is forearm plasma flow, NE_a is arterial plasma norepinephrine, NE_v is venous plasma norepinephrine and

$$f = (^3\text{H-NE}_a - {}^3\text{H-NE}_v) / {}^3\text{H-NE}_a.$$

The function f represents the fractional extraction of the tracer in the forearm. The forearm plasma flow, in units of ml/min/100 ml, was calculated from the forearm blood

flow and haematocrit. The clearance of NE in the forearm (ml/min per 100 ml) was calculated by multiplying the forearm plasma flow by the function f.
The clearance of EPI from arterial plasma and the estimated rate of appearance of endogenous EPI into arterial plasma were calculated according to similar formulas.
Results are expressed as means ± SEM unless indicated otherwise. To test the effects of doxazosin on baseline plasma kinetic variables, the Wilcoxon signed rank test was used. This test was also used to test for the effects of LBNP on each variable. $P < 0.05$ (two-sided) was considered significant.

Results

Hemodynamic data

Doxazosin decreased systolic blood pressure from 147 ± 3 to 135 ± 4 mmHg and diastolic pressure from 95 ± 2 to 85 ± 3 mmHg ($P<0.05$). The heart rate decreased slightly, from 77 ± 3 to 70 ± 2 beats/min, but this was not significant. Forearm vascular resistance decreased significantly ($P <0.05$), from 89 ± 7 to 64 ± 6 AU.
Doxazosin had no effect on the blood pressure and heart rate response to a LBNP of -15 mmHg but the increase in forearm vascular resistance during LBNP at -15 mmHg was smaller after doxazosin treatment (+7 ± 2 AU) than before (+31 ± 8 AU; $P<0.05$). The blood pressure and heart rate responses to LBNP at -40 mmHg were also unaffected by doxazosin, but the increase in forearm vascular resistance was again significantly smaller after doxazosin treatment (+5 ± 2 AU) than before (+32 ± 9 AU; $P<0.05$).

Plasma norepinephrine kinetics

After treatment with doxazosin, all subjects had an approximately 20% higher plasma NE level than before (Table 1). This increase in arterial plasma NE appeared to be due to an increase in total body NE spillover of about 20% (Fig. 1), since total body clearance of NE was not significantly altered (Table 1). Forearm NE spillover was also significantly increased after doxazosin treatment (Fig. 1) while the local clearance of NE by the forearm was not significantly affected (Table 1).
The changes in plasma NE, total body and forearm NE spillover and total body and forearm NE clearance in response to LBNP (-15 mmHg) were not affected by doxazosin. However, with -40 mmHg LBNP, the increase in plasma NE was significantly larger after doxazosin (+1.33 ± 0.11 nmol/l) than before (+0.88 ± 0.10 nmol/l; $P<0.05$). Total body and forearm NE spillover increases were also significantly higher during -40 mmHg LBNP after doxazosin treatment than before (Fig. 2). The decreases in total body NE clearance during LBNP at -40 mmHg were not significantly affected by doxazosin and the same applied to the reductions in forearm NE clearance (Fig. 2).

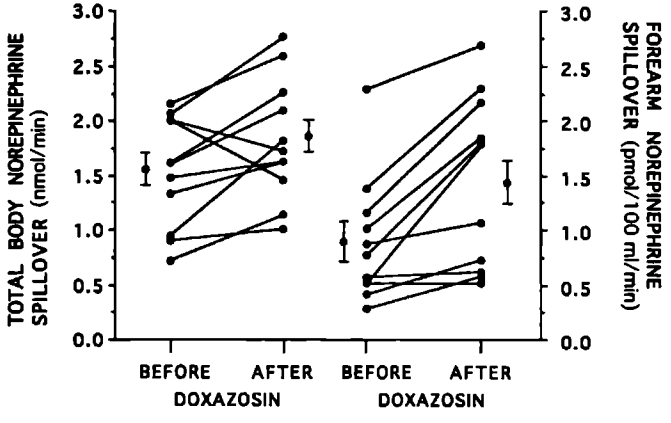


Figure 1. Individual responses by total body and forearm norepinephrine spillover before and after treatment with doxazosin.

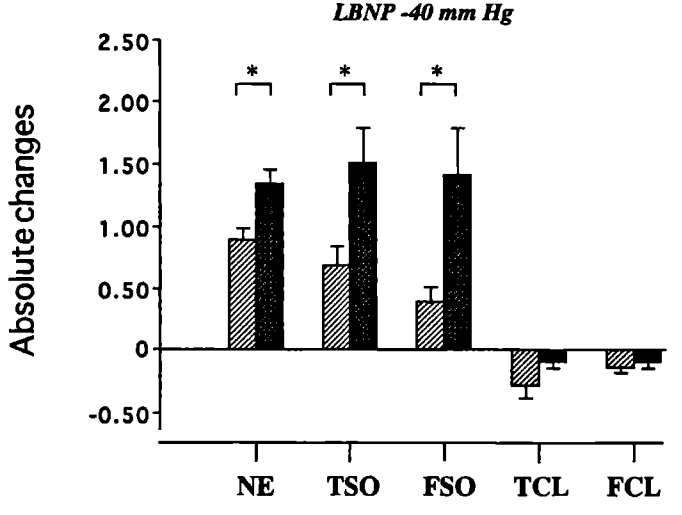


Figure 2. Mean ± SEM arterial plasma norepinephrine (NE), total body (TSO) and forearm (FSO) spillover and total body (TCL) and forearm (FCL) clearance of norepinephrine in response to lower body negative pressure at -40 mmHg before (light bar) and after (dark bar) doxazosin

Table 1. Plasma levels and kinetics of norepinephrine and epinephrine before and after treatment with doxazosin.

	Before doxazosin	After doxazosin
Norepinephrine		
Arterial plasma level (nmol/l)	0.97 ± 0.07	1.21 ± 0.07*
Total body spillover (nmol/l)	1.54 ± 0.15	1.84 ± 0.16*
Total clearance (l/min)	1.57 ± 0.10	1.52 ± 0.09
Forearm spillover (pmol/100ml per min)	0.89 ± 0.18	1.48 ± 0.23*
Forearm clearance (ml/100ml per min)	0.58 ± 0.06	0.82 ± 0.10
Epinephrine		
Arterial plasma level (nmol/l)	0.21 ± 0.03	0.15 ± 0.02
Total body spillover (nmol/l)	0.42 ± 0.07	0.27 ± 0.04
Total clearance (l/min)	1.94 ± 0.10	1.81 ± 0.11
Forearm clearance (ml/100ml per min)	0.70 ± 0.07	0.89 ± 0.12

* $P < 0.05$ versus before doxazosin.

Plasma epinephrine kinetics

After the administration of doxazosin, basal arterial plasma EPI was lower than before doxazosin treatment but the difference did not reach significance (Table 1). Total body EPI spillover was also lower after doxazosin but, again, the decrease was not significant. Total body clearance of EPI was not affected by doxazosin (Table 1).
The changes in EPI kinetics in response to LBNP of -15 and -40 mmHg were not significantly altered by doxazosin.

Discussion

The main new finding of the present study is that longterm antihypertensive treatment with the α_1-adrenoceptor blocker doxazosin elicits a selective increase in sympathetic but not adrenomedullary activity. This was demonstrated by the increase in plasma NE and total body and forearm NE spillover with no concurrent increase in plasma levels and total body spillover of EPI.
Chronic antihypertensive treatment with the α_1-adrenoceptor blocker doxazosin resulted in a significant decrease in blood pressure and forearm vascular resistance. The heart rate did not increase, as shown previously for prazosin. The following three explanations for the unchanged heart rate have been put forward: vasodilation through α_1-adrenoceptor blockade does not induce a reflexive increase in sympathetic activity because of resetting of the arterial baroreceptor reflex; blockade of central α_1-adreno-

ceptors [4]; selective α_1-adrenoceptor blocking drugs preserve the normal presynaptic α_2-adrenoceptor-mediated auto-inhibition of neuronal NE release by NE [3].
However, in the present study plasma NE levels were increased, as were total body and forearm NE spillover. This is in accord with previous studies [5-7] on prazosin. An increase in the spillover of NE into the blood compartment can be caused by an increase in sympathetic nerve traffic, by a decrease in neuronal uptake of NE or by decreased inhibition of NE release through presynaptic α_2-adrenoceptors. The last mechanism is unlikely to have occurred in the present study, since the affinity of doxazosin for α_2-adrenoceptors is about 400 times lower than that for α_1-adrenoceptors [12]. Alternatively, presynaptic α_1-adrenoceptors might be considered a possibility; however, although present in some species [13,14], no presynaptic α_1-adrenoceptors are functionally present in human blood vessels. Thus, an effect of doxazosin on presynaptic α_2-adrenoceptors or on eventual presynaptic α_1-adrenoceptors has to be rejected as a possible explanation for the increase in NE spillover. The most likely explanation for the increase in NE spillover, both systemically and in the forearm vascular bed, is that there was reflexive increase in sympathetic nerve traffic due to the blood pressure reduction. This argues strongly against a resetting of the arterial baroreceptor reflex during chronic treatment with doxazosin.
If there is, indeed, an increase in baroreflex-mediated sympathoneural activity during chronic α_1-adrenoceptor blockade, then why is there no increase in the heart rate? Since cardiac β_1-adrenoceptors are equally sensitive to NE as to EPI *in vitro*, the increased NE spillover may be expected to elicit an increase in the heart rate. However, *in vivo*, NE usually induces a decrease in the heart rate because of the baroreflex-mediated increase in vagal nerve activity. An alternative explanation for the absence of an increase in the heart rate could be provided by the absence of an increase in plasma EPI and in total body EPI spillover. Total body EPI spillover reflects adrenomedullary secretion of EPI. Apparently, the blood pressure reduction induced by doxazosin does not stimulate adrenomedullary secretion of EPI but only the sympathoneuronal release of NE. This differentiated response to a reduction in blood pressure has also been described for other vasodilating antihypertensive drugs and is therefore not a specific effect of α_1-adrenoceptor blockers [15].
During LBNP at -40 mmHg, both arterial and cardiopulmonary baroreceptors were deactivated and a marked increase in sympathoadrenomedullary activity ensued, as illustrated by the increase in forearm vascular resistance, in plasma NE and forearm NE spillover and in total body NE and EPI spillover. During doxazosin treatment, the blood pressure and heart rate responses to LBNP were not significantly altered but forearm vascular resistance response was lower than before doxazosin. This is at variance with a previous study with prazosin, demonstrating unaltered responses by peripheral vascular resistance to isometric exercise and exposure to cold [16].
Taken together, these data indicate that doxazosin is a very powerful α_1-adrenoceptor antagonist that blocks postsynaptic α_1-adrenoceptors adequately, even during strong

sympathetic stimulation. During chronic treatment with doxazosin there is a clear increase in baroreflex-mediated sympathoneural activity whereas adrenomedullary activity is not increased. This latter finding might provide a partial explanation for the absence of an increase in the heart rate during chronic treatment with doxazosin.

References

1. Young RA, Brogden RN. Doxazosine: a review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in mild or moderate hypertension Drugs 1988,35.525-541
2. Lund-Johansen P, Omvik P, Haugland H Acute and chronic haemodynamic effects of doxazosin in hypertension at rest and during exercise. Br J Clin Pharmacol 1986;21(suppl 1)·45S-54S.
3. Reid JL, Rubin PC. Catecholamines and blood pressure. In Catecholamines II. Edited by Trendelenburg U, Weiner N Berlin: Springer-Verlag, 1989:319-356.
4. Ramage AG A comparison of the effects of doxazosin and alfuzosin with those of urapidil on preganglionic sympathetic nerve activity in anaesthesized cats Eur J Pharmacol 1986;129·307-314
5. Mulvihill-Wilson J, Graham RM, Pettinger W, Muckleroy C, Anderson S, Gaffney FA, et al Comparative effects of prazosin and phenoxybenzamine on arterial blood pressure, heart rate and plasma catecholamines in essential hypertension. J Cardiovasc Pharmacol 1979;1(suppl 1):S1-S7.
6. Izzo JL, Horwitz D, Keiser HR. Physiologic mechanisms opposing the hemodynamic effects of prazosin Clin Pharmacol Ther 1981,29:7-11.
7. Eklund B, Hjemdahl P, Seideman P, Atterhog J-H. Effects of prazosin on hemodynamics and sympathoadrenal activity in hypertensive patients. J Cardiovasc Pharamacol 1983;5.384-391.
8. Esler M, Jennings G, Lambert G, Meredith I, Horne M, Eisenhofer G. Overflow of catecholamine neurotransmitters to the circulation. Source, fate, and functions. Physiol Rev 1990,70:963-985.
9. Brakkee AJM, and Vendrik AJH. Strain gauge plethysmography, theoretical and practical notes on a new design. J Appl Physiol 1966;21:701-7044.
10. Lenders JWM, Janssen GJ, Smits P, Thien Th. Role of the wrist cuff in forearm plethysmography Clin Sci 1991;80·413-417.
11. Van der Hoorn FAJ, Boomsma F, Man in 't Veld AJ, Schalekamp MADH. Determination of catecholamines in human plasma by high-performance liquid chromatography· Comparison between a new method with fluorescence detection and an established method with electrochemical detection. J Chrom 1989;487:17-28.
12. Alabaster VA, Davey MJ. The alpha-1-adrenoceptor antagonist profile of doxazosin: preclinical pharmacology Br J Clin Pharmacol 1986,21(suppl 1):9S-17S.
13. Story DF, Standford-Starr CA, Rand MJ. Evidence for the involvement of α_1-adrenoceptors in negative feedback regulation of noradrenergic transmitter release in rat atria Clin Sci 1985;68(suppl 10)·111S-115S.
14. Rump LC, Majewski H Modulation of norepinephrine release through α_1-and α_2-adrenoceptors in rat isolated kidney. J Cardiovasc Pharmacol 1987;9·500-507.
15. Lindqvist M, Kahan T, Melcher A, Hjemdahl P. Acute and chronic calcium antagonist treatment elevates sympathetic activity in primary hypertension Hypertension 1994,24:287-296.
16. Mancia G, Ferrari A, Gregorini L, Ferrari MC, Bianchini C, Terzoli L, et al. Effects of prazosin on autonomic control of circulation in essential hypertension. Hypertension 1980;2.700-707

Chapter 7

Chronic β_1-adrenergic blockade restores adrenomedullary activity in primary hypertension

M.C. Jacobs
J.W.M. Lenders
P. Smits
J.J. Willemsen
C. Tack
Th. Thien

Chronic β_1-adrenergic blockade restores adrenomedullary activity in primary hypertension

Abstract

In this study we examined the effects of chronic treatment of nineteen patients with primary hypertension with the β_1-adrenoceptor antagonist atenolol on norepinephrine and epinephrine kinetics, at rest and during sympathoadrenal stimulation by lower body negative pressure. Norepinephrine and epinephrine kinetics were measured using the radio-isotope dilution technique by steady state infusion of tritiated norepinephrine and epinephrine. The patients were studied before and at the end of 3 months treatment with atenolol (50 or 100 mg daily). A control group of four normotensive subjects was studied before and after 3 months without any drug treatment. In this group, only arterial blood samples were collected without infusion of the tritiated catecholamines. Atenolol decreased blood pressure and heart rate while forearm vascular resistance was not affected by atenolol. During atenolol, baseline arterial plasma epinephrine decreased from 0.23 ± 0.02 to 0.17 ± 0.01 nmol/L ($P<0.05$) and this was accompanied by a decrease in total body epinephrine spillover from 0.50 ± 0.05 to 0.35 ± 0.04 nmol/min ($P<0.05$). In the control group, arterial plasma epinephrine had not decreased after 3 months. In addition, the increment of arterial plasma epinephrine during lower body negative pressure at -40 mmHg was attenuated during atenolol. Atenolol had no effect on total body and forearm norepinephrine spillover rates, neither at rest, nor during lower body negative pressure. Clearance rates of epinephrine and norepinephrine were not significantly affected by atenolol.

These results suggest that treatment of patients with primary hypertension with the β_1-adrenoceptor blocker atenolol inhibits the adrenomedullary secretion of epinephrine, while it does not affect the biochemical indices of sympathoneural activity. It remains speculative whether this selective effect of atenolol on epinephrine secretion contributes to its hypotensive action and to its cardioprotective effects on the long term.

Introduction

Since a long time, β_1-adrenoceptor blocking agents have been widely used as effective antihypertensive drugs. The mechanism how they reduce blood pressure is still incompletely understood. The antihypertensive action is presumed to be dependent on a competitive antagonism with endogenous catecholamines at the β-adrenoceptor sites. These β-adrenoceptors are found pre- and postsynaptically in many tissues, like the

heart, the brain, the adrenal medulla and the arterial resistance arteries. Several mechanisms have been suggested to be implicated in the blood pressure reduction during β_1-blockade: resetting of the baroreflex, central nervous system mechanisms, decreased peripheral sympathetic discharge due to presynaptic β-receptor inhibition, and reduction of cardiac output [1,2]. Chronic treatment of patients with hypertension with β_1-blockers might have an effect on sympathetic nervous system activity but plasma norepinephrine (NE) levels are unsuited for a reliable assessment of sympathetic nervous system activity. Plasma NE levels during chronic treatment have been reported to be increased, unchanged or decreased [1]. Plasma catecholamine levels are determined both by their spillover into the circulation as by their clearance from the circulation. The clearance, depending on cardiac output, might be affected by β_1-adrenoceptor blocking agents. To assess the effects of β_1-blockade on catecholamine spillover and clearance rates, the isotope dilution method can be used [3]. Previous studies, reporting on NE kinetics during chronic β-blockade, showed decreased or unchanged NE spillover rates, or decreased NE clearance rates [4,5]. Epinephrine (EPI) kinetics during β_1-blockade have never been examined in humans. In this study we investigated the effects of three months treatment of patients with primary hypertension with atenolol on clearance and sympathoneural and adrenomedullary spillover of NE and EPI. This was carried out both at rest and during sympathoadrenal stimulation by lower body negative pressure (LBNP).

Subjects and methods

Subjects

Nineteen patients with primary hypertension (12 males and 7 females; mean ± SD age 40.9 ± 5.2 years) participated in the study. All participants had a normal physical examination before entry in the study. Secondary hypertension was excluded according to standard clinical criteria, and all subjects had a normal renal function. The mean ± SD Quetelet index was 25.2 ± 2.2 kg · m^{-2}. Thirteen of these nineteen participants used antihypertensive medication which was withdrawn at least four weeks before the study. After stopping the antihypertensive medication blood pressure was measured three times at 2-week intervals. The mean ± SD basal systolic/diastolic blood pressure before treatment with atenolol was 156 ± 16/100 ± 8 mmHg and the heart rate was 70 ± 11 bpm. Four normotensive subjects (mean ± SD basal systolic/diastolic blood pressure:125 ± 6/82 ± 5 years) served as a control group (mean ± SD age 45.9 ± 2.1 years). All subjects gave their written informed consent. The study protocol was approved by the Hospital Ethics Committee.

Study protocol

All patients with hypertension were studied twice: before and after treatment with atenolol for three months. Each patient started with atenolol at 50 mg a day and blood pressure was recorded every three weeks. The dose was increased to 100 mg a day after six weeks unless heart rate had decreased by more than 25% or below 45 beats per minute. After three months 8 subjects were taking 50 mg atenolol a day and eleven subjects were taking 100 mg atenolol a day. The control group of four normotensive subjects were also studied twice with an interval of 3 months. This group was enclosed to control for a time effect, so they did not receive atenolol. However in this group we only studied the reproducibility of the arterial plasma catecholamine levels and we did not apply the tracer infusions in this group.
On each study day the subjects were allowed a light breakfast. All participants were required to abstain from alcohol, nicotine, and caffeinated foods and beverages for at least 24 h before each study day. All studies were carried out in the morning in a temperature-controlled room. During the study the subjects remained supine in a LBNP box that was used to stimulate sympathoadrenomedullary activity. After instrumentation, radiotracer infusions (see below) were started and the subjects rested for 30 minutes. During the last three minutes, baseline recordings of blood pressure, heart rate and forearm blood flow were obtained. Then arterial and venous blood samples were drawn simultaneously to determine plasma concentrations of endogenous and tritiated catecholamines. Thereafter, LBNP was applied at -15 mmHg for 15 minutes. Blood pressure, heart rate and forearm blood flow recordings and blood samples were collected in sequence beginning after 12 minutes of LBNP. A rest period of 30 minutes ensued and then another 15 minutes of LBNP at -40 mmHg was applied, and blood pressure, heart rate and forearm blood flow recordings and blood samples were obtained as before.

Procedures

A brachial artery was cannulated with a cannula to monitor blood pressure and heart rate (Hewlett Packard GmbH, Böblingen, Germany) and to draw arterial blood samples. An intravenous catheter was inserted into a deep brachial vein in the ipsilateral arm to collect venous blood samples. A forearm venous catheter in the contralateral arm was used for simultaneous infusion of ^{3}H-NE and ^{3}H-EPI. Forearm blood flow was recorded in the arm with the sampling catheters, by venous occlusion strain-gauge plethysmography with air-filled cuffs [6]. During the measurement of forearm blood flow and the sampling of the blood samples, the hand circulation was excluded by inflation of a wrist cuff to 100 mmHg above systolic blood pressure [7].

Radiotracer infusion

^{3}H-NE (levo-[ring-2,5,6-^{3}H]-NE and ^{3}H-EPI (levo-[N-methyl-^{3}H]-EPI, with high specific activity, were infused intravenously to assess catecholamine kinetics. Tritiated catecholamines were obtained from Du Pont New England Nuclear ('s Hertogenbosch, the Netherlands), sterilized using a micropore filter (0.22 μm) and diluted in NaCl 0.9%, containing acetic (0.2 mol/l) and ascorbic (1 mg/ml) acid. The vials were stored until usage at -80 °C for maximal 3 months. Sterilization, dilution and storage took place under nitrogen. Just prior to the study an aliquot of each radiotracer was diluted in normal saline.
After a bolus injection of each radiotracer at 15 μCi/m^2, both tracers were infused for 90 minutes at a continuous rate of 0.35 μCi/m^2/min. The weight of the two syringes containing the radiotracers was measured before and after the infusion, to verify the infusion rate. Samples of the infusate were taken at the end of the infusion and stored at -80 °C until assayed.

Analytical methods

The blood samples were collected in prechilled tubes containing 0.25 mol/l EGTA and 0.2 mol/l glutathione in distilled water (pH=7.4). The blood samples were placed on melting ice. Plasma was separated by refrigerated centrifugation and frozen until assayed, which occurred within two months after collection. The samples were analyzed for concentrations of both unlabelled and tritium-labelled NE and EPI, using high performance liquid chromatography (HPLC) with fluorometric detection after selective precolumn derivatization of the catecholamines with the fluorescent agent 1,2- diphenylethylenediamine [8]. Using a Gilson fraction collector (model 201-202), connected to an automatic sample injector (Wisp 710B), we collected ^{3}H-NE and ^{3}H-EPI into scintillation vials, starting at the beginning of the peaks of NE and EPI in the standard mixture.

Data analysis

Forearm vascular resistance (FVR) was calculated by dividing mean arterial blood pressure (MAP) by forearm blood flow (FBF) and was expressed in Arbitrary Units (AU). The average of the hemodynamic data during three minutes was calculated.
The clearance rate of NE (L/min) from arterial plasma was calculated by dividing the infusion rate of ^{3}H-NE (dpm/min) by the steady state arterial plasma concentrations of ^{3}H-NE (dpm/L). Total body NE spillover rate (nmol/min), the estimated rate of appearance of endogenous NE in arterial plasma, was calculated by multiplying the steady state arterial plasma NE concentration (nmol/L) by the clearance (L/min). Analogously, NE spillover in the forearm was estimated as:

$$\text{Forearm NE spillover (pmol/min per 100 ml)} = FPF \times NE_a \times f + [FPF \times (NE_a - NE_v)]$$

where FPF is forearm plasma flow (ml/min per 100 ml), NE_a is arterial plasma norepinephrine (nmol/L), NE_v is venous plasma norepinephrine (nmol/L) and f is the fractional extraction = (^{3}H-NE_a – ^{3}H-NE_v) / ^{3}H-NE_a. The forearm plasma flow was calculated from the forearm blood flow and hematocrit. The clearance of NE from the forearm (ml/min per 100 ml) was calculated by multiplying the forearm plasma flow by the fractional extraction. The clearance of EPI from arterial plasma and the estimated rate of appearance of endogenous EPI into arterial plasma were calculated according to similar formulas.

Results are expressed as mean ± SEM unless indicated otherwise. To test the effects of atenolol on baseline plasma kinetic variables, the Wilcoxon signed rank test was performed. This test was also used to test for the effects of LBNP and to compare the responses to LBNP before and during atenolol. A P-value of less than 0.05 (two-sided) was considered to be significant.

Results

Haemodynamic data

After treatment with atenolol, systolic (SBP) and diastolic (DBP) blood pressure decreased significantly from 166 ± 4/89 ± 2 to 146 ± 4/75 ± 3 ($P<0.05$). Heart rate decreased from 60 ± 3 to 49 ± 2 ($P<0.05$). Forearm blood flow (FBF) and forearm vascular resistance (FVR) were not significantly affected by atenolol (1.91 ± 0.25 and 1.72 ± 0.22 ml/min/100 ml and 81.4 ± 8.7 and 80.9 ± 9.4 AU respectively).

LBNP at -15 mmHg had no effects on SBP, DBP and HR and this was the case both before and after atenolol. The increase in FVR at LBNP of -15 mmHg was not affected by atenolol. The blood pressure responses to LBNP at -40 mmHg were not altered, while the increase in HR was impaired (+8 ± 1 before and +5 ± 1 beats/minute after atenolol ($P<0.05$). The increase in FVR to LBNP at -40 mmHg was similar before and after atenolol.

Plasma epinephrine kinetics

After treatment with atenolol, the subjects had an approximately 35% lower basal arterial plasma EPI than before treatment (Table 1). This decrease in arterial plasma EPI was accompanied by a significant decrease in total body EPI spillover (Fig 1), while total body clearance and forearm clearance of EPI were not altered by atenolol (Table 1). In the control group we could not demonstrate a decrease in basal arterial plasma EPI when the subjects were restudied after 3 months.

The responses of total body EPI spillover and clearance and of forearm EPI clearance to LBNP at -15 mmHg were not affected by atenolol. However, atenolol did attenuate the

increase in total body EPI spillover to LBNP at -40 mmHg (+0.77 ± 0.26 nmol/min before and 0.21 ± 0.04 nmol/min after atenolol (P<0.05) (Fig 2), whereas the responses of total body and forearm clearance rates were not affected by atenolol. The attenuated response in total body EPI spillover to LBNP at -40 mmHg after atenolol was reflected by an attenuated increase in arterial plasma EPI concentration to LBNP at -40 mmHg after atenolol although the difference was not significant (+0.52 ± 0.14 nmol/L before and +0.23 ± 0.03 nmol/L after atenolol, P=0.11).

Plasma norepinephrine kinetics

Baseline arterial plasma NE, total body NE spillover and total body NE clearance were not affected by treatment with atenolol. Also forearm NE spillover (Fig 1) and forearm NE clearance were not changed by atenolol (Table 1). The responses of forearm NE spillover and total body NE spillover as well as forearm clearance and total body clearance of NE to LBNP at -15 mmHg and at -40 mmHg were not altered by atenolol.

Table 1. Plasma levels and kinetics of epinephrine and norepinephrine before and after treatment with atenolol.

	Before atenolol		After atenolol
Epinephrine			
Arterial plasma level (nmol/L)	0.23 ± 0.02	*	0.17 ± 0.01
Total body spillover (nmol/min)	0.50 ± 0.05	*	0.35 ± 0.04
Total body clearance (L/min)	2.20 ± 0.11		2.10 ± 0.12
Forearm clearance (ml/100ml/min)	0.83 ± 0.09		0.69 ± 0.07
Norepinephrine			
Arterial plasma level (nmol/L)	0.98 ± 0.10		0.92 ± 0.09
Total body spillover (nmol/min)	1.82 ± 0.16		1.75 ± 0.26
Total body clearance (L/min)	1.95 ± 0.13		1.85 ± 0.11
Forearm spillover (pmol/100ml/min)	0.87 ± 0.09		0.82 ± 0.10
Forearm clearance (ml/100ml/min)	0.78 ± 0.08		0.63 ± 0.06

* P<0.05; Mean ± SEM are given

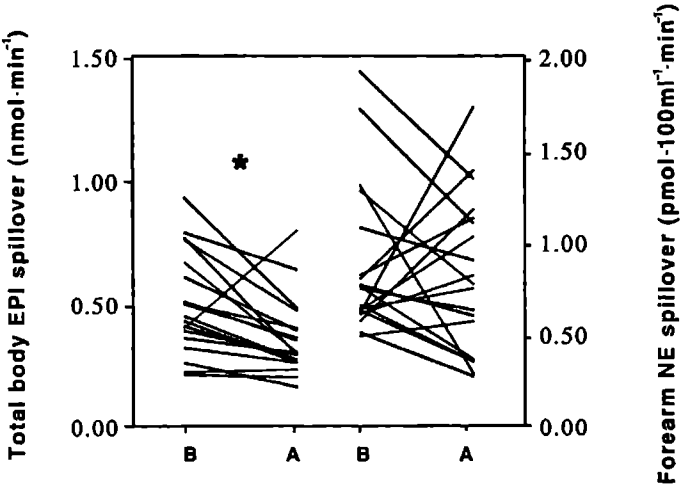


Figure 1. Individual values of total body epinephrine (EPI) spillover (nmol/min) and forearm norepinephrine (NE) spillover before (B) and after (A) three months treatment with atenolol. * P<0.05

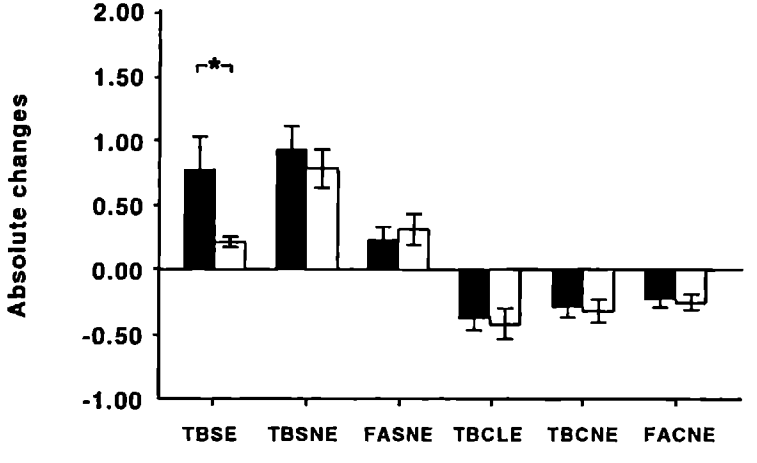


Figure 2. The responses of total body spillover of epinephrine (TBSE, nmol/min) and norepinephrine (TBSNE, nmol/min), forearm spillover of norepinephrine (FASNE, pmol/100ml/min), total body clearance of epinephrine (TBCLE, l/min) and norepinephrine (TBCNE, l/min) and forearm clearance of norepinephrine (FACNE, ml/100 ml forearm volume/min) to lower body negative pressure at -40 mmHg before (black bars) and after (open bars) chronic treatment with atenolol. Mean ± SEM are given.

Discussion

The principal finding of the present study is that chronic treatment with the β_1-adrenoceptor blocking agent atenolol decreases total body EPI spillover, while it does not affect total body NE spillover. The plasma clearance rates of both NE and EPI are not decreased by atenolol. Since the total body EPI spillover reflects the release of EPI from the adrenal medulla, these data imply that chronic β_1-adrenergic blockade attenuates adrenomedullary EPI release, but does not affect sympathoneural activity. In addition, EPI and NE spillover and clearance during sympathoadrenal stimulation by LBNP at -15 mmHg is not affected by atenolol, whereas the adrenomedullary secretion of EPI during LBNP at -40 mmHg is attenuated. So, chronic administration of atenolol does reduce baseline as well as stimulated release of EPI from the adrenal medulla in patients with primary hypertension.

Several mechanisms should be discussed to explain the differentiated response of the sympathetic nervous system during longterm treatment with atenolol, leading to diminished EPI release from the adrenal medulla with a concomitant unchanged NE spillover. First, diminished adrenomedullary EPI release might be a direct effect of blockade of facilitatory β_1-adrenoceptors on the adrenomedullary chromaffin cell membranes, as was shown in rat adrenal medulla cells in vitro [9]. In contrast, the presynaptic β-adrenoceptors at the sympathetic nerve endings appear to be of the β_2-subtype and therefore the NE spillover is not expected to decrease during atenolol administration [2]. Second, a decreased circulating EPI concentration might result from a decreased sympathetic impulse nerve traffic to the adrenal medulla. This however, would not be consistent with the unchanged NE spillover, unless atenolol would affect exclusively the sympathetic outflow to the adrenal medulla. Finally, it could be possible that the decreased arterial plasma EPI levels and the decreased EPI release from the adrenal medulla could be a time effect, or be just the expression of an attenuated defense reaction to the stress of the experimental procedure. However, the plasma EPI levels in the control group were not reduced at the second study after three months, although this also involved an arterial cannulation. Although we have no kinetic data in this control group, it is highly unlikely that the reduced arterial plasma EPI level after three months is aspecific and not related to the atenolol treatment.

Data on catecholamine spillover and clearance rates during chronic β_1-adrenergic blockade in human hypertension are not available from previous studies. EPI kinetics during β-blockade in humans has never been studied at all. NE kinetics were studied after acute administration of β_1-selective [5] and non-selective β-blockers [4] or chronic administration of non-selective β-blockers [10]. These data are hard to compare with our data on chronic β_1-blockade, since acute administration of β-blocking agents or use of non-selective β-blockade show important differences with regard to sympathetic nervous activity, organ blood flow or β-receptor occupation, thus influencing catecholamine kinetics.

Acute administration of a selective or non-selective β-blocker causes a reduction of cardiac output and a baroreflex-mediated increase in total peripheral resistance, whereas peripheral resistance generally returns to the pretreatment level during chronic β-blockade [1]. The increased intraneuronally measured muscle sympathetic activity after a bolus injection with a β_1-blocker is restored during chronic β_1-blockade [11,12] Moreover, baroreflex sensitivity is increased in chronic β-blocker treatment but not in acute administration [13,14]. In addition to baroreceptor-mediated effects on sympathetic outflow, catecholamine kinetics are influenced at the synaptic level by local blood flow, since catecholamine clearance is blood-flow-dependent [5]. A non-selective β-blocker has different sites of actions than a β_1-selective blocker, which may affect catecholamine kinetics as well. NE release from the sympathetic nerve terminals is modulated by presynaptic β_2-adrenoceptors, which can be blocked by a non-selective blocker, but probably not by β_1-selective blockers. Also, NE clearance is more β_2-adrenoceptor dependent than β_1-adrenoceptor dependent, since in rats NE clearance is decreased by propranolol but not by atenolol [15]. In a study with intra-arterial β-blocker infusions, thus preventing systemic hemodynamic effects, both metoprolol and propranolol decreased NE spillover [5]. There are however no inhibitory β_1-adrenoceptors on sympathetic nerve terminals. Alternatively it remains possible that the high local dose of metoprolol that was used was not β_1-selective anymore.

The EPI spillover response to LBNP at -40 mmHg was attenuated, though the NE spillover response was not changed. The neurohumoral responses to sympathoadrenal stimulation during β_1-adrenoceptor blockade have never been assessed in previous studies. Our data on NE kinetics confirm and extend earlier observations using microneurography that demonstrated that responses of sympathetic nerve traffic to graded LBNP were not attenuated by propranolol [16].

In conclusion, the results of this study suggest that treatment of patients with primary hypertension with the β_1-adrenoceptor blocker atenolol inhibits the basal and stimulated adrenomedullary secretion of EPI, while it does not affect the biochemical indices of sympathoneural activity. It remains speculative whether this selective effect of atenolol on EPI secretion contributes to its hypotensive action and to its cardioprotective effects on the long term.

References

1. Man in t Veld AJ , Schalekamp MADH. Effects of 10 different β-adrenoceptor antagonists on hemodynamics, plasma renin activity, and plasma norepinephrine in hypertension: the key role of vascular resistance changes in relation to partial agonist activity. J Cardiovasc Pharmacol 1983;5(suppl 1):S30-S45.
2. Langer SZ, Cavero I, Massingham R. Recent developments in noradrenergic neurotransmission and its relevance to the mechanism of action of certain antihypertensive drugs. Hypertension 1980;2:372-382.

3. Esler M, Leonard P, O'Dea K, Jackman G, Jennings G, Korner P. Biochemical quantification of sympathetic nervous activity in humans using radiotracer methodology. fallibility of plasma noradrenaline measurements. J Cardiovasc Pharmacol 1982;4.S152-S157.
4. Rosen SG, Supiano MA, Perry TJ, Linares OA, Hogikyan RV, Smith MJ, Halter JB β-adrenergic blockade decreases norepinephrine release in humans. Am J Physiol 1990;258:E999-D1005.
5. Chang PC, Grossman E, Kopin IJ, Goldstein DS On the existence of functional beta-adrenoceptors on vascular sympathetic nerve endings in the human forearm. J Hypertension 1994,12 681-690.
6. Brakkee AJM, Vendrik AJH. Strain gauge plethysmogaphy, theoretical and practical notes on a new design J Appl Physiol 1966;21:701-704
7. Lenders JMW, Janssen GJ, Smits P, Thien Th. Role of the wrist cuff in forearm plethysmography. Clin Sci 1991,80:413-417.
8. Willemsen JJ, Ross HA, Jacobs MC, Lenders JWM, Thien Th, Swinkels LMJW, Benraad TJ. Highly sensitive and specific HPLC with fluorometric detection for determination of plasma epinephrine and norepinephrine applied to kinetic studies in humans Clin Chem 1995,41·1455-1460
9. Gutman Y, Boonyavroj P Activation of adrenal medulla adenylate cyclase and catecholamine secretion. Naunyn-Schmiedeberg's Arch Pharmacol 1979,307·39-44.
10. Esler M, Jackman G, Leonard P, Skews H, A Bobik, G Jennings. Effect of propranolol on noradrenaline kinetics in patients with essential hypertension Br J Clin Pharmac 1981;12·375-380.
11. Sundlof G, Wallin BG, Stromgren E, Nerhed C Acute effects of metoprolol on muscle sympathetic activity in hypertensive humans. Hypertension 1983;5 749-756
12 Wallin BG, Sundlof G, Stromgren E, Åbeg H Sympathetic outflow to muscles during treatment of hypertension with metoprolol. Hypertension 1984;6:557-562.
13. Parati G, Mutti E, Frattola A, Castiglioni P, di Rienzo M, Mancia G β-Adrenergic blocking treatment and 24-hour baroreflex sensitivity in essential hypertensive patients. Hypertension 1994;23 .992-996.
14. Watson RDS, Stallard TS, Littler WA Effects of beta-adrenoceptor antagonists on sinoaortic baroreflex sensitivity and blood pressure in hypertensive man Clin Sci 1979,57.241-247.
15. Kent Keeton T, Biediger AM. Propranolol and atenolol inhibit norepinephrine spillover rate into plasma in conscious spontaneously hypertensive rats. Naunyn-Schmiedeberg's Arch Pharmacol 1991;344·47-55.
16. Jacobsen TN, Converse RL, Victor RG Contrasting effects of propranolol on sympathetic nerve activity and vascular resistance during orthostatic stress. Circulation 1992;85 1072-1076.

Chapter 8

General discussion and conclusions

Over the past decades a large amount of research has been carried out to clarify the role of the sympathetic nervous system in the pathogenesis of primary hypertension. An increased sympathetic reactivity or responsiveness to a variety of stimuli was presumed to contribute to the pathophysiology of hypertension. However, it appeared extremely difficult to measure sympathetic nervous system activity accurately because the system has a widespread distribution with a non-uniform and a highly variable outflow. In addition, it is affected by numerous circulating and local interfering compounds at the site of neurotransmission in the target organ or at a central level.

In the studies described in this thesis we assessed sympathetic nervous system activity by using the isotope dilution technique. This means that steady state infusions with tracer-labeled catecholamines are required. Plasma concentrations of epinephrine (EPI), norepinephrine (NE) and the radiolabeled NE and EPI concentrations have to be measured by an assay technique that is sensitive and specific enough to measure simultaneously the very low plasma concentrations of these compounds. This enables the calculation of spillover and clearance rates of the endogenous catecholamines. The catecholamine kinetics can be calculated for the whole body without taking into account the contribution of specific organs. From arterial and venous plasma samples and the blood flow through a specific organ like for instance the human forearm, one can calculate the local kinetics of the forearm i.e. the skeletal muscles. The EPI secretion by the adrenal medulla can be assessed by calculation of the total body EPI spillover.

In chapter 2 we describe a modification of a specific and sensitive HPLC-method with fluorometric detection for the analysis of plasma concentrations of endogenous EPI and NE and of tritiated EPI and NE. The detection limits for EPI and NE are 3.2 and 2.2 pmol/L respectively. The detection limit of ^{3}H-NE and ^{3}H-EPI is 6 dpm. Particularly the sensitivity for EPI is considerably higher than that can be attained with other analytical methods like HPLC with electrochemical detection.

Sympathetic and adrenomedullary activity have to be assessed not only in the basal state but also during sympathetic stimulation. In the present studies we used the lower body negative pressure (LBNP) technique to induce sympathetic stimulation. The applied

negative pressure to the lower body evokes a shift of blood from the upper to the lower part of the body. The decreased venous return and the subsequent decreased cardiac filling pressures will unload the cardiopulmonary and/or arterial baroreceptors, depending on the intensity of the applied negative pressure. The ensuing increase in sympathetic activity will lead to vasoconstriction, thereby preserving the arterial blood pressure level. At a LBNP intensity of less than -20 mmHg, cardiopulmonary baroreceptors will be unloaded selectively whereas at a LBNP intensity of more than -20 mmHg, both cardiopulmonary and arterial baroreceptors will be unloaded. Application of different levels of LBNP makes it possible to examine selectively the effects of unloading of cardiopulmonary baroreceptors.

In chapter 3 we describe the effects of LBNP on catecholamine kinetics in normotensive subjects. Cardiopulmonary baroreceptor unloading was achieved by non-hypotensive LBNP at -15 mmHg, and combined arterial and cardiopulmonary baroreceptor deactivation was attained by hypotensive LBNP at -40 mmHg. Forearm NE spillover and total body EPI spillover increased during LBNP at -15 mmHg but total body NE spillover did not change. During LBNP at -40 mmHg forearm NE spillover, total body EPI spillover, and total body NE spillover increased. Clearances of NE and EPI were unchanged during LBNP at -15 mmHg but decreased during LBNP at -40 mmHg. Apparently, selective deactivation of the cardiopulmonary baroreceptors during non-hypotensive LBNP increases sympathoneural activity to forearm skeletal muscles and increases adrenomedullary activity without a generalized increase in sympathoneural activity. Simultaneous deactivation of cardiopulmonary and arterial baroreceptors during hypotensive LBNP elicited a generalized increase in sympathoneural activity, concomitant with a further increase in adrenomedullary activity. Our data indicate that forearm sympathoneural and adrenomedullary outflow are increased during decreases in cardiac filling pressures, with augmented and more generalized responses to systemic hypotension.

During the pilot studies it appeared that several subjects were prone to vasodepressor syncope during prolonged hypotensive LBNP at -40 mmHg. The neurohumoral regulation of such an unexpected event is poorly understood because of its suddenness. Chapter 4 deals with the sympathoneural and adrenomedullary regulation in the time period that precedes a vasodepressor syncope. Healthy normotensive subjects underwent 30 minutes of low reduction of cardiac filling during non-hypotensive LBNP at -15 mmHg, followed by 30 minutes of more pronounced reduction of cardiac filling during hypotensive LBNP at -40 mmHg. Non-hypotensive LBNP at -15 mmHg was well tolerated by all participants without any symptom, whereas hypotensive LBNP at -40 mmHg provoked a presyncope in 11 of the 26 subjects, with sudden hypotension, nausea and dizziness. In the latter group, forearm NE spillover failed to increase during LBNP at -15 mmHg, whereas the arterial plasma EPI response to LBNP at -15 mmHg was exaggerated. These findings suggest that a neuroendocrine pattern of decreased sympathoneural activity and increased adrenomedullary activity precedes a vasodepressor

syncope. The cause of the exaggerated plasma EPI responses has to be elucidated but is probably of central origin. A causal relationship between the enhanced adrenomedullary activity and the subsequent syncope has to be examined by further research.

In chapter 5 we describe the NE and EPI kinetics in 47 patients with primary hypertension and 43 normotensive control subjects, at rest and during LBNP at -15 and -40 mmHg. Hypertensives showed a slightly higher basal arterial EPI concentration than the normotensive controls. We could demonstrate that the increased circulating arterial EPI concentration is due to an increased adrenomedullary release of EPI and not to diminished clearance. Venous and arterial NE concentrations at rest, as well as NE spillover in the forearm and in the whole body were similar in hypertensives and normotensives. The noradrenergic and adrenergic responses to cardiopulmonary and arterial baroreceptor unloading were similar in the two groups. These results confirm and extend earlier reports on increased plasma EPI concentrations in hypertension. Increased circulating EPI concentrations in hypertensives provide support for the "EPI hypothesis". In the EPI hypothesis it has been suggested that circulating EPI may induce hypertension by stimulating prejunctional β_2-adrenoceptors and thus facilitating NE release from the sympathetic nerve terminals. On the longterm, this could lead to hypertension by increasing peripheral vascular resistance. Our data could provide some support for the EPI hypothesis despite the absence of increased NE spillovers in the hypertensives. It should be kept in mind that the absence of an increased NE spillover into the circulation does not prove that there is no increase in neuronal NE release.

Two subgroups of hypertensive patients were studied before and after treatment with an α_1-adrenoceptor antagonist or a β_1-adrenoceptor antagonist for three months. These results are described in chapter 6 and 7 respectively. Eleven patients were treated with the selective α_1-blocker doxazosin. Doxazosin lowers blood pressure by reducing peripheral vascular resistance without concomitant tachycardia. The absence of reflex tachycardia seems paradoxical to the vasodilation-induced sympathetic activation. Our catecholamine kinetic studies demonstrated that arterial plasma NE increased as a consequence of increased NE spillover in the forearm and in the whole body. In contrast, arterial plasma EPI concentrations and EPI spillovers were not affected by doxazosin. Apparently, the vasodilation induced a reflex-mediated increase in sympathoneural outflow but not in adrenomedullary activity. The absence of tachycardia might be explained by the absence of an adrenomedullary response. It remains to be seen whether the increased plasma levels of NE that are found during chronic α_1-adrenoceptor blockade are innocent on the longterm.

Nineteen patients were treated with the β_1-adrenoceptor antagonist atenolol. Atenolol did not affect total body and forearm spillover rates of NE, whereas arterial plasma EPI levels and total body EPI spillover rate were reduced. The decreased circulating EPI concentrations might result in attenuation of presynaptic β_2-adrenergic facilitation of neuronal NE release. It remains speculative whether this selective effect of atenolol on

EPI secretion contributes to its hypotensive action and to its cardioprotective effects on the longterm.

Conclusions

1. The excellent sensitivity and specificity of high performance liquid chromatography with fluorometric detection for the measurement of low physiological concentrations of norepinephrine and epinephrine, combined with simultaneous measurement of tritiated norepinephrine and epinephrine, permits reliable assessment of norepinephrine and epinephrine kinetics in clinical studies.
2. Cardiopulmonary baroreceptor deactivation elicits a selective increase in sympathetic outflow to skeletal muscles and adrenal medulla.
3. Arterial baroreceptor deactivation in combination with cardiopulmonary baroreceptor deactivation evokes a diffuse increase in sympathetic outflow to the whole body.
4. Normotensive subjects, prone to vasodepressor syncope during lower body negative pressure at -40 mmHg, show adrenomedullary activation and sympathoneural inhibition in response to decreased cardiac filling. This abnormal neurohumoral regulation can already be demonstrated in the absence of any symptoms indicative of an imminent syncope.
5. Patients with mild primary hypertension have increased arterial plasma epinephrine levels and this can be ascribed to an increased adrenomedullary release and not to diminished plasma clearance of epinephrine.
6. Normal plasma norepinephrine concentrations in patients with mild hypertension are due to normal spillover and normal clearance of norepinephrine, suggesting normal sympathoneural activity in mild primary hypertension.
7. During treatment with the α_1-selective adrenoceptor antagonist doxazosin, sympathoneural outflow is increased without a concomitant adrenomedullary activation, indicating vasodilation-induced and baroreflex-mediated sympathoneural activation. The absence of adrenomedullary activation might explain the paradoxal absence of reflex tachycardia.
8. The selective β_1-adrenoceptor antagonist atenolol has no effect on the plasma norepinephrine concentration or on total body spillover of norepinephrine, but it reduces plasma epinephrine levels by reducing total body epinephrine spillover. It is unclear whether this selective effect of atenolol on epinephrine secretion contributes to its hypotensive action and to its cardioprotective effects on the longterm.

Chapter 9

Algemene discussie en conclusies

De laatste decennia is er veel onderzoek verricht naar de rol van het sympatisch zenuwstelsel in de pathogenese van verhoogde bloeddruk ofwel primaire hypertensie. Het sympatisch zenuwstelsel speelt een belangrijke rol bij de regulatie van de bloeddruk en het is dus niet verwonderlijk dat men veronderstelde dat een verhoogde activiteit of reactiviteit van het sympatisch zenuwstelsel zou kunnen bijdragen aan het ontstaan of zelfs de oorzaak zou kunnen zijn van een verhoogde bloeddruk. In de loop der jaren zijn er inderdaad nog al wat aanwijzingen gevonden dat er mogelijk iets mis is met de functie van het sympatisch zenuwstelsel, met name bij jonge patiënten met een verhoogde bloeddruk. Er zijn twee belangrijke redenen waarom het nog steeds onduidelijk is of een abnormale sympaticusactiviteit wel een directe oorzaak is van een verhoogde bloeddruk. In de eerste plaats is het praktisch onmogelijk om bij de mens een oorzaak-gevolg relatie onomstotelijk vast te stellen. Het is niet uitgesloten dat een abnormale sympaticus-activiteit niet de oorzaak maar een gevolg is van de verhoogde bloeddruk. In de tweede plaats is de activiteit van het sympatische zenuwstelsel buitengewoon variabel waarbij er bovendien geen gelijke activiteitsverdeling is over de verschillende organen. Sympaticusactiviteit wordt gegenereerd in het centrale zenuwstelsel en de meeste beschikbare meet-methoden richten zich juist op de activiteit in het perifere deel van het zenuwstelsel. Het is derhalve moeilijk om een representatieve maat te vinden voor de globale activiteit van het systeem.

In dit proefschrift staat één biochemische onderzoeksmethode om sympaticusactiviteit te meten centraal, namelijk de zogenaamde isotoopdilutie methode. Bij deze techniek wordt gebruik gemaakt van 'steady-state' infusies van radioactief-gemerkte catecholamines. Met behulp van een gevoelige bepalingsmethode kunnen zowel endogene als radioactief-gemerkte catecholamines gemeten worden, waarna het mogelijk is om zowel de klaring als de spillover van catecholamines in de bloedbaan te berekenen. Zo kunnen als kinetische parameters niet alleen de totale lichaamsklaring en spillover berekend worden, maar is het ook mogelijk deze parameters te berekenen voor een specifiek orgaan, indien zowel arteriële als veneuze bloedmonsters afgenomen worden en de doorbloeding van dit orgaan gemeten wordt. In dit proefschrift zijn deze parameters

gemeten in het onderarmsvaatbed. Als radioactief-gemerkte catecholamines zijn ^{3}H-noradrenaline en ^{3}H-adrenaline gebruikt. De noradrenaline-kinetiek geeft specifieke informatie over de activiteit van het sympatische zenuwstelsel terwijl de adrenaline-kinetiek specifieke informatie geeft over de activiteit van het bijniermerg.
Om niet alleen de basale activiteit, maar ook de reactiviteit van het sympatisch zenuwstelsel inclusief bijniermerg te kunnen meten moet het sympatische zenuwstelsel gestimuleerd worden. In het onderzoek dat in dit proefschrift beschreven is hebben wij als stimulus van het sympatische zenuwstelsel gebruik gemaakt van de LBNP ("lower body negative pressure")-methode. Hierbij wordt een negatieve druk aangebracht rond het onderlichaam, waardoor er een redistributie van het bloed plaatsvindt: bloed verplaatst zich van de bovenste naar de onderste lichaamshelft. Door de verminderde veneuze terugstroom van bloed naar het hart zullen, afhankelijk van de intensiteit van de negatieve druk, de cardiopulmonale of cardiopulmonale en arteriële baroreceptoren gedeactiveerd worden. Dit leidt tot een toename van sympaticusactiviteit en de hierdoor teweeggebrachte vasoconstrictie zal helpen de bloeddruk te stabiliseren. Bij een negatieve druk van lager dan -20 mmHg zullen specifiek de cardiopulmonale baroreceptoren gedeactiveerd worden, terwijl bij een negatieve druk van hoger dan -20 mmHg zowel cardiopulmonale als arteriële baroreceptoren gedeactiveerd worden. Toepassing van LBNP met verschillende graden van intensiteit maakt het dus mogelijk om selectief het effect van deactivatie van cardiopulmonale baroreceptoren te onderzoeken.
In hoofdstuk 2 beschrijven wij een modificatie van een specifieke en sensitieve HPLC-bepaling met fluorimetrische detectie om lage plasma concentraties van endogene en radioactief-gemerkte catecholamines gelijktijdig te kunnen meten. De detectiegrens voor endogeen (ongemerkt) noradrenaline en adrenaline is respectievelijk 2.2 en 3.2 pmol/L. In het bijzonder voor adrenaline is dit een beduidend betere gevoeligheid dan die bereikt kan worden met HPLC met electrochemische detectie. De detectiegrens van radioactief-gemerkte plasma catecholamines is 6 dpm. Deze bepalingsmethodiek is dus bijzonder geschikt voor onderzoek naar de kinetiek van catecholamines.
In hoofdstuk 3 worden de effecten beschreven van LBNP op de catecholaminekinetiek bij normotensieve personen. In het bijzonder werd onderzocht of de veranderingen in catecholaminekinetiek door LBNP afhankelijk zijn van de intensiteit van de toegepaste stimulus. Selectieve deactivatie van cardiopulmonale baroreceptoren door middel van LBNP (-15 mmHg) veroorzaakte een toename van de noradrenaline spillover in de onderarm met ongeveer 50%, zonder toename van de totale lichaamsspillover van noradrenaline. Kennelijk leidt selectieve deactivatie van de cardiopulmonale baroreceptoren tot een selectieve sympaticusactivatie van de skeletspieren in de onderarm, zonder gegeneraliseerde toename van sympaticusactiviteit. Blijkens een toename van de totale lichaamsspillover van adrenaline van ongeveer 30% neemt hierbij ook selectief de secretie van adrenaline door de bijnieren toe. Effecten op de klaring van beide catecholamines werden niet waargenomen tijdens deze LBNP fase. Gecombineerde deactivatie van de cardiopulmonale en arteriële baroreceptoren tijdens LBNP van -40 mmHg leidde

wel tot een toename van de totale lichaamsspillover van noradrenaline met een verdere toename van de lokale noradrenaline spillover in de onderarm. Ook de totale lichaamsspillover van adrenaline nam verder toe terwijl de klaringen van adrenaline en noradrenaline daalden bij deze intensiteit van LBNP. Deze gegevens wijzen er op dat de arteriële baroreceptoren even belangrijk zijn voor de baroreflexcontrole van skelet-spiercirculatie als de cardiopulmonale baroreceptoren.
Tijdens de onderzoeken waarbij LBNP werd toegepast gedurende 30 minuten hadden sommige gezonde normotensieve personen een neiging tot collaberen tijdens LBNP van -40 mmHg. Deze collapsneiging had een duidelijk vasovagaal karakter: duizeligheid, misselijkheid, lage bloeddruk en polsfrequentievertraging. In hoofdstuk 4 worden de personen die wel en die geen collapsneiging hadden met elkaar vergeleken. Onze aandacht ging speciaal uit naar de catecholaminekinetiek tijdens de fase van LBNP van -15 mmHg. Bij deze lichte intensiteit van de LBNP had geen enkele proefpersoon klachten en werden ook geen afwijkingen in bloeddruk of hartfrequentie vastgesteld. Toch bleek dat de personen die later bijna collabeerden tijdens LBNP van -40 mmHg ook al afwijkingen vertoonden in de catecholaminekinetiek tijdens LBNP van -15 mmHg. Zo hadden degenen die bijna collabeerden een sterkere stijging van het plasma adrenaline tijdens LBNP van -15 mmHg dan diegenen die niet collabeerden. Het plasma noradrenaline en de lokale noradrenaline spillover in de onderarm stegen daarentegen bijna niet bij diegenen die later bijna collabeerden. Dit zou kunnen betekenen dat, voorafgaand aan een vasovagale collaps, de combinatie van een toegenomen adrenomedullaire activiteit en een verminderde sympaticusactiviteit mogelijk een rol speelt bij het ontstaan van deze reactie. Het is goed mogelijk dat de toegenomen adrenomedullaire activiteit een centrale oorzaak heeft. Verder onderzoek moet duidelijk maken of er inderdaad sprake is van een causale relatie tussen de toegenomen adrenomedullaire activiteit en het ontstaan van de collaps.
In hoofdstuk 5 worden de resultaten besproken van een onderzoek bij een grote groep patiënten met een lichte primaire hypertensie. Deze patiënten hadden basaal een hogere plasma adrenaline concentratie dan de normotensieve controlegroep. Dit bleek te berusten op een toegenomen secretie van adrenaline door de bijnieren en niet op een verminderde klaring van adrenaline. Verhoogde plasma adrenaline concentraties zijn al eerder beschreven en dit gegeven zou kunnen passen bij de door anderen geformuleerde "adrenaline-hypothese". Deze hypothese veronderstelt dat een, al dan niet intermitterend, verhoogde plasma adrenaline concentratie leidt tot stimulering van presynaptische β_2-adrenoreceptoren waardoor er een facilitatie optreedt van de neuronale noradrenaline afgifte met als uiteindelijk resultaat een toename van vasoconstrictie. Het circulerend adrenaline wordt bovendien ook opgenomen door het terminale deel van de sympatische neuronen om vervolgens weer samen met noradrenaline afgegeven te worden in de synaps waar opnieuw presynaptische β_2-adrenoreceptor stimulatie plaatsvindt. Deze vicieuze cirkel zou volgens deze hypothese dus leiden tot hypertensie. Alhoewel er sterke aanwijzingen zijn voor bepaalde delen van deze hypothese is het bewijs bij de mens nog

niet geleverd. De gevonden verhoogde plasma adrenaline concentraties bij de patiënten met hypertensie zouden deze hypothese echter kunnen steunen. Wij vonden echter geen belangrijke verschillen in noradrenalinekinetiek tussen beide groepen hetgeen suggereert dat er géén sprake is van een gegeneraliseerde of lokaal verhoogde sympaticusactiviteit in de door ons bestudeerde patiënten met hypertensie. Het ontbreken van een toename van de noradrenaline spillover in de onderarm ondergraaft de "adrenaline-hypothese" overigens niet, omdat de noradrenaline spillover in het bloed niet gelijk is aan de neuronale noradrenaline afgifte in de synaps.
In hoofdstuk 6 beschrijven wij de effecten van 3 maanden behandeling van een kleine groep hypertensiepatiënten met de selectieve α_1-adrenoreceptor antagonist doxazosine met betrekking tot de catecholaminekinetiek. Doxazosine verlaagde, zoals verwacht, de bloeddruk zonder dat er een reflectoire tachycardie werd waargenomen. Toch moet er sprake zijn van activatie van het sympatisch zenuwstelsel tijdens deze therapie, omdat na 3 maanden behandeling de plasma noradrenaline concentratie was toegenomen, hetgeen bleek te berusten op een toename van de noradrenaline spillover in zowel onderarm als in het gehele lichaam en niet op een verminderde klaring. Er was echter géén toename van de adrenomedullaire activiteit, hetgeen mogelijk verklaart waarom er geen reflectoire tachycardie werd waargenomen. Blijkbaar leidt vasodilatatie door een α_1-adrenoreceptor antagonist tot een selectieve reflectoire toename van sympaticusactiviteit maar niet van adrenomedullaire activiteit. Het is de vraag of de toegenomen plasma noradrenaline concentraties tijdens behandeling met een α_1-adrenoreceptor antagonist op de lange termijn onschadelijk zijn.
Een andere groep hypertensiepatiënten werd gedurende 3 maanden behandeld met de selectieve β_1-adrenoreceptor antagonist atenolol. In tegenstelling tot de behandeling met de α_1-adrenoreceptor antagonist doxazosine werd geen effect van atenolol waargenomen op de totale lichaamsspillover en onderarmsspillover van noradrenaline. Daarentegen bleek de plasma adrenaline concentratie gedaald, hetgeen bleek te berusten op een gedaalde adrenomedullaire secretie van adrenaline. Het is de vraag of dit selectieve effect van atenolol op de adrenalinesecretie bijdraagt aan het hypotensieve effect van deze β_1-blokker en aan het cardioprotectieve effect op de lange termijn.

Conclusies

1. De hoge sensitiviteit en specificiteit van de HPLC met fluorimetrische detectie maken deze techniek uitermate geschikt voor het meten van lage fysiologische plasma concentraties van catecholamines. In combinatie met de mogelijkheid om simultaan radioactief-gemerkte catecholamines te meten schept dit de mogelijkheid om met name kinetiekstudies van adrenaline betrouwbaar uit te voeren.

2. Cardiopulmonale baroreceptordeactivatie leidt tot selectieve sympaticusactivatie van skeletspieren en bijniermerg.
3. Simultane deactivatie van cardiopulmonale en arteriële baroreceptoren leidt tot een meer gegeneraliseerde sympaticusactivatie in het gehele lichaam.
4. Gezonde normotensieve proefpersonen die neigen tot vasovagale reacties tonen reeds een abnormale neurohumorale regulatie vóórdat er enig symptoom van een collaps aanwezig is. De abnormaal sterke stijging van de plasma adrenaline concentratie in combinatie met een verminderde sympaticusactivatie speelt mogelijk een rol bij de totstandkoming van de vasovagale reactie.
5. Patiënten met een primaire hypertensie hebben licht verhoogde plasma adrenaline concentraties en dit is het gevolg van een toegenomen adrenomedullaire adrenaline-secretie en niet van een verminderde klaring van adrenaline.
6. Patiënten met een primaire hypertensie hebben niet alleen normale plasma noradrenaline concentraties maar ook de spillover en de klaring van noradrenaline zijn ongestoord. Dit suggereert dat er bij patiënten met een primaire hypertensie sprake is van een normale sympaticusactiviteit.
7. Behandeling van hypertensiepatiënten met de α_1-adrenoreceptor antagonist doxazosine leidt tot toename van de sympatoneuronale activiteit maar niet tot toename van de adrenomedullaire activiteit. Dit verklaart mogelijk het ontbreken van een reflectoire tachycardie tijdens langdurige behandeling met dit antihypertensivum.
8. Behandeling van hypertensiepatiënten met de β_1-adrenoreceptor antagonist atenolol leidt niet tot verandering van sympatoneuronale activiteit maar wel tot afname van de adrenomedullaire activiteit. Het is onduidelijk of dit selectieve effect van atenolol op de secretie van adrenaline bijdraagt aan de hypotensieve werking en aan het cardioprotectieve effect op de lange termijn.

Bibliography

This thesis was based on the following articles:

JJ Willemsen, HA Ross, MC Jacobs, JWM Lenders, Th Thien, ThJ Benraad. Highly sensitive and specific HPLC method with fluorometric detection for determination of plasma epinephrine and norepinephrine applied to kinetic studies in humans. Clin Chem 1995;41:1455-1460

MC Jacobs, DS Goldstein, JJ Willemsen, P Smits, Th Thien, JWM Lenders. Differential effects of prolonged low- and high-intensity lower body negative pressure on noradrenaline and adrenaline kinetics in humans. Clin Sci 1995;30:337-343

MC Jacobs, DS Goldstein, JJ Willemsen, P Smits, Th Thien, JWM Lenders. Neurohumoral antecedents of vasodepressor reactions. Eur J Clin Invest 1995;25:754-776

MC Jacobs, JWM Lenders, JJ Willemsen, Th Thien. Adrenomedullary secretion of epinephrine is increased in mild primary hypertension. Hypertension (in press)

MC Jacobs, JWM Lenders, JJ Willemsen, Th Thien. Chronic α_1-adrenergic blockade increases sympathoneural but not adrenomedullary activity in patients with essential hypertension. J Hypertension 1995;13:1837-1841

MC Jacobs, JWM Lenders, P Smits, JJ Willemsen, C Tack, Th Thien. Chronic β_1-adrenergic blockade restores adrenomedullary activity in primary hypertension.

Other publications

AE Tuinenburg, M Bootsma, MJA Janssen, J de Bie, CA Swenne, MC Jacobs, JWM Lenders. Heart rate and heart rate variability during 10- and 30-minute episodes of Lower Body Negative Pressure. Computers in Cardiology 1991;p333-336, 1992

P Smits, JA Kapma, MC Jacobs, J Lutterman, Th Thien. Endothelium-dependent vascular relaxation in patients with type I diabetes mellitus. Diabetes 1993;42:148-53, 1993

GA Rongen, MC Jacobs, P Smits, Th Thien. Endothelium-dependent vasodilation in hypercholesterolaemic subjects (letter). Lancet 1993;341:490-491

MC Jacobs, JWM Lenders, JA Kapma, P Smits, Th Thien. The effect of chronic smoking on endothelium-dependent vascular relaxation in humans. Clin Sci 1993; 85:51-55

DP Veerman, CE Douma, MC Jacobs, Th Thien, GA van Montfrans. Effects of ACE inhibition on autonomic vascular control, as assessed by spectral analysis, and on haemodynamics. Blood Pressure 1994;3 (suppl 2): 92

G Vreugdenhil, GA van Montfrans, MC Jacobs, JHB de Bruijn, DP Veerman, PN van Es, B Mellein, C Guitard, Th Thien, PW de Leeuw. 24-Hour ambulatory blood pressure monitoring and spirapril in mild to severe essential hypertension: A randomized dose comparison. Blood Pressure 1994;3 (suppl 2): 23-30

JWM Lenders, HR Keiser, DS Goldstein, JJ Willemsen, P Friberg, MC Jacobs, PWC Kloppenborg, Th Thien, G Eisenhofer. Plasma metanephrines in the diagnosis of pheochromocytoma. Ann Int Med 1995;123:101-109

G Eisenhofer, B Rundquist, A Aneman, P Friberg, N Dakak, IJ Kopin, MC Jacobs, JWM Lenders. Regional release and removal of catecholamines and extraneuronal metabolism to metanephrines. J Clin Endocrinol Metab 1995;80:3009-3017

M Bootsma, CA Swenne, JWM Lenders, MC Jacobs, AVG Bruschke. Intravenous instrumentation alters the autonomic state in humans. Eur J Appl Physiol 1996;73:113-116

Curriculum vitae

Marie-Cécile Jacobs werd op 5 augustus 1963 geboren in Mechelen. Nadat zij aan het St Bernardinuscollege te Heerlen in 1982 cum laude haar gymnasium-β-diploma had gehaald, studeerde zij geneeskunde aan de Rijksuniversiteit Limburg te Maastricht. In maart 1989 behaalde zij haar arts-examen. Gedurende 12 maanden werkte zij als assistent-geneeskundige-niet-in-opleiding op de afdeling Interne Geneeskunde van het Diaconessenhuis te Eindhoven. In maart 1990 begon zij als arts-onderzoeker binnen de afdeling algemeen inwendige geneeskunde van het Academisch Ziekenhuis St Radboud te Nijmegen (Hoofd destijds: Prof. dr A. van 't Laar). Na een onderzoek naar de effecten van renine-remming bij patiënten met essentiële hypertensie, startte zij in juli 1990 met het onderzoeksproject "Het metabolisme van catecholamines: de rol van bloeddruk, geslacht en adrenoceptor-blokkerende antihypertensiva", hetgeen uiteindelijk tot deze dissertatie heeft geleid. De studies konden worden verricht dankzij een subsidie van de Nederlandse Hartstichting (no 89212). Sedert juli 1993 is zij in opleiding tot internist. De opleiding werd begonnen in het Bosch Medicentrum te 's Hertogenbosch (opleider: Dr J.Th.M. Burghouts). In december 1995 werd de opleiding voortgezet in het De Weverziekenhuis te Heerlen (opleider: Dr F.A.Th. Lustermans).

Dankwoord

Allen die hebben bijgedragen aan het tot stand komen van dit proefschrift wil ik op deze plaats hartelijk danken. Op de eerste plaats dank ik de patiënten en proefpersonen die bereid waren de belastende onderzoeken te ondergaan en het verwerven van de gegevens mogelijk maakten; zonder hen was deze studie niet mogelijk geweest.
Ik heb met veel plezier deel uitgemaakt van de circulatie-werkgroep in het St Radboud-ziekenhuis. Ik dank alle leden van deze werkgroep voor de zeer prettige en motiverende sfeer op de afdeling. Aan mijn co-promotor dr Jacques Lenders ben ik de meeste dank verschuldigd, voor zijn nooit aflatende toewijding en steun. Zijn kennis, enthousiasme, relativeringsvermogen en integriteit zijn een voorbeeld voor mij.
Prof. dr Theo Thien verstaat de kunst op de goede momenten de juiste adviezen te geven, en daarvan heb ik dankbaar gebruik gemaakt. Van onze circulatie-werkgroep vormt hij de altijd aandachtige, motiverende en opgewekte spil. Prof. dr Paul Smits prikt altijd raak, tijdens de vele discussies en ook als ik een arterie had gemist. Hij redde zodoende vele experimenten. Verder wil ik hier Marie-Louise Bartelink en Gerard Rongen noemen. Als "collega-jonge-onderzoekers" maakten we elkaars ups en downs van het doen van onderzoek mee, en zowel tijdens als buiten de reguliere werkuren hebben we een heel gezellige tijd gehad. Zeer veel dank ook aan Sjaak Willemsen. Hij zette met grote inzet de nieuwe catecholaminebepaling op en verrichtte met uiterste nauwgezetheid en toewijding alle analyses. Dr Alec Ross dank ik voor zijn steun bij het berekenen van de catecholamine-kinetiek-waarden. Verder bedank ik Joost den Arend, die altijd bereid was bij te springen als de computer weigerde. Eugenie Olde Rickerink dank ik voor haar zorg voor het reilen en zeilen op de afdeling klinische fysiologie.

Buiten het ziekenhuis zijn er een aantal mensen die tegen wil maar wel met dank bij dit proefschrift betrokken raakten. Ik dank mijn ouders, familie en vrienden voor alle geduld en medeleven in de afgelopen jaren, vooral in tijden dat weer een dead-line gehaald moest worden. Lieve Peter-Paul, jij had een heel bijzondere rol tijdens het voltooien van dit proefschrift; zonder al je zichtbare en onzichtbare hulp, was het niet gelukt.

STELLINGEN

behorende bij het proefschrift

Catecholamine Kinetics in Primary Hypertension

1. Catecholamine-kinetiek studies kunnen alleen betrouwbaar uitgevoerd worden met een zeer specifieke en sensitieve methode voor het bepalen van plasma concentraties van endogene en tritium-gelabelde catecholamines. (dit proefschrift)

2. Een verhoogde secretie van adrenaline kan voorafgaan aan een vasovagale collaps en is niet alleen een gevolg hiervan. Mogelijk speelt adrenaline een rol in de pathogenese van de vasovagale collaps. (dit proefschrift)

3. Deactivatie van cardiopulmonale baroreceptoren veroorzaakt een toename van de adrenalinesecretie door de bijnieren. (dit proefschrift)

4. Patiënten met een primaire hypertensie hebben een toegenomen adrenalinesecretie. (dit proefschrift)

5. Behandeling van primaire hypertensie met de α_1-adrenoceptor antagonist doxazosine veroorzaakt een toename van sympaticusactiviteit. (dit proefschrift)

6. Door behandeling van primaire hypertensie met de β_1-adrenoceptor antagonist atenolol neemt de secretie van adrenaline door de bijnieren af, hetgeen mogelijk bijdraagt aan het bloeddrukverlagend en cardioprotectieve effect van β_1-adrenoceptor antagonisten. (dit proefschrift)

7. Alvorens de α_1-adrenoceptor antagonisten een vaste plaats toe te kennen bij de behandeling van benigne prostaathypertrofie, dient er meer bekend te zijn over hun effecten op de bloeddruk bij de overgang van liggen of zitten naar staan.

8. In de wetenschap is het net zo belangrijk om een probleem te ontdekken, als om het op te lossen.

9. Toen Jacobus I van Engeland in 1603 uitriep: roken is een gruwel voor het oog, een walging voor de neus, schadelijk voor het verstand en gevaarlijk voor hart en longen, kon hij nog niet weten dat rokers mogelijk minder kans hebben op het krijgen van de ziekte van Alzheimer.

10. Gezien de toename van het aantal ouderen op de fiets en het verminderd waarnemen van hoge tonen bij het ouder worden, zouden racefietsen moeten worden uitgerust met een fietsbel met lage frequentie.

11. Neem nu de wetenschap; die gaat alsmaar vooruit, zodat wij steeds verder achterblijven. Olivier B. Bommel

12. Isotopenjaren tellen dubbel.

Marie-Cécile Jacobs 10 december 1996