Denervation of
carotid baro- and chemoreceptors
in humans

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carotid baro- and chemoreceptors
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The pure and simple truth is rarely pure and never simple.

(Oscar Wilde, 1854-1900)

Voor mijn ouders

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Chapter 1

General introduction and outline of the studies

Arterial baroreceptors

History

The Greek word καροτιδεσ derives from the verb καρο, to plunge into sleep. Rufus of Ephesus (98-117 AD), a physician in ancient Rome, was probably the first to describe a carotid sinus reflex (1). In his treatise *Names of the Parts of the Human Body* he wrote: "The ancients named the vessels traversing the neck, carotids, because when you press upon them, people become sleepy and speechless". However, he believed, that "this is an affliction of the sensitive nerves which lie near to the arteries, not to the arteries themselves". It took nineteen centuries to prove him wrong. Hering and Koch were the first to localize the origin of reflex changes in heart rate and blood pressure during external massage of the neck, to nerve endings in the arterial wall of the carotid bifurcation (2;3). With their experiments in the early nineteen-twenties, these investigators inaugurated the modern era of baroreflex research.

Anatomy and physiology

The arterial baroreflex buffers abrupt transients of blood pressure and originates from stretch sensitive receptors in the arterial wall of the carotid sinus and the aortic arch and the great vessels of the thorax (1;4). Mechanotransduction in the baroreceptor nerve terminals is not fully understood, but the presence of subunits of the DEG/EnaC family of cation channels (which enable touch sensation in Caenorhabditis elegans) provide a first clue for its molecular identity (5). Afferent fibers from carotid sinus baroreceptors join the glossopharyngeal nerve (ninth cranial nerve) and project to the nucleus tractus solitarii in the dorsal medulla, which is under cortical command and in turn projects to efferent cardiovascular neurones in the ventrolateral medulla (Figure 1 and 2). The extra-carotid arterial baroreceptors as well as the low-pressure cardiopulmonary baroreceptors transmit their afferent information to the same brain stem nuclei along with the vagal nerves. The efferent limbs of the baroreflex loop consist of sympathetic and parasympathetic fibers to the heart as well as to smooth muscles in the peripheral blood vessels. Arterial baroreceptor firing exerts a tonic inhibitory influence on the central generation of efferent sympathetic tone.

Denervation of baroreceptors in animals

The impact of baroreceptor denervation varies considerably among species and depends largely on which baroreceptor areas are abolished (4;6). Combined arterial (sino-aortic) and cardiopulmonary baroreceptor denervation in dogs produced a persistent increase in blood pressure level and variability (7). After selective sino-aortic denervation in dogs and baboons, the increase in blood pressure level and variability was reported to be either chronic (8;9) or temporary (7). After selective carotid baroreceptor denervation, blood pressure was found to increase markedly, but returned to intact levels within 7 to 14 days in dogs and baboons respectively (10;11). Changes in blood pressure variability paralleled those of blood pressure level with an initial large increase and a gradual return to

baseline. These animal studies learn, that baroreceptor afferent activity importantly determines both short- and long-term blood pressure control and that extra-carotid baroreceptor areas have a large ability to compensate for the loss of carotid baroreceptors.
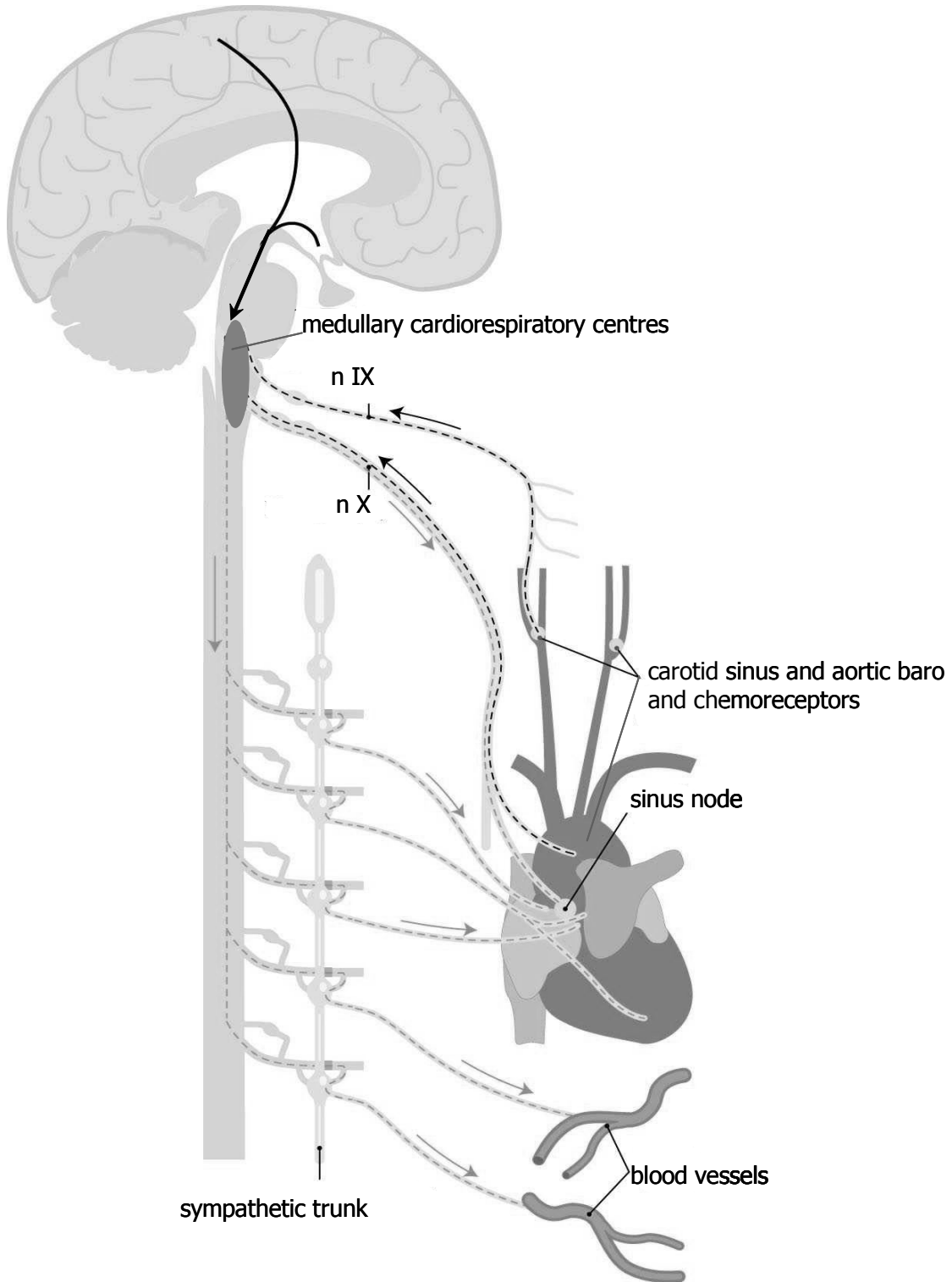
Figure 1. Arterial baro- and chemoreceptors
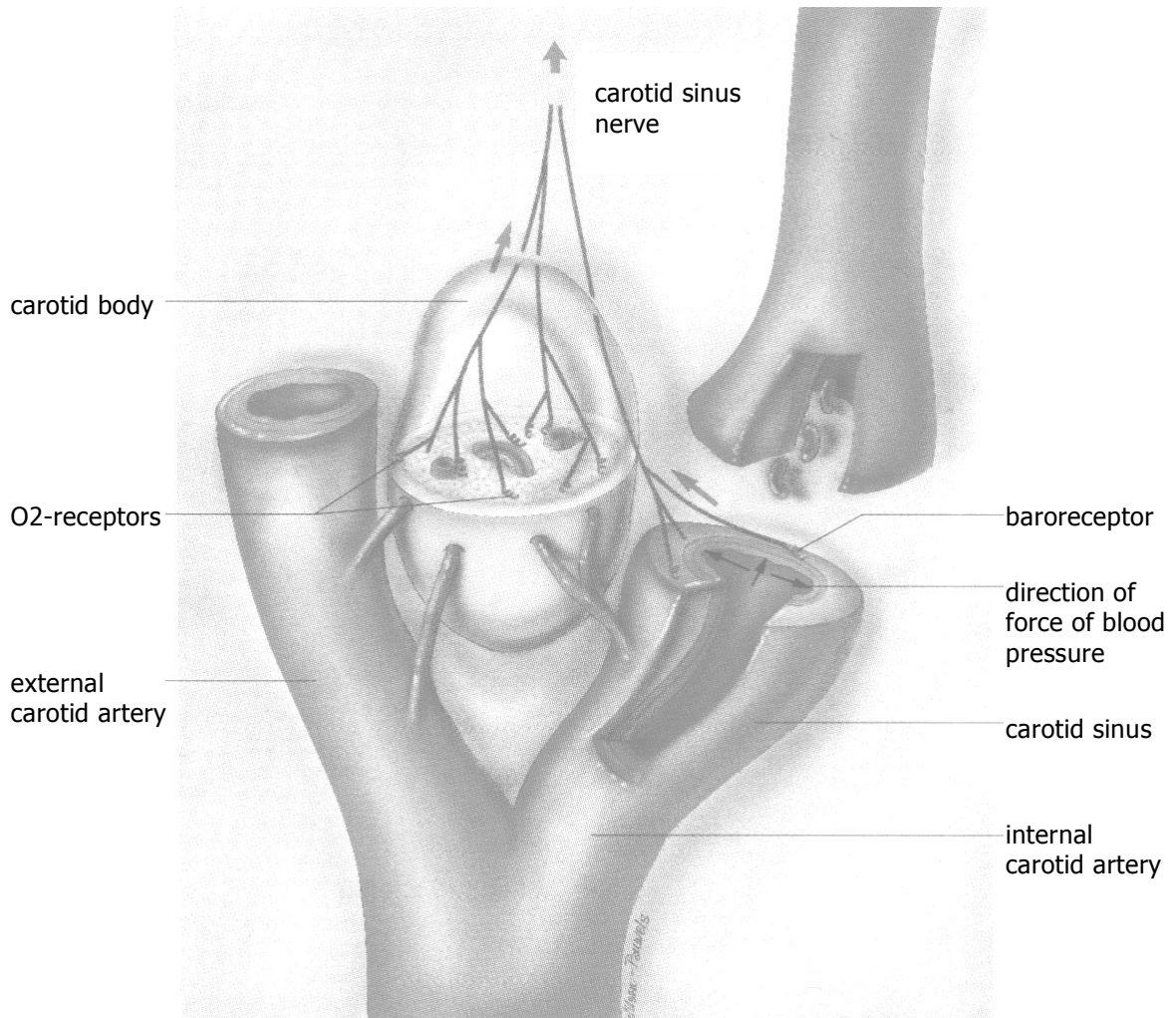
Figure 2. Schematic presentation of the carotid body (adapted from L. Wilson Pauwels)

Peripheral chemoreceptors

History

The carotid bodies were first mentioned by Taube ("ganglion minutem") in 1743, (12), although there is some uncertainty as to whether his Professor, Von Haller, who called it "ganglion exiguum" was the actual discoverer. They were described as organs with the size and shape of a grain of rice, nestled within the adventitia of the carotid bifurcation. Their function, however, remained unclear for decades. In 1926, De Castro stated, that they were able to "taste the blood" (13). Heymans was the first to demonstrate unequivocally their chemoreceptor function in the late nineteen-twenties (14), for which he was rewarded the Nobel Prize in 1938. Comroe further confirmed the above work by quantitive evaluation of the chemosensitivities of both aortic and carotid bodies in dogs (15).

Anatomy and physiology

Adjustment of respiration in response to alterations in levels of oxygen, carbon dioxide and hydrogen

ions in the body fluids are mediated by a complex interplay between centrally and peripherally located chemoreceptors (16). The peripheral arterial chemoreceptors, located in the carotid and aortic bodies, are responsible for the immediate ventilatory and arterial pressure increments during acute hypoxia (14) (Figure 2). Carotid and aortic bodies contain glomus (type I) cells, which release neurotransmitters (for instance catecholamines) in response to hypoxia, causing depolarisation of nearby afferent nerve endings (17). Apart from hypoxemia, peripheral chemoreceptors are also capable of sensing changes in arterial carbon dioxide tension (pCO_2) and pH. Other glomus tissues (glomus jugulare, trigeminale, pulmonare etc.) are not relevant for chemoreflex function in humans. Carotid and aortic bodies are supplied with sensory fibers, which course in carotid sinus/ glossopharyngeal and vagus nerve respectively towards medullary respiratory centers. Central chemoreceptive areas located at the rostral ventrolateral medulla respond to changes in the hydrogen ion concentration in the interstitial fluid in the brain and are chiefly responsible for ventilatory and circulatory adjustments during hypercapnia and chronic disturbances of acid-base balance.

Denervation of chemoreceptors in animals

In general, acute effects of carotid body chemoreceptor denervation in experimental animals include hypoventilation, apnea, a variable decrease in hypoxic ventilatory responsiveness and lower sensitivity for carbon dioxide. (Partial) restoration of chemoreflex function varies between species, but is more likely in neonatal than in adult animals and effects are more marked following bilateral than after unilateral denervation (18). In carotid body denervated rats, hypoxic responsiveness is first abolished, but may return to about half of normal within weeks (19). Superimposed aortic denervation had no effect in these animals, suggesting that the aortic body has little chemoreceptor function. In carotid body denervated dogs, hypoventilation and CO_2 hyposensitivity persisted through the 3 week follow-up period (20), whereas in goats, there was a near normalization of breathing and CO_2 sensitivity within days to weeks (21). In carotid sinus denervated ponies, arterial CO_2 levels did not normalize until two years after denervation (22). These animal studies suggest, that carotid bodies are the predominant mediators of hypoxic ventilatory drive and that they also contribute to CO_2 sensitivity. Nevertheless, most mammals show a considerable redundancy with regard to loss of carotid chemoreflex function.

Denervation of baro- and chemoreceptors in humans; aim of the studies

For obvious reasons, there are no human counterparts of the well-controlled denervation experiments in animal studies. For our knowledge of the impact of carotid sinus denervation on baro- and chemoreflex function, we predominantly rely on case studies of patients with inadvertent damage to baro- and/ or chemoreceptors following medical interventions of the neck. Iatrogenic lesions of the arterial baroreflex and/ or peripheral chemoreflex have been reported to occur as a result of carotid body tumor resection (23;24), radiotherapy of the neck (23;25) and carotid endarterectomy (26-29).

The effects on baro- and chemoreflex function of these interventions have not been systematically investigated in non-selected, consecutive patients who have undergone these interventions. In the studies presented in this thesis, we performed a retrospective evaluation of baro- and chemoreflex function in patients that had undergone bilateral carotid body tumor surgery, bilateral radiotherapy for laryngeal or pharyngeal carcinoma and unilateral carotid endarterectomy.

Our aim was to get insight into the long-term effects of denervation of carotid baro- and chemoreceptors on the control of circulation and ventilation in humans.

Patients

Carotid body tumor resection
Neoplastic growth of the carotid body is a rare disease. It occurs either as sporadic tumor or as part of the familial paraganglioma syndrome (30). In patients with this syndrome, one or more paragangliomas, also called glomus tumors or chemodectomas, may arise from the carotid, jugular, vagal and tympanic glomus tissue. In one third of patients these tumors occur bilaterally. During surgical removal of carotid body paragangliomas, branches of the carotid sinus nerves cannot be selectively spared (Figure 3). As a consequence, carotid sinus baroreceptors may well become (partially) denervated (31). In addition, radical resection of bilateral carotid body tumors by definition means removal of all carotid body chemoreceptive tissue.

Radiation therapy of the neck
Irradiation of the neck has a well established role in the treatment of head- and neck tumors. Bilateral radiotherapy of the neck with a curative intent is most commonly applied in patients with locally advanced laryngeal or pharyngeal cancer. Radiotherapy may or may not be preceded by surgical resection of the primary tumor and/ or radical lymph node resection. The target volume for radiation therapy depends on tumor size and involvement of regional lymph nodes. Usually, it consists of the larynx or pharynx and subdigastric and midjugular lymph nodes, which automatically includes the carotid bifurcation and its baro- and chemoreceptors. The receptors as well as their afferent innervation may be damaged by irradiation (23;25).

Carotid endarterectomy
Carotid endarterectomy is performed in selected patients with a history of transient ischemic attacks or ischemic stroke in addition to a hemodynamically significant stenosis of the common or internal carotid artery on the ipsilateral side of the affected cerebral hemisphere. The aim of carotid endarterectomy is to reduce the recurrence rate of ischemic stroke. In the individual patient, the likelihood of a beneficial effect of endarterectomy has to be carefully weighted against the perioperative mortality and morbidity of the procedure (32). Denervation of both carotid baroreceptors (26-29) and chemoreceptors (33) due to carotid endarterectomy have been reported.
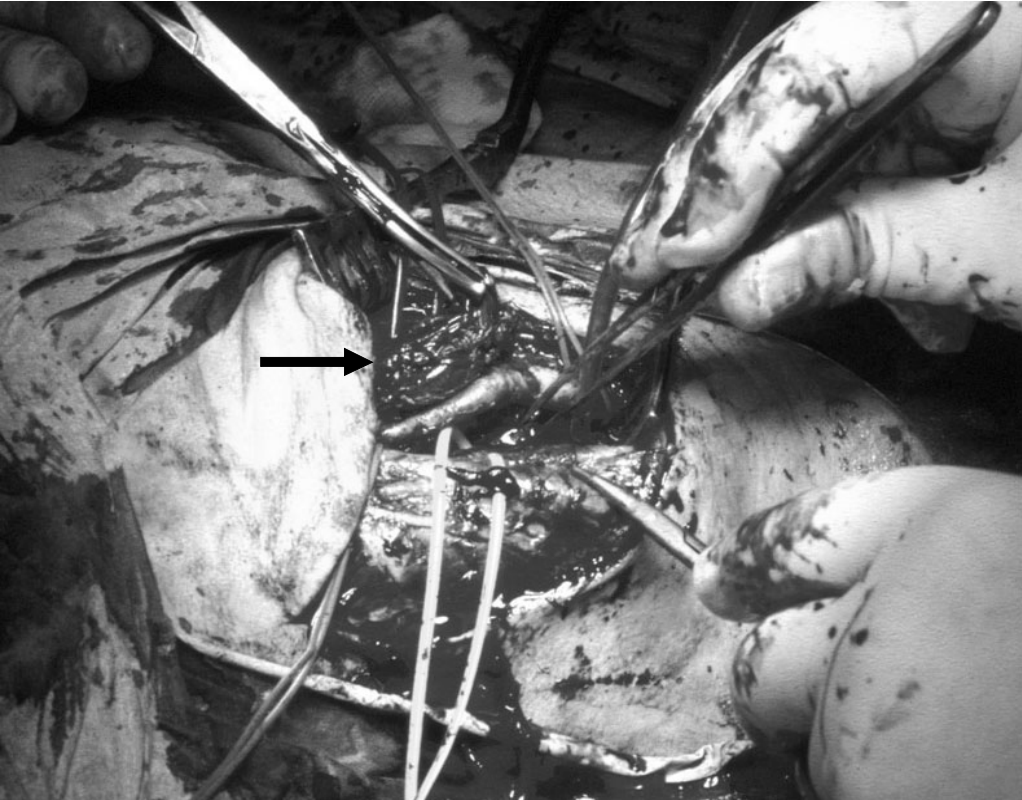
Figure 3. (A) Angiographic localisation of a right-sided carotid body tumor (Adapted from Velebit et al. New Engl J Med 2001 345:587) **(B)** surgical resection of a right-sided carotid body tumor (marked by arrow)

Methods applied

Blood pressure measurements

During laboratory investigations of autonomic function, continuous finger arterial BP was measured by Finapres device (model 5, TNO-BMI, Amsterdam, the Netherlands (34-36)). Off-line beat-by-beat blood pressure and heart rate values were derived from the arterial waveform using the FAST-System software package (37). Blood pressure level was assessed by means of repeated standardized sphygmomanometric measurements as well as from 24 hours intermittent ambulatory blood pressure recordings (SpaceLabs, Redmond, Washington, USA). Standard deviations and coefficients of variance of these home ambulatory readings were taken as estimates of blood pressure and heart rate variability (38). In addition, beat-by-beat blood pressure variability was measured using the Portapres, a portable version of the Finapres (TNO-BMI, Amsterdam, the Netherlands (34;36)). The ambulatory Portapres protocol consisted of five hours of strictly scheduled activities within the hospital.

Sympathetic nerve recordings

Multi-unit microneurographic recordings of post-ganglionic muscle sympathetic nerve activity (MSNA) were obtained with a unipolar tungsten electrode inserted selectively into a muscle-nerve fascicle of the right peroneal nerve, posterior to the fibular head as originally described by Sundlöf and Wallin (Figure 4 and 5) (39). A reference electrode was inserted subcutaneously 1 to 3 cm from the recording electrode. After amplification and filtering, a mean voltage neurogram of MSNA was obtained. MSNA is characterized by pulse-synchronous bursts of neural discharge, which are under baroreceptor control.

A   B
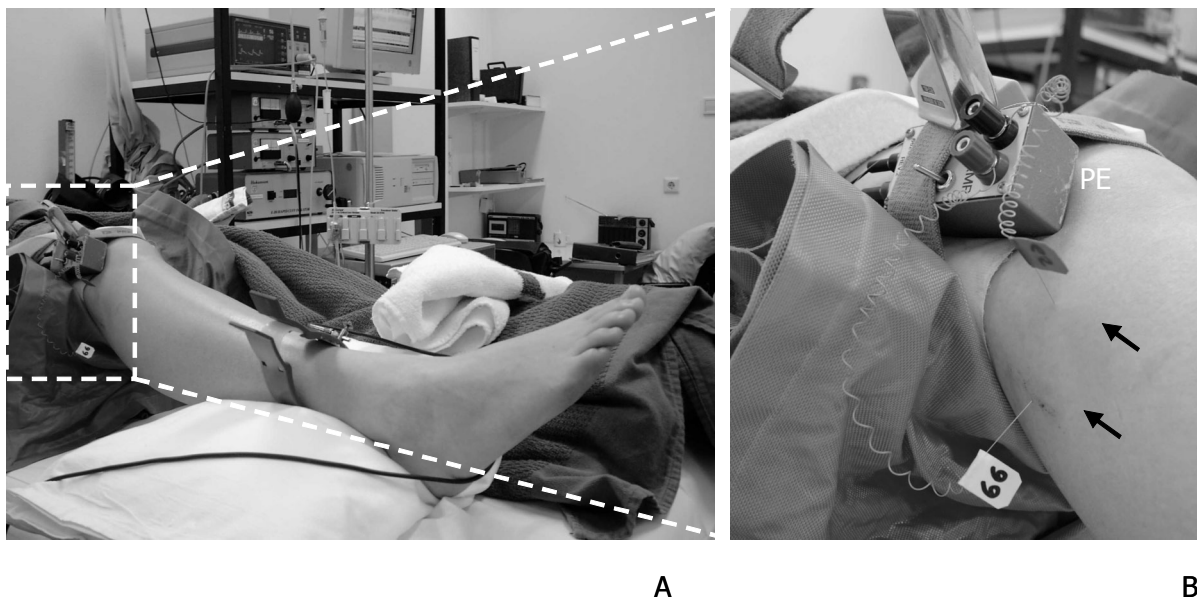
Figure 4. Experimental set-up for microneurographic recording of muscle sympathetic nerve activity (MSNA) from an electrode placed in the right peroneal nerve (marked by lower arrow). The reference electrode is marked by the upper arrow. Electrodes are connected to the pre-amplifier (PE).
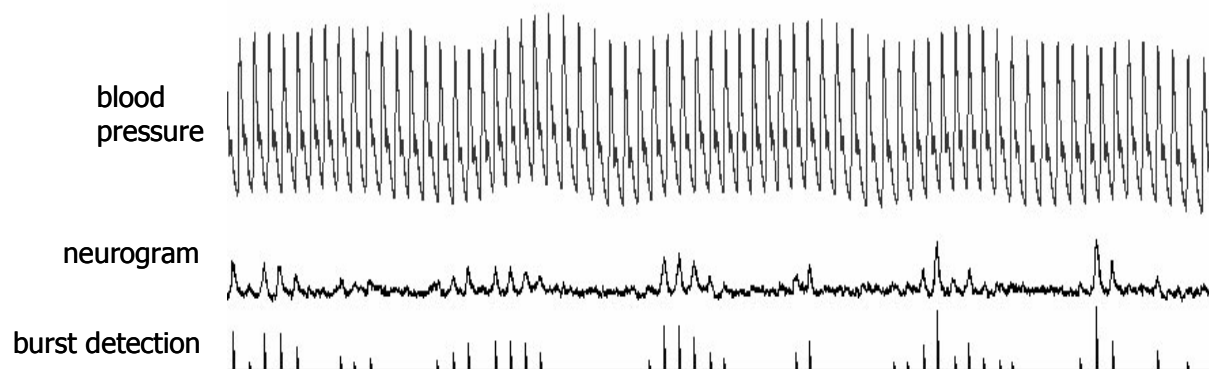
Figure 5. Simultaneous registration of blood pressure (Finapres) and integrated neurogram of muscle sympathetic nerve activity (MSNA) during supine rest. Note that changes in blood pressure elicit reciprocal changes in burst frequency and amplitude, which is mediated by the arterial baroreflex

Cardiovascular reflex tests and baroreflex sensitivity

Investigations were performed after an overnight fast in the morning in a room with a temperature of 22-24 °C. Subjects had abstained from caffeine, alcohol and smoking for 12-24 hours, depending on the protocol. Cardiovascular reflex tests were performed to investigate the overall baroreflex mediated heart rate and vasomotor control (Valsalva's maneuver, standing up (40)), efferent cardiovagal control (forced breathing, cold face test) and efferent sympathetic vasomotor control (cold pressor test, mental arithmetic) (4;40). Vagal and sympathetic baroreflex sensitivities were calculated from reflex changes in RR-interval and muscle sympathetic nerve activity relative to changes in blood pressure induced by intravenous infusion of vasopressor and vasodepressor drugs (i.e. phenylephrine, sodium-nitroprusside) (41;42).

Peripheral chemoreflex function

Peripheral chemoreflex function was assessed by the ventilatory responses to hypoxia and hypercapnia. Subjects were connected to a closed spirometric circuit in sitting position. Finger arterial oxygen saturation (Nellcor 200, Hayward, CA, USA) and end tidal pCO_2 (Gould Godart Mark II capnograph, Bilthoven, The Netherlands) at the mouth were continuously monitored. The ventilatory response to hypoxia was assessed during stable normocapnic as well as during stable hypercapnic conditions, using a rebreathing method (43).

Outline of the studies

Clinical characteristics of the syndrome of baroreflex failure, (differential) diagnosis, treatment and prognosis are described in *chapter 2*. *Chapter 3* elaborates on the course of acute baroreflex failure due to bilateral carotid body tumor resection. We describe the long-term effects of carotid sinus denervation on arterial blood pressure. Apart from studies, performed in selected patients with acute

baroreflex failure, subsequent investigations described in the following chapters were carried out in unselected patients. These patients had undergone invasive interventions of the neck, that carry a risk of injury to the carotid baro- and chemoreceptors. In *chapter 4* we report the long term effects of bilateral carotid body tumor resection on baroreflex and chemoreflex function. The impact of surgery on vagal baroreflex sensitivity, blood pressure level and variability, responses to standard autonomic reflex tests and ventilatory responsiveness to hypoxia and hypercapnia was assessed. In addition, microneurography of the peroneal nerve was performed in these patients for investigation of the baro-control of muscle sympathetic nerve activity. Results of this study are described in *chapter 5*. *Chapter 6 and 7* address the question, whether baro- or chemoreflex function is chronically affected by radiation therapy for locally advanced laryngeal or pharyngeal carcinoma and unilateral carotid endarterectomy respectively. *Chapter 8* contains our findings on the role of carotid chemoreceptors in the sympathetic activation by the purine nucleotide adenosine. In these microneurography studies, carotid body tumor resection served as a model for the absence of carotid chemoreflex function. The acute and chronic effects of carotid baro- and chemoreceptor denervation in humans are discussed and summarized in *Chapters 9 and 10*.

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Baroreflex failure syndrome

H.J.L.M. Timmers

W. Wieling

J. M. Karemaker

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Abstract

The arterial baroreflex buffers abrupt transients of blood pressure and prevents pressure from rising or falling excessively. In experimental animals, baroreceptor denervation results in temporary or permanent increases in blood presse level and variability, depending on the extent of denervation. In humans, the clinical syndrome of baroreflex failure may arise from denervation of carotid baroreceptors following carotid body tumor resection, carotid artery surgery, neck irradiation and neck trauma. The syndrome is characterized by acute malignant hypertension and tachycardia followed by labile hyper- and hypotension. Baroreflex failure can be a cause of secondary hypertension and should also be considered in the differential diagnosis of pheochromocytoma. Patients suspected for baroreflex failure should be referred to specialized centres for diagnostic testing and treatment.

Baroreceptors

In the late nineteen twenties, H.E. Hering and E. Koch were the first to recognize the reflex nature of changes in heart rate and blood pressure evoked by external massage of the neck. The afferents were tracked as nerve endings at the carotid bifurcation (1;2). The baroreflex buffers abrupt transients of blood pressure and originates from stretch sensitive receptors in the arterial wall of the carotid sinus and the aortic arch and the large vessels of the thorax (3;4). Afferent fibres from carotid sinus baroreceptors join the glossopharyngeal nerve (ninth cranial nerve) and project to the nucleus tractus solitarii in the dorsal medulla, which in turn projects to efferent cardiovascular neurones in the medulla. In addition to carotid baroreceptors, stretch-sensitive baroreceptors are also located in the aortic arch, the heart and large pulmonary vessels. The extra-carotid baroreceptors transmit their afferent information along with the vagal nerves to the same brain stem nuclei. The efferent limbs of the baroreflex loop consist of sympathetic and parasympathetic fibres to the heart as well as to blood vessels.

Experimental and iatrogenic denervation of baroreceptors

Arterial baroreceptors provide a tonic inhibitory influence on sympathetic tone, thus controlling peripheral vasoconstriction and cardiac output (1;2). Therefore, baroreceptor denervation would be expected to result in a sustained increase in sympathetic tone and, as a consequence, a sustained increase in blood pressure. Indeed, experimental denervation of carotid, aortic and cardiopulmonary baroreceptor denervation in dogs produces a persistent increase in blood pressure level and variability (5). Following selective carotid and/ or aortic baroreceptor denervation, the increase in blood pressure and heart rate is usually temporary (5-8).

The first report on baroreceptor denervation in humans appeared in the nineteen-thirties (9). Unilateral section of the glossopharyngeal nerve in five patients with glossopharyngeal neuralgia

produced a prompt and pronounced rise in blood pressure, which lasted from 5 to 12 days. In 1956, a patient died from a fatal hypertensive crisis following unilateral carotid sinus denervation, which had been performed for the relief of recurrent syncope due to a hypersensitive carotid sinus syndrome (10). In 1985 Fagius and Wallin reported the effects of experimental chemical denervation of carotid baroreceptors in humans (11). These authors performed bilateral anaesthetic blockade of vagus and glossopharyngeal nerves upon each other, which resulted in an elevation of blood pressure and tachycardia, accompanied by a strong increase in muscle sympathetic nerve activity.

Apart from these experimental studies, inadvertent baroreceptor denervation may occur as a complication of bilateral carotid body tumor resection (12-14), radiotherapy and surgery for laryngeal/ pharyngeal carcinoma (12;15;16), bilateral (17;18) and unilateral (19;20) carotid endarterectomy and trauma of the neck (12). Disruption of the baroreflex has also been reported in the event of ischemic or neurodegenerative lesion of the nucleus tractus solitarii (21). It was from the complications of these conditions, that the clinical syndrome of baroreflex failure has been characterized as a separate clinical entity (12;22).

Baroreflex failure syndrome

The acute form of baroreflex failure is encountered following loss of glossopharyngeal or carotid sinus nerve function due to surgical intervention or accidental injury (17-20). It is characterized by severe, unremitting hypertension, tachycardia, and headache (Table 1). The systolic blood pressure typically exceeds 250 mmHg (22), which may lead to hypertensive encephalopathy (17;19) and (fatal) cerebral haemorrhage (10;20). Hypertensive crisis may evolve over days and weeks into the more chronic volatile hypertensive phase (12;14;18;23;24). In addition, volatile hypertension may result from a gradual decline in baroreflex function due to neck irradiation (12;16). Irradiation may affect the stretch-induced afferent baroreceptor activity due to direct trauma of baroreceptors or by inducing atherosclerosis and fibrosis of the carotid sinus arterial wall (25-27). Volatile hypertension due to baroreflex failure is characterized by paroxysms of abrupt sympathetic activation, including excessive increments in plasma catecholamine levels (12;22). Surges of blood pressure and tachycardia may occur spontaneously or are elicited by mental stress or physical stimuli like exercise, cold and sexual arousal (28). These bouts of sympathetic activation may be accompanied by severe headaches, palpitations, diaphoresis, lightheadedness and anxiety (12). Intraocular pressure may be increased (29). In addition, emotional instability appears to be a prominent feature in this phase of baroreflex failure. Apart from hypertensive surges, hypotensive valleys may occur during sleep (16). In rare cases, inadequate baroreflex buffering of cardiovagal efference is the most prominent feature, resulting in malignant vagotonia with hypotension, bradycardia, and asystole (30). Accompanying symptoms of this so-called "selective baroreflex failure" include fatigue and dizziness, with possible progression to frank syncope.

Table 1. Symptoms and signs of baroreflex failure

Acute phase following baroreceptor denervation (days-weeks)

severe sustained elevation of blood pressure (systolic pressure typically >250 mmHg)

tachycardia

elevation of plasma catecholamines

headache

complications of hypertension

  encephalopathy

  cerebral haemorrhage

Chronic phase following baroreceptor denervation (weeks-years)

common:

volatile hypertension and tachycardia

paroxysm of

  palpitations

  headache

  diaphoresis

  light headedness

  anxiety

emotional instability

rare:

hypotension (during sleep)

bradycardia, asystole

fatigue

(orthostatic) dizziness

syncope

increased intraocular pressure

When should baroreflex failure be considered?

A history of prior (iatrogenic) trauma of the neck is the most important clue in suspecting the diagnosis of baroreflex failure. The diagnosis of baroreflex failure should be considered in patients with an unsuccessful work-up for pheochromocytoma, since this carries strong resemblance with baroreflex failure. Apart from pheochromocytoma, which should be ruled out by the proper investigations, the differential diagnosis of baroreflex failure includes paroxysmal tachycardia, migraine, hyperthyroidism, renovascular hypertension, alcohol withdrawal, drug use (amphetamines or cocaine), mastocytosis, carcinoid syndrome, tetanus, intracranial lesions and psychological disorders (panic attack, generalized anxiety disorder)(22).

Labile hyper- and hypotension can be demonstrated by a 24 hours ambulatory blood pressure recording (16). Patients with a suspicion of baroreflex failure should be referred to a specialised centre for tachycardia, migraine, hyperthyroidism, renovascular hypertension, alcohol withdrawal, drug use (eg, the evaluation of autonomic cardiovascular function. Disruption of the baroreflex arch is demonstrated by absence of reflex bradycardia and tachycardia in response to intravenous injection of

pressor drugs like phenylephrine and depressor drugs like nitroprusside, respectively (12;31). The baroreflex modulation of muscle sympathetic nerve activity can be assessed by microneurography of sympathetic fibres within the peroneal nerve (32;33) (Figure 1). Additional cardiovascular reflex tests such as Valsalva's maneuver, standing up, forced breathing, cold face test, cold pressor test and mental arithmetic (34) can be used to tease out the localisation of the baroreflex lesion (Table 2) (34).
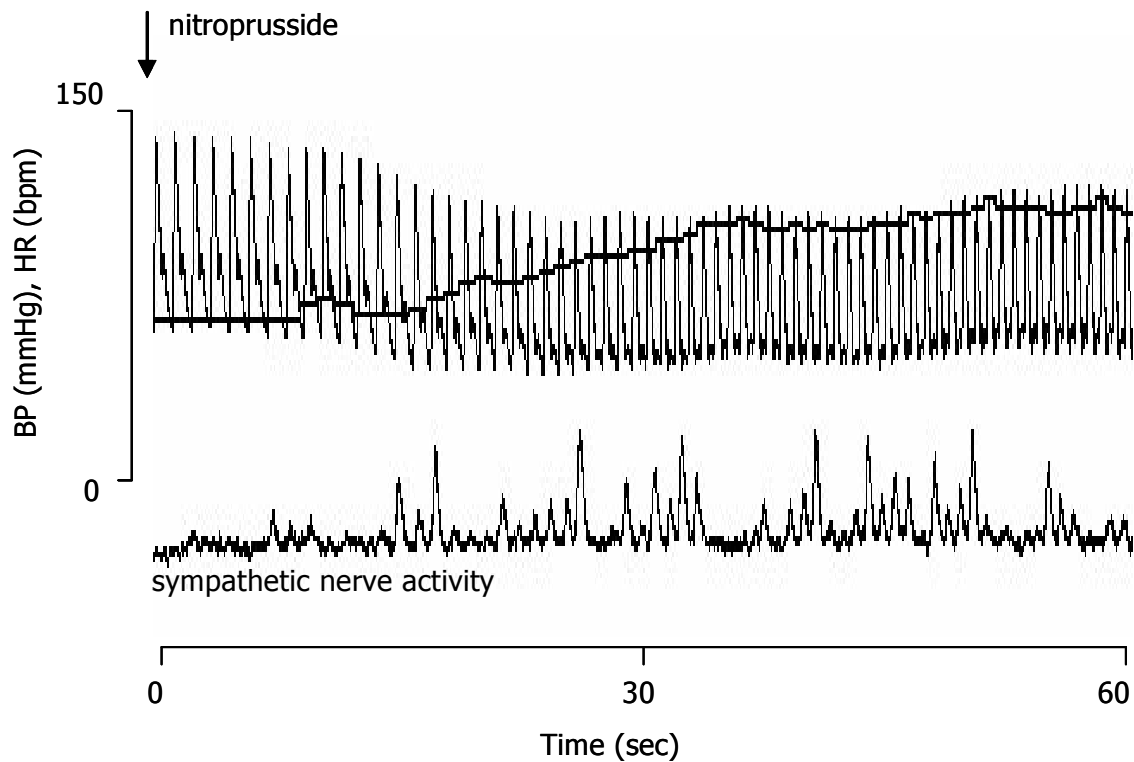
Figure 1. Assessment of baroreflex control of heart rate (upper panel) and muscle sympathetic nerve activity (lower panel) in a normal subject. Nitroprusside induced hypotension elicits a baroreflex mediated increase in heart rate and muscle sympathetic nerve activity. BP= blood pressure, HR= heart rate.

Baroreflex failure is essentially different from autonomic neuropathy which is either primary (pure autonomic failure, multiple system atrophy) or secondary (diabetes mellitus). In contrast to baroreflex failure which is caused by lesions of the afferent innervation of baroreceptors, autonomic neuropathy is characterized by abnormal efferent nerve activity to the heart and resistance vessels. The key feature of autonomic neuropathy is (severe) orthostatic hypotension (35). In addition, baroreflex failure represents the opposite of the hypersensitive carotid sinus syndrome, in which stimulation of the carotid baroreceptors elicits an excess of afferent nerve impulses towards the brainstem, causing cardioinhibition and/ or vasodepression resulting in syncope (36).

Table 2. Diagnostic scheme for diagnosis of baroreflex failure

Method of investigation	Findings fitting baroreflex failure
*baroreflex sensitivity (key feature)**	
bolus injections of phenylephrine, nitroprusside	vagal / sympathetic baroreflex sensitivity decreased or absent
cross power spectrum / sequence analysis	vagal baroreflex sensitivity decreased or absent
*efferent sympathetic vasomotor control**	
cold pressor test	excessive increase in blood pressure, heart rate and muscle sympathetic nerve activity
mental arithmetic	excessive increase in blood pressure, heart rate and muscle sympathetic nerve activity
*efferent cardiovagal control**	
forced breathing	normal inspiratory- expiratory difference in heart rate
cold face test	normal initial bradycardia
ambulatory blood pressure measurements (beat-by-beat recording by Portapress preferred)	blood pressure variability increased

* Recording of non-invasive beat-to-beat blood pressure and heart rate (Finapres), muscle sympathetic nerve activity (multi fibre microneurography of the peroneal nerve), breathing frequency (nose thermistor) and expiratory pressure. Measurements are performed in the supine position, except for Valsalva's maneuver (sitting position).

Treatment and prognosis

Information on treatment of acute, post surgical baroreflex failure is scarce and relies on observational case studies. As in any form of hypertensive emergency, antihypertensive treatment in baroreflex failure is aimed at the prevention of hypertensive encephalopathy, (cerebral) haemorrhage, myocardial infarction, heart failure and hypertensive retinopathy. Haemodynamic monitoring of these patients in a medium or intensive care unit is warranted in the acute phase. Theoretically, intravenous administration of drugs with a short half-life is preferred in view of the strong blood pressure lability. Drugs that have been used in this setting include nitroprusside, phentolamine and labetalol (37;38). Apart from antihypertensive treatment, adequate analgetic and sedative therapy for relief of post-surgical discomfort and baroreflex failure related symptoms like headache and palpitations are indicated.

In the phase of labile hypertension, the primary goal of therapy is to reduce the frequency and magnitude of surges in blood pressure and heart rate. Clonidine, a centrally and peripherally acting alpha-adrenoreceptor and imidazoline agonist, was shown to reduce both frequency and severity of pressor surges (12;20-22). Both central inhibition of noradrenergic neurotransmission and

sedative effects may contribute to the beneficial effect of clonidine in baroreflex failure. If tolerated, high daily doses up to 1.2 to 2.4 mg may be required. The alpha-adrenoreceptor blocker phenoxybenzamine was also shown to reduce the magnitude of blood pressure surges (12). In patients who have been well controlled for months to years, clonidine may be tapered off and replaced by high doses of benzodiazepines, such as diazepam (22). Apart from these agents, experimental treatment of baroreflex failure include (non-registered) inhibitors of norepinephrine release.

Agents that increase synaptic norepinephrine concentrations, and thereby elicit profound pressor responses are probably better avoided in baroreflex failure (22). These include tricyclic antidepressants, amphetamines, monamine oxidase A inhibitors, cocaine, prednisone and tyramine-containing food and beverages. Additional non-pharmacological strategies include avoidance of individual factors that evoke sympathetic surges and (relaxation-) biofeedback training (39-41). In the rare patients with malignant vagotonia due to selective baroreflex failure, pacemaker placement may be necessary (30). In the rare patients with predominant hypotension, low doses of fludrocortisone and increased dietary salt may be indicated (22). Regarding the polar shifts in blood pressure, treatment of baroreflex failure can be challenging and frequent follow-up of patients is warranted.

The clinical expression of baroreflex failure varies considerably among patients, probably depending on the extent of denervation of baroreceptors. In addition, the natural course of baroreflex failure in individual patients is unpredictable. In most cases, the pressor surges moderate over time, but in some cases, volatile hypertension is permanent (12).

Conclusion

Baroreceptors are essential to buffering of acute changes in blood pressure and to prevent it from rising or declining excessively. The syndrome of baroreflex failure results from denervation of arterial and/ or cardiopulmonary baroreceptors and is characterized by labile hyper- and rarely hypotension. Baroreflex failure is particularly likely to develop following carotid body tumor surgery, carotid artery surgery, neck irradiation and injury. Baroreflex failure should be considered in the differential diagnosis of pheochromocytoma. Referral to a specialised centre for correct diagnosis and specific therapy is warranted.

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Long-term effects of carotid sinus denervation on arterial blood pressure in humans

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Abstract

Background: After experimental carotid sinus denervation in animals, blood pressure (BP) level and variability increase markedly but normalize to preoperative levels within 10-14 days. We investigated the course of arterial BP level and variability after bilateral denervation of the carotid sinus baroreceptors in humans.

Methods: We studied four females (aged 41-63 years) that were referred for evaluation of arterial baroreflex function because of clinical suspicion of carotid sinus denervation due to bi-lateral Carotid Body Tumor Resection. The course of BP level and variability was assessed from repeated office and 24 hours ambulatory measurements (Spacelabs/ Portapres) during 1-10 years of (retrospective) follow-up. Rapid cardiovascular reflex adjustments to active standing and valsalva's maneuver were assessed.

Results: Office BP level increased from 132/86 mmHg (range 118-148/80-92 mmHg) prior to bilateral surgery to 160/105 mmHg (range 143-194/90-116 mmHg) 1-10 years after surgery. During continuous 24-h non-invasive BP recording (Portapres) a marked BP variability was apparent in all four patients. Initial symptomatic hypotension upon change to the upright posture and abnormal responses to Valsalva's maneuver were observed.

Conclusions: Acute carotid sinus denervation, as a result of bilateral carotid body tumor resection, has a chronic effect on the level, variability and rapid reflex control of arterial BP. Therefore, in contrast to earlier experimental observations, the compensatory ability of the baroreceptor areas outside the carotid sinus appears to be of limited importance in the regulation of BP in humans.

Introduction

Bilateral carotid sinus denervation in dogs and baboons increases BP and BP variability with severe hypertensive surges during agitation (1-3). Normalization of averaged daily BP and variability (10 minute averages) was observed within two weeks after surgery (3). The aortic and cardiopulmonary baroreceptors were stated to compensate for the lack of carotid baroreceptor function in those experimental animals (3-5).

In humans, bilateral carotid sinus denervation may result in acute baroreflex failure, producing severe, labile hypertension, headache, diaphoresis and emotional instability (6). Baroreflex failure may result from bilateral carotid body tumor resection (bCBTR) due to damage of the adjacent carotid sinus or glossopharyngeal nerves (7). Data on the long-term effect of bilateral carotid sinus denervation on arterial BP are limited and controversial. Reports vary from normalization of BP (8;9) to increased BP variability (6;7) and sustained hypertension in individual patients (10).

The aim of this study was to assess the chronic effects of carotid sinus baroreceptor denervation in humans on the level and variability of BP. Repeated office BP readings during follow-up were documented and ambulatory (beat-to-beat) BP measurements were carried out in four patients with

acute baroreflex failure following bilateral carotid body tumor resection. Functional baroreflex integrity was assessed by means of standard cardiovascular reflex testing.

Methods

Patients

Four patients were referred to the department of Internal Medicine for the evaluation and management of symptoms and signs suggestive of baroreflex failure following bCBTR. All results presented in this study were obtained when the patients were off medication. The study protocol was approved by the ethics committee of the Academic Medical Center Amsterdam and all subjects gave their informed consent. Followed procedures were in accordance with institutional guidelines.

Patient A, a 45-year-old, normotensive female with a positive history for familial paragangliomas underwent bCBTR at age 35 with an interval of 2 months between the first (left-sided) and second (right-sided) operation. Pathologic examination revealed complete excision and no signs of malignancy. Immediately after the second operation the patient had an unbearable headache with a diastolic BP up to 140 mmHg. Despite antihypertensive treatment during the following 2 years, she was referred to our institution for labile hypertension and orthostatic lightheadedness. Physical examination and routine laboratory examination were unremarkable except for BP values as described below.

Patient B, a 63-year-old female, had undergone radical excision of a right-sided carotid body tumor at the age of 23. Forty years later, a contralateral left-sided carotid body tumor was resected. Surgery was radical and no malignancy was found. The second (left-sided) operation was followed by attacks of severe headache evoked by mental stress and physical exercise and of postural lightheadedness. In addition she had developed dysphagia and voice changes due to surgical damage to the superior laryngeal nerve (11).

Patient C, a 63-year-old normotensive female underwent left- and right-sided bCBTR respectively within 3 months. Pathologic examination revealed non-radical excision of the right-sided tumor. During the first postoperative day after bCBTR a BP up to 140/100 mmHg were measured. During a 5-year follow-up period without medication, episodic lightheadedness upon standing in the morning persisted. Chronic attacks of headache with a red face and perspiration in the neck during mental or physical exercise were present.

Patient D, a 41-year-old normotensive female underwent right- and left-sided bCBTR within one year because of a globus feeling in the neck and difficulty at swallowing. Immediately after the second operation, she developed a severe headache accompanied by flushes of the head and upper trunk, nervousness, and tremulousness. During these episodes BP reached 210/120 mmHg. These attacks occurred during emotional events and a marked emotional lability was present. Occasionally the patient experienced lightheadedness upon standing. She was treated with moxonidine, atenolol and chlorthalidone.

The effect of carotid sinus denervation on BP level

The course of arterial BP level was evaluated by means of retrospective analysis of office BP readings obtained from the medical records on pre- and post-surgery follow-up. Sphygmomanometric measurements were performed in sitting position. BP level was also assessed by means of a 24 hours intermittent ambulatory BP recording (SpaceLabs, Redmond, Washington, USA) after the second resection. Values were obtained every 15 and 30 minutes during daytime (7am-11pm) and night-time (11pm-7am) respectively. The first three automatic BP readings in the doctor's office were taken as a second measure of casual BP. Normotension was defined as a mean BP ≤135/85 mmHg during daytime and ≤120/70 mmHg during night-time and a day-night difference equal to or more than 10% was considered normal (12;13). Spacelabs recordings were performed 2 years (patient A and C) and 3 months (patient D) after the second operation respectively. In patient B, it was performed shortly before as well as 1 year after removal of the second tumor.

The effect of carotid sinus denervation on 24 hour BP variability

Arterial BP variability was assessed by means of a 24 hours beat-to-beat registration of finger arterial pressure using the Portapres device (model 1, TNO-BioMedical Instrumentation, Amsterdam, The Netherlands). This device is suitable for analysis of BP variability during daily activities (14-16). Readings were taken from the third and second finger alternating every half hour. The continuous ambulatory recording was stored on a built-in tape cassette recorder along with a marker signal superimposed to a hydrostatic height correcting signal for off-line AD conversion. A 24 hour Portapres recording started at 12 a.m. and comprised strictly scheduled standardized activities: supine rest without sleep for 1.5 h (siesta, starting at 2 p.m.), cycling at 50 W/50-60 rpm on a bicycle ergometer for 20 min (starting at 4.30 p.m.), 2 periods of walking for 30 min (patient A: starting at 10 a.m. and 11 a.m.; patient B,C,D: starting at 4 p.m. and 10 a.m.). When no standardized activities were performed, patients were left free to perform non-fatiguing daily activities. They stayed in bed between 11 p.m and 6.30 a.m. and kept a diary to report the time of non-standardized activities. Five minute averages were calculated and used for presentation of 24 hour BP profiles and BP frequency distributions. Portapres recordings were performed 2 years (patient A and C) and 3 months (patient D) after the second operation respectively. In patient B, it was performed shortly before as well as 1 year after removal of the second tumor.

The effect of carotid sinus denervation on rapid cardiovascular reflex adjustments

Functional baroreflex integrity was assessed by measuring the beat-to-beat BP and heart rate responses to active standing, Valsalva's maneuver and forced breathing. Patients were non-smokers, abstained from caffeine and food 2-4 hours before reflex testing. Investigations were performed in a room with an ambient temperature of 22-24ºC. Continuous finger arterial BP was measured by the Fina-pres device (model 5, TNO-BioMedical Instrumentation) (17). BP was obtained from the mid-phalanx of the third finger of the left hand, which was held at heart level.

The BP and heart rate responses to active standing and Valsalva's maneuver were used to assess overall baroreflex mediated heart rate and vasomotor control (18;19). Normal baroreflex mediated vasomotor control was defined as an initial BP decrease less than 40/25 mmHg with return of BP to prestanding levels, and by the presence of a late phase II recovery and phase IV overshoot during the Valsalva's maneuver (18;19). The initial maximal heart rate increase upon standing (dHR_{max}) and the highest heart rate in phase II divided by the lowest heart rate in phase IV of the Valsalva's maneuver (Valsalva-ratio) were calculated to assess baroreflex mediated heart rate control (19). The inspiratory-expiratory difference in heart rate (I-E difference) during forced breathing was used as a selective test for efferent cardiovagal innervation. The dHR_{max}, Valsalva ratio and the I-E difference were compared with age matched normotensive data (19). Cardiovascular reflex testing was carried out after removal of the second tumor at intervals of 2 years (patient A) 3 days and 2 years (patient C) and 3 months (patient D) respectively. In patient B, it was performed shortly before as well as 1 month and 1 year after the second operation.

Results

Effect of carotid sinus denervation on BP level
Before any surgery was carried out, averaged office BP obtained by sphygmomanometry was normal in patients A, B and D (Table 1). In patient C BP was slightly increased (148/92 mmHg, normal < 140/90). Following the first carotid body tumor resection, BP level increased to 177/ 98 mmHg in patient B and 152/ 99 mmHg in patient D (Table 1). Compared with pre-operative values, BP levels were elevated immediately after the second operation as well as on the long term after bCBTR in patients A, B and D (Table 1).

The first three office BP readings by the ambulatory monitor were in agreement with those obtained by sphygmomanometry (Table 1). Compared with office BP readings, averaged ambulatory daytime values were lower in all patients, but still above the proposed values for normotension in patient A, B and D. In patient B, mean daytime ambulatory values had increased from 131/87 mmHg before bCBTR to 156/100 mmHg after bCBTR.

Effect of carotid sinus denervation on 24 hour BP variability
Prospective evaluation of BP variability by means of repeated Portapres recordings was assessed in patient B only. One month after the operation the variability of BP had increased compared to before the operation (Figure 1). As can be observed in the BP distribution curves the increase in BP variability was due to the occurrence of more high BP values. For example, after the operation 75% of mean arterial BP values were found between 66 to 110 mmHg, while 75% of BP values occurred between 69 to 97 mmHg before the operation. The marked BP increments were observed during scheduled activities like walking (from 111/66 to 160/79 mmHg) and cycling (from 139/68 to 160/80 mmHg). But also non-scheduled activities like undressing before going to bed and dressing the next morning were associated

with marked increases in BP (212/99 and 180/100 mmHg, respectively). During mental activity like watching television or telephone conversation BP rose to BP values between 192/96 and 232/123 mmHg. During supine rest, mean arterial BP was significantly lower than the remaining day time (siesta 115/56 mmHg versus ambulation 134/72 mmHg, p < 0.001, Students-t test). In Figure 2, the 24-hour BP recordings of all patients after bCBTR are shown. It is apparent that BP during the day and night is markedly variable in these patients, especially when compared with a healthy normotensive subject (left panel Figure 1).

Table 1. Averaged casual and ambulatory BP before and after uni- and bilateral carotid body tumor resection (CBTR)

Patient	Before CBTR	After unilateral CBTR	After bilateral CBTR	After bilateral CBTR ABPM[1]				
	Casual BP mm Hg (number of visits)	Casual BP mm Hg (number of visits)	Casual BP mm Hg (number of visits)	Number of recordings	BP of first 3 measurements	Day time mm Hg	Night time mm Hg	Day time night time diff (%)
A	130/85 (2)	(0)	143/104 (10)	3	142/104	131/95	118/83	10/13
B	normal	177/98 (6)	194/116 (11)	3	164/119	156/100	108/67	31/33
C	148/92 (2)	130/83 (2)	144/90 (9)	2	139/101	129/86	113/71	12/17
D	118/80 (2)	152/99 (3)	157/108 (3)	2	146/105	133/93	117/80	12/14

[1]Ambulatory blood pressure measurements (ABPM) obtained by Oxford or SpaceLabs. Normotension is defined as averaged daytime BP equal to or below 135/85 mm Hg and at night equal to or below 120/70 mm Hg (12).
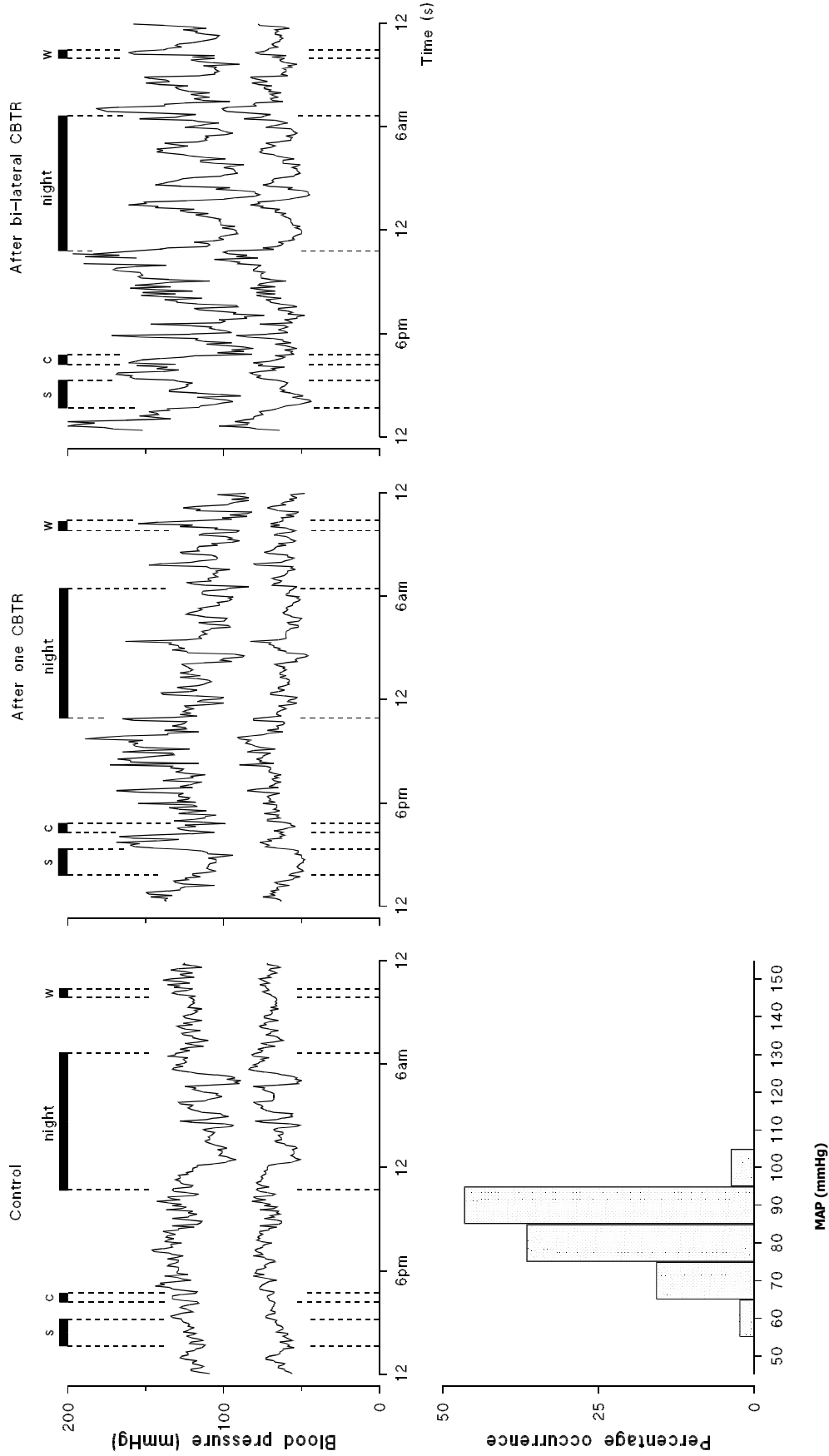
Figure 1. The effect of carotid sinus denervation on 24h BP. Profiles in a healthy subject (*top-left*), and a patient with carotid body tumors before (*top-middle*) and after (*top-right*) the second resection. BP variability for mean BP, as shown in the histograms (*bottom*) had increased in the patient after the second carotid body tumor resection.
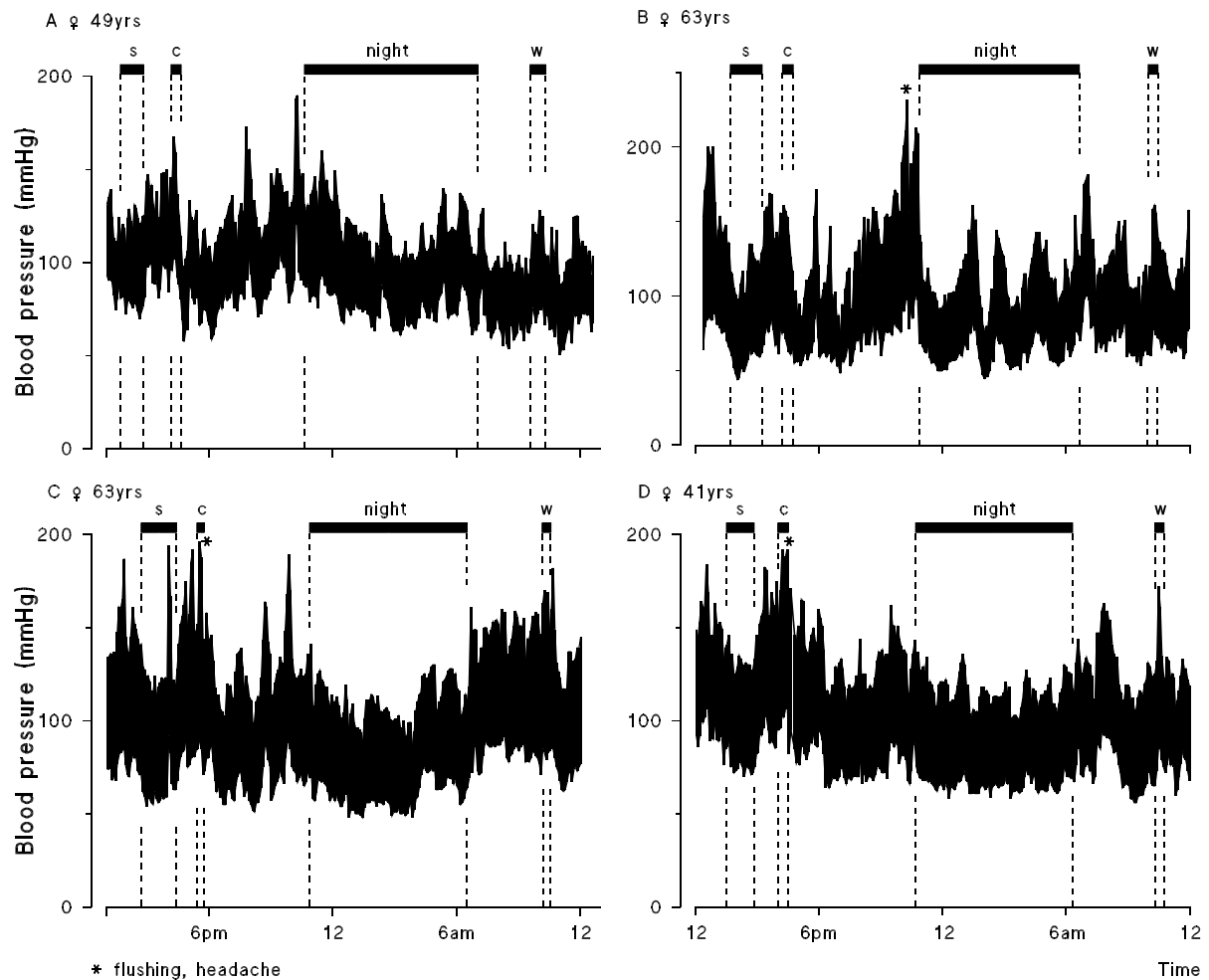
Figure 2. Twenty-four hour BP profiles in four patients after bilateral carotid body tumor resection. (s) denotes siesta, (w) denotes walking, (c) denotes cycling. A pronounced variability in all recordings was found as compared to the healthy subject in Figure 1.

Effect of carotid sinus denervation on rapid cardiovascular reflex adjustment

In response to standing up *patient A* showed a large initial decrease in BP from 142/92 to 77/55 mmHg with a slow and incomplete recovery to 108/79 mmHg after 1 minute, 2 years after bCBTR. The maximal initial increase in heart rate (dHR_{max}) was abnormally low (10 beats/min, normal > 15) (19). Valsalva's maneuver provoked a progressive decrease in BP during strain without overshoot after release of strain. The heart rate response during Valsalva's maneuver was abnormal. These findings were indicative of impaired baroreflex mediated vasomotor and heart rate control (18-20). During forced breathing a normal inspiratory-expiratory difference in heart rate was observed, indicative of normal efferent vagal heart rate control (Table 2). Presently (10 years post-operative), no improvement in the BP response upon standing or Valsalva's maneuver are present.

Patient B was evaluated shortly before the second operation. Upon standing, BP decreased from 150/80 mmHg to 100/43 mmHg within 12 seconds. At 1 minute standing, BP had returned to 135/65 mmHg. The maximal initial heart rate increase was abnormally low (5 beats/min, normal > 13). The inspiratory-expiratory difference in heart rate at forced breathing was just above the lower limit of normal (10 beats/min, normal > 9) at a basal heart rate of 70 beats/min. One month after the second

operation the initial decrease in BP was more pronounced (170/110 to 90/75 mmHg) with a sluggish recovery (Figure 3 *top-left*). At 1-year follow-up, the BP response to standing had improved but the initial BP decrease was still marked (55/35 mmHg, normal < 40/25) (Figure 3, top right, Table 2). At that time, Valsalva's maneuver showed a progressive decrease in BP during straining without a BP overshoot after release of the strain. Heart rate did not change during this procedure (Table 2). The heart rate response to forced breathing was abnormally low (5 beats/min; normal, >9). These findings indicated the persistence of impaired baroreflex mediated vasomotor and heart rate control.

Patient C, was evaluated within 3 days after the second operation. Upon standing, BP showed a marked initial decrease from 122/61 to 78/48 mmHg with a low increment in heart rate (dHR$_{max}$ 16 beats/min) in respect to the fall in BP (Figure 3 *bottom left*). After 2 years follow-up normal cardiovascular responses to standing (Figure 3 *bottom right*), Valsalva's maneuver, and forced breathing (Table 2), were found indicating a normal baroreflex functioning.

Three months after bCBTR, *patient D* showed a large a-symptomatic initial decrease in BP from 153/90 to 94/64 mmHg (59/26 mmHg, normal < 40/25) with a normal heart rate response. The cardiovascular responses to Valsalva's maneuver and forced breathing were normal.

Table 2. The chronic effects of bCBTR on cardiovascular reflex responses

Patient	Postoperative period	Active standing-up		FRSA	Valsalva' maneuver	
		BP	dHr (bpm)	I-E diff (bpm)	BP	Valsalva ratio
A	2 years	a	10 (> 15)	17 (> 10)	a	1.19 (≥1.20)
B	1 year	a	5 (> 12)	5 (> 8)	a	1.07 (≥1.08)
C	2 years	a	23 (> 12)	16 (> 8)	n	1.66 (≥1.08)
D	3 months	a	34 (> 15)	12 (> 10)	n	1.24 (≥1.20)

(n) denotes a normal BP response and (a) an abnormal response. Age matched reference values (19) are given between brackets. Abbreviations used: FRSA= forced respiratory sinus arrhythmia, I-E diff (bpm): inspiratory- expiratory heart rate difference in beats/min; dHR$_{max}$ (bpm): maximal initial heart rate increment in beats/min.
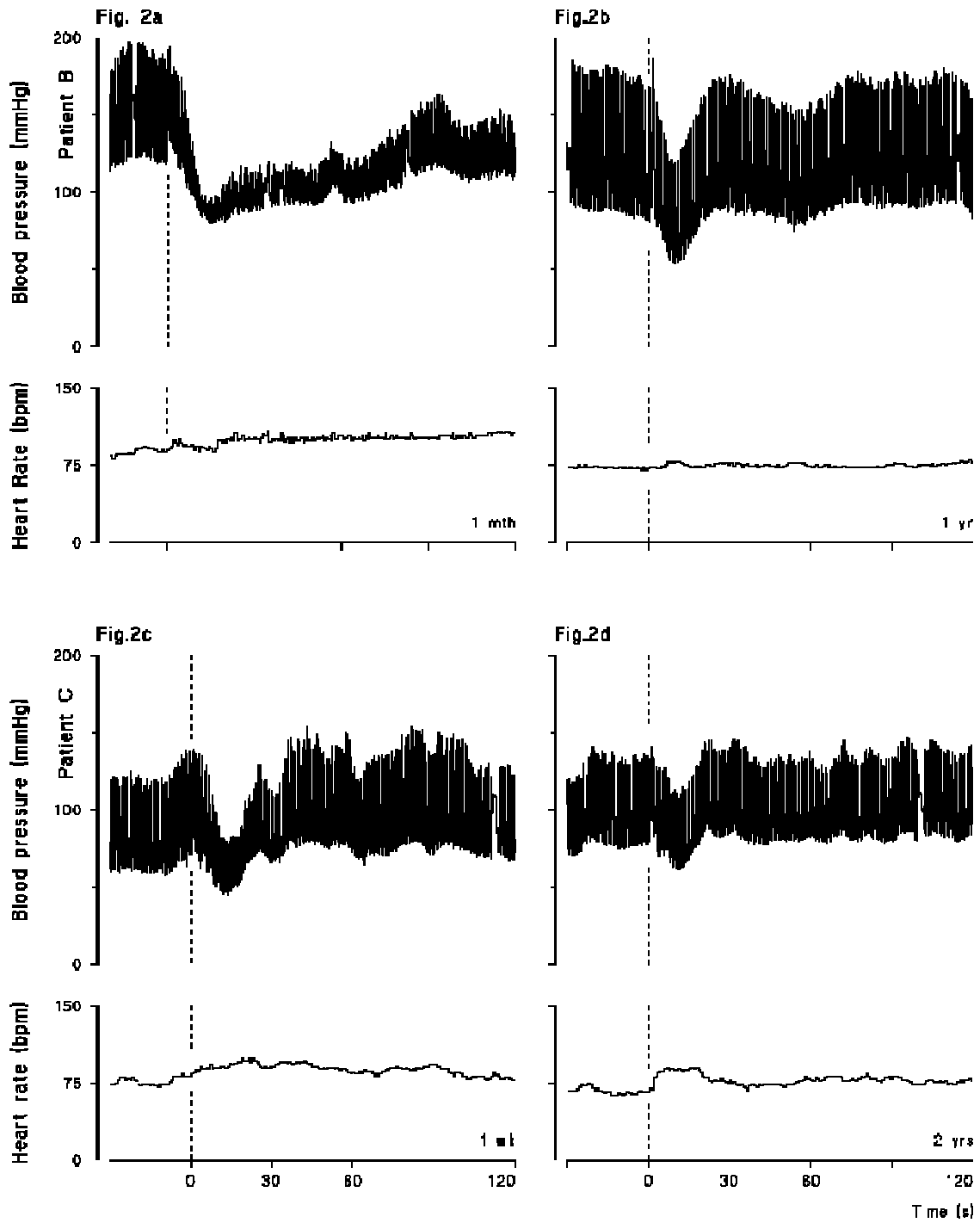
Figure 3. BP and heart rate responses to standing up in patient B: 1 month *(top-left)* and 1 year *(top right)* after bCBTR and patient C: 1 week *(bottom left)* and 2 years *(bottom right)* after bCBTR.

Discussion

The results of the present study document the chronic effects of carotid sinus denervation in humans. It appears to have a definite influence on the level of BP, BP variability and rapid reflex adjustments.

Effect of carotid sinus denervation on the level of arterial BP

Shortly after the second operation an increase in BP was observed (Table 1) in all patients. This is consistent with the short-term effect of carotid sinus denervation both in experimental animals (1-3) and in humans (6;8;9;21). In animal studies, arterial BP has been shown to return to preoperative levels within 14 days postoperatively (3). In humans, knowledge relies on office BP measurements where both normalization of BP (6;8) and sustained hypertension (6;10;22) have been observed.

Our study suggests that these different observations on the chronic effects of bCBTR on BP in humans can be explained by differences in completeness of denervation. In patient C, histologic evaluation revealed incomplete resection of the glomus body tumor which may imply that baroreceptor afferents from the carotid sinus on one side were left intact as well. Normal BP and heart rate responses during cardiovascular reflex testing support this view. In this patient almost normal averaged daytime and night time BP were observed after one year. In patient B, dysphagia, impaired rima glottis closure and a decrease in inspiratory-expiratory heart rate difference during forced breathing were indicative of iatrogenic vagus nerve impairment (11;19;23). In addition to efferent cardiac vagal fibers, afferent baroreceptor fibers from the aorta, afferent low pressure baroreceptor fibers and afferent lung fibers, could have been affected by the operative procedure as well, giving rise to a more extensive baroreceptor denervation than a selective carotid sinus denervation. Baroreflex mediated vasomotor control was still abnormal after one year follow up. The highest outpatient BP values were measured in this case.

Effect of carotid sinus denervation on the variability of arterial BP

Arterial baroreceptors provide the central nervous system with a continuous stream of information on changes in BP, on the basis of which efferent autonomic neural activity is dynamically modulated. Arterial baroreceptors are especially sensitive to abrupt transients in BP, whereas they adapt within 10-15 minutes to a persisting change in BP (18). Their main role appears to be the limitation of excursions in heart rate and BP in response to short challenges like orthostatic challenge, mental stress and exercise during daily life. Disruption of this dynamic reflex control of blood pressure explains our observation of an increased BP variability after bCBTR during these challenges. The BP level in patients after bCBTR seem to depend largely on the use of either office or ambulatory measurements. Casual BP measurements in the doctors office on long-term follow-up after bCBTR suggest overt hypertension in the patients presented here (Table 1). This finding is in line with the observations of Holton and Wood (in 2 patients) (6), Palatini (in one patient) (10) and Sleight (in one patient) (22). In these reports, it was concluded from office BP measurements that carotid sinus

denervation produces chronic hypertension. However, the effect on averaged ambulatory BP readings appears to be less pronounced. Averaged ambulatory BP values during daytime and night-time in the patients presented here were only slightly elevated with a normal day-night-time difference. The marked discrepancy between office and ambulatory values indicate that these patients are particularly sensitive to the pressor effect of mental stress caused by the measurement of BP in the doctors office. The magnitude of this pressor effect is similar to that observed in patients with white coat hypertension (24). Indeed, more pronounced elevations of BP provoked by mental and physical stress were observed in the Portapres recording in patient B after bCBTR as compared to before the second operation (Figure 1). A marked variability in continuous 24-hour BP was obvious in all patients (Figure 2).

Lability of BP observed after bCBTR is not solely determined by episodic BP elevations but also by episodic hypotension. After the second glomus resection patients complained of postural lightheadedness. The large BP decrease upon change of posture explains the periodic orthostatic lightheadedness in these patients. The disturbed initial adjustment of arterial pressure to the orthostatic posture becomes apparent by beat-to-beat analysis of circulatory transients and cannot be assessed sufficiently by sphygmomanometry (19). This might explain why initial orthostatic disturbances are not consistently reported in patients with baroreflex failure (7). Our study indicates that the carotid sinus baroreceptors are of great importance to provide rapid adjustment of BP and heart rate to standing. In the three patients with intact cardiac vagal control (patient A,C,D), the reciprocal relationship between heart rate and BP on standing, provides circumstantial evidence for mediation of the heart rate by the (unaffected) aortic baroreceptors (18). But the abnormally small heart rate peak in relation to the pronounced decrease in BP, despite a normal cardiovagal innervation (19) suggests a low sensitivity of these receptors compared to the carotid baroreceptors.

Limitations of the study

Since bilateral carotid body paraganglioma is a rare disorder, we studied the effects of carotid sinus baroreceptor denervation in only four patients. Except for one prospectively studied patient, BP data were obtained retrospectively from the individual clinical follow-up, which ranged from 1 to 10 years. In addition, there were considerable differences in the intervals between bCBTR and additional investigations of the baroreflex. Systematic prospective evaluation of baroreflex function in these patients is needed.

Conclusion

Our data provide support for the view that after carotid sinus denervation in humans, BP remains slightly elevated over normotensive values. In addition, BP variability remains markedly increased. Apparently, in contrast to some experimental observations in animals the lack of carotid baroreceptors cannot be compensated for by other reflex mechanisms on the long term. This leads to elevated BP during mental stress and might explain the isolated office hypertension that can be observed in these patients. Initial

orthostatic lightheadedness can be observed and results from a large initial orthostatic fall in BP, insufficiently buffered by the aortic baroreceptors. The hydrostatic position of the carotid sinuses in the upright posture in humans gives these baroreflex afferent areas a major role in defense of upright BP and, thereby, brain perfusion. In addition, it seems that the sensitivity of this part of the baroreflex cannot be compensated for by other (aortic) baroreceptor areas. This is different from observations in experimental animals.

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Baroreflex and chemoreflex function following bilateral carotid body tumor resection

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Abstract

Objective: Acute baroreflex and chemoreflex failure after bilateral carotid body tumor resection may result in severe hypertension (1) and apneic spells respectively (2). We investigated whether bilateral carotid body tumor resection invariably and chronically affects arterial baroreflex and/ or peripheral chemoreflex function.

Methods: We studied 8 consecutive patients (2m:6f, age: 48.1±11.8 years), 3.4 years (median; range 1.3-20.6) after bilateral carotid body tumor resection and 12 healthy controls (8m:4f, 53.7±10.1 years). Baroreflex sensitivity (phenylephrine), blood pressure level and variability (24 hours Spacelabs and 5 hours Portapres recordings), responses to standard cardiovascular reflex tests and the ventilatory responses to normocapnic and hypercapnic hypoxia were assessed.

Results: Baroreflex sensitivity was lower in patients (6.4±7.2 ms/mmHg) than in controls (14.7±6.6 ms/mmHg, p=0.011). Mean office blood pressure and heart rate were normal in patients (123.3±11.9/ 79.0±7.3 mmHg, 67.5±9.4 bpm) and controls (117.8±10.6/ 74.0±6.8 mmHg, 61.1±9.2 bpm). Blood pressure variability was increased during ambulatory measurements. Three patients exhibited orthostatic hypotension. The Valsalva-ratio, an index of baroreflex mediated cardiovagal innervation, was lower in patients (1.4±0.2) than in controls (1.8±0.5, p=0.008). The normocapnic ventilatory response to hypoxia was absent in all patients, whereas a small residual response to hypoxia was observed under hypercapnic conditions in two patients.

Conclusions: Bilateral carotid body tumor resection results in heterogeneous expression of arterial baroreflex dysfunction, while the normocapnic hypoxic drive is invariably abolished due to peripheral chemoreflex failure.

Introduction

Baroreflex and chemoreflex mechanisms play an important role in the dynamic adjustments of circulation and ventilation to perturbations during daily life. The arterial baroreflex, which originates from carotid sinus and aortic mechanoreceptors, buffers abrupt transients of blood pressure (BP) (3). Iatrogenic lesions of the afferent limb of the baroreflex may result in baroreflex failure, producing labile hypertension, headache, diaphoresis and emotional instability (1;4). Peripheral chemoreceptors mediate the immediate ventilatory and circulatory response to hypoxemia. In humans, peripheral chemoreceptor function is predominantly attributable to the carotid bodies (5), which lie in close approximity to the carotid sinus baroreceptors. The afferent signals from both carotid sinus baro- and chemoreceptors travel up the carotid sinus nerve to join with the glossopharyngeal nerve towards the brainstem (6). Bilateral carotid body resection, which has been performed as an experimental treatment for bronchial asthma, results in abolishment of the ventilatory response to normocapnic hypoxia (7;8).

Neoplastic growth of the carotid body is a rare disease. It occurs either as sporadic tumor or as part of the familial paraganglioma syndrome (9). In patients with this syndrome, one or more paragangliomas may arise from the carotid, jugular, vagal and tympanic glomus tissue and in one third of patients these tumors occur bilaterally. During surgical removal of carotid body paragangliomas, branches of the carotid sinus nerves cannot be selectively spared and bilateral carotid body tumor resection (BCBR) has been reported to cause acute baroreflex and chemoreflex failure in individual cases (1;10;11). In these reports, reflex function was evaluated in selected patients with severe labile hypertension and/ or apneic spells immediately following BCBR. There are no long-term data pertaining to the extent of baro- and chemoreflex dysfunction in unselected patients after BCBR.

The aim of this study was to investigate whether BCBR has a chronic effect on arterial baroreflex and peripheral chemoreflex function in consecutively operated patients. We hypothesized that chronic impairment of the baroreflex due to BCBR results in hypertension, increased blood pressure variability and abnormal cardiovascular reflexes. Secondly we hypothesized that BCBR abolishes ventilatory responsiveness to hypoxia. This unique group of patients offers an opportunity to study baro- and chemoreflex physiology in humans.

Methods

Patients and controls

Between 1978 and 1998, thirteen patients had consecutively undergone BCBR at the Department of Otorhinolaryngology/ Head and Neck Surgery of the University Medical Center Nijmegen, the Netherlands. They had been referred either because of a neck mass or, as a result of paraganglioma screening among relatives of index patients. Paragangliomas were diagnosed by means of angiography and/ or magnetic resonance imaging. Surgical techniques for BCBR have been described in detail elsewhere (9). A final histological diagnosis of benign paraganglioma was made in all cases. Of the thirteen consecutively operated patients, we excluded two patients: a 86 year old male, since his general physical condition was insufficient to carry out the study protocol and a male patient with atrial fibrillation. These two patients, together with three patients who chose not to participate in the experiments did agree to fill out an inventory of symptoms and signs fitting baroreflex failure following surgery, including bouts of headache, flushing, palpitations, diaphoresis, emotional instability, elevated blood pressure and gave permission to review their medical files of pre- and post-surgery follow-up. From both sources, there were no clues for baroreflex failure following BCBR in any of these 5 patients. Eight BCBR patients (2 males, 6 females) were included for evaluation of baroreflex and chemoreflex function. The median interval between the first and second operation was 11 months (range: 1 week-14 months) and 3.4 years (1.3-20.6 years) between the second operation and the study. Patients were free of neurological, cardiovascular and pulmonary disease and diabetes. Antihypertensive treatment of two patients (patient 1: atenolol/ chloorthalidon/ moxonidine, patient 8:

atenolol) was discontinued one week before the study. Individual information on tumor size, additional tumor localizations and surgical details are summarized in table 1. Twelve (8 males, 4 females) healthy control subjects were recruited through advertisement in a local newspaper. Groups were matched for age (BCBR: 48.1±11.8; controls: 53.7±10.1 years), body mass index (23.8±1.8; 24.5±2.2 kg/m^2) and alcohol intake (6.4±6.7; 4.3±5.3 units/ week). The study protocol was approved by the regional ethics committee. All subjects gave their informed consent. All institutional guidelines were followed.

Table 1. Characteristics of individual patients

	BCBR 1	BCBR 2	BCBR 3	BCBR 4	BCBR 5	BCBR 6	BCBR 7	BCBR 8
sex (m/f) age (y)	f44	f58	f44	f35	f62	m65	f41	m36
first CBR:								
time since (y)*	4.1	17.4	4.8	2.3	2.3	21.1	3.2	4.4
side	right	left	left	left	left	right	right	left
tumor size (max cm)	2.5	2.0	4.0	3.0	3.0	4.0	2.0	2.0
radical resection	yes	no	yes	yes	yes	yes	yes	Yes
(cranial) nerve damage	-	-	nIX(?)	-	-	nX, nXII	-	-
second CBR:								
time since (y)*	3.1	17.3	3.7	1.3	1.4	20.6	2.2	3.6
side	left	right	right	right	right	left	left	right
tumor size (max cm)	1.0	1.5	3.0	3.0	2.0	4.0	5.0	1.5
radical resection	yes	no	yes	yes	no	yes	yes	yes
(cranial) nerve damage	-	cervical plexus	-	-	-	-	symp chain, nXII	-
symptoms of baroreflex failure	persistent	-	-	-	-	-	transient	transient
additional paraganglioma: therapy	-	right skull basis: radiotherapy	-	-	-	left jugular fossa: radiotherapy	left tympanic: resection	-
family history of paraganglioma	yes	yes	no	yes	yes	no	yes	Yes
medication	chloorthalidon, atenolol, moxonidine							

(B)CBR= (bilateral) carotid body tumor resection, *interval between surgery and investigation

Blood pressure level and variability

24 hour ambuatory BP measurement (ABPM, Spacelabs, Redmond USA) was performed during normal activities at home, at reading intervals of 15 and 30 minutes during day and night-time respectively. Hypertension was defined as a mean ambulatory daytime BP (9:00AM-9.00PM) of ≥135/85 mmHg (12). The standard deviation of ABPM readings was taken as a measure of BP variability (13). BP variability was also assessed from a 5 hour beat-to-beat Portapres recording of finger arterial BP (TNO-BMI, Amsterdam, the Netherlands (14;15)). During the ambulatory Portapres recording, the following standardized activities were strictly scheduled: 12.00h: lunch, 13.30-14.30h: siesta, 15.00: 20 minutes of bicycle exercise (50 Watt, 50-60 rounds per minute), 16.00h: 30 minutes of quiet walking, 17.00h: end. In between of these activities, subjects were asked to sit down and relax. Off-line beat-by-beat BP and HR values were derived from the arterial waveform using the FAST-System software package (16).

Cardiovascular reflex tests and baroreflex sensitivity

Investigations were performed during morning time after an overnight fast in a room with a temperature of 22-24 °C. Subjects had abstained from caffeine, alcohol and smoking for at least 12 hours. Beat-by-beat finger arterial BP was measured by Finapres (14). Cardiovascular reflex tests were performed to investigate the overall baroreflex mediated HR and vasomotor control (Valsalva's maneuver, standing up (17)), efferent cardiovagal control (forced breathing, cold face test) and efferent sympathetic vasomotor control (cold pressor test, mental arithmetic) (3;17). Orthostatic hypotension was defined as a SBP decrease of ≥20 mmHg within 1-3 min of standing (18). Venous blood samples, drawn after 15 min supine rest and 10 min standing, were assayed for concentrations of epinephrine and norepinephrine using high performance liquid chromatography (HPLC) (19). Mental arithmetic was performed in the lying position after 5 minutes of rest, by repeatedly urging the subjects to subtract 17 from 5000, then from the remainder and so on for a period of 5 minutes (20). Cold pressor test and cold face test were carried out in supine position by placing the right hand in ice water for 60 sec and by covering the forehead and eyes with an ice water soaked compress for 30 sec respectively (17;21). After 15 minutes of supine rest, baroreflex sensitivity (BRS) was assessed from the response to graded bolus injections of phenylephrine (25-50-100-150 µg) (22). The dose producing a SBP increase 15-40 mmHg was repeated thrice. Linear regression analysis was performed on the RR intervals and SBP between the beginning and the end of the first significant SBP increase. BRS (msec/ mmHg), was obtained by calculating the mean of at least three slopes of statistically significant regression lines.

Peripheral chemoreflex function

To investigate peripheral chemoreflex function, the ventilatory response to hypoxia was assessed during stable normocapnic as well as during stable hypercapnic conditions. Subjects were connected to a closed spirometric circuit in sitting position. Finger arterial oxygen saturation (SaO_2 : pulse

oximetry, Nellcor 200, Hayward, CA, USA) and partial carbon dioxide pressure (P_{CO2}: Gould Godart Mark II capnograph, Bilthoven, The Netherlands) at the mouth were continuously monitored. The end-tidal carbon dioxide level (P_{ETCO2}) was controlled by adjusting a three-way valve, partially short-circuiting the CO_2 absorber in the inspiratory limb of the circuit.

The ventilatory response to hypoxia was assessed by a rebreathing method (23). SaO_2 decreased to a minimum of 80% within 3-4 minutes, while P_{ETCO2} was kept constant at the initial resting level. Ventilation was plotted against SaO_2. The inverse slope of a (statistically significant, p<0.05) regression line was taken as a measure of normocapnic ventilatory response to hypoxia (nVRH, $L*min^{-1}*[\%SaO_2]^{-1}$ (23). The hypercapnic ventilatory response to hypoxia (hVRH) was measured in a similar fashion. After breathing room air during 15 minutes, P_{ETCO2} was gradually raised until 1 kPa above the individual initial resting level. After reaching a new steady state in which both P_{ETCO2} and ventilation were stable for at least 3 minutes, progressive hypoxia was again induced and hVRH was calculated (23).

Statistics

Results are given as mean±sd unless indicated otherwise. Differences between patients and controls with respect to nominal variables were compared using the Chi-square test. Other variables were compared using the Student t-test or Mann-Whitney rank-sum test, depending on the (normal) distribution of data. A two-sided p<0.05 was taken as the level of significance. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS for Windows 6.1.3).

Results

Clinical follow-up after BCBR

Patient 1 had experienced a pulsating headache, flushing of the upper trunk and head, excitation, and diaphoresis immediately after BCBR, accompanied by severe hypertension (210/120 mmHg) and tachycardia (90 bpm). Symptoms gradually declined within three days and BP decreased to 160/110 mmHg. During the years until the study, similar attacks were evoked by exercise and mental stress. She reported complaints of emotional instability, initial orthostatic and post exercise lightheadedness. *Patient 3* had gradually developed daily complaints of initial orthostatic dizziness since BCBR and occasional complaints of near-syncope following cycling. *Patient 7* had an immediate onset of hypertension (200/120 mmHg) after BCBR and was severely invalidated by complaints of palpitations, headache, orthostatic lightheadedness and emotional irritability. Symptoms resolved within four months. Two months after surgery, BP level had decreased to pre-operative values (102/72 mmHg). *Patient 8* had a mild primary hypertension before surgery (BP:150/92 mmHg). Temporary symptoms of headache and initial orthostatic dizziness were documented following BCBR, which resolved within weeks. BP rose from 132/90 to 160/108 mmHg immediately after BCBR. During the two years until

the study, BP remained slightly elevated (146/96 mmHg). *In patients 2, and 4-6*, symptoms fitting baroreflex failure were absent and BP data were not available.

BP/ HR level and variability

Mean ABPM values were similar between groups (table 2). None of the patients met the criteria for hypertension. Standard deviations of ambulatory BP(17) were higher in patients than in controls (sd daytime DBP, sd night-time DBP and sd night-time SBP: P<0.05), whereas HR variability did not differ. Absolute day-night time differences were similar. A higher BP variability in BCBR patients is illustrated by broader BP distibutions, calculated from the Portapres recordings (figure 1). HR variability during Portapres recordings was similar. (sd HR BCBR 15.6±2.2 bpm vs controls 15.2±4.2 bpm, ns).

Baroreflex sensitivity (figure 2)

BRS was significantly lower in patients than in controls: 6.4±7.2 ms/ mmHg and 14.7±6.6 ms/ mmHg respectively (p=0.011). In 6 of 8 patients BRS was less than 5 ms/ mmHg versus in 1 of 12 controls.

Table 2. Blood pressure level and variability: 24 hours Spacelabs

	patients			controls		
BP level						
daytime						
SBP	120.8	±	8.0	123.8	±	10.7
DBP	78.7	±	6.3	78.4	±	7.9
HR	79.8	±	4.8	75.7	±	9.3
night-time						
SBP	104.1	±	11.1	106.1	±	15.7
DBP	63.6	±	6.6	62.3	±	11.2
HR	68.3	±	5.9	59.6	±	7.5
BP variability						
daytime						
sd SBP	12.8	±	3.2	10.3	±	3.3
sd DBP	10.0	±	1.9*	8.2	±	1.6
sd HR	11.1	±	2.3	11.4	±	3.6
night-time						
sd SBP	10.6	±	2.6*	7.0	±	1.4
sd DBP	9.1	±	1.2*	6.1	±	1.6
sd HR	8.2	±	5.1	4.7	±	1.7

Sd= standard deviation, SBP= systolic blood pressure (mmHg), DBP= diastolic blood pressure (mmHg), HR= heart rate (beats/min), daytime: 9 am-9pm, night-time: 1am-7am, *p<0.05
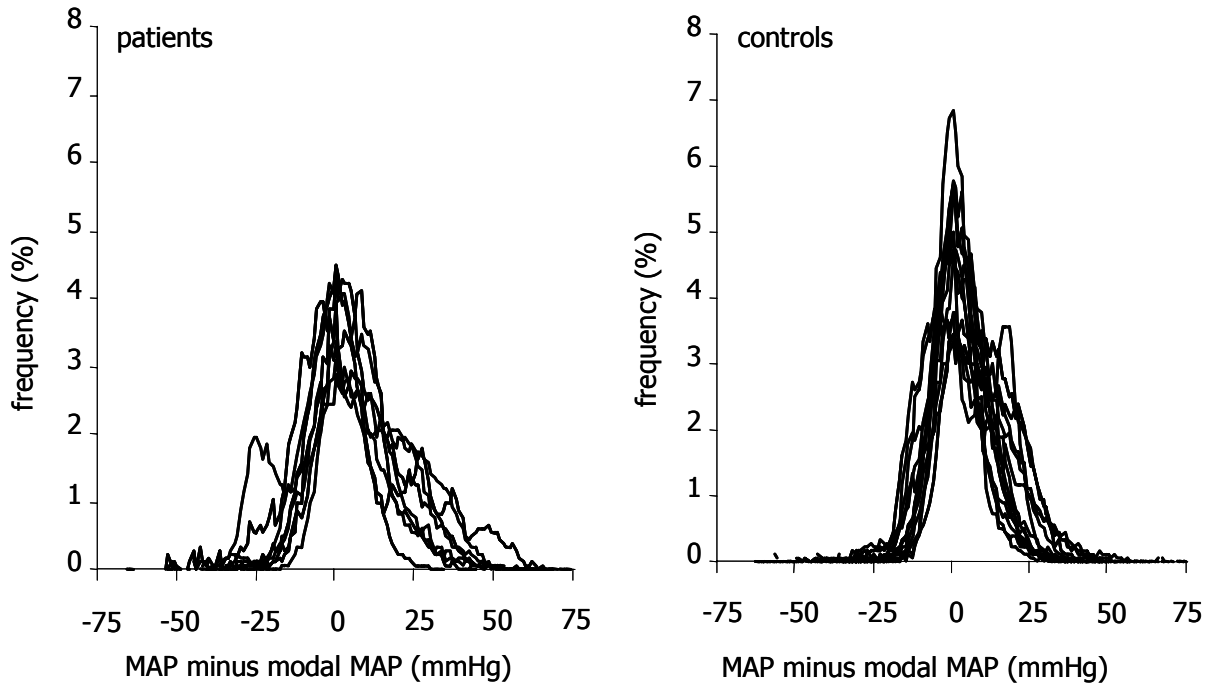
Figure 1. Frequency histograms of BP; 5 hours Portapres registration. MAP = mean arterial pressure, y-axis: frequency of MAP level as percentage of total number of frequencies
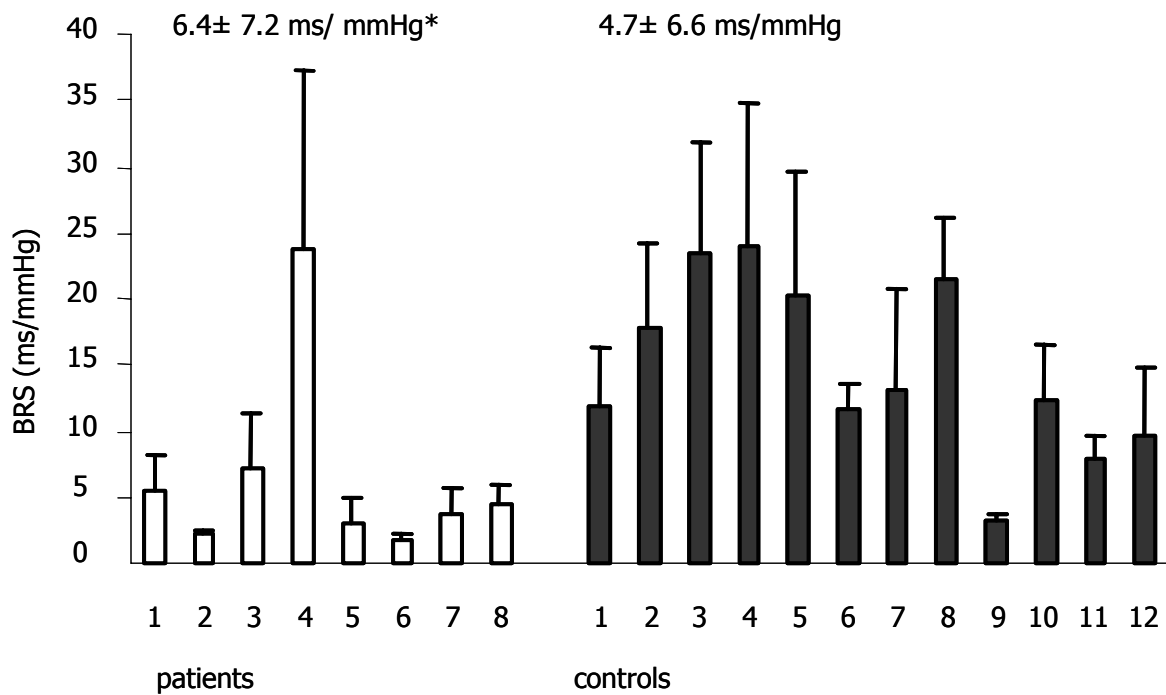
Figure 2. Baroreflex sensitivity; individual mean±sd responses to phenylephrine. BRS= baroreflex sensitivity, *p=0.011

Cardiovascular reflex tests (table 3)

In response to Valsalva's maneuver, the maximal HR was not different between groups, whereas the Valsalva-ratio was lower in patients (1.4±0.2) than in controls (1.8±0.5, p=0.008). BP levels during phase 2 and 4 were similar. In response to standing up, groups exhibited a similar initial fall in BP and a similar HR increase. An overshoot was absent in 6 of 8 patients (75%) and 3 of 12 controls (25%, p=0.028). During prolonged standing, three patients (4, 6, 8) exhibited persisting orthostatic hypotension. Values for BRS in these patients were 23.7, 1.7 and 4.4 ms/mmHg respectively (figure 2). Patient 4 reported lightheadedness during the first 4 min, which then gradually resolved, although orthostatic hypotension persisted. In this particular patient, the mean increase in HR from baseline was +20.6±7.0 bpm during 10 minutes of standing. Patient 6 and 8 did not experience lightheadedness. They both showed an abnormally low increase in HR of +5.7±1.7 and +5.7±2.2 bpm respectively. Baseline levels of venousplasma (nor)epinephrine were not different between groups. Standing-up elicited similar catecholamine responses (figure 3). Catecholamine responses were normal in patients with orthostatic hypotension. Inspiratory- expiratory HR differences were normal in all patients and controls. A reflex bradycardia was evoked by cold face test in all subjects. BP and HR responses to both mental arithmetic and cold pressor test did not differ between groups (figure 4). There was a large inter-individual variability however. Patients 6, 7 and 8 exhibited an exaggerated BP response to both mental arithmetic (dSBPmax> 40 mmHg) and cold pressor test (dSBPmax> 50 mmHg). An exaggerated response to cold pressor test was observed in controls 1 and 6.

Peripheral chemoreflex function (figure 5)

At baseline, mean SaO_2 did not differ (BCBR: 97.4±2.1% versus controls: 97.0±2.3%) nor did ventilation (6.1±1.5 versus 7.6±1.8 l/min), whereas P_{ETCO2} was higher in patients (6.6±0.7 versus 5.4±0.8 kPa, p=0.013). The 1kPa increase in P_{ETCO2} induced an increase in ventilation of 6.4±5.2 l/min (patients) and 9.2±3.4 l/min (controls, ns). The nVRH was completely absent in all patients. Patient 5 and 8 showed a low hVRH of 0.21 and 0.17 $L*min^{-1}*[\%SaO_2]^{-1}$, whereas hVRH was absent in the other patients. Averages for nVRH and hVRH were lower in patients (0.00±0.00 and 0.05±0.09 $L*min^{-1}*[\%SaO_2]^{-1}$ than in controls (0.22±0.14 and 1.28±0.83 $L*min^{-1}*[\%SaO_2]^{-1}$, p<0.01).

Table 3. Cardiovascular reflex tests

	patients			controls		
Valsalva's maneuver						
dHRmax	+26.8	±	10.7	+32.1	±	15.7
Valsalva ratio	1.4	±	0.2*	1.8	±	0.5
dSBPmax	+29.9	±	19.5*	+26.6	±	18.2
dDBPmax	+19.3	±	11.8*	19.2	±	9.3
standing up						
dHRmax	+21.9	±	13.7	+24.8	±	11.7
HRmax/min ratio	1.2	±	0.15	1.32	±	0.23
dSBPmin	-37.8	±	10.3	-32.5	±	10.3
dDBPmin	-20.5	±	7.1*	-18.6	±	5.4
dSBPmax	-6.0	±	10.6*	+4.9	±	15.1
dDBPmax	+1.4	±	7.4*	+7.8	±	8.2
forced breathing						
I-E diference in HR	17.0	±	5.8	14.9	±	7.5
cold face test						
dHRmin	-16.1	±	11.4	-7.4	±	8.7

Finapres measurements; SBP= systolic blood pressure (mmHg), DBP= diastolic blood pressure (mmHg), HR= heart rate (beats/min), d-max= maximal increase from baseline, I-E= inspiratory- expiratory, mean± sd, *$p<0.05$
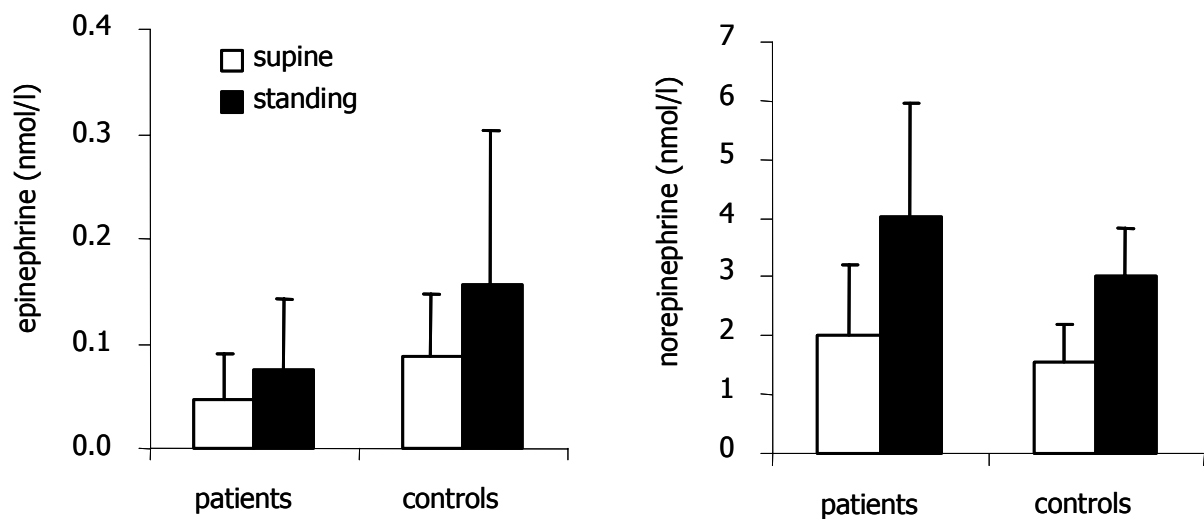
Figure 3. Venous plasma catecholamine levels.
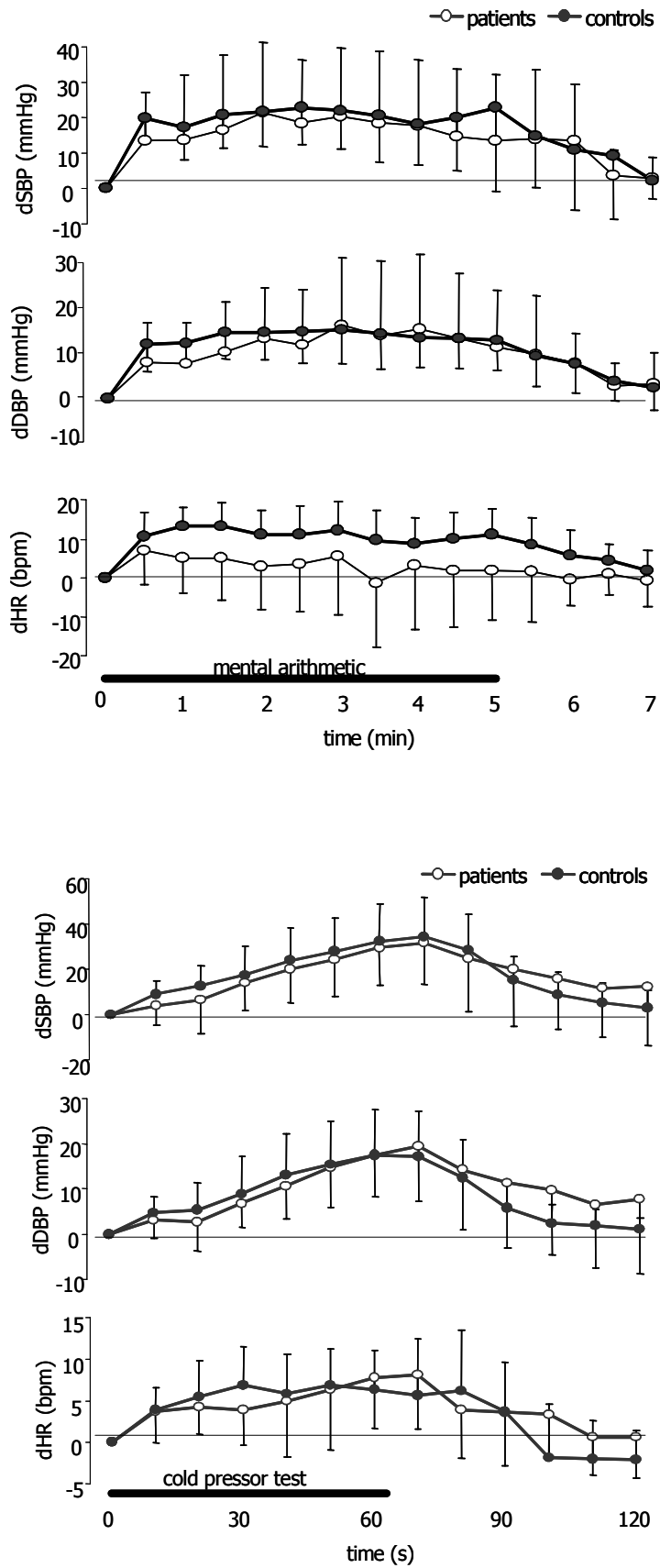
Figure 4. BP and HR response to mental arithmetic **(A)** and cold pressor test **(B)**. Changes in SBP= systolic blood pressure, DBP= diastolic blood pressure and , HR= heart rate
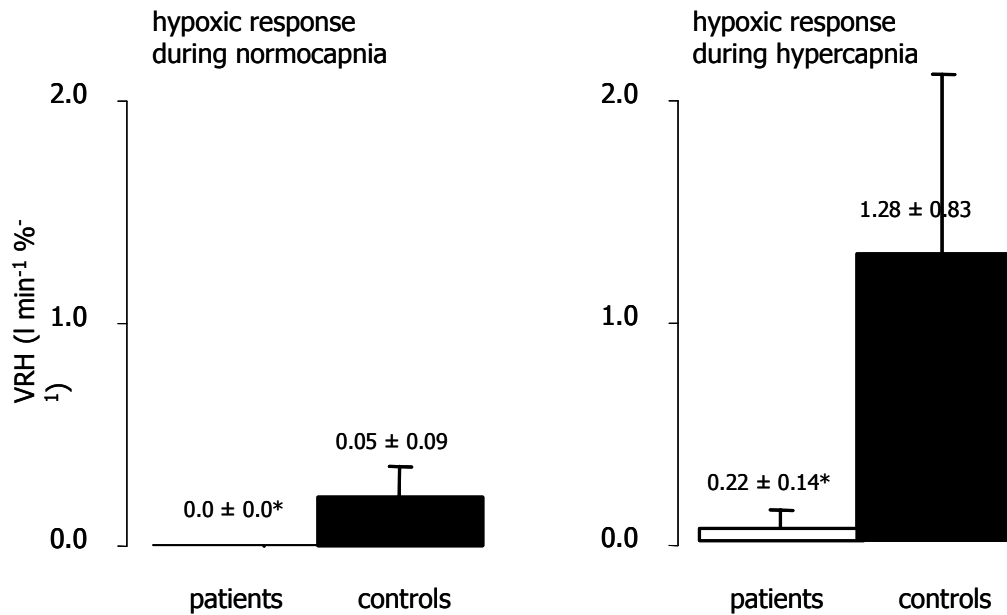
Figure 5. Ventilatory response to hypoxia. Responses were assessed during simultaneous normocapnia (left panel) and hypercapnia ($PetCO_2$+1 kPa, right panel) *patients versus controls: $p < 0.01$. VRH= ventilatory response to hypoxia.

Discussion

We investigated the chronic effects of BCBR on baro- and chemoreflex function. BCBR elicited acute baroreflex failure syndrome in three of thirteen patients. Symptoms and signs in these patients gradually resolved within months, except in one patient in whom symptoms and signs persisted for years. Irrespective of symptoms and signs, BCBR patients exhibited an attenuated BRS and an increased BP variability, but no hypertension. Three patients exhibited orthostatic hypotension and three BCBR patients exhibited an exaggerated BP response to mental arithmetic and cold pressor tests. After BCBR, the normocapnic ventilatory response to hypoxia was absent in all patients, while a small response to hypoxia was observed under hypercapnic conditions in two patients.

Arterial baroreflex function after BCBR

Carotid sinus denervation in humans may occur as a complication of BCBR (1;10), radiotherapy and surgery for pharyngeal carcinoma (1;4), carotid endarterectomy (24), trauma of the neck (1) and ischemic or neurodegenerative lesion of the nucleus tractus solitarii (1;25). The resulting clinical syndrome of baroreflex failure is characterized by paroxysms of hypertension and tachycardia with elevation of plasma catecholamines (1). It has been suggested, that BCBR invariably leads to (partial) denervation of the carotid sinus, resulting in decreased BRS, increased BP level and variability and abnormal cardiovascular reflexes (26). Previous studies have been focussed on selected patients with full-blown iatrogenic baroreflex failure. There are no prospective follow-up studies on baroreflex function following BCBR. The present retrospective study, suggests that clinically relevant baroreflex failure occurs in a minority of patients and is usually temporary. Therefore, the risk of this – undoubtedly very important- complication seems to be overemphasized in the literature due to patient

selection. On the other hand, in the absence of fullblown baroreflex failure, baroreflex function does appear to be affected by BCBR in a more subtle fashion.

Baroreflex sensitivity: The finding of a decreased BRS in BCBR patients suggests impairment of reflex control of HR due to (partial) denervation of the carotid sinus baroreceptors. The absence of a reflex HR response to exogenous vasoactive substances is considered as a key feature of full-blown baroreflex failure (1). However, our data indicate, that the relationship between an attenuated BRS and clinical baroreflex dysfunction is gradual. One patient with symptoms and signs of baroreflex failure had a BRS of 5.4 ms/mmHg. Two patients in whom clinical baroreflex failure had resolved at the time of the study had even lower low values of BRS (3.7 and 4.4 ms/mmHg). Unfortunately, pre-operative and short-term follow-up data on BRS are lacking. However, the isolated value of BRS is a poor indicator of overall baroreflex function in the individual patient, since BRS varies considerably among healthy subjects. BRS ranged from 3 to 24 ms/mmHg among healthy controls. In addition, BRS only reflects the integrity of the baroreflex modulation of the efferent cardiovagal effector mechanisms, whereas hypertensive and tachycardic paroxysms in baroreflex failure result from desinhibition of the efferent sympathetic limb of the baroreflex. It has been shown, that deafferentiation of arterial baroreceptors may selectively affect the modulation of efferent sympathetic and parasympathetic pathways (27).

BP level and variability: After bilateral carotid sinus denervation in animals, BP level and variability initially increase markedly but normalize to preoperative levels within 10-14 days (28;29). In humans, sustained hypertension appeared to be limited to days to months after surgical carotid baroreceptor denervation, whereas episodic surges of hyper- as well as hypotension may persist for a longer period (1;8;10). Our findings are in agreement with these observations. In three of eight patients presented here, hypertension was documented consistently during several months after BCBR. However, at the time of this study, at least 1 year after surgery, ambulatory BP levels were normal in all patients. On the other hand, ambulatory BP measurements show, that BP variability remains markedly increased after BCBR. This can be ascribed to the persistent decrease in BRS. In hypertensives, BRS was shown to be negatively correlated with BP variability and positively to HR variability (30) (31). In the present study, BRS was not significantly correlated with either BP or HR variability. This might be due to the small sample size.

Cardiovascular reflexes: In line with a decreased BRS, the compensatory changes in HR during Valsalva's maneuver were attenuated in BCBR patients. Normal I-E differences during forced breathing, and the normal reflex bradycardia during the cold face test prove an intact cardiovagal innervation in all patients. Thus, the low mean Valsalva ratio can only be explained by an abnormality in the afferent limb of the baroreflex (17). A progressive decrease of BP during phase 2 as reported in baroreflex failure patients (4;11) was not observed. Orthostatic hypotension was originally claimed not to be part of the baroreflex failure syndrome (32). However, compensatory mechanisms were shown to fail after squatting (4) and orthostatic hypotension was even reported as a presenting symptom in primary (idiopathic) afferent arterial baroreflex failure (33). In other cases it may not become

apparent until years after baroreceptor denervation (26). Our findings of an abnormal orthostatic response in 3 of 8 BCBR patients reiterates, that baroreflex failure may indeed cause orthostatic hypotension. Three patients exhibited an abnormally large increase in BP to both cold pressor test and mental arithmetic. Excessive increments of sympathetic tone including a marked elevation of catecholamine plasma levels in response to physical (and emotional) stress are explained by central desinhibition of efferent sympathetic pathways due to lack of arterial baroreceptor input (1;34).

Peripheral chemoreflex function after BCBR

The present study is the first report on the effect of carotid paraganglioma surgery on ventilatory control in humans. The role of the carotid bodies in human ventilatory control has been studied in patients after bilateral resection of normal carotid bodies as an experimental treatment of bronchial asthma or chronic obstructive pulmonary disease (7;8;35;36). These subjects do not hyperventilate in response to experimentally induced hypoxemia (8;36). In addition, they do not show a decline in ventilation following the abrupt and surreptitious administration of 100% oxygen against a hypoxic background (36). This abnormality was shown to persist on the long-term after bilateral carotid body resection (7). In contrast to previous investigations, data on the effect of carotid body resection in the present study are not hampered by the possible confounding of chronic pulmonary disease, which itself may alter chemoreflex function (37). We demonstrated an abolishment of the normocapnic ventilatory response to progressive hypoxia after BCBR in all patients.

Only two patients did have an attenuated ventilatory response to hypoxia under hypercapnic conditions, suggesting slight residual peripheral chemoreflex function. It is generally accepted, that an increased arterial P_{CO_2} enhances peripheral hypoxic chemosensitivity. In line with our observations, Honda (7) and Swanson (38) demonstrated a small component of hypoxic ventilatory drive during simultaneous hypercapnia in patients after carotid body resection for chronic pulmonary disease. It was suggested that residual responsiveness to hypoxemia in these patients might originate from the aortic bodies, which subserve a minor role in the modulation of spontaneous respiratory activity, but may generate a discernible response when their gain is increased by hypercapnia (39). The normoxic transition from normocapnia to 1 kPa hypercapnia increased ventilation by 6.4±5.2 l/min in patients versus 9.2±3.4 l/min in controls, which was not statistically significant. Taking into account the large standard deviations and small sample size, attenuation of hyperventilation in response to hypercapnia in BCBR patients cannot be ruled out. In addition, resting end tidal PCO_2 was slightly higher in the BCBR group. From a previous study it was concluded that 30% of the ventilatory drive to hypercapnia is modulated by peripheral chemoreceptor function (36). BCBR may cause relative hypoventilation resulting in increased resting P_{ETCO_2} levels and a blunted CO_2 response.

Conclusions

BCBR elicits the full-blown clinical syndrome of baroreflex failure only in a minority of cases and when it does, it is usually temporary. This risk tends to be overestimated in literature due to patient selection. Nevertheless, BRS appears to be chronically attenuated after BCBR. This results in a heterogeneous expression of mild arterial baroreflex dysfunction, including increased day and night-time BP variability, unopposed sympathetic activation in response to physical and mental stress and orthostatic hypotension. Differences in the extent of carotid sinus denervation due to BCBR, the activation of central and residual aortic baroreceptor mediated compensatory mechanism and the regeneration of carotid sinus afferent innervation may account for this heterogeneity.

The findings of an abnormal hypoxic ventilatory drive following bilateral carotid body tumor surgery are in line with the observations after experimental carotid body resection in asthmatic patients. The present study, lacking the possible confounder of chronic pulmonary disease, emphasizes the importance of carotid relative to aortic chemoreceptor function in humans.

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Baroreflex control of muscle sympathetic nerve activity following carotid body tumor resection

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Abstract

Bilateral carotid body tumor resection causes a permanent attenuation of vagal baroreflex sensitivity. We retrospectively examined the effects of bilateral carotid body tumor resection on the baroreflex control of sympathetic nerve traffic.

Muscle sympathetic nerve activity was recorded in five patients after bilateral carotid body tumor resection (BCBR, 1m:4f, 51± 11 years) and six healthy controls (2m:4f, 50±7 years). Baroreflex sensitivity was calculated from changes in RR interval and muscle sympathetic nerve activity in response to bolus injections of phenylephrine and nitroprusside. In addition, sympathetic responses to Valsalva's maneuver, and cold pressor test were measured.

The integrated neurogram of patients and controls contained a similar pattern of pulse synchronous burst of nerve activity. Baroreflex control of both heart rate and sympathetic nerve activity were attenuated in patients as compared to controls: Heart rate baroreflex sensitivity: 3.68±0.93   vs 11.61±4.72 ms/mmHg (phenylephrine, p=0.011) and 2.53±1.36 vs 5.82±1.94 ms/mmHg (nitroprusside, p=0.05). Sympathetic baroreflex sensitivity: 3.70±2.90 vs 7.53±4.12 activity/100beats/mmHg, (phenylephrine, p=0.10) and 3.93±4.43 vs 15.27±10.03 activity/100beats/mmHg (nitroprusside, p=0.028). Valsalva's maneuver elicited normal reflex changes in muscle sympathetic nerve activity, whereas heart rate responses were blunted in the BCBR patients. Maximal sympathetic responses to cold pressor test did not differ between the two groups.

Denervation of carotid sinus baroreceptors due to bilateral carotid body tumor resection produces chronic impairment of baroreflex control of both heart rate and sympathetic nerve activity. During Valsalva's maneuver, loss of carotid baroreflex control of heart rate is less well compensated for by the extra carotid baroreceptors than the control of muscle sympathetic nerve activity.

Introduction

Efferent sympathetic nerve traffic is strongly governed by the restraining effects of arterial and cardiopulmonary baroreceptors (1). Direct stimulation of carotid sinus baroreceptors in humans causes a reduction of muscle sympathetic nerve activity (MSNA) (2). In contrast, anaesthetic deafferentiation of arterial and cardiopulmonary baroreceptors results in a strong increase in MSNA accompanied by hypertension and tachycardia (3). In addition, normal cardiac rhythmicity of MSNA is lost following baroreceptor denervation. Apart from experimental denervation, iatrogenic denervation of baroreceptors may occur as complication of bilateral carotid body tumor resection (BCBR) (4;5), neck or mediastianal irradiation (4;6;7) and carotid endarterectomy (8). The resulting clinical syndrome of baroreflex failure is characterized by recurrent bouts of unrestrained sympathetic excitation, manifesting as severe hypertension, headache and diaphoresis. The findings of excessive rises in plasma catecholamines during these attacks and of exaggerated pressor responses to cold and mental stress in these patients suggest the loss of baroreflex-mediated inhibition of efferent sympathetic

nerve activity (4;6). In a previous study, we have demonstrated that although BCBR elicits the full-blown syndrome of baroreflex failure only in a minority of patients (9), baroreflex control of heart rate is impaired and blood pressure variability is increased on the long-term following BCBR (9;10). Whether BCBR also affects baroreflex control of sympathetic outflow has not yet been established in humans.

The aim of this study was to examine the chronic effects of BCBR on the baroreflex control of sympathetic nerve activity. In this cross-sectional, retrospective study of BCBR patients and age-matched healthy controls, sympathetic baroreflex sensitivity was calculated from MSNA responses to (de-)activation of baroreceptors by phenylephrine and nitroprusside bolus injections. In addition, MSNA responses to Valsalva's maneuver and cold pressor test were assessed.

Methods

Patients and controls
Five patients (1 male, 4 females) who had undergone two stage BCBR at the Department of Otolaryngology of the University Medical Center Nijmegen, the Netherlands, were included in this study. Individual information on tumor size, additional tumor localizations and surgical details of these five patients are shown in table 1. The median interval between the second operation and the study was 6.7 years (range: 4.4-20.3 years). Patients were free of diabetes, neurological, cardiovascular and pulmonary disease. Six healthy subjects (2 males, 4 females) served as controls. Full medical history and physical examination including blood pressure measurements revealed no abnormalities. Groups were matched for age (BCBR: 51.2±10.8 versus controls: 50.0±6.5 years), body mass index (24.8±1.1 versus 25.7±3.8 kg/m^2) and alcohol intake (7.8±8.5 versus 9.0±7.4 units/ week). The study protocol was approved by the institutional ethics committee and all subjects gave their informed consent.

Blood pressure, heart rate, breathing frequency
Investigations were carried out during morning time after an overnight fast in a room with an ambient temperature of 22-24°C. Subjects had abstained from caffeine, tea, alcohol, chocolates and smoking for at least 24 hours. Office systolic and diastolic blood pressure (SBP/ DBP) was determined from the mean of 3 supine sphygmomanometric measurements after 10 minutes of rest. Continuous finger arterial blood pressure was monitored by a Finapres device (TNO, The Netherlands, model 5)(11) and heart rate (HR) by surface ECG connected to a Hewlett Packard 378341A monitor. Respiratory rate was monitored from changes in inspiratory and expiratory air temperature by means of a nose thermistor (Fysicon Medical Technology, Oss, The Netherlands). An intravenous line was placed in an antecubital vein for collection of blood samples and administration of vasoactive drugs. All measurements were done in the supine position.

Table 1. Characteristics of individual BCBR patients

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Sex/ age	F44	F58	F44	F62	M36
First CBR:					
Time since (y) *	7.1	20.4	7.8	5.3	7.4
Right/ left	right	left	left	left	left
Tumor size (max cm) †	2.5	2.0	4.0	3.0	2.0
Radical resection †	yes	no	yes	yes	yes
(Cranial) nerve damage †	-	-	nIX	-	-
Second CBR:					
Time since (y) *	6.1	20.3	6.7	4.4	6.6
Right/ left	left	right	right	right	Right
Tumor size †	1.0	1.5	3.0	2.0	1.5
Radical resection †	yes	no	yes	no	yes
(Cranial) nerve damage †	-	Cervical plexus	-	-	-
Symptoms/ signs of baroreflex failure	persistent	-	-	-	transient
Additional paraganglioma localisations/ therapy		Right side skull basis: radiotherapy			
Family history of paraganglioma	yes	yes	no	yes	yes
Medication		chloorthalidon, atenolol, moxonidine			atenolol

CBR: carotid body tumor resection, * interval between surgery and investigation, † according to surgical and pathological reports

Sympathetic nerve recordings

Multi-unit microneurographic recordings of post-ganglionic MSNA were obtained with a unipolar tungsten electrode inserted selectively into a muscle-nerve fascicle of the right peroneal nerve, posterior to the fibular head as originally described by Sundlöf and Wallin (12). Recordings were made with tungsten microelectrodes with a 200µm shaft diameter, tapering to a 1 to 5 µm uninsulated tip. A reference electrode was inserted subcutaneously 1 to 3 cm from the recording electrode. Electrodes were connected to a preamplifier with a gain of 1000 and an amplifier with a gain that could be varied from 30 to 90 as required in a subject. Amplification was constant throughout the study in each subject. Neural acvtivity was fed through a bandpass filter with a bandwidth of 700 to 2000 Hz. The filtered neurogram was routed through an amplitude discriminator to a storage oscilloscope and a loudspeaker. For recording and analysis, the filtered neurogram was fed through a resistance-capacitance integrating network (time constant 0.1 sec) to obtain a mean voltage neurogram of

MSNA. Acceptable recordings met the following criteria: spontaneous bursts of neural discharge, no response to arousal stimuli or skin stroking, an increase in nerve burst frequency with apnea and an amplitude to noise ratio of 3:1. In contrast to the usual criteria for identification of MSNA, the criterium of pulse-synchronicity was initially omitted, since cardiac rhythmicity of MSNA may be lost after deafferentiation of baroreceptors (3).

Study protocol

After 20 minutes of supine rest, 10 minutes baseline MSNA was recorded. Baroreflex control of heart rate and sympathetic nerve traffic was assessed from RR-interval and MSNA responses to increments and decrements in blood pressure induced by phenylephrine (PHE) and sodium nitroprusside (SNP) respectively (13). After a 20-minute baseline period, graded bolus injections of phenylephrine (25-50-100-150 μg) followed by injections of SNP (12.5-25-50-100 μg) were given intravenously at intervals of 10 minutes. The dosage producing an increase (PHE) or decrease (SNP) in arterial pressure of ≈15 mmHg was repeated thrice. After 15 minutes of supine rest baroreflex control of HR and MSNA during Valsalva's maneuver was examined (14;15). Subjects were asked to maintain an expiratory pressure of 40 mmHg during 15 sec, by means of forced expiration into a mouthpiece connected to a pressure transducer. Closure of the glottis was prevented by a small leak to maintain a flow of air. A cold pressor test was carried out by placing the right hand in ice water for 120 sec (16;17).

Data analysis

A computer-assisted method was applied for automatic detection and quantification of individual bursts of sympathetic nerve activity by means of a curve-fitting method. During a pre-scan of the tracing, the neurogram was correlated with a triangular signal by applying a least squares algorithm. In order to define "reference bursts", the 100 largest triangular waves detected during the pre-scan were taken. After discarding the 20 largest waves for possible artifacts, the remaining 80 were taken as a reference. Their mean amplitude and delay from the corresponding R-wave on the ECG were determined. During a subsequent scan, the individual amplitudes and time delays of all triangular waves were compared with the mean amplitude and delay of the reference waves. Waves were accepted and marked as sympathetic bursts on two conditions: an amplitude of >20% of the mean reference amplitude and a time delay of <200 ms beneath or above the mean reference delay. Automatically calculated burst amplitude correlated well (r>0.9) with manual burst detection (unpublished data). MSNA was expressed as number of bursts per minute and per 100 beats, total integrated activity (=summed amplitude of bursts) per minute (TIA/min) and total integrated activity per 100 beats (TIA/100beats).

Baroreflex control of heart rate (hBRS) was assessed by means of linear regression analysis between changes in SBP and RR-interval during PHE- and SNP-induced BP ramps. The mean slope of at least three statistically significant regression lines of PHE and SNP trials were taken as $hBRS_{PHE}$ and $hBRS_{SNP}$ respectively.
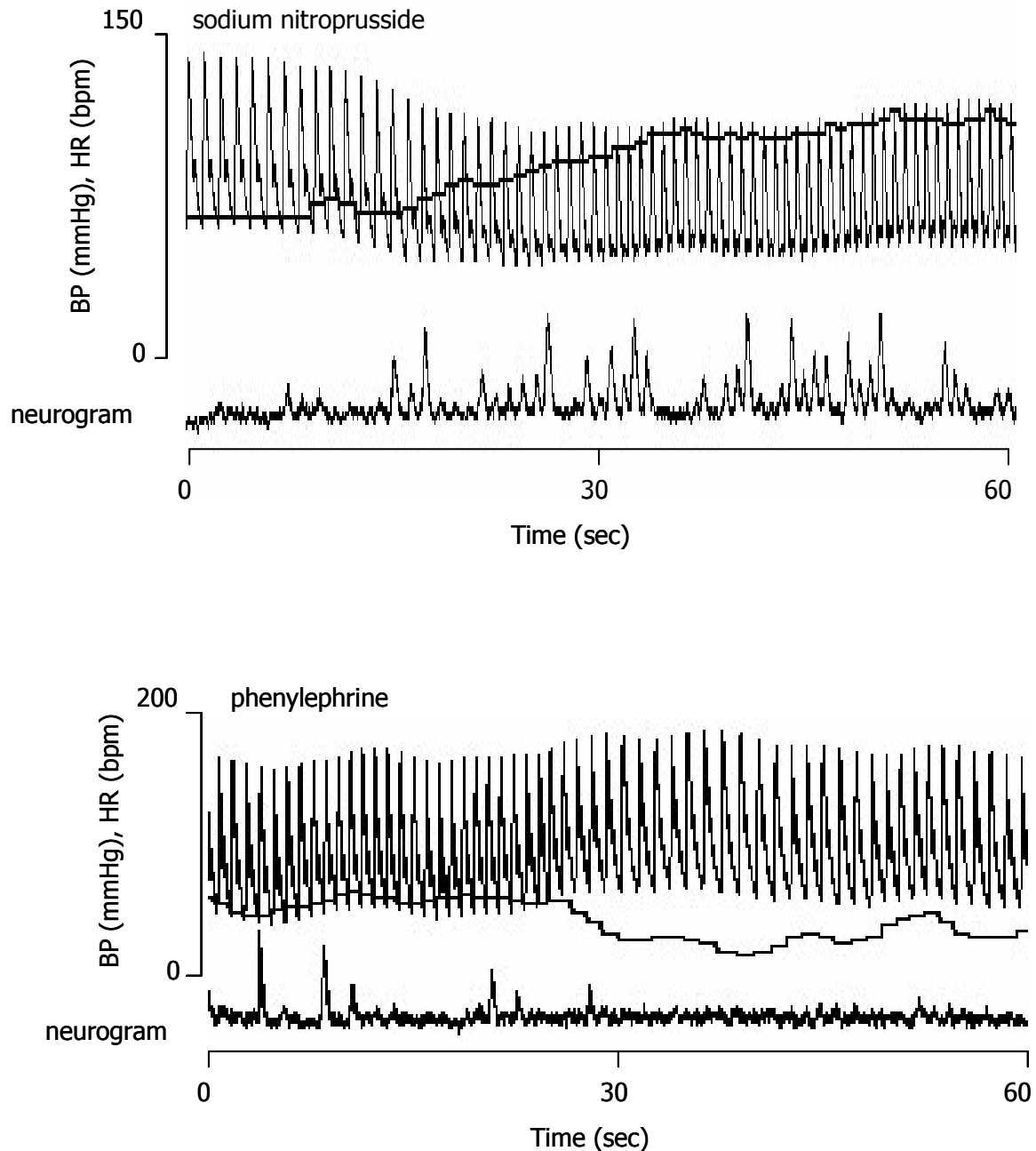
Figure 1. Sodium nitroprusside and phenylephrine injections in control subject #1. Tracings of arterial pressure, heart rate and muscle sympathetic nerve activity; injection at t=0s

Sympathetic baroreflex sensitivity (sBRS) was calculated from changes in MSNA evoked by absolute changes in DBP induced by PHE and SNP, since diastolic blood pressure correlates more closely to MSNA than systolic blood pressure (18). After combining MSNA and pressure data of the 3 SNP trials, relative changes in total integrated MSNA activity (%TIA/min, %TIA/100beats) were pooled over 3-mmHg pressure ranges (13;19). Any heart beat not followed by a burst was assigned an MSNA activity of zero. Linear regression analysis between relative changes in MSNA and DBP was performed. The slope of a statistically significant regression line was taken as sBRS$_{SNP}$. Values for sBRS$_{PHE}$ were calculated in a different way, since the relation between changes in MSNA and DBP is

not necessarily linear. In our experience, PHE-induced rises in BP often elicit abrupt silencing of MSNA. Therefore, we compared mean MSNA during the BP ramp after PHE injection with the mean MSNA during 20 sec at baseline. $sBRS_{PHE}$ was calculated as the mean decrease in MSNA (%TIA/min, %TIA/100beats) of 3 PHE trials divided by the mean increase in DBP during the ramp.

The HR response to Valsalva's maneuver was expressed as the maximal increase in HR during phase 2 and the ratio between the highest and lowest heart rate during phase 2 and 4 respectively (Valsalva ratio) (15). The maximal phase 2 decrease in BP was calculated as the difference between the maximal and minimal BP during phase 2. Phase 4 BP overshoot was calculated as the maximal increase in SBP and DBP as compared to baseline. The MSNA response to Valsalva's maneuver was expressed as the mean increase in MSNA during late phase 2 and the mean decrease in MSNA during the first 15 seconds of phase 4 overshoot, relative to baseline (14).

Relative changes in BP, HR and MSNA in responses to cold pressor test were expressed as 1-minute averages during the stimulus and during 2 minutes of recovery as compared to 1 minute at baseline.

Statistics

Results are given as mean±sd unless indicated otherwise. Differences between patients and controls with respect to nominal variables were compared using the Chi-square test. Other variables were compared using the Student t-test or Mann-Whitney rank-sum test when appropriate. A two-sided $p < 0.05$ was taken as the level of significance. Statistical analysis was performed using SPSS (Statistical Package for the Social Sciences) for Windows 6.1.3.

Results

MSNA at baseline

At baseline, sphygmomanometric SBP/DBP and HR did not differ between patients and controls: 120.8±11.1/ 79.0±5.9 mmHg, 66.8±8.5 bpm vs 123.3±11.9/ 81.5±7.6 mmHg, 66.2±6.5 bpm. On visual inspection, the neurograms of both patients and controls were characterized by a regular burst pattern and cardiac rhythmicity. The neurograms contained no skin sympathetic nerve (-like) activity (1). The mean time delay between reference bursts and corresponding R-waves was not different between patients (1.342±88 ms) and controls (1.337±82 ms). In patient neurograms, 34.1±24.9% of the triangular waves were discarded as possible bursts, since they did not meet the time-delay (and amplitude) criterium, which was similar to a proportion of 31.0±18.7% in controls. Sympathetic burst frequency at baseline did not differ between patients and controls: 39.6±20.0 vs 38.8±11.5 bursts/min and 49.3±21.5 vs 59.0±19.8 bursts/100 beats.
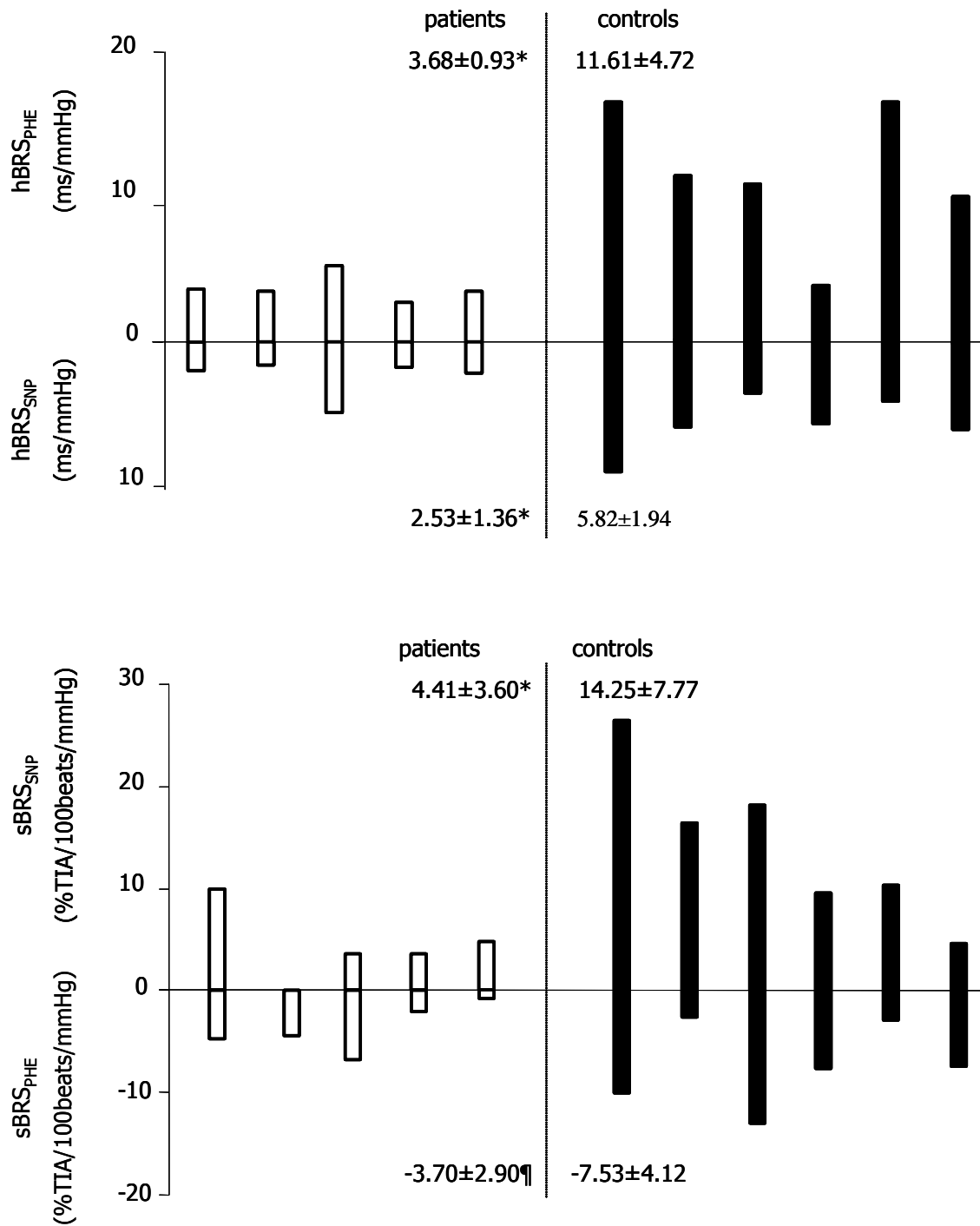
Figure 2. Vagal and sympathetic baroreflex sensitivity. In the upper panel, bars indicate individual values for vagal baroreflex sensitivity calculated from responses to phenylephrine ($hBRS_{PHE}$) and sodium nitroprusside ($hBRS_{SNP}$) expressed in milliseconds/ millimeter of mercury (ms/ mmHg). In the lower panel, individual values for sympathetic baroreflex sensitivity ($sBRS_{PHE}$ and $sBRS_{SNP}$). Group averages are shown. TIA= total integrated activity. *$p<0.05$, ¶ $p=0.10$

Baroreflex sensitivity

Tracings of an individual BP, HR and MSNA response to PHE and SNP in control subject #1 are shown in figure 1. Baroreflex control of heart rate was lower in patients than in controls: $hBRS_{SNP}$: 2.53±1.36 vs 5.82±1.94 ms/mmHg (p=0.05); $hBRS_{PHE}$: 3.78±0.93 vs 11.61±4.72 ms/mmHg (p=0.011) (figure 2). Sympathetic BRS calculated from SNP responses was also lower in patients than in controls: $hBRS_{SNP}$: 3.93±4.43 vs 15.27±10.03 %TIA/min/mmHg, p=0.028 and 4.41±3.60 vs 14.25±7.77 %TIA/100beats/mmHg, p=0.028. PHE resulted in a similar mean increase in DBP (patients: 9.52±2.93 mmHg vs controls: 9.75±2.71 mmHg, ns). $sBRS_{PHE}$ tended to be lower in patients than in controls: 3.74±2.92 vs 7.87±3.96 %TIA/min/mmHg (p=0.097) and 3.70±2.90 vs 7.53±4.12 %TIA/100beats/mmHg (p=0.10).

Valsalva's maneuver

In response to Valsalva's maneuver, the maximal increase in HR during phase 2 was lower in patients than in controls (11.0±8.3 versus 38.7±18.1 bpm, p=0.004), as was the Valsalva ratio (1.28±0.15 vs 2.04±0.31, p=0.004). The maximal phase 2 decrease in SBP and DBP was similar in patients and controls: -51.0±7.3 and -23.4±6.1 mmHg vs -48.5±25.0 and -18.3±11.5 mmHg (figure 3). Phase 4 SBP and DBP overshoot was lower in patients than in controls: +10.9±15.4 and +5.5±10.8 mmHg vs +36.2±19.0 and +21.2±9.6 mmHg (p=0.05/ 0.031). Patients and controls showed an increase in MSNA during phase 2 of +76.3±75.3 vs +104.4±71.7 %TIA/min and +74.4±71.1 vs +44.9±68.6 %TIA/100beats, which was not significantly different. Phase 4 decrease in MSNA was also similar in patients and control: -55.6±20.5 vs -61.0±15.8 %TIA/min and -56.2±21.4 vs -64.1±12.7 %TIA/100beats.

Cold pressor test

In both patients and controls, cold pressor test elicited increases in BP, HR and MSNA, which were not significantly different between groups (figure 4).
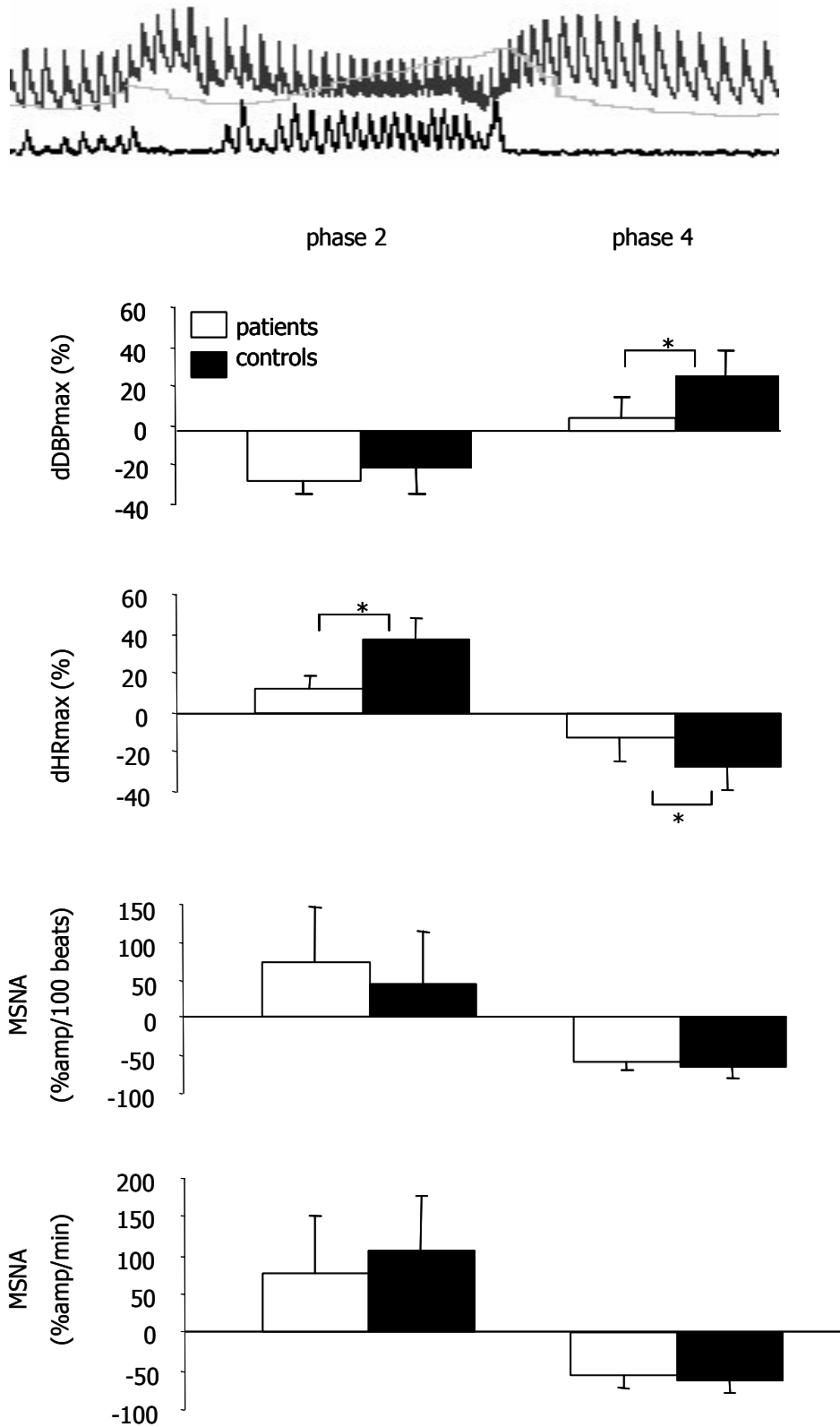
Figure 3. Valsalva's maneuver. Individual tracing of arterial blood pressure (BP), heart rate (HR) and MSNA in control subject #1 (upper panel). (MSNA was offset 1.3 seconds to account for nerve conduction delays). Group averaged responses during phase 2 (left bar pairs) and phase 4 (right bar pairs), presented as changes relative to baseline. dDBPmax and dHRmax: maximal change in diastolic blood pressure and heart rate; dMSNA: phase averaged change in MSNA; TIA= total integrated activity, * p<0.05.
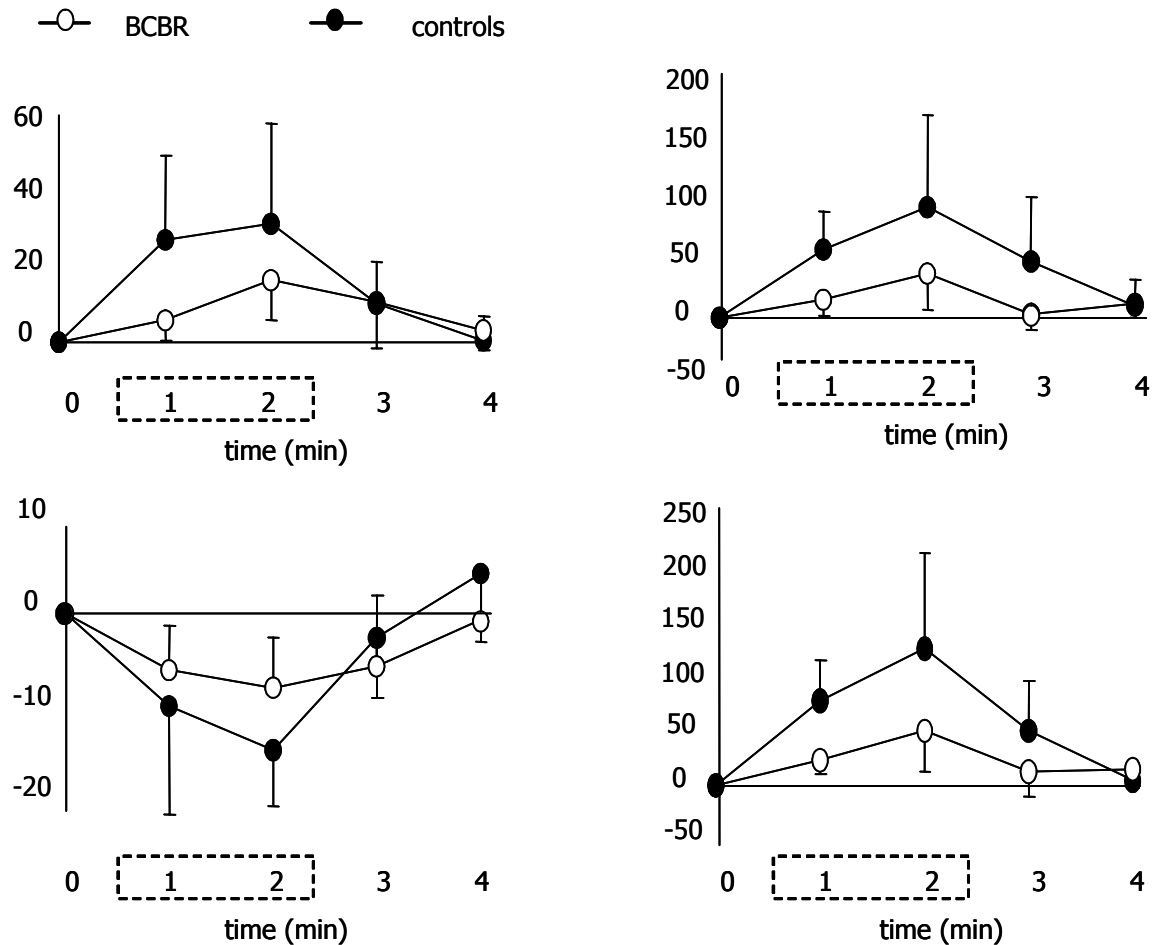
Figure 4. Cold pressor test. Relative changes from baseline in mean arterial pressure (MAP, top left), RR-interval (bottom left) and muscle symapthetic nerve activity (MSNA) expressed as total intergrated activity (TIA) per 100 beats (top right) and per minute (bottom right). Data are presented as minute averages during 2 minutes of cold stimulation (dotted square) and 2 minutes of recovery.

Discussion

Summary

Patients who had BCBR showed no permanent effects on burst incidence or pulse synchronicity of supine resting MSNA at baseline. However, there is a chronic decrease in the baroreflex adjustments of both heart rate and MSNA, as indicated by attenuated responses to both PHE-induced hypertension and SNP-induced hypotension. In contrast to an abnormal heart rate response to Valsalva's maneuver, normal compensatory changes in MSNA were observed in BCBR patients.

Effect of BCBR on baseline MSNA

Fagius et al. have shown that chemical deafferentiation of arterial and cardiopulmonary baroreceptors in man has a profound effect on the characteristics of MSNA (3). Following a local anaesthetic block of vagus and glossopharyngeal nerves, pulse synchronicity of MSNA disappeared and the resulting neurogram was characterized by bursts of impulses of variable duration occurring in a slow, irregular

rhythm. Disruption of the phase lock between sympathetic discharges and the cardiac rhythm has also been demonstrated after sino-aortic denervation of arterial baroreceptors in cats (20). In the present study, neurograms of BCBR patients exhibited a normal burst pattern, including a normal cardiac rhythmicity and an unaltered mean reflex latency of sympathetic bursts of 1.3 seconds (21). The heterogeneous expression of clinical and physiological baroreflex dysfunction suggests, that the extent to which the carotid sinus baroreceptors become denervated by BCBR differs considerably among patients (9;10). Therefore, the presence of cardiac rhythmicity of MSNA after BCBR may originate from residual carotid baroreceptors and/or unaffected aortic and cardiopulmonary baroreceptors. Absence of pulse synchronicity of MSNA in a patient after selective sinoaortic denervation due to bilateral carotid bypass surgery and mediastinal irradiation (6) suggests that MSNA rhythmicity is not generated by cardiopulmonary mechanoreceptors.

BCBR does not result in a permanent elevation of resting MSNA (this study), nor does it result in chronic hypertension (10). The acute phase of iatrogenic carotid sinus denervation, is characterized by sympathoexcitation which may persist for hours to days (4;4;5;7;8). Unselective anesthetic deafferentiation of arterial and cardiopulmonary baroreceptors in humans was shown to cause a strong rise in MSNA accompanied by hypertension and tachycardia, which persisted during the nerve block (3). In patient #1, an immediate onset of severe hypertension and tachycardia was observed following BCBR, which had gradually declined within three days. At the time of investigation, 6 years after surgery, she had an unremarkable sympathetic burst incidence of 20.3 bursts/ 100 beats and normal supine venous concentrations of epinephrine (0.24 nmol/l) and norepinephrine (1.74 nmol/l). Resting MSNA appears to be down-regulated in time, possibly by residual baroreceptor and/ or central mechanisms. Our observations are in line with animal studies. Sinoaortic denervation in rats results in acute hypertension and an increase in renal sympathetic nerve activity (22;23). Although attenuation of the baroreflex control of sympathetic outflow persists in the chronic phase (20 days after denervation), basal blood pressure level and renal sympathetic nerve activity returns to normal. This normalisation was suggested to be result from the net effect of the abolishment of inhibitory (baroreceptor deafferentiation) and excitatory (chemoreceptor deafferentiation) influences.

Despite a normal resting level of MSNA, paroxysms of sympatho-excitation due to inadequate buffering of spontaneous fluctuations in sympathetic activity may persist after baroreflex denervation (4;7). However, we were unable to provoke an excessive sympathetic response by a cold pressor test, which may be present in patients with the full-blown clinical syndrome of baroreflex failure (4).

Effect of BCBR on baroreflex sensitivity

Our previous report on an attenuated baroreflex control of heart rate in BCBR patients (9) was confirmed by the present study. In addition, we demonstrated that BCBR causes a chronic decrease in sympathetic BRS as well. However, reflex changes in heart rate and MSNA still did occur in response to both PHE and SNP injections in all patients, except in patient #2, in whom repeated blood pressure decreases of 15 mmHg did not elicit any change in MSNA (figure 2). In contrast our findings in

patients with presumed carotid-selective denervation, deafferentiation of both carotid and aortic baroreceptors causes complete abolishment of MSNA responses to PHE and SNP (6). This suggests, that both carotid and aortic receptors are important modulators of sympathetic nerve traffic in humans.

MSNA responses to PHE were analyzed in a different manner than SNP ramps, since pressure elevations usually provoked sudden silencing of MSNA in controls. In line with previous reports (24;25), linear regression analysis between pressure and MSNA during PHE ramps yielded no significant correlation coefficients. However, this does not imply a larger gain of sympathetic BRS during pressure increases than during decreases. Brusque MSNA silencing by PHE is explained by the fact that resting blood pressure level is near the threshold for sympathetic activation on the sigmoid relationship between MSNA and blood pressure (26). Therefore, SNP administration is a more sensitive tool for the evaluation of baroreflex control of sympathetic outflow than PHE. In addition we prefer bolus injections over step-wise infusion of SNP, since MSNA is determined by changes, rather than absolute levels of baroreceptor activity (18).

Effect of BCBR on functional baroreflex performance during Valsalva's maneuver

Phase 2 and 4 changes in blood pressure during Valsalva's maneuver elicited normal reciprocal adjustments of MSNA in BCBR patients and controls (14), whereas heart rate responses were blunted in patients. This discrepancy suggests a differential effect of BCBR on the functional baroreflex modulation of heart rate and sympathetic nerve activity. The relative roles of carotid sinus and aortic baroreceptors in the reflex control of heart rate and vascular resistance during changes in arterial blood pressure have been investigated by denervation experiments in rabbits (27). It was shown, that reflex heart rate responses to PHE were impaired significantly by denervation of either carotid or aortic baroreceptors. In contrast, reflex vascular responses in the hindlimb (perfused at constant blood flow) were preserved except for a slight impairment of reflex vasoconstriction after aortic baroreceptor denervation. In line with these observations, (partial) denervation of carotid baroreceptors due to BCBR is not compensated by aortic receptors with respect to heart rate control. In contrast, residual aortic baroreceptors after BCBR, are capable of reflex modulation of MSNA to a certain extent.

Previous studies on the relative importance of carotid and aortic baroreceptors in the reflex modulation of heart rate have yielded contrasting results. Experiments on selective (un)loading of aortic baroreceptors by simultaneous infusion of vasoactive substances and application of neck suction/ pressure in order to maintain a stable carotid sinus transmural pressure indicated, that aortic baroreceptors are dominant in the baroreflex control of heart rate, with the carotid baroreceptors contributing only about 30% (28;29). In line with these observations, baroreflex control of heart rate is determined by the distensibility of the aortic arch rather than of the carotid sinus (30). In contrast, combined neck suction/ pressure with non-pharmacological (un)loading of aortic baroreceptors,

indicate, that carotid baroreceptors are the principal contributors to baroreflex control of heart rate (31). Our finding are in favor of the latter study.

Despite their potent baroreceptor properties, the contribution of cardiopulmonary baroreceptors to reflex adjustments during Valsalva's maneuver are probably limited, since reflex heart rate responses to changes in airway pressure are abolished in conscious dogs with denervated arterial baroreceptors and intact cardiopulmonary reflexes (32). Acute MSNA silencing during the short pressure rise in phase 1 of the Valsalva's maneuver has been attributed to increased carotid baroreceptor firing, since simultaneous reduction of aortic cross-sectional area (and therefore decreased wall tension) during this phase (14) (33) suggests an opposing input to aortic baroreceptors, which would increase MSNA. MSNA silencing during early phase 2 was clearly present in BCBR patient #1, 3 and 4 (data not shown), indicating again, that, some residual carotid sinus baroreceptor function is present in these patients.

During phase 4 of the Valsalva's maneuver, blood pressure overshoot was lower in patients than in controls. This cannot be explained by an attenuated reflex increase in MSNA during phase 2, since MSNA responses were shown to be normal. Attenuation of phase 4 blood pressure overshoot might be explained by a lower reflex increase in *cardiac* sympathetic nerve activity (and thereby stroke volume), which is not measured by peripheral microneurography. Baroreceptor denervation may have a differential effect on the reflex control of muscle vs cardiac sympathetic nerve activity.

In conclusion, on the long-term following BCBR, patients exhibit a normal pulse synchronous burst pattern of muscle sympathetic nerve activity. Denervation of carotid sinus baroreceptors due to BCBR produces a chronic decrease in baroreflex mediated adjustments of both heart rate and sympathetic nerve activity. However, this impairment of carotid baroreceptor function in humans has differential effects with regard to heart rate and MSNA. During Valsalva's maneuver, sympathetic nerve activity seems to be controlled by both carotid and extra-carotid baroreceptors, whereas baroreflex modulation of heart rate outflow appears to largely depend on the integrity of carotid baroreceptors.

Perspectives

Our knowledge of the relative contribution of carotid receptors to baroreflex function is mainly derived from experimental denervation studies in animals. For obvious reasons, no human counterparts for these well controlled prospective studies are available. Inadvertent damage to the carotid sinus as a complication of surgical treatment of rare bilateral carotid body tumors may serve as a human model of carotid sinus denervation. Despite evidence for permanent abnormalities in the reflex regulation of sympathetic nerve activity and heart rate in these patients, full-blown baroreflex failure with unrestrained sympathetic activation, hypertension and tachycardia is mainly limited to the acute phase following denervation. Prospective studies before and after the surgical denervation might improve our understanding of the importance of carotid baroreflex function in humans.

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Arterial baroreflex and peripheral chemoreflex function after radiotherapy for laryngeal or pharyngeal cancer

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Abstract

Purpose: Denervation of the carotid sinus causes baroreflex and chemoreflex failure, resulting in labile hypertension and loss of hypoxic responsiveness. We investigated whether radiation therapy for laryngeal or pharyngeal cancer affects baroreflex and chemoreflex function.

Methods and materials: 12 patients were studied after radiation therapy for locally advanced laryngeal or pharyngeal cancer (11m:1f, age: 56.0±7.9 years), 3.3 years (median; range 1.0-4.7) after radiotherapy and 15 healthy controls (11m:4f, 53.4±9.2 years). We measured baroreflex sensitivity (phenylephrine), blood pressure level and variability (24h Spacelabs and 5h Portapres recordings), responses to cardiovascular reflex tests and the ventilatory responses to normocapnic and hypercapnic hypoxia.

Results: Baroreflex sensitivity was lower in patients (9.7±7.8 ms/mmHg) than in controls (17.5±10.3 ms/mmHg, p=0.011). Mean office blood pressure was significantly higher in patients (141.5±27.8/ 89.2±10.6 mmHg, 63.3±12.3 bpm) than in controls (117.3±10.1/ 75.1±6.8 mmHg, 61.8±10.8 bpm). Blood pressure variability was not different between groups, nor were the responses to reflex tests. The normo/ hypercapnic ventilatory response to hypoxia was similar in patients (0.21±0.10/ 1.37±0.60 l/min/%) and controls (0.22±0.16/ 1.19±0.78 l/min/%).

Conclusions: Radiation therapy for laryngeal or pharyngeal carcinoma does not affect chemoreflex function, but results in an attenuated baroreflex sensitivity. Clinically relevant blood pressure lability is absent however.

Introduction

Baroreflex and chemoreflex mechanisms mediate the dynamic adjustments of circulation and ventilation to transients in blood pressure and hypoxemia respectively (1;2). In humans, these reflexes mainly originate in the carotid sinus baroreceptors and the carotid body chemoreceptors. Afferent fibers from both receptors join the glossopharyngeal nerves, which terminate in the nucleus tractus solitarii, which in turn projects to cardiovascular and respiratory centers in the medulla. The efferent limbs of the baroreflex loop consist of sympathetic and parasympathetic fibers to the heart as well as to smooth muscles in the peripheral blood vessels (1;3).

Carotid sinus denervation in humans has been reported as a complication of bilateral carotid body tumor surgery (4;5), carotid endarterectomy (6) and radiotherapy for head and neck cancer (4;7;8). The resulting clinical syndrome of baroreflex failure is characterized by disabling labile hyper- and hypotension, headache, diaphoresis and emotional instability (4;7). The absence of reflex changes in heart rate in response to the vasoactive substances phenylephrine and nitroprusside and to cardiovascular reflex tests like Valsalva's maneuver are the main diagnostic features (4). Chemoreflex failure, caused by resection or denervation of the carotid body chemoreceptors, results in an abolishment of the ventilatory response to normocapnic hypoxia (9-11).

Apart from case studies on full-blown baroreflex failure after radiotherapy of the neck (4;5;7), the impact of neck irradiation on baro- and chemoreflex function has not been systematically studied. The aim of this study was to systematically investigate whether bilateral irradiation of the jugular fields, covering the carotid baroreceptors and chemoreceptors, has a chronic effect on arterial baroreflex and peripheral chemoreflex function. We studied twelve patients after radiotherapy for laryngeal or pharyngeal carcinoma and fifteen healthy controls. Baro- and chemoreflexes were examined by measuring baroreflex sensitivity, hemodynamic responses to cardiovascular reflex tests, blood pressure variability and hypoxic ventilatory drive respectively.

Methods

Patients and control subjects

Patients had undergone bilateral radiotherapy of the neck (RAD) for (locally advanced) laryngeal or pharyngeal cancer at the Institute for Radiotherapy of the St. Radboud University Medical Center between January 1995 and July 1998. From a database of 109 patients that were free of recurrent and metastatic disease according to standard follow-up including laryngo-pharyngoscopy and palpation of the neck, we consecutively excluded 27 patients (25%) because of preexistent cardiovascular disease including hypertension, 14 (13%) because of chronic pulmonary disease requiring medication and 14 (13%) because of alternative comorbidity including diabetes, neurological disease or a second malignancy. Twenty-eight (26%) were excluded because of a history of chronic alcohol abuse or a self reported consumption of alcohol exceeding 3 units a day. Fourteen (13%) refused participation. Finally 12 RAD patients (11%) were investigated. Conventionally fractionated radiotherapy with curative intent was given to a target volume consisting of the larynx or pharynx and subdigastric and midjugular lymph nodes, using a linear accelerator (4 or 6 MV photons). The total dose received by the carotid sinus baroreceptors and the carotid body chemoreceptors varied from 40 to 68 Gy, depending on whether they were included in the boost volume or not. Dose per fraction was always 2 Gy. In case of nodal metastases, the target volume included the lower neck nodes as well. Radiotherapy was preceded by unilateral radical lymph node resection in three patients. The median interval between completion of radiotherapy and time of investigation was 3.3 years (range: 1.0-4.4 years). Patients were free of drugs affecting autonomic control of the cardiovascular system. Individual information on diagnosis and treatment is summarized in table 1.

Sex- and age matched healthy control subjects without a history of hypertension were recruited through advertisement in a local newspaper. From a database of 28 suitable candidates, 15 (11 males, 4 females) were randomly selected for participation on the basis of sex- and age criteria only. Full medical history and physical examination including blood pressure measurements revealed no abnormalities. Groups were matched for age (RAD: 56.0±7.9 years versus controls: 53.4±9.2 years), body mass index (25.0±2.9 kg/m^2 versus 24.7±2.7 kg/m^2) and alcohol intake (8.5±6.7 versus

4.5±5.5 units/ week, table 2). At the time of the study, 4 RAD patients were smokers whereas none of the controls were (p=0.015). Ten of 12 RAD patients had a history of smoking.

The study protocol was approved by the ethics committee of the University Medical Center Nijmegen and all subjects gave their informed consent.

Table 1. Characteristics of individual patients

patient	sex age (y)	tumor	surgery	Time since RAD (y)	RAD dose (Gy)
RAD1	m 50	T2N0M0) larynx		4.4	50
RAD2	m 73	T1N0M0 larynx		1.3	50
RAD3	m 47	T2N2M0 oropharynx		1.0	R 68, L 44
RAD4	m 67	T2N0M0 larynx		2.8	50
RAD5	m 50	T2N0M0 larynx		2.2	50
RAD6	m 61	T2N0M0 larynx		4.0	50
RAD7	f 51	T3N3M0 nasopharynx	right RLNR	3.8	R 70, L 64
RAD8	m 53	T2N1M0 oropharynx	resection+ left RLNR	1.7	50
RAD9	m 62	T2N0M0 larynx		1.3	50
RAD10	m 52	T1N2M0 nasopharynx	right RLNR	3.8	R 64, L 50
RAD11	m 54	T2N0M0 larynx		3.2	50
RAD12	m 52	T2N2M0 larynx		3.3	64

RAD= radiotherapy patient, R/L LNR= right/ left lymph node resection

Table 2. Group characteristics

	patients			controls		
n		12			15	
male: female	11	:	1	11	:	4
age (y)	56.0	±	7.9	53.4	±	9.2
BMI (kg/m2)	25.0	±	2.9	24.7	±	2.7
smoker: non smoker	4	:	8	0	:	15
alcohol intake (U/w)	8.5	±	6.7	4.5	±	5.5

BMI= body mass index, *p=0.015

Blood pressure level and variability

Office blood pressure level was determined from the mean of 3 supine sphygmomanometric measurements after 10 min of rest as well as from the average of 12 consecutive automatic (Dinamap) readings during 1 h of supine rest. All measurements were performed during morning time. In addition, blood pressure was monitored for 24 h (Spacelabs, Redmond, Washington, US) during normal daily activities at home. Automatic readings were taken at intervals of 15 and 30 min during day and night-time respectively. Subjects were considered hypertensive if mean systolic/ diastolic sphygmomanometric blood pressure exceeded 140/ 90 mmHg and if mean ambulatory daytime blood pressure (9:00AM-9.00PM) exceeded 135/ 85 mmHg (12). The coefficient of variance of blood pressure during ambulatory monitoring was taken as a measure of variability of blood pressure (13).

Higher frequency oscillations of blood pressure under ambulatory conditions were detected by a continuous beat-to-beat recording of finger arterial pressure by the Portapres device, a portable version of Finapres (TNO-BioMedical Instrumentation, Amsterdam, the Netherlands (14;15)). The five h Portapres recording comprised the following strictly scheduled standardized activities: 12.00h: lunch, 13.30-14.30h: siesta, 15.00: 20 min of bicycle exercise (50 Watt, 50-60 rounds per min), 16.00h: 30 min of quiet walking, 17.00h: end of measurements. In between of these scheduled activities, subjects were asked to sit down, relax and read. Off-line beat-by-beat blood pressure and heart rate values were derived from the arterial waveform using the FAST-System software package (TNO-BMI) (16).

Cardiovascular reflex tests and baroreflex sensitivity
This part of the study was performed during morning time after an overnight fast in a room with an ambient temperature of 22-24 °C. Subjects had abstained from caffeine, alcohol and smoking for at least 12 h. Continuous finger arterial blood pressure (Finapres (14)) was measured. Standard cardiovascular reflex tests were performed to investigate the overall baroreflex mediated heart rate and vasomotor control (Valsalva's maneuver, standing up), efferent cardiovagal control (forced breathing, cold face test) and efferent sympathetic vasomotor control (cold pressor test, mental arithmetic) (1) (17). Forced breathing, standing up and the Valsalva's maneuver were subsequently performed as previously described (17). Orthostatic hypotension was defined as a decrease in systolic blood pressure of 20 mmHg or more after 1-3 min of standing (18). Blood samples taken after 15 min of supine rest and after 10 min of standing were assayed for concentrations of epinephrine and norepinephrine using high performance liquid chromatography (HPLC) (19). Mental arithmetic was performed in the lying position after 5 min of rest, by repeatedly urging the subjects to subtract 17 from 5000, then from the remainder and so on for a period of 5 min (20). Cold pressor test and cold face test were carried out in the supine position after 5 min of supine rest. The right hand was placed in ice water for 60 seconds and the ophthalmic area of the trigeminal nerve with an ice water soaked compress for 30 seconds respectively (17) (21).

After 15 min of supine rest, baroreflex sensitivity was assessed by means of graded bolus injections of phenylephrine (25-50-100-150 μg) (22). The dosage producing an increase in systolic pressure of 15-40 mmHg was repeated thrice. Inter-beats (RR) intervals were plotted against the systolic blood pressure of the preceding beat and a linear regression analysis was performed for those points included between the beginning and the end of the first significant increase in systolic arterial pressure. Baroreflex sensitivity (msec/ mmHg), was obtained by calculating the mean of at least three slopes of statistically significant regression lines (p<0.05).

Peripheral chemoreflex function
To investigate peripheral chemoreflex function, the ventilatory response to hypoxia was assessed during stable normocapnic as well as during stable hypercapnic conditions. Subjects were connected

to a closed spirometric circuit in sitting position. Finger arterial oxygen saturation (SaO_2 : pulse oximetry, Nellcor 200, Hayward, CA, USA) and partial carbon dioxide pressure (P_{CO2}: Gould Godart Mark II capnograph, Bilthoven, The Netherlands) at the mouth were continuously measured during the experiments. The end-tidal carbon dioxide level (P_{ETCO2}) was controlled by adjusting a three-way valve, partially short-circuiting the CO_2 absorber in the inspiratory limb of the circuit.

The ventilatory response to hypoxia was assessed by rebreathing into the spirometer filled with room air until SaO_2 reached a minimum of 80% within about 3-4 min, while P_{ETCO2} was kept constant at the initial resting level (23). Ventilation was plotted against SaO_2 and a linear regression analysis was performed. The slope of a (statistically significant, $p<0.05$) regression line was taken as a measure of normocapnic ventilatory response to hypoxia (nVRH), expressed as $L/min*\%SaO_2$ (23). The hypercapnic ventilatory response to hypoxia (hVRH) was measured in a similar fashion. After breathing room air during 15 min, P_{ETCO2} was gradually raised until 1 kPa above the individual initial resting level. After reaching a new steady state in which both P_{ETCO2} and ventilation were stable for at least 3 min, progressive hypoxia was induced and hVRH was calculated (23).

Statistics

Results are given as mean±SD unless indicated otherwise. Differences between patients and controls with respect to nominal variables were compared using the Chi-square test. Other variables were compared using the Student t-test or Mann-Whitney rank-sum test when appropriate. Hemodynamic changes during prolonged standing were compared by means of ANOVA for repeated measurements. A two-sided $p<0.05$ was taken as the level of significance. Statistical analysis was performed using SPSS (Statistical Package for the Social Sciences) for Windows 6.1.3.

Results

Symptoms and signs of baroreflex failure

According to a standardized inventory of symptoms and signs fitting baroreflex failure, none of the RAD patients had experienced any attacks of headache, flushing, palpitations or diaphoresis related to exercise and/ or mental stress following radiotherapy. One patient (RAD 6) had occasional complaints of initial orthostatic and post exercise dizziness since treatment. Five of twelve (42%) RAD patients had experienced mood swings and feelings of fear, anger and/or sadness in the course of or after completing fractionated radiotherapy. However, these signs of emotional lability were considered to be an unreliable marker of baroreflex failure in view of the (preceding) malignancy.

Blood pressure level and variability

Mean systolic and diastolic office blood pressure was significantly higher in patients than in controls (table 3). This applies to both sphygmomanometric and automatic (Dinamap) blood pressure values. Systolic/ diastolic sphygmomanometric blood pressure values exceeding 140/ 90 mmHg were

observed in patients 1,2,4,5,6 and 7, not in controls. Mean 24 h, daytime and night-time diastolic blood pressure was significantly higher in patients than in controls as was night-time systolic blood pressure. A mean daytime ambulatory systolic/ diastolic blood pressure exceeding 135/ 85 mmHg was observed in patient 4, 6 and 7, not in controls.

**Table 3**. Blood pressure level

	patients			controls			P value
sphygmomanometry							
SBP	141.5	±	27.8	117.3	±	10.1	0.004
DBP	89.2	±	10.6	75.1	±	6.8	0.001
HR	63.3	±	12.3	61.8	±	10.8	
Dinamap							
SBP	136.5	±	27.3	111.4	±	11.2	0.003
DBP	82.0	±	10.4	69.6	±	7.5	0.001
HR	60.1	±	10.4	57.6	±	9.6	
ABPM 24 hours							
SBP	129.9	±	15.3	119.5	±	10.4	
DBP	81.6	±	5.0	75.0	±	7.8	0.016
HR	74.1	±	11.3	71.8	±	7.7	
ABPM daytime							
SBP	133.3	±	15.9	124.1	±	10.0	
DBP	84.8	±	5.5	79.1	±	7.8	0.045
HR	77.9	±	11.4	77.3	±	9.7	
ABPM night-time							
SBP	118.7	±	13.8	105.7	±	14.1	0.024
DBP	72.6	±	6.2	62.9	±	10.4	0.009
HR	65.6	±	10.0	59.1	±	7.0	

ABPM= ambulatory blood pressure measurements, SBP= systolic blood pressure (mmHg), DBP= diastolic blood pressure (mmHg), HR= heart rate (beats/min), daytime: 9 am-9pm, night-time: 1am-7am, *p<0.05

Blood pressure variability, expressed as coefficients of variance of mean arterial pressure, was not different between groups, neither during 24 h Spacelabs measurements (patients: 11.3±2.3% versus controls: 12.5±3.2%), nor during 5 h Portapres measurements (patients: 11.6±2.4% versus controls: 12.0±1.5%). Patients and controls show a similar distribution in the individually calculated frequency histograms of mean arterial pressure (figure1), indicating similar blood pressure variabilities.
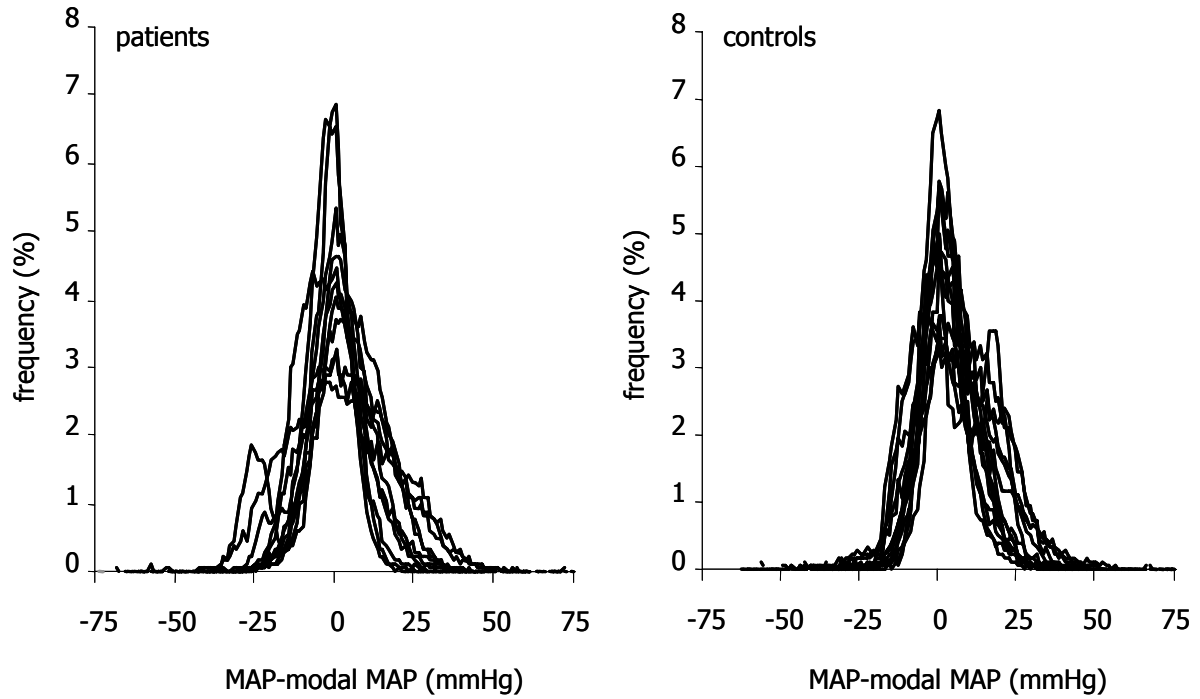
Figure 1. Individual frequency histograms of blood pressure during 5 h Portapres registrations (standardized activities). x-axis: MAP (= mean arterial pressure) relative to modal MAP, y-axis: frequency of MAP level as percentage of total
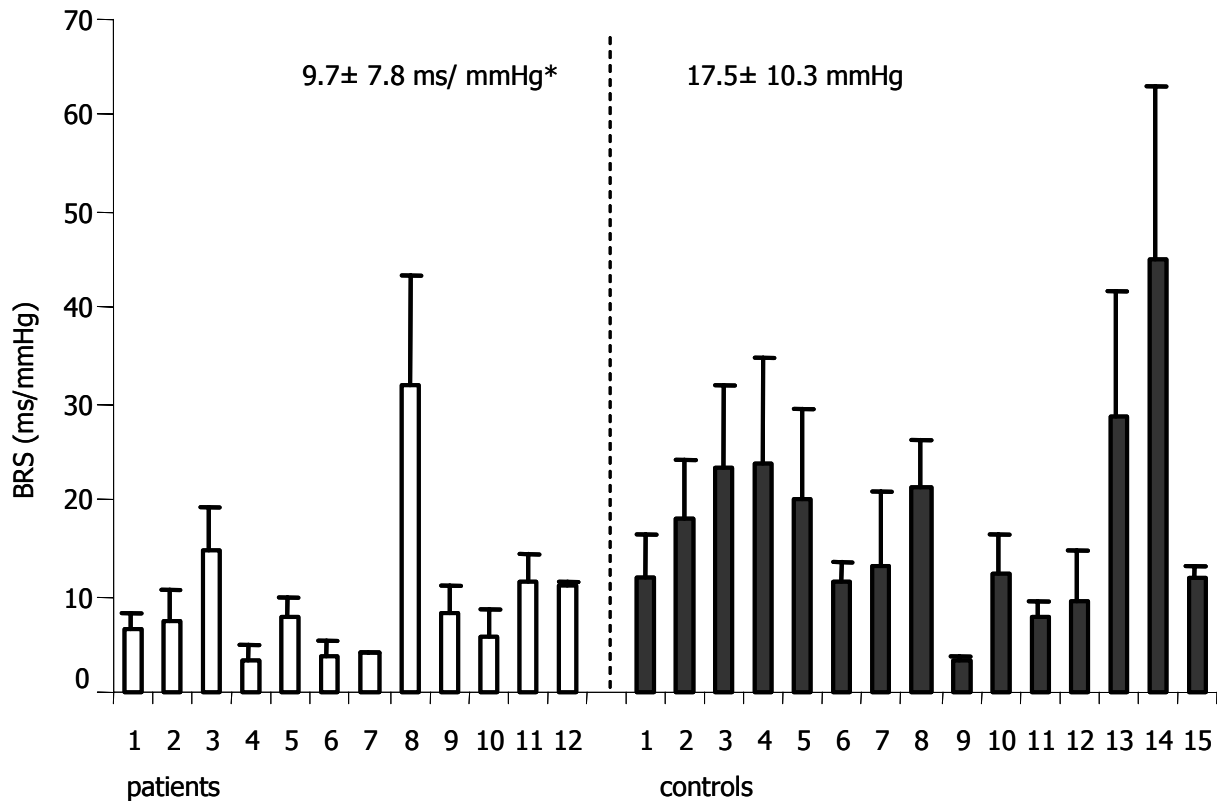
Figure 2. Individual baroreflex sensitivity (BRS in ms/ mmHg= milliseconds per millimeter of Mercury) *mean± sd group averages

Baroreflex sensitivity

All patients and controls exhibited a reflex decrease in heart rate. Baroreflex sensitivity was significantly lower in patients than in controls: 9.7±7.8 ms/ mmHg and 17.5±10.3 ms/ mmHg respectively (p=0.011). In 3 of 12 patients versus 1 of 15 controls a baroreflex sensitivity of less than 5 ms/ mmHg was measured (figure 2).

Cardiovascular reflex tests

Heart rate and blood pressure responses to *Valsalva's maneuver* and *standing up* were not different between groups (table 4). None of the patients exhibited orthostatic hypotension. Mean changes in blood pressure and heart rate during prolonged standing were not different among groups (figure 3). At baseline, plasma epinephrine (E) and norepinephrine (NE) levels in patients were not different from controls (patients: E: 0.10±0.06, NE: 1.44±0.76 nmol/l versus controls: E: 0.08±0.06, NE: 1.60±0.65 nmol/l), nor were levels after ten min of standing (patients: E: 0.17±0.14, NE: 3.01±1.30 nmol/l versus controls: E: 0.15±0.14, NE: 3.02±0.80 nmol/l). *Forced breathing* resulted in similar inspiratory-expiratory differences in patients and controls. Reflex bradycardia in response to *cold face test* was present in all patients and not different between groups. *Mental arithmetics* resulted in similar increases in blood pressure and heart rate in the two groups, with a large inter-individual response variability (table 4). The same applies to the blood pressure and heart rate increase in responses to *cold pressor test* (table 4).

Peripheral chemoreflex function

At baseline, mean SaO_2 did not differ (patients: 95.5±1.9% versus controls: 97.0±2.1%) nor did ventilation (7.9±1.8 versus 7.6±1.9 l/min) and P_{ETCO2} (5.9±0.6 versus 5.5±0.8 kPa). The normocapnic ventilatory response to hypoxia (nVRH) did not differ between groups , nor did the hypercapnic ventilatory response to hypoxia (figure 4).

Table 4. Cardiovascular reflex tests

	patients			controls		
Valsalva's maneuver						
dHRmax	+25.5	±	11.9	+32.1	±	14.8
Valsalva ratio	1.61	±	0.42	1.84	±	0.44
dSBPmax*	+27.1	±	15.3	+26.6	±	18.2
dDBPmax*	+12.7	±	9.7	+19.8	±	10.0
standing up						
dHRmax	+19.7	±	10.7	+27.4	±	11.8
HRmax/min ratio	1.22	±	0.14	1.35	±	0.24
dSBPmin	-37.3	±	16.5	-33.9	±	14.2
dSBPmax	-3.8	±	17.1	+5.1	±	13.5
forced breathing						
I-E diference in HR	14.4	±	4.6	15.5	±	7.0
cold face test						
dHRmin	-11.3	±	8.6	-8.7	±	8.4
mental arithmetic						
dSBPmax	+22.2	±	17.8	+24.7	±	9.5
dDBPmax	+13.8	±	10.8	+15.7	±	7.8
dHRmax	+14.5	±	7.0	+17.1	±	9.4
cold pressor test						
dSBPmax	+34.8	±	11.0	+31.1	±	18.6
dDBPmax	+20.3	±	7.3	+18.3	±	10.7
dHRmax	+7.2	±	5.6	+9.9	±	11.5

Finapres measurements; SBP= systolic blood pressure (mmHg), DBP= diastolic blood pressure (mmHg), HR= heart rate (beats/min), d-max= maximal increase from baseline, d-min= maximal decrease from baseline, *during overshoot, I-E= inspiratory- expiratory, mean± sd
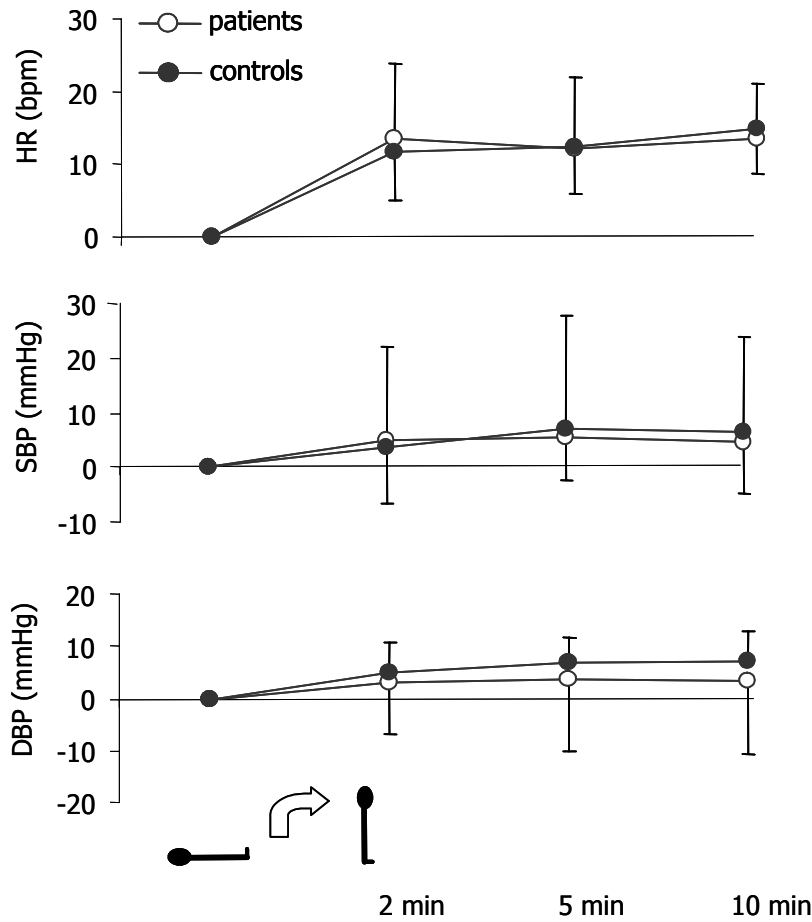
Figure 3. Blood pressure and heart rate response to prolonged standing. Changes from baseline (supine) in HR (=heart rate), SBP (=systolic blood pressure) and DBP (=diastolic blood pressure) at 2, 5 and 10 minutes of standing, mean± sd
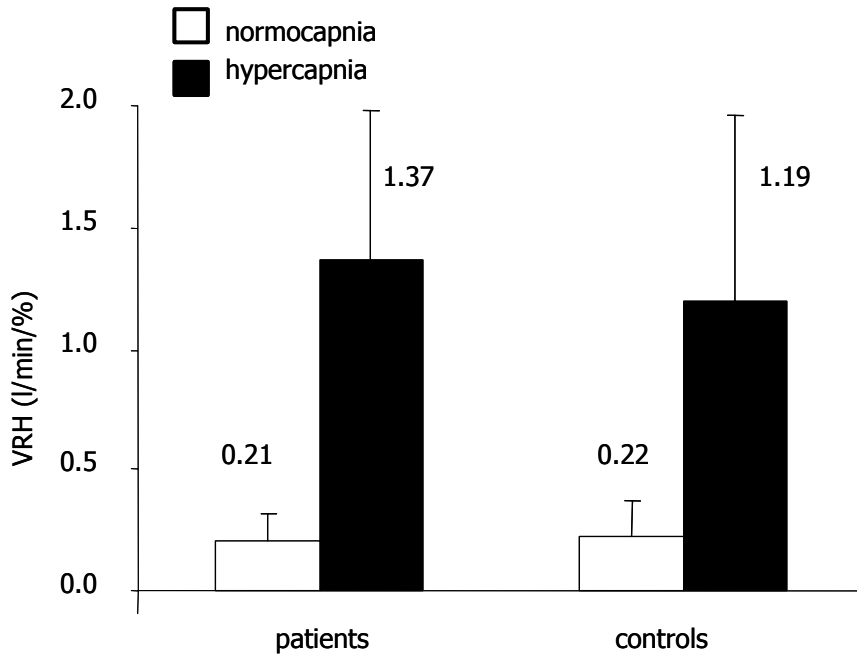
Figure 4. Ventilatory response to hypoxia. Ventilatory response to hypoxia under normocapnic (nVRH, open bars) and hypercapnic (baseline+ 1 kPa) conditions (hVRH, closed bars), mean± sd

Discussion

We investigated the chronic effects of bilateral neck irradiation for laryngeal or pharyngeal cancer on arterial baroreflex and peripheral chemoreflex function. Baroreflex sensitivity was significantly lower in RAD patients than in controls. However, relative blood pressure variability during ambulatory measurements was not different between groups and normal hemodynamic responses to cardiovascular reflex tests were observed in both patients and controls. Mean office as well as ambulatory blood pressure levels were higher in patients than in controls. Ventilatory responsiveness to hypoxia under both normocapnic and hypercapnic conditions was similar between patients and controls.

Arterial baroreflex function

Radiotherapy of the neck for head and neck cancer (4;7;8) may in individual cases be complicated by baroreflex failure due to impaired carotid sinus baroreceptor function. The full-blown clinical syndrome of baroreflex failure is characterized by paroxysms of severe hypertension and tachycardia, which result from excessive increments of sympathetic tone including marked elevations of catecholamine plasma levels. These bouts of sympathetic activation may be accompanied by headache, palpitations, diaphoresis and pale flushing (4). They may occur spontaneously or are elicited by physical, emotional or sexual stimuli. Symptoms and signs bear a strong resemblance to that of a pheochromocytoma. In baroreflex failure, unopposed central activation of efferent sympathetic pathways arises from the absence of tonic inhibitory baroreceptor input to the vasomotor centers of the brainstem (4;24). Centrally acting sympatholytic agents like clonidine may reduce the frequency and severity of the attacks (4;25;26).

The full blown clinical picture of baroreflex failure was not observed in any of the patients described in the present study. All patients showed a reflex heart rate response to vasoconstriction by phenylephrine. Absence of this response is a key feature of the syndrome (4). However, the finding of a decreased baroreflex sensitivity in patients as compared to controls suggests impairment of reflex control of heart rate due to decreased baroreceptor function. Changes in carotid sinus baroreceptor function after radiotherapy may have been caused by damage to the baroreceptor or its afferent innervation, consisting of the carotid sinus nerves of Hering and subsequently the glossopharyngeal nerves (1). Cranial nerve palsies are uncommon complications after radiotherapy to the neck (27). Damage of the lower cranial nerves (nIX-nXII) has been reported in particular as a complication of radiotherapy for nasopharyngeal cancer (28;29). The hypoglossal nerve appears to be the most commonly affected while the vagal nerve and especially the glossopharyngeal nerve are only seldom involved (28). The incidence of lower cranial neuropathy after radiotherapy is probably underestimated however. Minor nerve damage, not producing obvious signs or symptoms can easily be missed because a comprehensive neurological examination is generally not part of the routine follow-up of these patients. In particular unilateral glossopharyngeal nerve palsy may remain

undetected. The radiation dose after which cranial nerve palsies occur are relatively high, mostly above an equivalence of 70 Gy with conventional fractionation(28;29). The majority of the patients in the present study received much lower doses to the carotid bodies and afferent nerve. Functional integrity of cardiovagal innervation was demonstrated by the normal I-E differences during forced breathing and the reflex bradycardia induced by the cold face test.

Another potential mechanism may be encasement of small afferent nerve fibers by connective tissue fibrosis resulting from radiotherapy and/ or neck surgery. Thirdly, arterial baroreflex function may have been altered by irradiation induced structural changes of the internal carotid artery wall. Patients who have received radiotherapy to the head and neck have a higher risk of developing carotid atherosclerosis (30;31). In addition, irradiation injury to the vasa vasorum of the carotid artery and the consequent ischaemic lesions of the arterial wall may induce necrotizing vasculitis, occlusive thrombosis and periadventitial fibrosis (32). Atherosclerosis and fibrosis may result in a decreased distensability of the carotid sinus and thereby may reduce stretch-induced afferent carotid sinus nerve activity (33). In line with these observations, removal of an atherosclerotic plaque from the carotid artery in some patients enhances baroreflex function (34). However, we did not investigate this possible explanation for the reduced baroreflex sensitivity in the RAD patients by means of carotid ultrasonography. Whichever of these mechanisms is a major contributor, baroreceptor dysfunction typically is a late effect of neck irradiation which explains the slow and gradual development of symptoms and signs of baroreflex failure following radiotherapy (4;7) which is in contrast to the immediate onset after bilateral carotid body tumor surgery (4) (5) or carotid endarterectomy (6).

Apparently, the impairment of reflex control of heart rate after radiotherapy, as indicated by the attenuated response to phenylephrine, is too mild to cause abnormal blood pressure adjustments to Valsalva's maneuver, standing up as well as to cold and mental stress. Alternatively, reflex control of efferent sympathetic nerve pathways may have been selectively spared (35) and/ or compensation by the residual aortic and cardiopulmonary baroreceptor areas may occur (36). This may also explain the normal blood pressure variability observed in RAD patients. Blood pressure level, however, was significantly elevated in the patients group. After bilateral carotid sinus denervation in animals, blood pressure and its variability initially increase markedly but normalize to preoperative levels within 10-14 days (37) (38). In humans, sustained hypertension appears to be limited to days to months after sudden surgical carotid baroreceptor denervation, whereas episodic surges of hyper- as well as hypotension may persist for a longer period (4) (10) (5). Therefore, the observations of a sustained elevation of blood pressure level in the presence of normal blood pressure variability in RAD patients does not fit the typical pattern that is usually observed after baroreceptor denervation. If by coincidence this study sample includes patients with not previously recognized primary hypertension, the lower baroreflex sensitivity may even be a consequence in stead of the cause of hypertension; hypertension is accompanied by a resetting of the baroreceptor to a higher blood pressure level and a decreased sensitivity of the baroreceptor to changes in blood pressure (39).

Peripheral chemoreflex function

The role of the carotid bodies in human ventilatory control has been studied in patients after bilateral resection of (normal) carotid bodies as an experimental treatment of bronchial asthma or chronic obstructive pulmonary disease (9) (10) (40). In these patients, the hyperventilation response to experimentally induced hypoxemia is abolished (10). As the carotid body chemoreceptors share the same afferent pathways towards the brainstem (1), we hypothesized that irradiation induced carotid sinus denervation may also lead to peripheral chemoreflex dysfunction. In addition, the carotid body tissue itself may be sensitive to irradiation injury, as radiotherapy is used for the control of chemodectomas of the head and neck (41). However, a normal ventilatory response to progressive hypoxia, similar to that observed in control subjects, was present in all patients. The compensatory role of aortic chemoreceptors in humans after loss of carotid body function is considered to be of limited importance (4). Therefore, carotid body chemoreflex function does not seem to be impaired by moderate doses of radiotherapy for laryngeal or pharyngeal cancer.

Conclusion

The clinical syndrome of arterial baroreflex failure resulting from carotid sinus baroreceptor denervation is a rare complication of radiotherapy for head and neck tumors. The decreased baroreflex sensitivity observed after irradiation of the neck in patients without the full blown syndrome does not have functional implications for the control of arterial blood pressure. In addition, peripheral chemoreflex function is not altered by this treatment.

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Long-term effects of unilateral carotid endarterectomy on arterial baroreflex function

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Abstract

Background and Purpose: Carotid endarterectomy (CE) may be complicated by the clinical syndrome of baroreflex failure. Alterations of baroreflex function may also account for the frequently observed blood pressure lability in the first hours following surgery. We investigated the long-term effects of unilateral CE on baroreflex control of function and blood pressure.

Methods: We investigated 14 patients after unilateral CE (13m:1f, 64.8±6.5 years), 9 patients with a surgically untreated uni-/bilateral carotid stenosis (CS, 7m:2f, 57.6±10.7 years) and 12 healthy controls (HC, 11m:1f, 60.9±7.9 years) by means of Valsalva's maneuver, active standing, forced breathing, cold face test, cold pressor test and mental arithmetic. Ambulatory blood pressure level and variability were determined from 24 hours Spacelabs and 5 hours beat-to-beat Portapres recordings.

Results: Baroreflex sensitivity (derived from phase IV Valsalva's maneuver) was significantly lower in CE (1.53±0.83 ms/mmHg) than in CS (4.39±2.27, p=0.002) and HC (5.34±3.78, p=0.003). CE patients exhibited a decreased reflex control of heart rate in response to Valsalva's maneuver and active standing without orthostatic hypotension. Office blood pressure levels before and after endarterectomy were similar, as were ambulatory blood pressure levels in the three groups. Ambulatory blood pressure variability was higher in CE and CS than in HC, but not different between CE and CS.

Conclusions: Unilateral CE causes a long-term impairment of baroreflex function, resulting in an attenuated reflex control of heart rate, but no hypertension or blood pressure lability.

Introduction

The recognition of a beneficial effect of carotid endarterectomy (CE) on the recurrence rate of ischemic stroke in selected patients has led to dramatic resurgence in the rates of this procedure in the United States and Canada during the past decade (1). Despite a reduction in perioperative mortality and morbidity, CE still carries a small risk of inducing hemorrhagic or ischemic stroke. In some cases, the CE-induced neurological deficit coincides with an elevation of blood pressure level during or following CE (2-6). Lability of blood pressure following either unilateral or bilateral CE has been attributed to abnormal autonomic cardiovascular control by the arterial baroreceptors (3;7-9). The arterial baroreflex, which originates from carotid sinus and aortic mechanoreceptors, buffers abrupt transients of blood pressure (10). Iatrogenic lesions of the afferent limb of the baroreflex may result in baroreflex failure, producing labile hypertension, headache, diaphoresis and emotional instability (11;12). Carotid sinus trauma due to endarterectomy is thought to be the causative mechanism of the occurrence of this syndrome in individual cases (3;8;9).

Apart from these case reports, the acute effects of CE on arterial baroreflex function have been evaluated in patients without full-blown clinical baroreflex failure. These studies were performed during or shortly after surgery and have yielded contrasting results; baroreflex sensitivity was reported

to be increased, decreased or unaltered by CE (13-17). In a longer term study with a follow-up of six months, no uniform effect of CE on baroreflex sensitivity was found (18).

The aim of this study was to investigate the long-term effects of unilateral CE on arterial baroreflex function and blood pressure. We hypothesized that CE results in permanent attenuation of baroreflex sensitivity, abnormal cardiovascular reflexes and an increased blood pressure variability. Patients with a surgically untreated carotid stenosis and healthy subjects served as control groups.

Materials and methods

Patients and controls

Between 1989 and 2000, 173 patients had undergone unilateral CE at the Department of Vascular Surgery of the University Medical Center Nijmegen, the Netherlands. Indications for CE included (recurrent) transient ischemic attack (TIA) or stroke in the past 6 months in addition to a carotid stenosis of at least 70% (19). Surgery had been carried out in a standardized fashion and by experienced surgeons. No attempt was made to selectively spare carotid sinus innervation. Of these 173 patients, 9 had died between CE and the study. We excluded patients with an age above 75 years (n=62) and patients with preexisting hypertension that were on antihypertensive medication before and after CE (n=51) since both old-age and the use of antihypertensives impede reliable assessment of cardiovascular reflexes (20). In addition we excluded patients with diabetes (n=9), cardiac diseases including instable angina pectoris, heart failure and arrhythmia (n=19), malignant disease (n=2) as well as patients who were physically or mentally unable to participate for several reasons (n=11). Five patients refused participation. Finally 14 patients (13 males, 1 female) were included for evaluation of baroreflex function following CE. In the acute phase folowing surgery, (baroreflex related) hemodynamic instability was had not been observed in any of these patients. The median interval between CE and the study was 4.3 years (range 0.5-11.2). Individual patient characteristics, including details on carotid stenosis and surgery are summarized in table 1.

We included two control groups in this study. The first control group consisted of 9 patients (7 males, 2 females) with a uni- or bilateral carotid stenosis (CS) in whom CE was not indicated due to either insufficient hemodynamic or clinical significance of the stenosis (table 1)(19). The second group consisted of 12 healthy controls (HC, 11 males, 1 female), who were recruited through advertisement in a local newspaper. A comprehensive history was taken, physical examination was performed and an electrocardiogram was made in order to investigate whether the subjects met the in- and exclusion criteria. Groups were matched for age (CE 64.8±6.5, CS 57.6±10.7, HC 60.9±7.9 years) and body mass index (CE 26.6±3.2, CS 27.2±3.4, HC 26.1±2.1 kg/m^2). A similar number of CE and CS patients were on aspirin and lipid-lowering drugs (table 1). The study protocol was approved by the ethics committee of the University Medical Center Nijmegen. All subjects gave their informed consent and all institutional guidelines were followed.

Table 1. Patient characteristics

	sex/ age	atherosclerosis	stenosis (localisation; %)	endarterectomy	interval(y)	medication
CE patients						
# 01	m68	TIA	rICA75	rICA	9.3	a/s/d
# 02	m60	IS	lICA50; rICA50	rICA	4.1	a/s/d
# 03	m65	IS	lICA75	lICA	4.0	a/s
# 04	m62	TIA, PAS	lECA50; rICA75; rECA75	rICA; rECA	2.3	a
# 05	m56	TIA	lICA75	rICA	9.1	a/s
# 06	m61	IS	lICA75; rICA100	lICA; lCCA	11.2	a
# 07	m74	TIA	rICA75	rICA	6.5	a
# 08	f 70	IS	lICA100; rICA75	rICA; rCCA	7.6	a/s
# 09	m79	TIA	rICA75	rICA	5.9	a
# 10	m73	IS	lICA75	lICA	0.7	a
# 11	m54	TIA	lICA75; rICA100	lICA	0.3	a
# 12	m68	IS	lICA75	lICA	1.3	a/s
# 13	m64	IS, AMI	lICA75	lICA	0.5	a/d
# 14	m73	TIA	lICA75	lICA; lECA	4.5	a
CS patients						
# 01	f 60	TIA, FH	lICA<50; rICA<50			a/s
# 02	m43	FH	lICA<50; rICA<50			s
# 03	m51	AMI FH	lICA50			s
# 04	m43	FH	lICA<50; rICA<50			s
# 05	m53	TIA FH	lICA50; rICA75			a/s
# 06	m73	TIA, AAA	lICA50			a
# 07	f 69	PAS	lICA75			a
# 08	m64	IS	lICA<50			a
# 09	m62	IS	lICA100; lECA75; rICA75; rECA75			a/p

CE=unilateral carotid endarterectomy, CS=carotid stenosis, m=male, f=female, atherosclerosis= history of atherosclerotic disease, interval= time between endarterectomy and study, TIA=transient ischemic attack, IS=ischemic stroke, AMI=acute myocardial infarction, PAS= peripheral arterial stenosis, AAA=abdominal aortic aneurysm, r(ight)/l(eft) I(nternal)/E(xternal)/C(ommon) C(arotid) A(rtery) % stenosis, a=aspirin, s=statin, d=dipyridamol

Cardiovascular reflex tests

Investigations were performed during morning time after an overnight fast in a room with a temperature of 22-24 °C. Subjects had abstained from caffeine, alcohol and smoking for at least 12 hours. Beat-by-beat finger arterial BP was measured by Finapres (21).

Cardiovascular reflex tests were performed to investigate the overall baroreflex mediated heart rate and vasomotor control (Valsalva's maneuver, standing up (20)), efferent cardiovagal control (forced breathing, cold face test) and efferent sympathetic vasomotor control (cold pressor test, mental arithmetic) (10;20). During Valsalva's maneuver the patient maintained an expiratory pressure of 40 mmHg during 15 sec in sitting position, by means of forced expiration into a mouthpiece connected to a pressure transducer. Closure of the glottis was prevented by a small leak to maintain a flow of air (20). Orthostatic hypotension was defined as a SBP decrease of >20 mmHg within 1-3 min

of standing. Blood samples, drawn after 15 min supine rest and 10 min standing, were assayed for concentrations of epinephrine and norepinephrine using high performance liquid chromatography (HPLC) (22). Mental arithmetic was performed in the lying position after 5 minutes of rest, by repeatedly urging the subjects to subtract 17 from 5000, then from the remainder and so on for a period of 5 minutes (23). Cold pressor test and cold face test were carried out in supine position by placing the right hand in ice water for 60 sec and by covering the forehead and eyes with an ice water soaked compress for 30 sec respectively (20;24).

Baroreflex sensitivity

Noninvasive estimates of baroreflex sensitivity (BRS) were derived from changes in heart rate and blood pressure during phase 4 of Valsalva's maneuver by applying least-squares regression analysis (25). BRS was calculated as the slope of a statistically significant regression line between systolic blood pressure and one-beat-lagged RR-intervals. This estimate of BRS has previously been shown to be significantly correlated with invasive measures of baroreflex control of heart rate (25).

Blood pressure level and variability

Office blood pressure was measured thrice by a random zero mercury sphygmomanometer in the supine position after 10 minutes of rest. The average blood pressure was compared to the pre-operative office BP readings obtained from the medical records. 24 hour ambulatory BP measurement (ABPM, Spacelabs, Redmond USA) was performed during normal activities at home, at reading intervals of 15 and 30 minutes during day and night-time respectively. Hypertension was defined as a mean ambulatory daytime BP (9:00AM-9.00PM) of >135/85 mmHg (26). The coefficient of variance of ABPM readings was taken as a measure of BP variability (27).

BP variability was also assessed from a 5 hour beat-to-beat Portapres recording of finger arterial BP (TNO-BMI, Amsterdam, the Netherlands (28)). During the ambulatory Portapres recording, the following standardized activities were strictly scheduled: 12.00h: lunch, 13.30-14.30h: siesta, 15.00: 20 minutes of bicycle exercise (50 Watt, 50-60 rounds per minute), 16.00h: 30 minutes of quiet walking, 17.00h: end. In between these activities, subjects were asked to sit down and relax.

Statistics

Results are given as mean±sd unless indicated otherwise. The coefficient of variance was calculated as the standard deviation as percentage of mean. Differences between patients and controls with respect to nominal variables were compared using the Chi-square test. Pre- and post-operative blood pressure values were compared using the paired-T-test. Other variables were compared using the Student t-test or Mann-Whitney rank-sum test when appropriate. A two-sided p<0.05 was taken as the level of significance. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS for Windows 6.1.3).

Results

Baroreflex sensitivity and cardiovascular reflex tests

BRS (ms/mmHg) was significantly lower in CE (1.53±0.83) than in CS (4.39±2.27, p=0.003) and HC (5.34±3.78, p=0.003, CS vs HC: ns, figure 1). In response to Valsalva's Maneuver, groups showed a similar maximal phase 2 reflex increase in heart rate and a similar phase 4 blood pressure overshoot (table 2). The Valsalva ratio between maximal and minimal heart rate, a measure of the baroreflex modulation of vagal efferent nerve activity, was lower in both CE and CS as compared to HC. In response to active standing, CE showed attenuation of the maximal initial increase in heart rate, the maximal/ minimal heart rate ratio and blood pressure overshoot, whereas the initial blood pressure decrease was larger than in CS and HC. Orthostatic hypotension was not observed in any patient. Groups exhibited similar heart rate responses to forced breathing and cold face test, indicating normal cardiovagal innervation. Blood pressure and heart rate responses to both cold pressor test and mental arithmetic did not differ between groups. Supine plasma epinephrine and norepinephrine levels were not different between groups: epinephrine: CE 0.10±0.04 nmol/l; CS 0.07±0.05 nmol/l; HC 0.07±0.05 nmol/l and norepinephrine: CE 1.89±0.83 nmol/l ; CS 1.97±0.66 nmol/l; HC 1.87±0.79 nmol/l, nor were standing levels: epinephrine: CE 0.14±0.06 nmol/l ; CS 0.08±0.05 nmol/l; HC 0.09±0.06 nmol/l and norepinephrine: CE 3.42±1.37 nmol/l ; CS 4.18±1.24 nmol/l; HC 3.13±1.06 nmol/l.
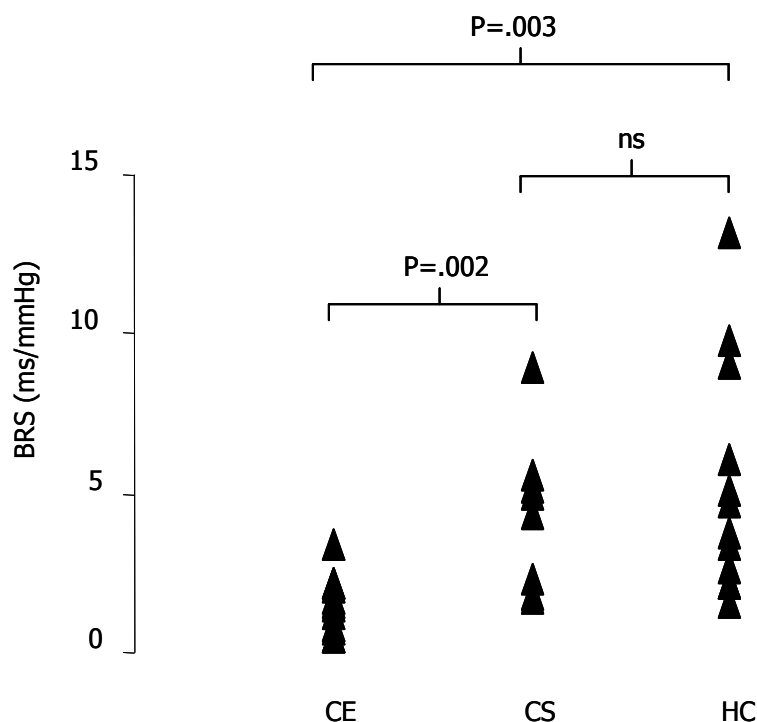
Figure 1. Individual baroreflex sensitivity (BRS) CE=unilateral carotid endarterectomy, CS=carotid stenosis, HC=healthy controls; The indicated P values refer to Mann-Whitney rank-sum testing.

Table 2. Cardiovascular reflex tests

	CE	CS	HC	P
Valsalva's maneuver				
maximal HR increase phase-II (bpm)	23.4±9.7	18.2±7.6	28.3±9.0	CS vs HC: 0.014
Valsalva ratio	1.4±0.3	1.4±0.2	1.8±0.5	CE+ CS vs HC: <0.05
SBP overshoot phase-IV (mmHg)	21.1±22.4	18.7±27.2	23.2±15.3	
standing up				
maximal HR increase (bpm)	15.8±5.9	19.6±8.1	25.5±11.3	CE vs HC: 0.01
maximal/ minimal HR ratio	1.15±0.06	1.23±0.20	1.32±0.23	CE vs HC: 0.013
maximal initial SBP decrease (mmHg)	-53.6±21.1	-38.4±16.4	-36.7±14.8	CE vs HC: 0.029
SBP overshoot (mmHg)	-8.9±15.6	-0.7±15.1	3.1±13.6	CE vs HC: 0.05
orthostatic hypotension *	0	0	0	
forced breathing				*forced breathing*
inspiratory-expiratory difference (bpm)	12.8±9.3	14.8±8.3	13.7±3.6	inspiratory-expiratory difference (bpm)
cold face test				
maximal HR decrease (bpm)	4.7±3.5	7.3±7.0	8.7±8.8	
cold pressor test				*cold pressor test*
mean SBP increase (mmHg)	22.3±15.8	3.2±15	17.4±12.5	mean SBP increase (mmHg)
mean DBP increase (mmHg)	9.9±7.1	5.5±3.2	9.9±7.5	mean DBP increase (mmHg)
mean HR increase (bpm)	2.3±5.7	3.3±9.6	4.9±5.0	mean HR increase (bpm)
mental arithmetic				*mental arithmetic*
mean SBP increase (mmHg)	16.3±10.1	9.1±1.5	18.1±9.4	mean SBP increase (mmHg)
mean DBP increase (mmHg)	9.5±4.3	5.0±0.3	12.0±6.4	mean DBP increase (mmHg)
mean HR increase (bpm)	7.1±9.2	8.7±1.2	8.8±5.5	mean HR increase (bpm)

CE= unilateral carotid endarterectomy, CS= carotid stenosis, HC= healthy controls, S/DBP= systolic/ diastolic blood pressure, HR= heart rate

Blood pressure level and variability

None of the individual CS or CE patients were hypertensive. In CE patients, mean office (systolic/ diastolic) blood pressure at time of the investigation did not differ from pre-operative values (140.1±17.0/ 80.1±6.2 mmHg versus 140.8±15.1/ 84.4±7.5 mmHg, table 3). Mean ABPM values were similar in CE, CS and HC. Night-time heart rate was slightly higher in CE patients than in HC. Coefficients of variance during ABPM were higher in CE and CS than in HC, but not different between CE and CS. This applies to both Spacelabs and beat-to-beat Portapres measurements. A higher BP variability in CE patients and CS as compared to HC is illustrated by broader BP distributions (figure 2) and larger coefficients of variance (table 3), both calculated from the Portapres recordings.

Table 3. Ambulatory blood pressure level (Spacelabs) and variability (Spacelabs, Portapres)

	CE	CS	HC	P
level				
daytime				
SBP (mmHg)	139.7±15.8	136.2±11.6	130.7±11.4	
DBP (mmHg)	84.8±8.1	86±16.3	82.0±6.6	
HR (bpm)	79.1±7.7	76.1±8.7	77.4±9.4	
night-time				
SBP (mmHg)	124.1±18.6	118.5±11.2	114.0±12.5	
DBP (mmHg)	73.8±10.4	73.9±16.7	67.9±6.9	
HR (bpm)	67.8±5.2	67.1±10.1	61.0±9.4	CE vs HC: 0.039
variability (cov)				
daytime				
SBP (%)	9.8±2.2	8.8±2.2	7.8±3.0	CE vs HC: 0.078
DBP (%)	11.9±2.3	12.1±2.3	9.6±2.3	CE vs HC: 0.022, CS vs HC: 0.024
HR (%)	13.2±4.8	12.6±3.7	15.6±4.3	
night-time				
SBP (%)	8.9±2.1	10.5±3.2	7.2±2.0	CE vs HC: 0.059, CS vs HC: 0.01
DBP (%)	12.0±3.1	15.0±5.6	9.2±3.2	CE vs HC: 0.041, CS vs HC: 0.01
HR (%)	8.3±3.3	8.3±6.5	7.6±2.2	
portapres				
SBP (%)	16.5±3.5	17.0±4.4	14.3±2.4	
DBP (%)	16.2±3.5	18.7±5.2	13.2±3.2	CE vs HC: 0.034, CS vs HC: 0.01
HR (%)	16.1±4.1	20.1±8.8	17.5±3.5	
MAP (%)	15.2±3.5	16.2±4.7	12.2±2.3	CE vs HC: 0.028, CS vs HC: 0.028

S/DBP= systolic/ diastolic blood pressure, HR= heart rate, daytime: 9.00 am- 9.00 pm,

night-time: 1.00 am - 7.00 am, COV=coefficient of variance (%).
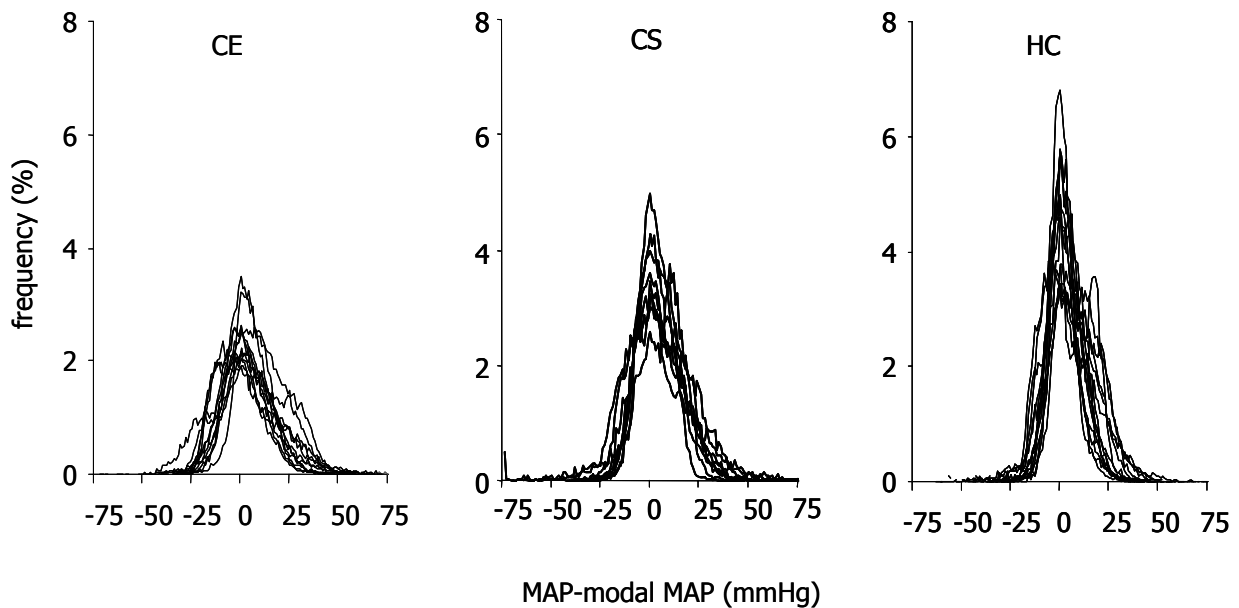
Figure 2. Individual frequency histograms of blood pressure 5 hours Portapres registration. MAP =mean arterial pressure, y-axis: frequency of MAP level as percentage of total number of frequencies; 0 mmHg refers to the modal blood MAP, i.e. the MAP with the highest frequency of occurrence. CE=unilateral carotid endarterectomy, CS=carotid stenosis, HC=healthy controls

Discussion

We investigated the long-term effects of unilateral CE on arterial baroreflex function and blood pressure. Baroreflex sensitivity was significantly lower in CE patients as compared to CS patients and healthy controls. CE patients exhibited a decreased reflex control of heart rate in response to Valsalva's maneuver and active standing. Orthostatic hypotension was not observed. Efferent cardiovagal activity was unimpaired as shown by normal heart-rate responses during forced breathing and cold face test. Mean ambulatory blood pressure levels were not different between groups. Office blood pressure levels before and after CE were similar. Blood pressure variability during ambulatory measurements was similar in CE and CS patients.

Bilateral (3;29) as well as unilateral (8;9) carotid endarterectomy may in individual cases be complicated by baroreflex failure, a syndrome that results from carotid sinus baroreceptor denervation (11). Baroreflex failure is characterized by excessive increments of sympathetic tone including marked elevations of catecholamine plasma levels (11). Unrestrained central activation of efferent sympathetic pathways arises from the absence of tonic inhibitory baroreceptor input to the vasomotor centers of the brainstem (11). This explains the bouts of severe hypertension and tachycardia in patients with this syndrome following CE (3;8;9;29), which in turn may result in hypertensive encephalopathy (3;8) and cerebral hemorrhage (9). Apart from these severe cases of persistent baroreflex failure, temporary hyper- and hypotension are frequently observed during the hours to days following CE (5;7;30-32). These hemodynamic changes were also attributed to changes in arterial baroreflex control.

The full-blown clinical picture of baroreflex failure as a complication of CE appears to be rare and was not observed in any of the patients described in the present study. However, the finding of a decreased baroreflex sensitivity in patients as compared to CS patients and healthy controls suggests that CE induces long-term impairment of arterial baroreceptor function. Prior studies on the effect of CE on arterial baroreflex function were mainly focused on the acute phase following surgery (13-17). These investigations, which yield contrasting results, vary considerably with respect to patient selection and methods for baroreflex testing. In a classic study by Wade et al. (13), responses to Valsalva's maneuver were studied in 8 patients before and after bilateral CE. A baroreflex dependent phase 4 overshoot was minimal before and showed no consistent change as a result of surgery. In addition, peripheral chemoreflex function, which depends on the integrity of the same afferent innervation as carotid baroreceptors, was markedly impaired by bilateral CE. Intraoperative studies in 6 patients ondergoing unilateral CE showed an increase in baroreflex sensitivity following removal of the atherosclerotic plaque (14). Baroreflex gain was calculated from the reflex effect on systemic pressure of carotid artery occlusion. Interpretation of these closed-loop baroreflex trials was complicated by different levels of blood pressure at baseline before and after CE. In a larger study by Hirschl et al. (16), baroreflex sensitivity was calculated before and shortly after unilateral CE from the heart rate responses to Valsalva's maneuver and intravenous injection of angiotensin-II and nitroglycerine in 50 patients, including 34 (68%) hypertensives. They reported a differential effect of CE on baroreflex function in hyper- and normotensive subjects. Compared to pre-operative values, sensitivity was increased in hypertensives versus no change in normotensives. However, the second baroreceptor trial was possibly biased by a post-operative reduction of antihypertensive medication. Dehn et. al carried out neck-suction studies in 25 patients before and 6 months after uni- or bilateral CE (18). They found baroreflex function to be unchanged in 15/25 and improved in 8/25 of patients after unilateral CE. A decrease was noted in 2 patients that had undergone bilateral CE. In another neck suction study in 8 patients, baroreflex sensitivity was similar before and after unilateral CE (15). It is unknown, whether carotid surgery induces structural changes in the soft tissue of the neck, that alter the tranduction of the externally applied negative pressure to the carotid sinus wall.

Altogether, the effect of CE on arterial baroreflex function appears to be heterogeneous among individuals. Our findings of an attenuated baroreflex control of heart rate in response to Valsalva's maneuver and active standing in CE patients suggest, that on average an unfavorable effect of unilateral CE on baroreflex sensitivity prevails. Suggested mechanisms of attenuated baroreflex sensitivity by CE include trauma to the carotid sinus baroreceptors or to the carotid sinus nerve (33) and a decrease in wall distensibility due to surgery-induced periarterial fibrosis (34). On the other hand, removal of an atherosclerotic plaque may have a beneficial effect on baroreflex function by means of changes in the mechanical properties of the carotid sinus arterial wall and reintegration of baroreceptor areas into circulatory regulation (33). Another important determinant of the net effect of unilateral CE on functional baroreflex integrity is the compensatory ability of the residual aortic and contralateral carotid baroreceptors. Compensation by residual baroreceptors probably accounts for the

fact that severe and acute baroreflex failure resolves within days to weeks in most cases (11;35). After unilateral CE, compensation by the contralateral carotid baroreceptors may be limited by atherosclerotic changes of the non-operated carotid artery. In atherosclerosis, distensibility of the carotid sinus vessel wall and sensitivity of baroreceptors are reduced (36;37).

Patients investigated in this study showed no long-term elevation of blood pressure level following CE. In the acute post-operative phase, none of the patients required prolonged hemodynamic monitoring in the intensive care unit for blood pressure lability. The absence of long-term hypertension following CE is not surprising, since carotid sinus denervation does not induce sustained hypertension in either experimental animals (38) or humans (35;39). In contrast to our findings in patients after unilateral carotid sinus trauma, blood pressure variability is chronically increased following bilateral denervation of carotid sinus baroreceptors due to carotid body tumor surgery (35;40). Bilateral carotid sinus denervation was shown to result in attenuation of both vagal and sympathetic barorereflex sensitivity (40;41). This discrepancy suggests that the unaffected carotid baroreceptors are largely capable of compensating for the loss of contralateral baroreceptors. In addition, cold and mental stress did not elicit exaggerated pressor responses in CE patients. This might also be due to a differential effect of baroreflex denervation on the vagal control of heart rate and the sympathetic control of blood pressure (42). Animal studies have shown, that carotid baroreceptor denervation can selectively impair baroreflex control of heart rate without influencing control of sympathetic nerve activity or vascular resistance (43).

The principle limitation of the present study is its retrospective design, which may conceal variable effects of CE on baroreflex function. In these post-intervention studies, it is difficult to find a suitable control group. Comparison between CE patients and CS controls is hampered by the fact, that unoperated CS patients have less severe carotid atherosclerosis. Prospective evaluation in a larger number of patients is warranted in order to be able to confirm an on average unfavorable effect on baroreflex function. In the mean time, clinicians should be aware of possible effects of CE on the baroreflex regulation of blood pressure. Large blood pressure swings, with the possible complication of cerebral hemorrhage, appears to be limited to the acute phase, i.e. hours to days, following surgery. The risk of clinically relevant baroreflex failure on the long term following carotid desobstruction appears to be small.

In conclusion, unilateral carotid endarterectomy causes a long-term impairment of baroreflex function, resulting in an attenuated reflex control of heart rate, without hypertension or blood pressure lability. Net baroreflex function after unilateral CE probably depends on the extent of carotid sinus denervation, compensation by residual contralateral carotid and aortic baroreceptors and changes in the elastic properties of the carotid sinus vascular wall.

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The role of carotid chemoreceptors in the sympathetic activation by adenosine in humans

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Abstract

The direct vasodilatory and negative chronotropic effects of adenosine in humans are counterbalanced by a reflex increase in sympathetic nerve traffic. Suggested mechanisms for this reflex include peripheral chemoreceptor activation. We, therefore, assessed the contribution of carotid chemoreceptors to sympatho-excitation by adenosine.

Muscle sympathetic nerve activity was recorded during adenosine infusion (140 microgram/kg/min, 5 min) in five patients lacking carotid chemoreceptors after bilateral carotid body tumor resection (1m:4f, 51±11yr) and in 6 healthy controls (2m:4f, 50±7yr). Sympathetic responses to nitroprusside injections were assessed to measure baroreceptor mediated sympathetic activation.

In response to adenosine, controls showed no change in blood pressure, +48.2±13.2% heart rate increase (p=0.003) and +195±103% increase in sympathetic nerve activity (p=0.022). In contrast, patients showed a -14.6±4.9/-17.6±6.0% blood pressure decrease (p<0.05), a +25.3±8.4% heart rate increase (p=0.032) and no significant change in sympathetic activity. Adenosine-induced hypotension in individual patients elicited less sympathetic activation than equihypotensive nitroprusside injections.

In humans lacking carotid chemoreceptors, adenosine infusion elicits hypotension due to the absence of significant sympatho-excitation. Chemoreceptor activation is essential for counterbalancing the direct vasodilation by adenosine. In addition, blunting of the baroreflex sympathetic response to adenosine-induced hypotension may indicate a direct sympatho-inhibitory effect of adenosine.

Introduction

The clinical use of adenosine as a therapeutic agent in supraventricular tachycardia (1) and as a diagnostic tool in adenosine-thallium stress testing for cardiac ischemia (2) is based on its negative chrono- and dromotropic (3) and vasodilatory (4) properties respectively. However, in conscious healthy subjects adenosine infusion results in a dose-related increase in heart rate and respiration without a change in mean arterial blood pressure (5-7). Hypotension and bradycardia are prevented by an increase in sympathetic nerve activity in response to adenosine (5;8). This reflex sympathoexcitation to vasodilation is responsible for the absence of hypotension and bradycardia since in patients with severe autonomic failure and in anaesthetized subjects, in whom autonomic reflexes are blunted, adenosine produces sustained hypotension (9;10).

Mechanisms that have been suggested to contribute to a reflex increase in muscle sympathetic nerve activity (MSNA) include activation of peripheral chemoreceptors (7), unloading of arterial and cardiopulmonary baroreceptors (8;11) and stimulation of adenosine-sensitive afferents in the heart (12), kidney (13) and forearm (14). Adenosine-induced activation of peripheral chemoreceptors has been demonstrated in animal studies (15;16). Indirect evidence for the involvement of peripheral chemoreceptors in the sympathetic activation by adenosine in humans was

provided by a differential effect of adenosine on blood pressure depending on the site of infusion. Adenosine (with a very short half-life of <1.5 s (17)) has pressor effects when administered in the aortic arch proximal to the origin of the carotid arteries, but depressor effects when the carotid body chemoreceptors are bypassed by administration at the level of the descending aorta (7;18). Furthermore, the MSNA and heart rate responses to adenosine are blunted when peripheral chemoreceptors are silenced by hyperoxia (11). However, the relative impact of chemoreceptor activation on sympatho-excitation by adenosine in humans remains to be elucidated.

In a previous study, we have demonstrated that carotid body chemoreflex function is abolished in patients after removal of a bilateral carotid paraganglioma (bilateral carotid body tumor resection, BCBR) as indicated by the absence of a ventilatory response to normocapnic hypoxemia in these patients (19). The aim of the present study was to examine the role of peripheral chemoreceptors in the sympathetic activation by exogenous adenosine, by comparing MSNA responses to continuous intravenous infusion of adenosine between BCBR patients (lacking carotid chemoreceptors) and healthy controls. In addition, MSNA responses to sodium nitroprusside injections were assessed for estimation of baroreceptor mediated sympathetic activation.

Methods

Patients and controls

Five patients (1 male, 4 females) who had undergone BCBR at the Department of Otolaryngology of the University Medical Center Nijmegen, the Netherlands, were included in this study. Individual information on tumor size, additional tumor localizations and surgical details of these five patients are shown in table 1. The median interval between the second operation and the study was 6.7 yr (range: 4.4-20.3 yr). Patients were free of diabetes, neurological, cardiovascular and pulmonary disease. Six healthy subjects (2 males, 4 females) served as controls. Full medical history and physical examination including blood pressure measurements revealed no abnormalities. Groups were matched for age (BCBR: 51.2±10.8 versus controls: 50.0±6.5 yr), body mass index (24.8±1.1 versus 25.7±3.8 kg/m^2) and alcohol intake (7.8±8.5 versus 9.0±7.4 units/ week). The study protocol was carried out in accordance with the declaration of Helsinky (2000) of the World Medical Association and was approved by the institutional ethics committee and all subjects gave their informed consent. The procedures followed were in accordance with institutional guidelines.

Blood pressure, heart rate, breathing frequency

Investigations were carried out during morning time after an overnight fast in a room with an ambient temperature of 22-24°C. Subjects had abstained from caffeine, tea, alcohol chocolates and smoking for at least 24 hours (20). Office systolic and diastolic blood pressure (SBP/ DBP) was determined from the mean of 3 supine sphygmomanometric measurements after 10 min of rest. Continuous finger arterial blood pressure was monitored by a Finapres device (model 5)(21) and heart rate (HR)

by surface ECG connected to a Hewlett Packard 378341A (Germany) monitor. Respiratory rate was monitored from changes in inspiratory and expiratory air temperature by means of a nose thermistor (Fysicon medical technology, Oss, The Netherlands). An intravenous line was placed in an anticubital vein for adenosine infusion.

Table 1. Characteristics of individual BCBR patients

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Sex/ age	F44	F58	F44	F62	M36
First CBR:					
Time since (y) *	7.1	20.4	7.8	5.3	7.4
Right/ left	right	left	left	left	left
Tumor size (max cm) †	2.5	2.0	4.0	3.0	2.0
Radical resection †	yes	no	yes	yes	yes
(Cranial) nerve damage †	-	-	nIX	-	-
Second CBR:					
Time since (y) *	6.1	20.3	6.7	4.4	6.6
Right/ left	left	right	right	right	Right
Tumor size †	1.0	1.5	3.0	2.0	1.5
Radical resection †	yes	no	yes	no	yes
(Cranial) nerve damage †	-	Cervical plexus	-	-	-
Symptoms/ signs of baroreflex failure	persistent	-	-	-	transient
Additional paraganglioma localisations/ therapy		Right side skull basis: radiotherapy			
Family history of paraganglioma	yes	yes	no	yes	yes
Medication		chloorthalidon, atenolol, moxonidine			atenolol

CBR: carotid body tumor resection, * interval between surgery and investigation, † according to surgical and pathological reports

Sympathetic nerve recordings

Multi-unit microneurographic recordings of post-ganglionic MSNA were obtained with a unipolar tungsten electrode inserted selectively into a muscle-nerve fascicle of the right peroneal nerve, posterior to the fibula head as originally described by Sundlöf and Wallin (22). A reference electrode was inserted subcutaneously 1 to 3 cm from the recording electrode. Electrodes were connected to a preamplifier with a gain of 1000 and an amplifier with a gain that could be varied from 30 to 90 as required in a subject. Amplification was constant throughout the study in each subject. Neural activity

was fed through a bandpass filter with a bandwidth of 700 to 2000 Hz. The filtered neurogram was routed through an amplitude discriminator to a storage oscilloscope and a loudspeaker. For recording and analysis, the filtered neurogram was fed through a resistance-capacitance integrating network (time constant 0.1 sec) to obtain a mean voltage neurogram of MSNA. Acceptable recordings met the following criteria: spontaneous bursts of neural discharge synchronous with heart rate, no response to arousal stimuli or skin stroking and an increase in nerve burst frequency with apnoe.

Study protocol

After instrumentation, subjects lay quietly for a stabilization period of 20 min. After a 5-10 min baseline recording, adenosine (adenocor® 6 mg/2ml) was administered intravenously at a dosage of 140 µg/kg/min during a 5-min period. This infusion rate is similar to the dose that is applied during diagnostic myocardial stress testing (23).

To assess the baroreflex control of sympathetic outflow, we calculated the increase in MSNA in response to a decrease in blood pressure induced by sodium nitroprusside (SNP) (24). After a 20-min baseline period, graded bolus injections of SNP (12.5-25-50-100 µg) were given intravenously. The dosage producing a decrease in arterial pressure of ≈15 mmHg was repeated thrice at intervals of 10 min.

Data analysis

A computer-assisted method was applied for automatic detection and quantification of individual bursts of sympathetic nerve activity by means of a curve-fitting method. During a pre-scan of the tracing, the neurogram was correlated with a triangular signal by applying a least squares algorithm. In order to define "reference bursts", the 100 largest triangular waves detected during the pre-scan were taken. After discarding the 20 largest waves for possible artifacts, the remaining 80 were taken as a reference. Their mean amplitude and delay from the corresponding R-wave on the ECG were determined. During a subsequent scan, the individual amplitude and time delay of all triangular waves were compared with the mean amplitude and delay of the reference waves. Waves were accepted and marked as sympathetic bursts on two conditions: an amplitude of >20% of the mean reference amplitude and a time delay of <200 ms beneath or above the mean reference delay. Automatically calculated burst amplitude correlated well (r>0.9) with manual burst detection during supine rest and adenosine infusion (unpublished data). MSNA was expressed as bursts per min, bursts per 100 beats, total integrated activity (TIA) per min, TIA per 100 beats and mean integrated activity per burst. Responses to adenosine infusion were expressed as relative changes in blood pressure, heart rate, breathing frequency and MSNA, calculated as 1-min averages of the 5 min during adenosine infusion and 5 min of recovery as compared to 1 min at baseline.

The relative increase in MSNA during a 20-s stable BP minimum after SNP injection was taken as an estimate of baroreflex control of sympathetic outflow during sustained hypotension. In subjects that exhibited hypotension in response to adenosine, the relative increase in MSNA was compared to

the increase in MSNA elicited by an equihypotensive dose of SNP in that particular patient. For comparison of MSNA responses to adenosine- and SNP-induced hypotension, we selected a 1-min interval during adenosine infusion in which the decrease in DBP was equal to the mean SNP-induced decrease in BP. The MSNA response to SNP was taken as the expected baroreflex-mediated sympathetic response to hypotension during adenosine infusion (8).

Statistics

Results are given as mean±sem unless indicated otherwise. Differences between patients and controls with respect to nominal variables were compared using the Chi-square test. Other variables were compared using the Student t-test or Mann-Whitney rank-sum test when appropriate. A two-sided p<0.05 was taken as the level of significance. Statistical analysis was performed using SPSS (Statistical Package for the Social Sciences) for Windows 6.1.3.

Results

Sympathetic responses to adenosine

At baseline, sphygmomanometric SBP/DBP and HR did not differ between groups: (patients: 120.8±11.1/ 79.0±5.9mmHg, 66.8±8.5bpm) versus controls: 123.3±11.9/ 81.5±7.6mmHg, 66.2±6.5bpm), nor did sympathetic burst frequency (patients: 39.6±20.0 bursts/min, 49.3±21.5 bursts/100 beats versus controls: 38.8±11.5 bursts/min, 59.0±19.8 bursts/100 beats).

Patients and controls retrospectively reported the following symptoms during adenosine infusion: chest pain (all patients and controls), dyspnea (patients #1-3, controls #1-4, #6), flushing (patient #1), headache (patient #1), nausea (patient #4, control #5) and nervousness (patient #1).

Representative tracings of blood pressure, heart rate and MSNA responses to adenosine as observed in BCBR control #4 and patient #4 are depicted in figure 1. Group averaged responses during the 5th min of adenosine infusion as compared to baseline are shown in figure 2. Controls show no significant change in SBP/DBP (+1.1±2.6/ -2.1±2.0%), a +48.2±13.2% increase in heart rate (p=0.003), a +195±103% increase in MSNA expressed as TIA/min (p=0.022) and a 38.0±12.0% increase in integrated activity per burst (p=0.036). The increase in MSNA expressed as bursts/min (+96±48%), bursts/100beats (+39.6±38%) and TIA/100 beats (+113±79%) in controls were not significant. BCBR patients showed a decrease in SBP/DBP of -14.6±4.9/-17.6±6.0% (p<0.05) an increase in HR of +25.3±8.4% (p=0.032) and (non-significant) increases in MSNA of +63.1±30.8% TIA/min, +7.4±7.8% mean integrated activity per burst, +50.6±27.5% bursts/min, +18.2±16.8% bursts/100beats and +27.5±19.3% TIA/100 beats. The responses differed significantly between patients and controls with respect to SBP (p=0.04), DBP (p=0.004) and mean amplitude per burst (p=0.021). There was a trend towards a smaller HR increase in patients as compared to controls (p=0.094). After the infusion, changes in BP, HR and MSNA returned to baseline in both groups within

3-5 min. Mean respiratory rate during adenosine infusion was not different from baseline in patients (14.4±4.4 and 16.4±1.9/ min respectively) nor in controls (13.8±2.6 and 14.3±2.2/min).
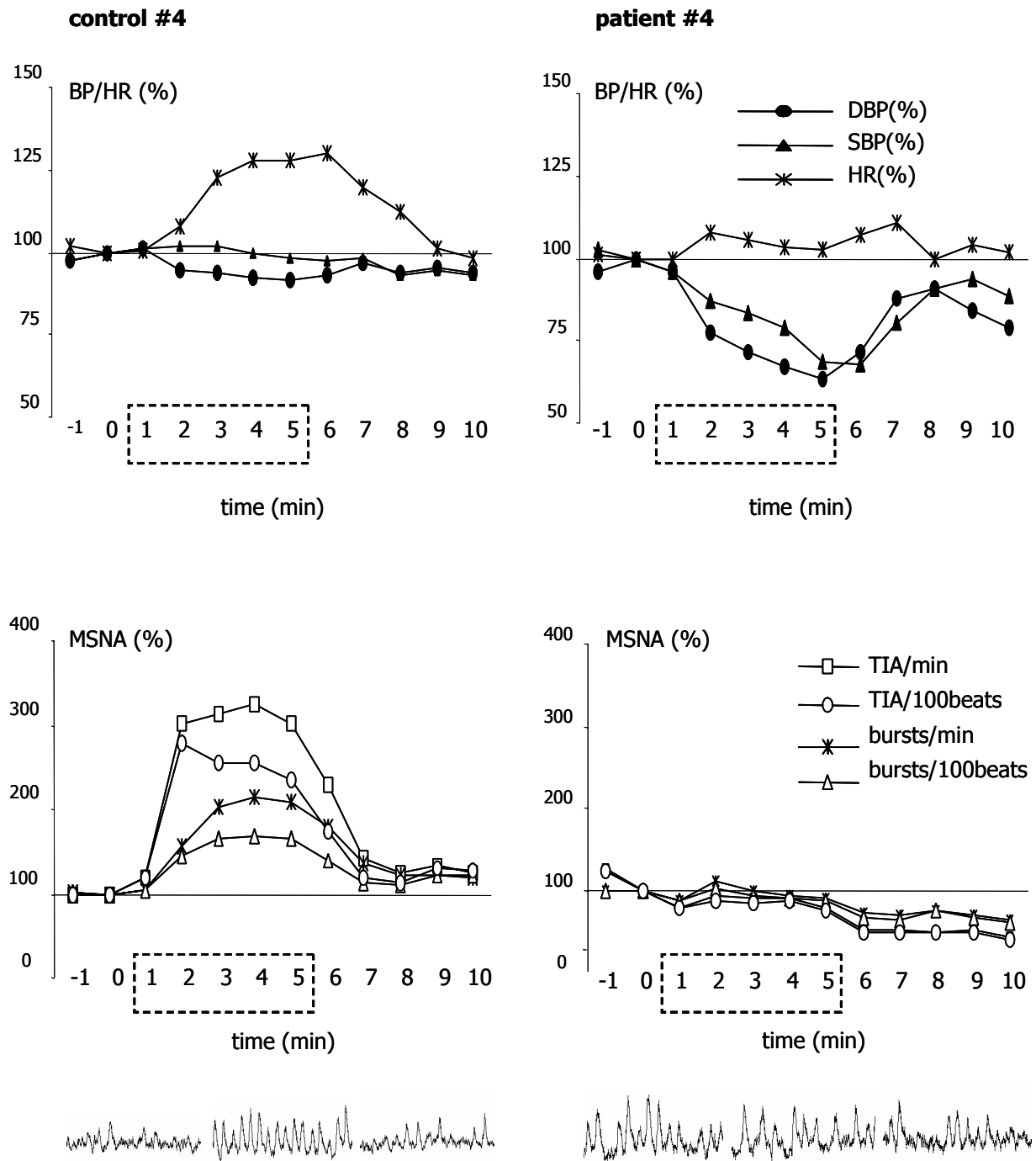

Figure 1. Individual responses to adenosine infusion in control #4 (left panel) and patient #4 (right panel). Presented as 1-min averaged relative changes in diastolic blood pressure (DBP), systolic blood pressure (SBP), heart rate (HR) and muscle sympathetic nerve acitvity (MSNA, TIA= total integrated activity) during (1-5 min, dotted square) and after (6-10 min) adenosine infusion as compared to baseline (-1-0 min). The neurogram of the last 10 s before, the 5th min during, and the 5th min after infusion are displayed at the bottom.
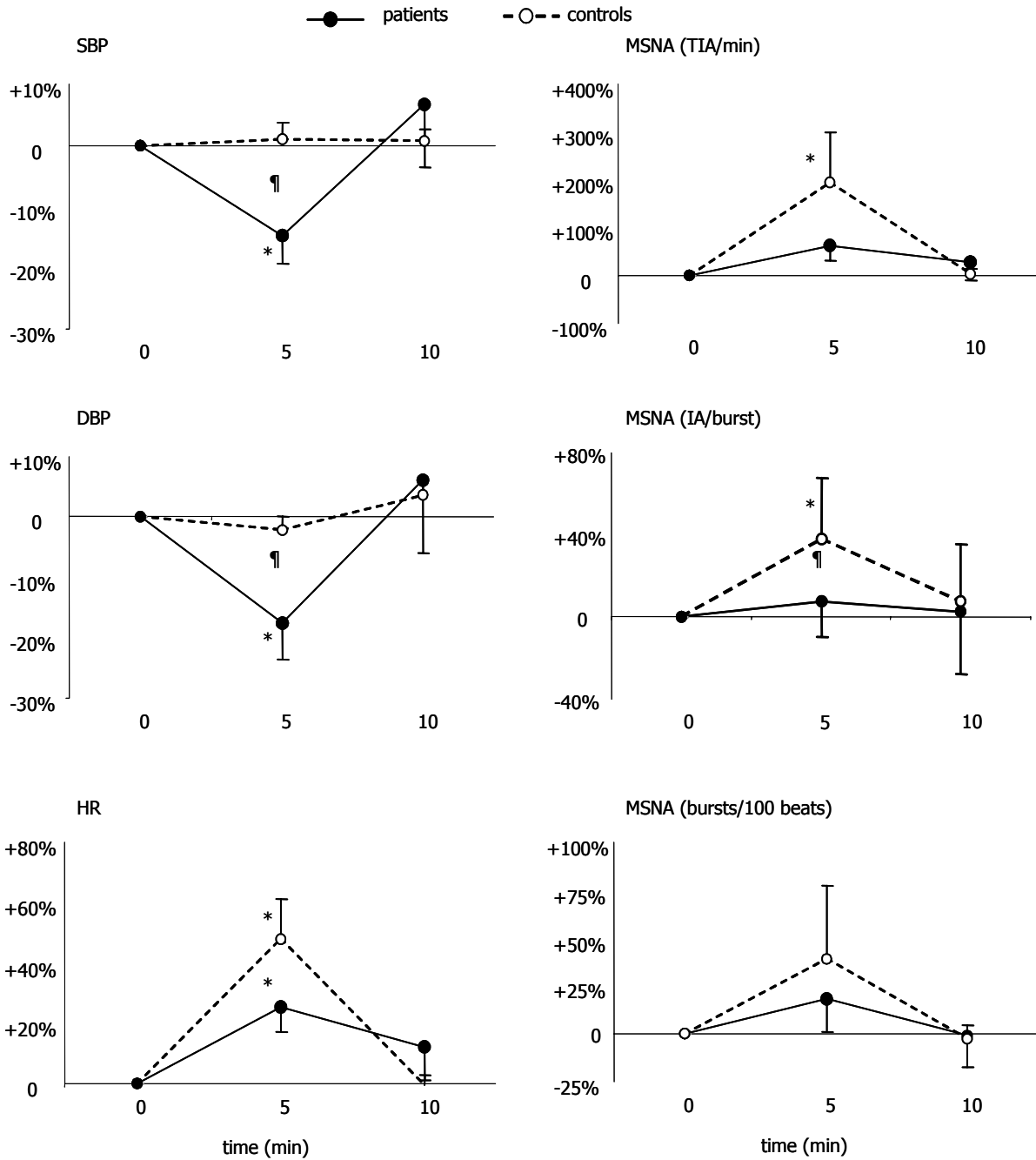
Figure 2. Group averaged responses to adenosine in patients and controls. Presented as (mean±sem) relative changes from baseline in systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR) and muscle sympathetic nerve activity (MSNA, (T)IA= (total) integrated activity) during the last min of adenosine infusion (t=5 min) and after return to baseline (t=10 min). * significant <u>within</u>-group change from baseline (p<0.05), ¶ significant <u>between</u>-group differences in 5th-min responses.

Sympathetic responses to sodium nitroprusside

MSNA responses to SNP induced hypotension as compared to changes in MSNA in the 4 BCBR patients that exhibited a BP decrease during adenosine infusion (patient # 2-5) are shown in figure 3. Adenosine elicited smaller increases in MSNA than equihypotensive doses of SNP.

	Patient # 2	# 3	# 4	# 5
delta DBP (mmHg)	-14.9 -14.9	-11.1 -13.4	-14.9 -15.8	-12.3 -12.6
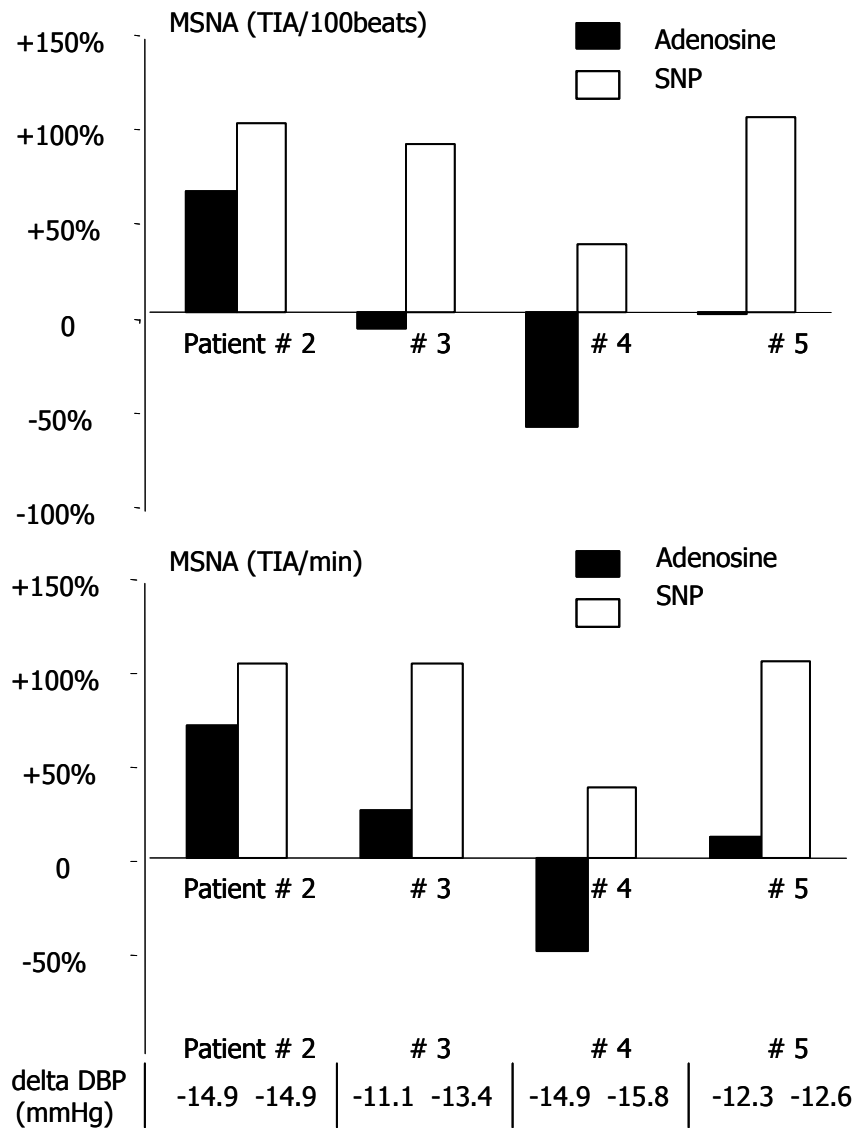
Figure 3. Changes in MSNA in response to equihypotensive doses of adenosine (closed bars) and sodium nitroprusside (open bars) in individual patients. Relative changes in total integrated activity (TIA) per 100 beats (upper panel) and TIA per min (lower panel) in response to changes in diastolic blood pressure (DBP) induced by adenosine and sodium nitroprusside (SNP). Please note, that within each patients, adenosine and SNP induce similar decreases in diastolic blood pressure (indicated in the table at the bottom of the figure) and that similar blood pressure reductions elicit smaller MSNA responses during adenosine infusion than during SNP infusion.

Discussion

We investigated the role of peripheral chemoreceptors in the sympathetic activation by exogenous adenosine, by comparing MSNA responses to continuous intravenous infusion of adenosine (140 µg/kg/min) between BCBR patients, lacking carotid chemoreceptors, and healthy controls. Our principle finding is that absence of carotid body chemoreflex function due to BCBR results in abolishment of significant MSNA and HR responses to adenosine. In the absence of chemoreceptor-dependent sympatho-excitation, adenosine infusion causes a 15% decrease in BP. Furthermore, the BP decrease induced by adenosine in BCBR patients, elicited a smaller increase in MSNA than expected from the observed individual baroreflex mediated MSNA responses to SNP.

It has been well established, that continuous adenosine infusion in healthy awake humans causes a dose-dependent increase in HR, MSNA and plasma norepinephrine levels without change in mean arterial blood pressure (5-8). The tachycardic response correlates significantly to increases in norepinephrine (5). In a previous study, spectral analysis of heart rate showed a reduced parasympathetic and increased sympathetic influence on the sinus node during intravenous infusion of adenosine in healthy volunteers (25). Our present observations of blunted MSNA and HR responses and hypotension in response to adenosine infusion in patients lacking carotid chemoreceptors indicate, that carotid chemoreceptors are indispensable to trigger autonomic reflexes during adenosine infusion which prevent hypotension that would otherwise occur because of the direct vasodilatory action of adenosine (26). Evidence for peripheral chemoreceptor activation by adenosine comes from animal studies in which intracarotid infusion of adenosine causes an A2-receptor-specific stimulation of afferent chemoreceptor nerve traffic and ventilation (15;16).

In humans, intravenous adenosine infusion produces a dose-related increase in ventilation, mostly due to increased depth of respiration (5;6) even at a dose below the threshold for symptoms (7;27). In line with these observations, we found no effect of adenosine on respiratory rate in either patients or controls. For practical reasons, we did not perform spirometry during the infusion, so tidal volume was not measured. Respiratory stimulation by adenosine is suggested to result from peripheral chemoreflex activation, as ventilatory stimulation only occurs when adenosine is injected proximal to the origin of carotid arteries (and carotid chemoreceptors) and not after administration in the descending aorta (7;18). The finding that adenosine increases the ventilatory response to hypoxia, not to hypercapnia, reiterates its stimulatory effect on peripheral chemoreceptors (27). Previous studies on the role of peripheral chemoreceptor activation in mechanism of sympatho-excitation by adenosine infusion in humans have yielded merely indirect evidence. Adenosine was shown to have a differential pressor and depressor effect, depending on infusion proximal or distal to the carotid chemoreceptors respectively (7;18). In addition, suppression of peripheral chemoreceptors by hyperoxia was shown to decrease MSNA and HR response to adenosine by one third as compared to the normoxic response (11) and adenosine infusion causes HR and MSNA responses similar to that observed during an equal ventilatory stimulation by hypoxia (8).

We have reported previously, that BCBR has a profound effect on peripheral chemoreflex function, as suggested by the complete abolishment of the normocapnic ventilatory responsiveness to hypoxia [28]. During simultaneous hypercapnia a small hypoxic response was observed in 2 of 8 patients (Patient #4 and 5 in this study). This residual chemoreflex function was attributed to the remaining aortic bodies. These subserve a minor role in the modulation of spontaneous respiratory activity in humans, but they may generate a discernible response when their gain is increased by hypercapnia (28). Apart from carotid body chemoreceptor stimulation, adenosine was shown to increase aortic chemoreceptor discharge in cats (29). However, blunted MSNA responses in BCBR patients suggest little, if any, contribution of aortic chemoreceptors to the sympathoexcitation by adenosine.

Other possible mechanisms for sympatho-excitation by adenosine in humans, besides chemoreceptor activation, include stimulation of adenosine-sensitive afferents in the coronary arteries (12), kidney (13) and forearm (14). In addition, a reflex increase in MSNA may be triggered by unloading of arterial and cardiopulmonary baroreceptors by the direct vasodilator action of adenosine (8;11). In healthy subjects, adenosine was shown to produce a greater increase in MSNA than SNP injections at doses that resulted in equivalent hypotension (8). Arterial baroreceptor unloading, therefore, could not totally explain the increase in sympathetic traffic produced by adenosine. On the other hand, mechanical baroreceptor unloading by −15 mmHg lower body negative pressure (LBNP) elicited an MSNA response similar to that observed during an equihypotensive dose of adenosine (11). However, this level of LBNP causes both arterial and cardiopulmonary baroreceptor unloading and the impact of LBNP and adenosine on central venous pressure (and thereby on cardiopulmonary mechanoreceptors) were not compared. Adenosine was reported to cause no change (8), or even a small increase (30) in central venous pressure, which makes unloading of cardiopulmonary receptors unlikely. Furthermore, caffeine abstinence was less well implemented in the LBNP study, which may have underestimated the autonomic response to adenosine (20).

In a previous study we have shown that BCBR results in attenuation of the baroreflex control of heart rate [28]. The decrease in baroreflex sensitivity after BCBR was suggested to be due to partial denervation of the carotid sinus baroreceptors. However, SNP-induced hypotension in these patients elicited a prominent baroreceptor mediated rise in MSNA, which was even larger than the MSNA response to an equivalent decrease in BP during adenosine infusion. We speculate, that this finding is explained by an inhibitory role of adenosine on ganglionic neurotransmission (31). Direct central depression of sympathetic tone by exogenous adenosine (32) is unlikely to occur, since adenosine crosses the blood-brain barrier very slowly (33). On the other hand, the sympathetic nuclei lie close to the area postrema, which was shown to be permeable to adenosine in animal studies (34). In healthy awake individuals this direct sympatholytic effect of adenosine might be overwhelmed by the reflex activation of sympathetic nerve traffic.

The findings of hypotension and absence of a significant MSNA increase during adenosine infusion in BCBR patients, versus the presence of a baroreflex mediated MSNA response to SNP,

suggests, that neither baroreceptor unloading nor stimulation of adenosine-sensitive afferents other than carotid body chemoreceptors contribute significantly to the mechanism of sympatho-excitation by intravenous adenosine. Interruption of the chemoreflex influence on sympathetic outflow and unopposed direct vasodilatory action probably also account for adenosine-induced hypotension during general anesthesia (9). The finding of an incidence of hypotension (decrease in SBP $\geq$-30 mmHg) of 10% (116/1000) in patients undergoing adenosine-thallium stress testing for cardiac ischemia may be due to the large prevalence of diabetes (25%) among these patients (23). Diabetic autonomic neuropathy may cause a disruption of the efferent sympathetic pathway by which adenosine brings about reflex vasoconstriction. This may also explain adenosine-induced hypotension in patients with severe autonomic failure (7).

Conclusions

The present study indicates, that carotid chemoreceptors play a key role in the sympatho-excitation during intravenous adenosine infusion in humans. This chemoreceptor-dependent increase in MSNA prevents adenosine-induced hypotension by counterbalancing its direct vasodilatory action. In addition, blunting of the baroreflex mediated MSNA response to adenosine-induced hypotension may indicate a direct sympatho-inhibitory effect of adenosine.

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General Discussion

Denervation of carotid baro- and chemoreceptors in humans

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Abstract

Experimental denervation in animals has shown, that carotid baro- and chemoreceptors play an eminent role in maintaining blood pressure and blood gas homeostasis. Denervation of carotid sinus baro- and chemoreceptors in humans may occur as a complication of invasive interventions on the neck or after experimental surgical treatment in asthma. In this topical review, the short- and long-term effects of carotid baro- and chemoreceptor denervation on the control of circulation and ventilation in humans are discussed.

Carotid baroreceptor denervation in humans causes a persistent decrease in vagal and sympathetic baroreflex sensitivity and an increase in blood pressure variability; however, carotid denervation does not lead to chronic hypertension. Therefore, although carotid baroreceptors contribute to short-term blood pressure control, other receptors are able to maintain normal chronic blood pressure levels in the absence of carotid baroreceptors. Conversely, carotid chemoreceptor denervation leads to permanent abolition of normocapnic ventilatory responses to hypoxia and reduced ventilatory responses to hypercapnia.

Introduction

H.E. Hering and E. Koch were the first to recognize the reflex nature of changes in heart rate and blood pressure evoked by external massage of the neck. The afferents were tracked as nerve endings at the carotid bifurcation (1;2). At about the same time, Heymans and coworkers unequivocally demonstrated chemoreceptor activity of the carotid bodies (3). With their experiments in the nineteen-twenties/ thirties, these investigators inaugurated the modern era of baro- and chemoreflex research.

The arterial baroreflex buffers abrupt transients of blood pressure and originates from stretch sensitive receptors in the arterial wall of the carotid sinus and the aortic arch and the large vessels of the thorax (Figure 1) (4) (5). Afferent fibres from carotid sinus baroreceptors join the glossopharyngeal nerve (ninth cranial nerve) and project to the nucleus tractus solitarii in the dorsal medulla, which is under cortical command and in turn projects to efferent cardiovascular neurones in the medulla and spinal cord. Extra-carotid baroreceptors consist of arterial baroreceptors in the aortic arch as well as stretch-sensitive receptors in the heart and pulmonary vessels, the latter two being lumped together as "cardiopulmonary" receptors despite their distinctive properties (6). The extra-carotid baroreceptors transmit their afferent information along with the vagus nerves to the same brain stem nuclei. The efferent limbs of the baroreflex loop consist of sympathetic and parasympathetic fibres to the heart as well as to smooth muscles in the peripheral blood vessels.

Adjustment of respiration in response to alterations in levels of oxygen, carbon dioxide and hydrogen ions in the body fluids are mediated by a complex interplay between central and peripheral chemoreceptors (7). The peripheral arterial chemoreceptors, located in the carotid and aortic bodies,

are responsible for the immediate ventilatory and arterial pressure increments during acute hypoxia (3) (Figure 1).
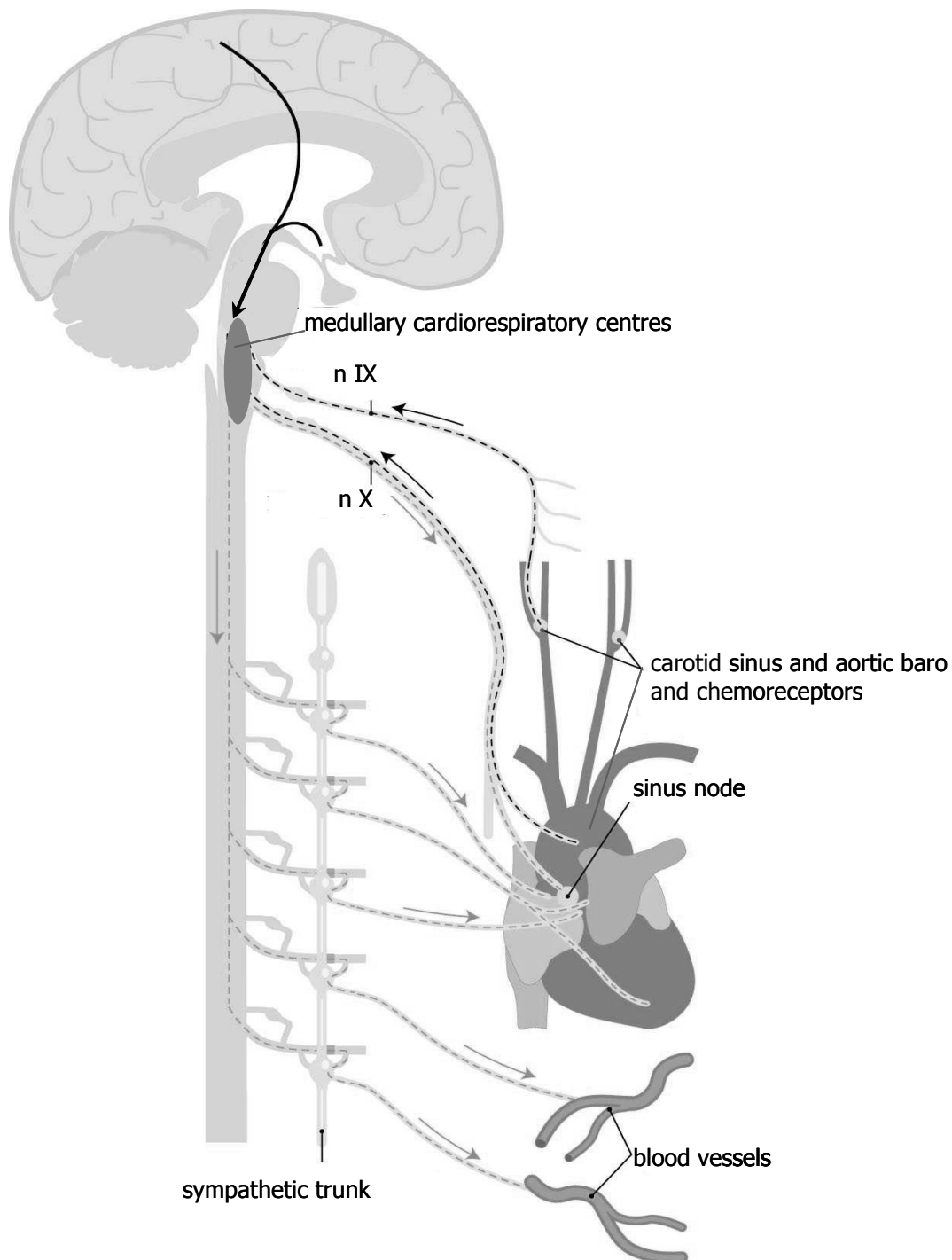
Figure 1. Arterial baro and chemoreceptors. Arterial baroreflex loops: 1) carotid sinus baroreceptors, nIX, medullary centres, sympathetic and parasympathetic fibres to heart and blood vessels. 2) aortic baroreceptors, nX, medullary centres, sympathetic and parasympathetic fibres to heart and blood vessels. Peripheral chemoreflex loops: 1) carotid body chemoreceptors, n IX, medullary respiratory centres, motor nerves to respiratory muscles. 2) aortic body chemoreceptors, n X, medullary respiratory centres, motor nerves to respiratory muscles. n IX= ninth cranial nerve (=glossopharyngeal nerve), n X= tenth cranial nerve (=vagus nerve); the arrows coming from the cortex signify the modulation of brainstem nuclei by higher, cortical centres. Adapted with permission from (83)

Carotid and aortic bodies contain glomus (type I) cells, which release neurotransmitters in response to hypoxia, causing depolarisation of nearby afferent nerve endings (8). Apart from hypoxaemia, peripheral chemoreceptors play a minor role in the sensing of changes in arterial carbon dioxide tension (pCO_2) and pH. Other glomus tissues (glomus jugulare, trigeminale, pulmonare etc.) are not relevant to chemoreflex function in humans. Carotid and aortic bodies are supplied with sensory fibres, which course through carotid sinus/ glossopharyngeal and vagus nerve respectively towards medullary centres, including the nucleus tractus solitarii (9). Central chemoreceptive areas located at the rostral ventrolateral medulla respond to changes in the hydrogen ion concentration in the interstitial fluid in the brain and are chiefly responsible for ventilatory and circulatory adjustments during hypercapnia and chronic disturbances of acid-base balance.

The relative contribution of carotid receptors to baro- and chemoreflex function as well as the compensation after functional loss of these receptors has been investigated extensively by well-controlled denervation studies in experimental animals. For obvious reasons, no human counterparts for the controlled prospective denervation studies in animals are available. Information on the impact of carotid sinus denervation in humans is limited and largely relies on investigations following iatrogenic damage to the carotid sinus as a complication of medical interventions like carotid body tumor surgery, jugular radiotherapy and carotid endarterectomy. Interpretation of these human studies is hampered by uncertainty regarding the completeness of denervation, differences in acute (surgical) versus gradual (radiation) denervation, additional changes in the mechanical properties of the carotid artery wall or surrounding tissue due to the interventions, and the lack of prospective studies. Most studies consist of retrospective, post-intervention assessment of reflex function in small numbers of patients and matched (healthy) control subjects.

We review the short- and long-term effects of carotid baro- and chemoreceptor denervation on the control of circulation and ventilation respectively. Whereas findings in animal studies are briefly mentioned, this review is focussed on data obtained from investigations in humans, including recent studies in this field by the authors. Although the effects of carotid baroreceptor and chemoreceptor denervation will be presented separately, the two conditions arise in parallel in most instances, due to the underlying anatomy.

Carotid baroreceptor denervation

Animal studies

Arterial baroreceptors provide a tonic inhibitory influence on sympathetic tone, thus controlling peripheral vasoconstriction and cardiac output (4) (5). Therefore, baroreceptor denervation would be expected to result in a sustained increase in sympathetic tone and, as a consequence, a sustained increase in blood pressure. The chronic effects of carotid and extra-carotid baroreceptor denervation on blood pressure control have been studied extensively in animals and have been reviewed by others (5;10;11). After selective carotid baroreceptor denervation, both blood pressure level and variability

increased markedly but returned to intact levels within 7 to 14 days in dogs and baboons respectively (12;13). Selective aortic baroreceptor denervation in baboons causes a mild temporary increase in blood pressure, whereas blood pressure variability was unchanged (14). Combined sino-aortic baroreceptor denervation in dogs produced an increase in blood pressure and heart rate in the acute phase, whereas permanent elevation of blood pressure was either present (15;16) or absent (17). Sino-aortic denervation in baboons -the investigated species that is closest to humans- resulted in a persistent increase in blood pressure level and variability and a decrease in heart rate variability (13). Combined sino-aortic, cardiac and pulmonary baroreceptor denervation in dogs produced a persistent increase in blood pressure level and variability (17).

These animal studies show, that extra-carotid baroreceptor areas have a large ability to compensate for the loss of carotid baroreceptors. In some species, chronic hypertension is evoked by combined sino-aortic and cardiopulmonary baroreceptor denervation and by sino-aortic denervation but not by selective carotid denervation.

Human studies

Acute unilateral carotid baroreceptor denervation

The first report on baroreceptor denervation in humans appeared in the nineteen-thirties (18). Unilateral section of the glossopharyngeal nerve in five patients with glossopharyngeal neuralgia produced a prompt and pronounced rise in blood pressure in four out of five patients, which lasted from 5 to 12 days. This phenomenon was recognised as an effect of disruption of "nervous impulses from the carotid sinus which have a reducing effect on blood pressure". In 1956 a patient died from a fatal hypertensive crisis following unilateral carotid sinus denervation, which had been performed for the relief of recurrent syncope due to a hypersensitive carotid sinus syndrome (19).

Lability of blood pressure in the hours following unilateral carotid endarterectomy for symptomatic carotid stenosis has been attributed to carotid baroreflex dysfunction (20;21). However, in the acute phase following carotid endarterectomy, baroreflex sensitivity has been reported to be increased, decreased or unaltered (22-24). Apart from trauma to the carotid sinus, baroreceptors or to the carotid sinus nerve (25), removal of an atherosclerotic plaque may have a beneficial effect on baroreflex function by means of changes in the mechanical properties of the carotid sinus arterial wall and reintegration of baroreceptor areas into circulatory regulation (25). In addition, the effect of unilateral carotid endarterectomy depends on the compensatory ability of the contralateral baroreceptor integrity. This may be limited by atherosclerotic changes in the non-operated carotid artery, since in atherosclerosis, distensibility of the carotid sinus vessel wall and sensitivity of baroreceptors are reduced (26;27).

Although evidence for lateralization of certain human autonomic control functions has been published (28), a differential effect of left- versus right-sided deafferentiation of carotid baroreceptors has not been reported.

Acute bilateral carotid baroreceptor denervation

Bilateral anaesthetic injections in the regions of the carotid sinuses in patients with malignant hypertension were shown to elevate blood pressures to even higher levels (29). Paroxysms of severe hypertension and tachycardia were reported following bilateral carotid body resection as an experimental treatment of asthma (30), carotid paraganglioma resection (31;32), carotid endarterectomy (20;33) and trauma of the neck (31).

Long term effects of unilateral carotid baroreceptor denervation

In a prospective study, the effects of unilateral carotid endarterectomy on carotid sinus baroreflex function were measured in 25 patients (34). Six months after surgery, no overall change in blood pressure was found. Baroreflex sensitivity decreased in 2, remained unchanged in 15 and increased in 8 patients. Thus, similar to the findings in the acute phase following surgery, the long-term effects on baroreflex function were heterogeneous among individuals.

In a retrospective study on the effects of unilateral carotid endarterectomy (35), at a median interval of 4.3 years after surgery, baroreflex sensitivity was significantly lower in endarterectomized patients than in patients with an untreated uni-/bilateral carotid stenosis and healthy controls. So in these patients an unfavourable effect on baroreflex sensitivity prevailed. Despite this, Ambulatory blood pressure level and variability did not differ between groups.

Long term effects of bilateral carotid baroreceptor denervation

In 1993 chronic failure of the baroreflex due to bilateral carotid denervation was described as a separate clinical syndrome, characterized by a limited blood pressure buffering capacity against excessive rises or falls in response to emotional and physical stimuli (31) (Figure 2A). Symptoms and signs included headache, palpitations, diaphoresis and pale flushing. They bear a strong resemblance to those of a pheochromocytoma. In baroreflex failure, desinhibition of central activation of efferent sympathetic pathways arises from the absence of tonic inhibitory baroreceptor input to the vasomotor centres of the brainstem (11;31). Apart from volatile hypertension, which is most common, baroreflex failure has a broad spectrum of other clinical presentations including predominant hypotension, orthostatic tachycardia and intolerance and malignant vagotonia with severe bradycardia, depending on the extent of baroreceptor denervation and concomitant destruction of autonomic structures (36-38). Centrally acting sympatholytic agents like clonidine may reduce the frequency and severity of the attacks (21;31;39).

Long term effects of bilateral baroreceptor denervation have been investigated in patients who had suffered from acute baroreflex failure following bilateral carotid body tumor resection (40). Ambulatory blood pressure level was found to remain slightly elevated as compared to pre-operative values. Overt hypertension, however, appears to be limited to days and months following surgical

carotid baroreceptor denervation, whereas episodic surges of hyper- as well as hypotension may persist for a longer period (30-32).
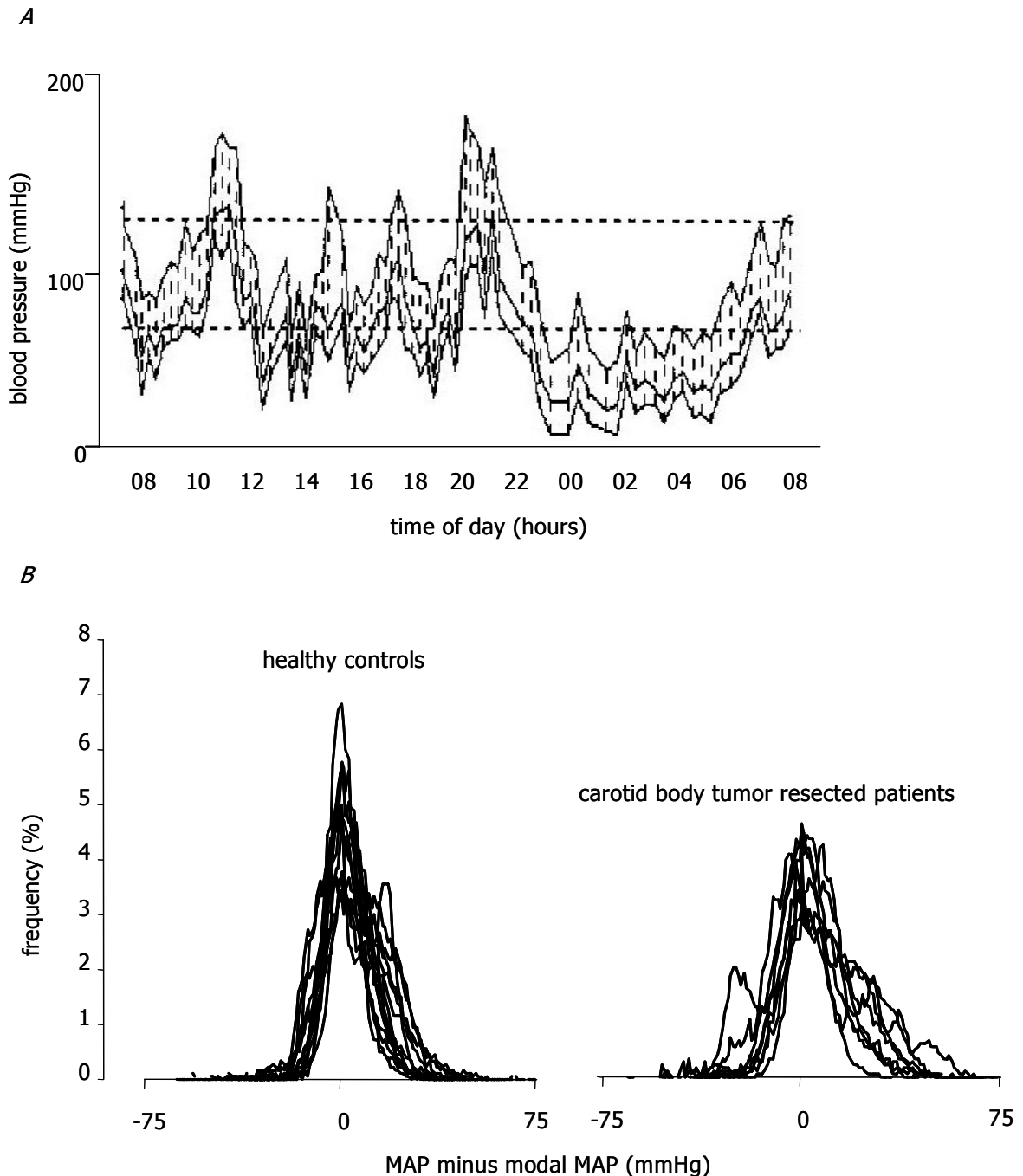
Figure 2. Blood pressure variability following carotid sinus baroreceptor denervation. **Panel A.** 24 hours ambulatory blood pressure profile during normal daily activities characterized by labile hyper- and hypotension in a patient with baroreflex failure due to radiotherapy of the neck for nasopharyngeal carcinoma (48). Dotted lines indicate upper levels for diastolic (<90 mmHg) and sytolic (<140 mmHg) normotension. **Panel B**. Individual frequency histograms of blood pressure calculated from 5 hours ambulatory beat-by-beat recordings in 12 healthy controls (bottom left) and 8 patients after bilateral carotid body tumor resection (bottom right). X-axis: MAP = mean arterial pressure, y-axis: frequency of MAP level as percentage of total number of frequencies. Carotid body resected patients exhibit a broader distribution of MAP, indicating higher blood pressure variability. "Modal MAP" refers to the MAP with the highest frequency during the individual blood pressure tracing.

Retrospective studies were performed in patients who had undergone similar surgery, with a mean interval between the second (i.e. contralateral) operation and the study of 3.4 years (41). At the time of the study, at least 1 year after surgery, ambulatory BP levels were normal in all patients. In the absence of chronic clinically overt baroreflex failure, vagal baroreflex sensitivity, calculated from the reflex changes in RR interval to phenylephrine injections (42), was approximately 50% lower in these patients than in healthy age matched controls. The subnormal vagal baroreflex gain also emerged from minimal reciprocal heart rate changes during phase II blood pressure decrease and phase IV blood pressure overshoot during Valsalva's maneuver (43;44). In addition, microneurography recording in a subgroup of five carotid body resected patients showed, that the baroreflex modulation of muscle sympathetic nerve activity (MSNA) was profoundly affected as well (45). These studies indicate that bilateral carotid sinus trauma causes permanent impairment of vagal as well as sympathetic baroreflex sensitivity. In these patients, the additional finding of an increased ambulatory blood pressure variability was explained by a lower baroreflex sensitivity (Figure 2B). In hypertensives, baroreflex sensitivity has been shown to be negatively correlated with blood pressure variability (46).

Chronic baroreflex failure has been reported as a late complication of radiotherapy of the neck (31;47;48). Changes in carotid sinus baroreceptor function may be induced by irradiation damage to carotid sinus and/or the glossopharyngeal nerves, although cranial nerve palsies are uncommon complications after radiotherapy to the neck (49). Alternatively, arterial baroreflex function may have been altered by structural changes of the internal carotid artery wall. Irradiation induced atherosclerosis (50) and fibrosis (51) may result in a decreased distensibility of the carotid sinus and thereby may reduce stretch-induced afferent carotid sinus nerve activity (52).

The impact of neck irradiation for laryngeal or pharyngeal carcinoma on baroreflex function was retrospectively studied in twelve patients who had undergone bilateral radiation therapy for locally advanced laryngeal or pharyngeal cancer (53). Irradiation fields included the carotid sinus area and the median interval between completion of radiotherapy and time of investigation was 3.3 years. Baroreflex sensitivity was 45% lower in patients than in matched healthy controls. Ambulatory blood pressure variability was not different from matched control subjects. Although baroreflex sensitivity was decreased after neck irradiation, blood pressure buffering was unaffected.

Summary

Labile hypertension due to baroreflex failure may arise from both uni- and bilateral carotid baroreceptor denervation. The incidence of this syndrome following carotid body tumor surgery, radiotherapy of the neck and carotid endarterectomy is low. Baroreflex dysfunction after unilateral denervation is usually mild and transient. On the long term following bilateral carotid denervation, the expression of baroreflex dysfunction is heterogeneous. Bilateral carotid denervation in humans does not elicit chronic hypertension, but in contrast to other investigated species, it causes a long-term increase of blood pressure variability. In humans, carotid baroreceptors are more important for the

dynamic than static blood pressure control. A chronic decrease in blood pressure buffering following carotid denervation suggest, that humans have less potent compensatory mechanisms for loss of carotid baroreflex function than other investigated species. This may be due to our upright position, whereby tonic sympathoinhibitory influences from cardiac and pulmonary baroreceptors have become less than in quadruped species (10). As a consequence of a minor role for cardiac and pulmonary baroreceptors, loss of arterial baroreceptor function in humans may have a larger impact on blood pressure homeostasis.

Previous studies on the relative importance of carotid versus aortic baroreceptors in intact humans have yielded contrasting results. Experiments on selective (un)loading of aortic baroreceptors by simultaneous infusion of vasoactive substances and application of neck suction/ pressure in order to maintain a stable carotid sinus transmural pressure indicated that aortic baroreceptors are dominant in the baroreflex control of heart rate, with the carotid baroreceptors contributing only about 30% (54;55). In line with these observations, baroreflex control of heart rate is more importantly determined by the distensibility of the aortic arch than of the carotid sinus (56). In contrast, combined neck suction/ pressure with non-pharmacological (un)loading of aortic baroreceptors, indicate, that carotid baroreceptors are the principal contributors to baroreflex control of heart rate (57). Our review of studies on iatrogenic denervation is in agreement with the latter study. These studies in intact humans should be interpreted with caution, however, since the baroreceptors respond to stretch and not pressure. The stimulus to be measured should be the diameter of the arteries and not blood pressure. Changes of dimensions of the baroreceptive arteries during the several interventions were not measured.

Denervation of carotid chemoreceptors

Aminal studies.

In general, acute effects of carotid body chemoreceptor denervation in experimental animals include hypoventilation, apnoea, a variable decrease in hypoxic ventilatory responsiveness and attenuation of CO_2 sensitivity. The occurrence of (partial) restoration of chemoreflex function varies among species, but is more likely in neonatal than in adult animals and effects are more marked following bilateral than after unilateral denervation (58). In carotid body denervated rats, hypoxic responsiveness is first abolished, but returns to about half of normal within weeks (59). Compensation was stated to result from inputs from either aortic or abdominal chemoreceptors or from central mechanisms. Superimposed aortic denervation had no effect in these animals, suggesting that the aortic body has little chemoreceptor function. In carotid body denervated dogs, hypoventilation and CO_2 hyposensitivity persisted through the 3 week follow-up period (60), whereas in goats, there was a near normalization of breathing and CO_2 sensitivity within days to weeks (61). In carotid sinus denervated ponies, arterial CO_2 levels did not normalize until two years after denervation (62). In these ponies, but also in cats (63), partial regain of hypoxic ventilatory responsiveness was attributed

to aortic body chemoreceptor function. Subsequent aortic denervation resulted in loss of chemoreflex function. However, this denervation was not necessarily aorta specific and may have also affected cardiac chemoreceptors (58).

These studies indicate, that most mammals show a considerable ability to compensate for the loss of carotid chemoreflex function. Compensatory mechanisms on a peripheral and/ or central level remain largely unclarified.

Human studies

Studies on the effect of peripheral chemoreceptor removal or denervation on human ventilatory control are limited. Experimental anaesthetic blockade of the glossopharyngeal and vagus nerves in healthy subjects was shown to result in abolition of the ventilatory response to hypoxia, without any depression of resting ventilation (64) (65). Information on selective abolition of carotid body chemoreflex function is mainly derived from studies in small numbers of patients who underwent bilateral resection of healthy carotid bodies as an experimental treatment of bronchial asthma or chronic obstructive pulmonary disease (30;66-69). Baroreflex function was presumed to be unaffected by this procedure. These subjects exhibit an on-average limited hypoxemia and hypercapnia response, with a large interindividual variability (68;70). They do not hyperventilate in response to sustained or progressive hypoxemia neither at rest or during exercise (30;66). In addition, they do not show a decline in ventilation following the abrupt and surreptitious administration of 100% oxygen against a hypoxic background (66). Abnormalities were shown to persist on the long-term after removal of carotid bodies and were more severe after bilateral than unilateral carotid body removal (67). In response to muscle exercise, there was a slower compensatory hyperpnoea, resulting in more profound hypoxia and metabolic acidosis (71). Sleep structure and frequency of nocturnal hemoglobin desaturation were found to be unaltered as shown by polysomnographic studies (68).

However, all of these observations are hampered by the possible confounding chronic pulmonary disease, which itself alters chemoreflex function (72). Peripheral chemoreflex function was assessed in eight patients who had undergone bilateral carotid body tumor resection and were free of pulmonary disease (41). The ventilatory response to hypoxia was assessed by a rebreathing method. Peripheral oxygen desaturation to a level of 80% within 3-4 minutes while alveolar pCO2 was kept constant. The ventilatory increase relative to the decrease in oxygen saturation was taken as a measure of hypoxic responsiveness. Hypoxic responsiveness was assessed at two constant levels of clamped alveolar pCO_2: normocapnia and 1 kPa above normocapnia. At baseline, oxygen saturation and ventilation did not differ between patients and controls. A slightly higher resting alveolar PCO_2 in patients than in controls however, suggested mild chronic hypoventilation. Whether this is due the absence of carotid bodies is uncertain. Subjects with severe chronic obstructive pulmonary disease who have undergone bilateral carotid body resection show a further hypoxemia and hypercapnia that is consistent with the removal of an ongoing hypoxic drive as result of the surgery (68;69). Long-term hypoventilation with increased levels of arterial PCO_2 were also demonstrated in patients with

inadvertent denervation of carotid chemoreceptors in patients undergoing carotid endarterectomy (73). On the other hand, normoventilation with no effect on arterial blood gas was found by others (66;67).

Complete abolition of normocapnic hypoxive responsiveness was observed in all carotid body tumor resected patients. Two of eight patients exhibited a slight ventilatory response to hypoxia under hypercapnic conditions. An increased arterial pCO2 enhances peripheral hypoxic chemosensitivity. In line with our observations a small component of hypoxic ventilatory drive during simultaneous hypercapnia in patients after carotid body resection for chronic pulmonary disease was demonstrated (7) (74). Residual responsiveness to hypoxemia in these patients may originate from the aortic bodies, which subserve a minor role in the modulation of spontaneous respiratory activity, but generate a discernible response when their gain is increased by hypercapnia (70). Alternative explanations for residual chemoreflex function include incomplete carotid body resection and regeneration of carotid chemosensitivity. The latter has been demonstrated in cats (75), but not in humans.

Carotid body resection was also shown to decrease the steady-state ventilatory response to hypercapnia, independently of the degree of concomitant hypoxemia (66;76). In normoxia, carotid chemoreceptors were estimated to modulate 20 to 30% of the ventilatory drive to hypercapnia. In our study, a 1 kPa rise in pCO2 induced an increase in ventilation of 6.4 ± 5.2 l min^{-1} in patients versus 9.2 ± 3.4 l min^{-1} in controls (ns). Taking into account the large standard deviation and small sample size, a blunted CO_2 response due to loss of carotid body function may well be present. Evaluation of chemoreflex function in patients who had undergone radiation therapy for laryngeal or pharyngeal cancer showed no abnormalities (53).

In contrast to baroreceptors, stimulation of peripheral chemoreceptors has a sympatho-excitatory effect in humans (77). Activation of peripheral chemoreceptors by hypoxemia accounts for the strong increase in blood pressure and MSNA that is observed during prolonged voluntary apnoea in awake healthy humans (78-80). In patients lacking carotid bodies, breath-hold time was appreciably longer than in either healthy subjects or asthmatic controls (81). Carotid bodies contribute to the sensation of breathlessness that results in the resumption of breathing. In these patients, hypoxia induced a decrease in blood pressure, which was ascribed to lack of chemoreceptor-dependent sympathetic activation (66;73). However, direct evidence from MSNA recordings during apnoea was lacking. Our preliminary microneurography studies in paraganglioma resected patients show, that increases in MSNA during apnoea occur despite the absence of carotid bodies. Therefore, carotid chemoreceptors do not seem to be the sole determinant of sympatho-excitation during voluntary apnoea.

Summary

Bilateral denervation or removal of carotid body chemoreceptors causes a permanent abolition of ventilatory responsiveness to hypoxia under normocapnic conditions. A small residual hypoxic response may be present during simultaneous hypercapnia. In addition, the condition causes a 20-30% decrease in CO_2 sensitivity. Long-term resting hypoventilation and hypercapnia may occur. The

impairment of chemoreflex function is less severe following unilateral than after bilateral carotid body resection. These observations emphasise the importance of carotid relative to aortic chemoreceptor function in humans. The aortic bodies subserve a minor role in the modulation of spontaneous respiratory activity, but may generate a discernible response when their gain is amplified by hypercapnia. In comparison to other species, compensation for the loss of carotid body chemoreceptor function is limited in humans. The impact of this chronic loss of chemoreflex function on the control of blood gas and acid-base status in response to chemoreflex challenges like sleep, exercise (82) and chronic hypoxia at high altitudes needs further investigation in subjects that lack the possible confounder of pulmonary disease. Carotid paraganglioma resection offers a unique opportunity for research in the field of chemoreceptor physiology in humans.

Conclusion

Inadvertent denervation of carotid sinus baro- and chemoreceptors in humans may occur as a complication of invasive interventions on the neck like carotid body tumor surgery, radiotherapy and endarterectomy. Carotid baroreceptor denervation in humans causes a persistent decrease in baroreflex sensitivity and an increase in blood pressure variability; however, carotid denervation does not lead to chronic hypertension. Therefore, although carotid baroreceptors contribute to short-term blood pressure control, other receptors can maintain normal chronic blood pressure levels, in the absence of carotid baroreceptors. Conversely, carotid chemoreceptor denervation leads to permanent abolition of normocapnic ventilatory responses to hypoxia and reduced ventilatory responses to hypercapnia.

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Chapter 10

Summary

The arterial baroreflex buffers abrupt transients of blood pressure and originates from stretch sensitive receptors in the arterial wall of the carotid sinus and the aortic arch (*Chapter 1*). Afferent fibres from carotid sinus baroreceptors join the glossopharyngeal nerve and project to the nucleus tractus solitarii in the dorsal medulla, which in turn projects to efferent cardiovascular neurones in the medulla. The extra-carotid baroreceptors transmit their afferent information along with the vagus nerves to the same brain stem nuclei. The efferent limb of the baroreflex loop consists of sympathetic and parasympathetic fibres to the heart and blood vessels.

Adjustment of respiration in response to alterations in levels of oxygen, carbon dioxide and hydrogen ions in the body fluids are mediated by a complex interplay between centrally and peripherally located chemoreceptors. The peripheral arterial chemoreceptors, located in the carotid and aortic bodies, are responsible for the immediate ventilatory and arterial pressure increments during acute hypoxia, but are also capable of sensing changes in arterial carbon dioxide tension and pH. Carotid and aortic bodies are supplied with sensory fibers, which course through the glossoparygeal and vagus nerve respectively towards medullary respiratory centers.

Experimental denervation experiments in animals have clearly indicated, that carotid baro- and chemoreceptors play an eminent role in maintaining blood pressure and blood gas homeostasis. For our knowledge on the relative contribution of carotid receptors to baro- and chemoreflex function in humans, we predominantly rely on case studies of patients with inadvertent damage to baro- and/ or chemoreceptors following medical interventions of the neck. The aim of the studies presented in this thesis, was to get insight in the short and long-term effects of denervation of carotid baro- and chemoreceptors on the control of circulation and ventilation in humans. For this purpose, we performed a retrospective evaluation of baro- and chemoreflex function in patients that had undergone surgery and/ or radiation therapy of the neck.

Inadvertent baroreceptor denervation may occur as a complication of carotid body tumor resection, radiotherapy and surgery for laryngeal or pharyngeal carcinoma, carotid endarterectomy and trauma of the neck. Disruption of the baroreflex has also been reported in the event of ischemic or neurodegenerative lesion of the nucleus tractus solitarii. It was from the complications of these conditions, that the clinical syndrome of baroreflex failure has been characterized as a separate clinical entity. The clinical syndrome of baroreflex failure is characterized by a limited buffering capacity of blood pressure, resulting in excessive rises or falls in response to emotional and physical stimuli like cold and sexual arousal *(Chapter 2)*. The most commonly encountered presentation of the syndrome consists of paroxysms of severe hypertension and tachycardia, lasting minutes to hours. These attacks result from excessive increments of sympathetic tone including marked elevations of catecholamine plasma levels. Desinhibition of central activation of efferent sympathetic pathways arises from the absence of tonic inhibitory baroreceptor input to the vasomotor centers of the brainstem. Patients may become severely disabled by recurrent headache, palpitations, diaphoresis and emotional instability. Apart from hypertensive surges, hypotensive valleys may occur during sleep. Baroreflex

failure should be considered in the differential diagnosis of pheochromocytoma, renovascular hypertension, paroxysmal tachycardia, migraine, hyperthyroidism, alcohol withdrawal, drug use, mastocytosis, carcinoid syndrome, tetanus, intracranial lesions and anxiety disorders.

Baroreflex sensitivity can be calculated from beat-by-beat changes in heart rate and muscle sympathetic nerve activity in response to phenylephrine and nitroprusside induced hyper- and hypotension. Multifiber muscle sympathetic nerve activity is recorded by microneurography of the peroneal nerve. Different levels of disruption of the baroreflex arch result in distinct patterns of abnormal responses to autonomic function tests (active standing, Valsalva's maneuver, forced breathing, cold face test, cold pressor test and mental arithmetic). Ambulatory blood pressure variability can be assessed, preferably by a beat-by-beat Portapres registration.

Treatment of baroreflex failure is aimed at the prevention of large blood pressure swings. Centrally acting sympatholytic agents like clonidine may reduce the frequency and severity of the hypertensive attacks. In clinical practice, a stable blood pressure level is difficult to achieve, since antihypertensive treatment carries a risk of recurrent and profound hypotension in these patients. Non-pharmacological strategies include avoidance of eliciting factors and relaxation or biofeedback training. Frequent follow-up of these patients is warranted. Data on the prognosis of baroreflex failure are limited. With time, the pressor peaks tend to attenuate spontaneously.

The long-term effects of carotid sinus baroreceptor denervation were investigated in patients with acute baroreflex failure following bilateral carotid body tumor surgery *(Chapter 3)*. Casual blood pressure measurements in the doctor's office suggest overt hypertension. However, averaged ambulatory blood pressure values during daytime and night-time were only slightly elevated with a normal day-night-time difference. The marked discrepancy between office and ambulatory values indicate that these patients are particularly sensitive to the pressor effect of mental stress caused by the blood pressure measurement in the doctors office (white coat hypertension). Twenty-four hours continuous Portapres registrations of blood pressure indicated, that blood pressure variability was markedly elevated in all patients. Rapid reflex adjustment of blood pressure and heart rate to active standing and Valsalva's maneuver remain abnormal, which explains the occurrence of orthostatic hypotension in these patients. It was concluded from our studies, that carotid sinus baroreceptor denervation in humans has a permanent effect on the reflex control of blood pressure variability.

Apart from these selected patients who had clinically overt baroreflex failure following surgery, baro- and chemoreflex function was also investigated in non-selected, consecutive patients who had undergone resection of bilateral carotid body tumors *(Chapter 4)*. Acute baroreflex failure syndrome with severe labile hypertension had occurred in three of thirteen patients in the post-operative period. Symptoms and signs in these patients gradually resolved within months, except in one patient in whom they persisted for years. In the same three patients, hypertension was documented consistently during several months, but at the time of the study, at least 1 year after surgery, ambulatory blood

pressure levels were normal in all patients. In the absence of chronic clinically overt baroreflex failure, vagal baroreflex sensitivity, calculated from the reflex changes in RR interval to phenylephrine induced hypertension, was approximately 50% lower in these patients than in healthy controls. The abnormal baroreceptor control of cardiovagal efference in these patients also emerges from minimal reciprocal heart rate changes during blood pressure changes during Valsalva's maneuver. The chronic decrease in baroreflex sensitivity in these patients results in a heterogeneous expression of mild arterial baroreflex dysfunction, including increased blood pressure variability, unopposed sympathetic activation in response to physical and mental stress and orthostatic hypotension. Differences in the extent of carotid sinus denervation due to bilateral carotid body tumor resection and the activation of central and residual aortic baroreceptor mediated compensatory mechanism may account for this heterogeneity.

Normocapnic hypoxive responsiveness was completely abolished in all carotid body tumor resected patients. Two of eight patients exhibited a slight ventilatory response to hypoxia under hypercapnic conditions. An increased arterial carbon dioxide enhances peripheral hypoxic chemosensitivity. Residual responsiveness to hypoxemia in these patients probably originates from the aortic bodies, which subserve a minor role in the modulation of spontaneous respiratory activity, but may generate a discernible response when their gain is increased by hypercapnia. Carotid body resection was also shown to decrease the steady-state ventilatory response to hypercapnia. Carotid chemoreceptors are estimated to modulate 20 to 30% of the ventilatory drive to hypercapnia. CO_2 responsiveness appeared to be blunted in carotid body resected patients, however, the small sample size of our study did not permit a definite conclusion. The findings of these studies emphasize the importance of carotid relative to aortic chemoreceptor function in humans.

It has been shown by others, that pharmacological blockade of arterial and cardiopulmonary baroreceptors in humans results in a strong increase in muscle sympathetic nerve activity and loss of its cardiac rhythmicity. The long-term effect of bilateral carotid body tumor resection on the baroreflex modulation of muscle sympathetic nerve activity is reported in *Chapter 5*.

In these patients, a normal burst incidence and pulse synchronicity of supine resting muscle sympathetic nerve activity was present. The presence of cardiac rhythmicity following carotid surgery may originate from residual carotid baroreceptors and/or unaffected aortic and cardiopulmonary baroreceptors.

In line with the observation of a decreased baroreflex control of heart rate, baroreflex adjustments of sympathetic nerve activity was also impaired in these patients, as indicated by attenuated sympathetic responses to pharmacologically induced alterations of blood pressure. Despite abnormal baroreflex control of both heart rate and sympathetic nerve activity, the hemodynamic control during Valsalva's maneuver is differentially affected by bilateral carotid body tumor resection. Valsalva's maneuver elicited normal compensatory changes in sympathetic nerve activity versus an attenuated heart rate response. These findings suggests, that during Valsalva's maneuver,

sympathetic nerve activity is controlled by both carotid and extra-carotid baroreceptors, whereas baroreflex modulation of heart rate appears to depend largely on the integrity of carotid baroreceptors.

Neck irradiation can affect carotid sinus baroreflex function through injury to carotid sinus afferent innervation and irradiation induced atherosclerosis. Atherosclerosis reduces the distensibility of the carotid sinus and thereby stretch-induced afferent carotid sinus nerve activity. The impact of neck irradiation on baro- and chemoreflex function was studied in patients who had undergone bilateral radiation therapy for locally advanced laryngeal or pharyngeal cancer *(Chapter 6)*. None of the patients had experienced any symptoms or signs of baroreflex failure since treatment. Baroreflex sensitivity, calculated from the heart rate responses to phenylephrine, was significantly lower in patients than in controls. Nevertheless, baroreflex mediated responses of heart rate and blood pressure during Valsalva's maneuver and active standing were unaffected. Ambulatory blood pressure variability was not different from matched control subjects. These findings suggest, that although baroreflex sensitivity of heart rate is decreased substantially after neck irradiation, it does not affect blood pressure buffering to a relevant level. In addition, the finding of a normal ventilatory responsiveness to hypoxia in these patients indicate, that peripheral chemoreflex function is unaffected by neck irradiation. Symptomatic baroreflex failure as a complication of modern radiotherapy for head and neck tumors is a rare condition.

Long-term effects on baroreflex function of unilateral carotid endarterectomy is reported in *Chapter 7*. Patients with an untreated uni-/bilateral carotid stenosis as well as healthy controls served as control groups. Chronic, full-blown baroreflex failure was not observed in any of the patients following unilateral endarterectomy. Baroreflex sensitivity of heart rate was significantly lower in endarterectomized patients as compared to both control groups. Endarterectomised patients exhibited a decreased reflex control of heart rate in response to Valsalva's maneuver and active standing. Orthostatic hypotension was not observed. Efferent cardiovagal activity was unimpaired as shown by normal heart-rate responses during forced breathing and cold face test. Ambulatory blood pressure level and variability did not differ between endarterectomised patients and patients with untreated carotid stenosis. Net baroreflex function after unilateral carotid endarterectomy depends on the extent of carotid sinus denervation, compensation by residual contralateral carotid and aortic baroreceptors and changes in the elastic properties of the carotid sinus vascular wall.

Carotid body tumor resected patients served as a human model for absence of carotid chemoreflex function in the pharmacologic study described in *Chapter 8*.

In healthy, awake subjects, the direct vasodilatory and negative chronotropic effects of the purine nucleotide adenosine are counterbalanced by a reflex increase in sympathetic nerve traffic. Previous studies had provided indirect evidence, that this sympatho-excitation results from the

activation of carotid chemoreceptors by adenosine. The role of carotid chemoreceptors in the sympathetic activation by adenosine, was investigated by comparing sympathetic responses during intravenous adenosine infusion between patients lacking carotid chemoreceptors due to bilateral carotid body tumor resection and healthy controls. In response to adenosine, healthy controls showed an increase in muscle sympathetic nerve activity, but no change in blood pressure. In patients, however, there was no significant change in sympathetic activity and a decrease in blood pressure. These findings indicate, that in humans lacking carotid chemoreceptors, adenosine infusion elicits hypotension due to the absence of significant sympatho-excitation and that carotid chemoreceptor activation is essential for counterbalancing the direct vasodilation by adenosine.

In *Chapter 9*, the short- and long-term effects of carotid baro- and chemoreceptor denervation on the control of circulation and ventilation in humans are reviewed from a physiologic point of view.

Conclusions

- Labile hypertension due to baroreflex failure may arise from both uni- and bilateral carotid baroreceptor denervation. The incidence of persistent baroreflex failure following carotid body tumor surgery, radiotherapy of the neck and carotid endarterectomy is low.
- Bilateral carotid denervation in humans causes a persistent decrease in baroreflex sensitivity in the control of both heart rate and sympathetic nerve activity. This causes a chronic increase in blood pressure variability. The expression of baroreflex dysfunction is heterogeneous among patients.
- Despite tonic inhibition of central sympathetic outflow by carotid baroreceptors, bilateral carotid denervation does not lead to a permanent increase in sympathetic nerve activity or chronic hypertension.
- Bilateral denervation or removal of carotid body chemoreceptors causes a permanent abolition of ventilatory responsiveness to hypoxia under normocapnic conditions. A small residual hypoxic response may be present during simultaneous hypercapnia. In addition, it causes a twenty to thirty percent decrease in carbon-dioxide sensitivity.

Perspective

The relative contribution of carotid receptors to baro- and chemoreflex function as well as the compensation after functional loss of these receptors has been investigated extensively by well-controlled denervation studies in experimental animals. For obvious reasons, no human counterparts for the controlled prospective denervation studies in animals are available. Information on the impact of carotid sinus denervation in humans, relies on investigations following iatrogenic damage to the carotid sinus, as discussed in the present thesis. Interpretation of these human studies is hampered

by uncertainty regarding the completeness of denervation, additional changes in the mechanical properties of the carotid artery by different interventions and differences in acute (surgical) versus gradual (radiation) denervation.

The presented studies mainly consist of retrospective, post-intervention assessment of reflex function in small numbers of patients and matched healthy control subjects. Prospective evaluation of baro- and chemoreflex function in patient who are at risk for carotid sinus injury is warranted in order to draw more definite conclusions on the impact of these interventions on the control of blood pressure and respiration. Nevertheless, the available studies indicate that on the long term, baroreflex control of blood pressure is only mildly impaired in the majority of patients. The impact of loss of hypoxic responsiveness observed following bilateral carotid body tumor surgery on the control of blood gas and acid-base status in response to chemoreflex challenges like sleep, exercise and hypoxic environments is unknown and needs further investigation.

Despite the apparent low incidence of baroreflex failure, clinicians should be aware of this syndrome, since it is a disabling and potentially life threatening condition. Surgeons and radiotherapists have to include inadvertent carotid sinus injury as a possible complication of their treatment. Surveillance of symptoms and signs of baroreflex failure and monitoring of blood pressure is warranted in the follow-up of these patients. Patients suspected for baroreflex failure should be referred to specialized centres for diagnostic testing and treatment.

Chapter 11

Nederlandse samenvatting

De arteriële baroreflex buffert abrupte veranderingen in de bloeddruk. Essentieel voor deze reflex zijn de rek-gevoelige receptoren in de arteriewand van de sinus caroticus en de aortaboog (*Hoofdstuk 1*). Afferente vezels vanuit de sinus caroticus baroreceptoren lopen via de nervus glossopharyngeus naar de nucleus tractus solitarii in de dorsale medulla oblongata. Deze kern is weer verbonden met de efferente cardiovasculaire neuronen in de medulla. De afferente innervatie van de overige baroreceptoren loopt via de nervus vagus naar dezelfde centraal gelegen kernen. Het efferente been van de baroreflex bestaat uit sympatische en parasympatische vezels die het hart en de bloedvaten innerveren.

Aanpassing van de ademhaling aan veranderingen in concentraties van zuurstof, koolzuur en waterstof-ionen in het lichaam geschiedt via een complexe interactie tussen centraal en perifeer gelegen chemoreceptoren. De perifere arteriële chemoreptoren, welke zich bevinden in het glomus caroticum en het glomus aorticum, zijn betrokken bij de toename van de ademhaling en de bloeddruk tijdens hypoxemie. Daarnaast zijn deze receptoren in staat om veranderingen in arteriële koolzuurconcentratie en pH te registreren. De afferente vezels vanuit het glomus caroticum en aorticum lopen via respectievelijk de nervus glossopharyngeus en de nervus vagus richting de medullaire respiratoire centra.

Denervatie-experimenten bij proefdieren hebben aangetoond, dat de baro- en chemoreceptoren in de carotisregio van eminent belang zijn voor de buffering van de bloeddruk en de bloedgashomeostase. Onze kennis over de relatieve bijdrage van de carotisreceptoren aan de baro- en chemoreflex bij de mens is voornamelijk gebaseerd op onderzoek naar de gevolgen van onbedoelde baro- en chemoreceptordenervatie ten gevolge van medische interventies in het halsgebied. Het doel van de studies die in dit proefschrift zijn opgenomen was het verkrijgen van inzicht in de korte- en langetermijneffecten van carotis baro- en chemoreceptordenervatie op de regulatie van circulatie en ventilatie bij de mens. In dit kader is retrospectief onderzoek gedaan naar het functioneren van de baro- en chemoreflex bij patiënten die een halsoperatie of halsbestraling hebben ondergaan.

Baroreceptordenervatie kan optreden als complicatie van glomus caroticumtumorresectie, radiotherapeutische en chirurgische behandeling van larynx- of farynxcarcinomen, carotisdesobstructie en halstrauma. Onderbreking van de baroreflex is eveneens gerapporteerd na ischemische of neurodegeneratieve lesies van de nucleus tractus solitarii. Dankzij beschrijving van de complicaties van deze interventies werd het klinische syndroom van baroreflexfalen herkend en gekarakteriseerd als een afzonderlijke klinische entiteit. Het syndroom wordt gekenmerkt door een beperkte capaciteit van het autonome zenuwstelsel om de bloeddruk te bufferen, met als gevolg het optreden van excessieve stijgingen en dalingen van de bloeddruk tijdens fysieke en emotionele stimuli zoals koude en seksuele opwinding (*Hoofdstuk 2*). De meest voorkomende verschijningsvorm van baroreflexfalen bestaat uit paroxysmen van ernstige bloeddrukstijging (systolische tensie >250 mmHg) en tachycardie, welke minuten tot uren kunnen aanhouden. Deze aanvallen ontstaan ten gevolge van

ongebreidelde toename in sympaticusactiviteit, die gepaard gaat met een sterke toename in plasma catecholamineconcentraties. Deze centrale desinhibitie van efferente sympaticusactiviteit is het gevolg van het ontbreken van tonische inhibitie van de sympatische kernen vanuit de baroreceptoren. Patiënten met dit syndroom kunnen ernstig geïnvalideerd raken door recidiverende hoofdpijn, hartkloppingen, zweetaanvallen en emotionele labiliteit. Naast hypertensieve episodes kan tijdens de slaap juist hypotensie optreden. Baroreflexfalen dient te worden overwogen in de differentiaaldiagnose van het feochromocytoom, renovasculaire hypertensie, paroxysmale tachycardie, migraine, hyperthyreoïdie, alcoholonttrekking, druggebruik, mastocytose, carcinoïdsyndroom, tetanus, intracraniële lesies en angststoornissen.

De baroreflexgevoeligheid kan worden berekend uit de slag op slag veranderingen in hartfrequentie en sympatische zenuwactiviteit als reactie op door fenylefrine en nitroprusside geïnduceerde hyper- en hypotensie. De activiteit van sympatische zenuwvezels richting spieren kan worden geregistreerd middels microneurografie in de nervus peroneus. Verschillende niveaus van onderbreking van de baroreflexboog resulteren in afzonderlijke patronen bij autonoom functieonderzoek (gaan staan, Valsalva manoeuvre, geforceerd ademen, duikreflex en geforceerd rekenen). Ambulante bloeddrukvariabiliteit kan worden bepaald met behulp van de Portapres, een niet-invasieve slag op slag vingerbloeddrukmeter.

De behandeling van baroreflexfalen is gericht op het voorkomen van excessieve bloeddrukschommelingen. Centraal werkende sympaticolytica zoals clonidine kunnen de frequentie en ernst van de bloeddrukstijgingen verminderen. In de klinische praktijk is het induceren van een stabiel bloeddrukniveau echter moeizaam vanwege het feit, dat antihypertensieve behandeling ernstige hypotensie kan veroorzaken bij deze patiënten. De niet farmacologische behandeling bestaat uit het vermijden van uitlokkende factoren en uit ontspannings- en biofeedbacktraining. Deze patiënten dienen intensief te worden vervolgd. Er zijn weinig gegevens beschikbaar over de prognose van baroreflexfalen. In het algemeen treedt er een spontane vermindering op van de bloeddrukpieken.

De langetermijneffecten van sinus caroticus baroreceptordenervatie werden onderzocht bij patiënten met acuut baroreflexfalen in aansluiting op dubbelzijdige resectie van glomus caroticumtumoren (*Hoofdstk 3*). Afgaande op de bloeddruk gemeten in de spreekkamer zou er bij deze patiënten sprake zijn van hypertensie. Echter, de gemiddelde ambulante bloeddrukwaarden waren slechts licht verhoogd met daarnaast een normaal dag-nachtritme. De discrepantie tussen de spreekkamerbloeddruk en de ambulante waarden suggereren, dat deze patiënten gevoelig zijn voor het bloeddrukverhogende effect van mentale stress tijdens de spreekkamermeting (witte jas hypertensie). Vierentwintig uurs continue Portapresregistraties tonen aan, dat er een duidelijke toegenomen bloeddrukvariabiliteit is bij de onderzochte patiënten. De snelle, baroreflex-gemedieerde veranderingen in bloeddruk en hartfrequentie tijdens gaan staan en de Valsalva manoeuvre zijn permanent abnormaal, hetgeen het optreden van orthostatische hypotensie bij deze patiënten

verklaart. Uit dit onderzoek werd geconcludeerd, dat sinus caroticus baroreceptordenervatie bij de mens een permanent effect heeft op de baroreflexsturing van de bloeddrukvariabiliteit.

Behalve bij deze geselecteerde patiënten met een post-operatief syndroom van baroreflexfalen werden baro- en chemoreflexfunctie ook onderzocht bij ongeselecteerde patiënten die een dergelijke bilaterale glomustumorresectie hadden ondergaan (*Hoofdstuk 4*). Een acuut syndroom van baroreflexfalen met ernstige labiele hypertensie was opgetreden bij drie van de veertien patiënten in de postoperatieve fase. De verschijnselen van baroreflexfalen waren bij twee van de drie patiënten binnen enkele maanden verdwenen, één patiënt hield jarenlang klachten. Bij dezelfde drie patiënten werd gedurende enkele maanden een verhoogde bloeddruk gemeten, maar ten tijde van de studie, minstens een jaar na chirurgie, waren de ambulante bloeddrukwaarden normaal. Ondanks het ontbreken van een permanent syndroom van baroreflexfalen bij de onderzochte patiënten, was de vagale baroreflexgevoeligheid, berekend uit de reflexgemedieerde veranderingen in het RR-interval tijdens fenylefrine geïnduceerde bloeddrukstijging, ongeveer de helft van de gemiddelde baroreflexgevoeligheid bij de gezonde deelnemers aan het onderzoek. De abnormale sturing van cardiovagale innervatie door de baroreflex blijkt eveneens uit de te lage reflexmatige veranderingen in hartfrequentie tijdens de Valsalva manoeuvre. De chronische verlaging van baroreflexgevoeligheid bij deze patiënten resulteert in een heterogene expressie van milde baroreflexdysfunctie, zoals een toename in bloeddrukvariabiliteit, ongelimiteerde sympatische activatie tijdens fysieke en mentale stress en orthostatische hypotensie. Verschillen in de mate van sinus caroticusdenervatie door glomuschirurgie alsmede in compensatie door centrale baroreceptoren en receptoren in de aortaboog vormen een mogelijke verklaring voor deze heterogeniciteit.

Normocapnische hypoxemie resulteerde bij geen enkele patiënt met een status na bilaterale glomustumorresectie in een compensatoire chemoreflex-gemedieerde toename van de ventilatie. Twee van de acht patiënten liet een kleine ventilatoire respons op hypoxemie zien tijdens gelijktijdige hypercapnie. Hypercapnie stimuleert de perifere chemoreceptorgevoeligheid. De geobserveerde residuale hypoxemierespons vindt zijn oorsprong mogelijk in het glomus aorticum, wat een kleine rol lijkt te spelen in de modulatie van spontane respiratoire activiteit. Dit kan wel van belang kan zijn wanneer de gevoeligheid wordt gestimuleerd door hypercapnie. Patiënten met een status na dubbelzijdige glomus caroticumresectie tonen een afname van de steady-state ventilatoire respons op hypercapnie. Van de chemoreceptoren in het glomus caroticum wordt aangenomen, dat zij verantwoordelijk zijn voor 20-30% van deze hypercapnische respons. Ook in de door ons onderzochte patiënten was deze respons in vergelijkbare mate beperkt. Het kleine patiëntenaantal laat echter hierover geen definitieve uitspraak toe. De bevindingen benadrukken het relatieve belang van het glomus caroticum ten opzichte van het glomus aorticum bij de mens.

In eerdere studies is aangetoond, dat farmacologische blokkade van de arteriële en cardiopulmonale baroreceptoren bij de mens resulteert in een sterke toename van sympatische activiteit richting

spiervaatbed. Daarnaast verdwijnt door de blokkade de synchroniciteit van de sympatische activiteit met de hartslag. De langetermijneffecten van bilaterale glomus caroticumtumorresectie op de baroreflex modulatie van sympatische zenuwactiviteit worden besproken in *Hoofdstuk 5*.

De sympatische activiteit in rust bij deze patiënten was normaal qua burst-incidentie en synchroniciteit met de hartslag. Deze intacte synchroniciteit kan worden verklaard door onvolledige denervatie van de sinus caroticus baroreceptoren, danwel door compensatie vanuit de aortale en/of cardiopulmonale baroreceptoren.

Bilaterale glomus caroticum tumorresectie beïnvloedt niet alleen de vagale baroreflexgevoeligheid, maar ook de sympatische baroreflexsensitiviteit. De patiënten tonen een verminderde sympatische reactie op farmacologisch geïnduceerde bloeddrukveranderingen. Ondanks een abnormale baroreflexregulatie van zowel de hartfrequentie als sympatische activiteit, worden de hemodynamische veranderingen tijdens de Valsalva manoeuvre op een gedifferentieerde manier beïnvloed. Tijdens de Valsalva manoeuvre treden er normale compensatoire veranderingen in sympatische activiteit op, terwijl hartfrequentierespons is verminderd. Deze bevindingen suggereren, dat de reflexmatige aanpassing van de sympatische activiteit tijdens de Valsalva manoeuvre niet afhankelijk is van de sinus caroticus baroreceptoren, terwijl de integriteit van deze receptoren wel essentieel is voor de baroreflexmodulatie van de hartfrequentie onder deze omstandigheden.

Halsbestraling kan de baroreflex beïnvloeden via beschadiging van de afferente innervatie van de sinus caroticus baroreceptoren en via bestraling-geïnduceerde atherosclerose. Atherosclerose vermindert de distensibiliteit van de sinus caroticus en daarmee de rek-afhankelijke baroreceptoractiviteit. De invloed van halsbestraling op het functioneren van de baro- en chemoreflex werd onderzocht bij patiënten die dubbelzijdige radiotherapie hadden ondergaan in verband met een locaal uitgebreid larynx- dan wel farynxcarcinoom (*Hoofdstuk 6*). Bij geen van de patiënten waren er verschijnselen van baroreflexfalen opgemerkt sinds de behandeling. De gemiddelde baroreflexgevoeligheid, welke werd berekend aan de hand van hartfrequentierespons op fenylefrine, was significant lager bij patiënten dan bij controles. De Valsalva manoeuvre en gaan staan leidden tot een normale baroreflex-gemediëerde aanpassing van de hartfrequentie en de bloeddruk bij de patiënten. Ook de ambulante bloeddrukvariabiliteit was niet verschillend van de controles. Blijkbaar leidt de vermindering in baroreflexgevoeligheid m.b.t. hartfrequentie na halsbestraling niet tot een relevante verstoring van de bloeddrukbuffering. Ook de respiratoire hypoxemierespons bij deze patiënten is ongestoord. Halsbestraling beïnvloedt de perifere chemoreflexfunctie dus niet. Symptomatisch baroreflexfalen is een zeldzame complicatie van de huidige halsbestraling voor hoofd- en halstumoren.

De langetermijneffecten van eenzijdige carotisdesobstructie worden besproken in *Hoofdstuk 7*. De controlegroepen bij deze studie bestonden enerzijds uit patiënten met een onbehandelde uni- of bilaterale stenose van de arteria carotis en anderzijds uit gezonde vrijwilligers. Bij geen van de

onderzochte patiënten waren er na eenzijdige carotisdesobstructie verschijnselen van baroreflexfalen opgemerkt. De gemiddelde baroreflexgevoeligheid m.b.t. hartfrequentie was bij geopereerde patiënten significant lager ten opzichte van beide controlegroepen. Bij patiënten met een status na endarterectomie was de baroreflexsturing van de hartfrequentie tijdens de Valsalvamanoeuvre en gaan staan gecompromitteerd. Dit ging niet gepaard met orthostatische hypotensie. De efferente cardiovagale innervatie was ongestoord, getuige de normale hartfrequentierespons tijdens geforceerd ademen en de duikreflex. Tijdens ambulante metingen waren noch het bloeddrukniveau, noch de bloeddrukvariabiliteit verschillend tussen geopereerden en patiënten met een onbehandelde stenose. De netto baroreflexfunctie na unilaterale carotisdesobstructie hangt blijkbaar af van de mate van denervatie van de sinus caroticus baroreceptoren, de compensatie vanuit de baroreceptoren in de contralaterale sinus caroticus en in de aorta, alsmede van verandering in de elastische eigenschappen van de arteriewand.

Patiënten met een status na dubbelzijdige glomus caroticum tumorresectie dienden als model voor afwezigheid van de perifere chemoreflex in de farmacologische studie die wordt beschreven in *Hoofdstuk 8*.

Bij gezonde mensen met een helder bewustzijn, worden de directe vasodilatoire en negatief chronotrope effecten van de purine-nucleoside adenosine tenietgedaan door een reflexmatige toename in de sympatische zenuwactiviteit. Eerdere studies leverden een indirect bewijs voor de hypothese, dat deze toename in sympaticusactiviteit het gevolg is van stimulatie van glomus caroticum chemoreceptoren door adenosine. De rol van deze chemoreceptoren in de sympaticusactivatie door adenosine werd onderzocht door de sympatische responsen tijdens intraveneuze infusie van adenosine te vergelijken tussen patiënten zonder glomus caroticum en gezonde vrijwilligers. Bij gezonde vrijwilligers resulteerde de infusie van adenosine in een toename van de sympatische zenuwactiviteit zonder dat er sprake was van een bloeddrukverandering. Bij de patiënten ontbrak een significante sympatho-excitatie en trad er een bloeddrukdaling op. Blijkbaar spelen de glomus caroticum chemoreceptoren een essentiële rol bij de toename in sympaticusactiviteit, welke normaal gesproken het directe vasodilatoire effect van adenosine tegengaat.

In *Hoofdstuk 9* worden de korte- en langetermijneffecten van denervatie van sinus caroticus baro- en chemoreceptoren op de sturing van circulatie en ventilatie bij de mens uiteengezet vanuit een fysiologische invalshoek.

Conclusies

- Zowel uni- als bilaterale denervatie van de sinus caroticus baroreceptoren kan leiden tot labiele hypertensie als uiting van baroreflexfalen. De incidentie van een chronisch

baroreflexfalensyndroom als complicatie van glomus tumorchirurgie, halsbestraling en carotis-desobstructie is laag.

- Dubbelzijdige denervatie van de sinus caroticus baroreceptoren bij de mens leidt tot een permanente aantasting van de baroreflex-gemedieerde sturing van zowel hartfrequentie als sympatische activiteit. Dit leidt tot een chronische toename in bloeddrukvariabiliteit. De mate waarin dysfunctie van de baroreflex tot expressie komt verschilt van patiënt tot patiënt.

- Ofschoon de sinus caroticus baroreceptoren normaalgesproken een tonisch remmende invloed hebben op de centraal gelegen sympatische kernen, leidt bilaterale denervatie van deze baroreceptoren niet tot een chronische verhoging van sympaticusactiviteit of tot hypertensie.

- Bilaterale denervatie of resectie van glomus caroticum chemoreceptoren leidt tot permanente afwezigheid van een ventilatoire respons op hypoxemie onder normocapnische omstandigheden. Tijdens simultane hypercapnie kan er een beperkte hypoxische respons optreden. Daarnaast geeft dubbelzijdige glomus caroticumextirpatie een daling in de koolzuurgevoeligheid van twintig tot dertig procent.

Perspectief

De relatieve bijdrage van sinus caroticus receptoren aan baro- en chemoreflexfunctie alsmede de compensatie voor het verlies van deze receptoren is uitgebreid onderzocht in prospectieve dierexperimentele denervatiestudies. Uiteraard zijn dergelijke denervatiestudies bij de mens niet mogelijk. In dit proefschrift worden de gevolgen van sinus caroticusdenervatie bij de mens beschreven aan de hand van patiënten die als gevolg van een interventie in het halsgebied het risico liepen op een iatrogene beschadiging van de sinus caroticus. Factoren die de interpretatie van deze studies bij de mens bemoeilijken zijn onduidelijkheid met betrekking tot de mate van denervatie bij verschillende interventie, bijkomende veranderingen in de mechanische eigenschappen van de arteria carotis en verschillen in acute (chirurgische) versus geleidelijke denervatie (in geval van bestraling).

De studies die besproken worden in dit proefschrift betreffen hoofdzakelijk retrospectief onderzoek bij kleine groepen patiënten en gezonde controle-personen. Prospectieve studies naar het effect van halsinterventies op het functioneren van de baro- en chemoreflex is aangewezen om definitieve conclusies te trekken over het risico van dergelijke interventies op de sturing van de circulatie en ventilatie. Desalniettemin tonen de beschikbare studies aan, dat er op de lange termijn bij de meerderheid van de patiënten slechts lichte afwijkingen zijn in de baroreflexregulatie van de bloeddruk. Daarnaast verdienen de consequenties van het verdwijnen van de hypoxische respons na glomustumorchirurgie voor de bloedgashomeostase onder omstandigheden waarbij de chemoreceptoren worden geprikkeld nader onderzoek. Dit betreft bijvoorbeeld slaap, lichamelijk inspanning en het verblijf in een omgeving met een verlaagde zuurstofspanning.

Ofschoon de incidentie van baroreflexfalen mee lijkt te vallen, dienen artsen alert te zijn op dit syndroom, omdat het een invaliderende en potentieel levensbedreigende aandoening is. Chirurgen en

radiotherapeuten dienen te beseffen dat beschadiging van de sinus caroticus een mogelijke complicatie is van hun handelen. Waakzaamheid met betrekking tot de verschijnselen van baroreflexfalen en controle van de bloeddruk bij de follow-up van deze patiënten is aangewezen. Patiënten bij er een verdenking bestaat op baroreflexfalen dienen te worden verwezen naar een gespecialiseerd centrum voor verdere diagnostiek en behandeling.

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Curriculum Vitae

Henri Timmers, de schrijver van dit proefschrift, werd op 10 november 1971 geboren in Veghel en groeide op in Boekel. Na het behalen van het VWO diploma op het Kruisheren College te Uden, startte hij in 1990 met de studie Geneeskunde aan de Katholieke Universiteit Nijmegen. Tijdens zijn studie werkte hij van september 1994 tot december 1995 als student-onderzoeker op het Research Laboratorium Morfologische Neurologie van het Universitair Medisch Centrum St. Radboud te Nijmegen en het Nederlands Instituut voor Hersenonderzoek van het Academisch Medisch Centrum te Amsterdam. Hij bestudeerde mechanismen van neuronale dood bij de ziekte van Huntington onder supervisie van prof. dr. H.P.H. Kremer en prof. dr. D.F. Swaab. In juli 1995 slaagde hij cum laude voor het doctoraal examen Geneeskunde. Na de coschappen behaalde hij in september 1997 het artsexamen cum laude. In oktober 1997 startte hij met de opleiding tot internist op de afdeling Algemeen Interne Geneeskunde van het Universitair Medisch Centrum St. Radboud te Nijmegen (opleider prof. dr. J.W.M. van der Meer). Daarnaast werkte hij vanaf 1998 aan het door de Nederlandse Hartstichting gesubsidieerde project wat geleid heeft tot dit proefschrift. In februari 2003 zette hij zijn opleiding tot internist voort in het Canisius Wilhelmina Ziekenhuis te Nijmegen (opleider dr. A.S. Dofferhoff). Per september 2004 start hij met het aandachtsgebied endocrinologie (opleider prof. dr. A.R.R.M. Hermus). Hij woont samen met Harald van Wordragen.