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Neural And Humoral Control Of Regional Vascular Beds Via A1 Adenosine Receptors Located In The Nucleus Of The Solitary Tract

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NEURAL AND HUMORAL CONTROL OF REGIONAL VASCULAR BEDS VIA A_1 ADENOSINE RECEPTORS LOCATED IN THE NUCLEUS OF THE SOLITARY TRACT

by

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DEDICATION

To my family and friends for all their love and support.

ACKNOWLEDGEMENTS

I would thank the members of my dissertation committee for their unending support.

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LIST OF ABBREVIATIONS

CGRP	calcitonin gene related peptide
CVLM	caudal ventrolateral medulla
GABA	gamma amino butyric acid
HDR	hypothalamic defense response
IBF	iliac blood flow
IML	intermediolateral column of the spinal cord
IVC	iliac vascular conductance
LSNA	lumbar sympathetic nerve activity
MAP	mean arterial pressure
MBF	mesenteric blood flow
MVC	mesenteric vascular conductance
NTS	nucleus of the solitary tract
pre-ASNA	preganglionic adrenal sympathetic nerve activity
PVN	paraventricular nucleus
RBF	renal blood flow
RSNA	renal sympathetic nerve activity
RVC	renal vascular conductance
RVLM	rostral ventrolateral medulla
SON	supraoptic nucleus

Experimental groups:	
INT	Intact
βX	β-aderenergic blockade
ADX	adrenalectomy
LX	lumbar sympathectomy
VX	V_1 vasopressin receptor blockade
ATX	AT_1 angiontensin II receptor blockade
LX+VX	lumbar sympathectomy + V_1 vasopressin receptor blockade
ADX+LX	adrenalectomy + lumbar sympathectomy
ADX+VX	adrenalectomy + V_1 vasopressin receptor blockade
ADX+GX	adrenalectomy + ganglionic blockade
ADX+GX+VX	adrenalectomy + ganglionic blockade + V_1 vasopressin receptor blockade
ADX+GX+VX+ATX	adrenalectomy + ganglionic blockade + V_1 vasopressin receptor blockade + AT_1 angiontensin II receptor blockade
SAD	sinoaortic denervation
SAD+ADX	sinoaortic denervation + adrenalectomy

Drug	Dose	Manufacturer
α-chloralose	80 mg/kg, iv	Aldrich
[β-mercapto-β,β-cyclopentylmethylenepropionyl,1-O-Me-Tyr^2,Arg^8]-vasopressin	20 µg/kg, iv	Sigma
artificial cerebrospinal fluid (ACF)	solvent	prepared in the laboratory
angiotensin II	300 ng/kg, iv	Sigma
arginine-vasopressin	50 mU/kg iv	Sigma
CPA, N^6-cyclopentlyadenosine	330 pmol in 50 nl of ACF microinjections into the NTS	Tocris
hexamethonium bromide	25 mg/kg, iv	Sigma
losartan	5 mg/kg, iv	Merck
phenylephrine (PE)	200 μg/1ml of 0.9% NaCl solution, 0.8-2ml/hour, iv	Sigma
propranolol	2 mg/kg, iv	Sigma
Urethane	500mg/kg, iv	Sigma

PREFACE

GENERAL INTRODUCTION

It is widely accepted that the nucleus tractus solitarii (NTS) is a major integrative center for processing sensory information involved in cardiovascular control and other autonomic reflexes. The NTS, through its reciprocal connections with medullary, pontine and hypothalamic centers, modulates reflex reactivity creating specific patterns of autonomic responses observed in physiological and pathological situations, for example, stress, the defense response, exercise, hemorrhage etc.

Tonic blood pressure regulation involves input from baroreceptor afferents to the NTS. Baroreceptors are stretch receptors, located in the arterial walls mainly in the aortic arc and the bifurcation of the carotid arteries [20]. These receptors increase their activity in response to increases of arterial pressure which distends the arterial walls. Baroreceptor afferents reach the NTS via the vagus and glossopharyngeal nerves and activate central neurons of this baroreflex pathway via glutamatergic mechanism (Figure 1). The same nerves also provide afferents from

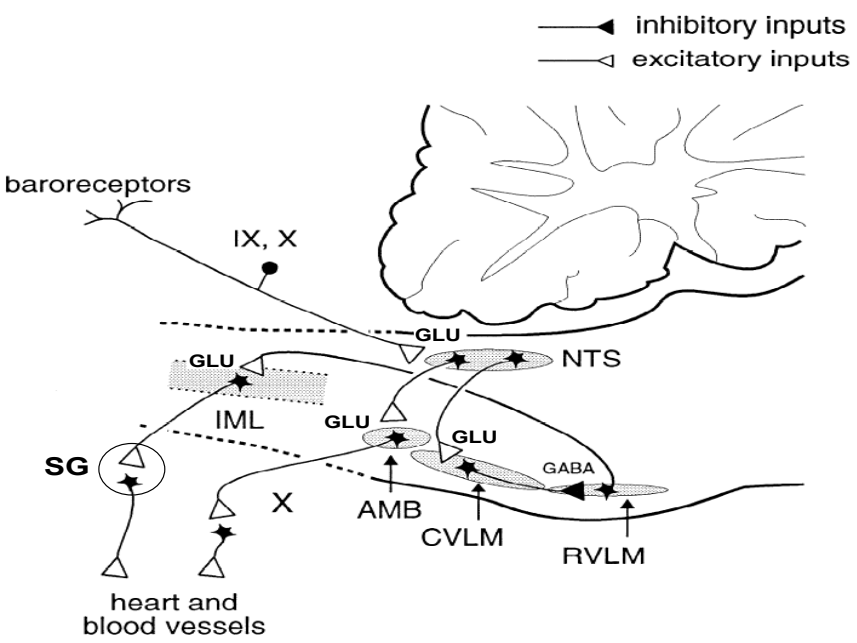

Figure 1. Medullary pathways of the baroreceptor reflex. Abbreviations: GABA, γ-amino-butyric acid; GLU, glutamate; NTS, nucleus of the solitary tract; CVLM, caudal ventrolateral medulla; iRVLM, rostral ventrolateral medulla; AMB, nucleus ambiguus; IML, intermediolateral column of spinal cord; SG, sympathetic ganglion; IX,. glossopharyngeal nerve; X, vagal nerve. Modified from Dampney RAL 1994 (20).

arterial chemoreceptors and cardiopulmonary baro- and chemoreceptors to the NTS.

Efferent control of the cardiovascular system is provided via two main factors: the resistance of the peripheral vasculature and cardiac output, which depends on cardiac contractility and heart rate (HR). According to Ohm's law applied to circulating fluids P=RxCO, where P is arterial pressure, R is total peripheral resistance and CO is cardiac output. Cardiac contractility and HR are increased by activation of efferent sympathetic nerves releasing norepinephrine, which acts via cardiac β_1- adrenergic receptors. HR is decreased by efferent vagal fibers releasing acetylcholine operating via cardiac muscarinic receptors[20]. Medium and small arteries increase and decrease peripheral resistance via vasoconstriction and vasodilation, respectively. Vascular control is mediated via efferent sympathetic vasoconstrictor nerves which are tonically active at resting conditions. The major vasoconstrictor mediator released from efferent sympathetic terminals is norepinephrine acting via α_1-adrenergic receptors located in vascular smooth muscles[20]. Sympathetic vasoconstrictor tone is generated in the rostral ventrolateral medulla (RVLM) (Figure 1). RVLM neurons activate sympathetic preganglionic neurons located in the intermediolateral column (IML) of the spinal cord via a glutamatergic mechanism. Efferent axons of the preganglionic neurons terminate in the sympathetic ganglia and activate ganglionic neurons via the release of acetylcholine operating via nicotinic receptors. The ganglionic neurons send their efferent, postganglionic fibers to the peripheral vasculature and cause vasoconstriction via α_1-adrenergic receptors. At rest the efferent sympathetic nerves generate tonic vasoconstriction. Increases and decreases in this efferent sympathetic tone result in vasoconstriction and vasodilation, respectively.

Tonic afferent traffic from arterial baroreceptors and tonic efferent sympathetic tone mediate constant regulation of arterial pressure via corrections of efferent sympathetic (and cardiac vagal) activity mediated by medullary cardiovascular centers illustrated in Figure 1. For example, increases in arterial blood pressure increase afferent baroreceptor traffic, which activate primary central neurons and interneurons located in the NTS via a glutamatergic mechanism. NTS neurons activate inhibitory GABA-ergic neurons located in the caudal ventrolateral medulla (CVLM) also via a glutamatergic mechanism. Activated CVLM neurons inhibit RVLM neurons via the release of GABA from their axons terminating in the RVLM; this decreases efferent sympathetic tone which allows peripheral arteries to dilate, decrease peripheral resistance and decrease arterial pressure toward normal values. In contrast, decreases in arterial pressure, cause unloading (relaxation) of arterial baroreceptors which decreases afferent activation of NTS neurons, decreases activation of CVLM and reduces tonic baroreceptor inhibition of sympathetic vasoconstrictor tone generated in the RVLM. This allows for the increase of efferent sympathetic vasoconstriction, increase in peripheral resistance and increase of arterial pressure back toward normal values.

Although most of the efferent sympathetic fibers supplying the peripheral vasculature mediate vasoconstriction (releasing norepinephrine and to a lesser extent ATP and NPY) some sympathetic efferents may produce active vasodilation in a regionally specific manner[10; 13; 20; 21; 43; 57]. For example, the iliac vasculature may be actively dilated via the release of NO from efferent sympathetic nerves[21; 57] or by epinephrine which was previously absorbed from the circulation by sympathetic terminals[10]. Sympathetic vasodilation may be provided also by calcitonin gene related

peptide (CGRP) or adenosine which is generated by ectonucleotidases from neuronally released ATP[13; 43; 94]. These vasodilatory mechanisms, which oppose the prevailing efferent sympathetic vasoconstriction contribute to the fine tuning of regional vasculature and to specific patterns of autonomic responses, which require the redistribution of blood between vascular beds.

An important sympathetic output which provides humoral control of regional vascular beds is the adrenal medulla. Chromaffin cells of the adrenal medulla are modified sympathetic ganglionic neurons and as such they are activated by preganglionic nerves that release acetylcholine operating via nicotinic receptors[20]. Although the activated adrenal medulla releases epinephrine and norepinephrine (in ratio of ~4:1), the main adrenergic effect is provided by circulating epinephrine[31]. The majority of circulating norepinephrine is generated by sympathetic nerve terminals, therefore, the relatively small amount of norepinephrine released from the adrenal medulla is negligible. Epinephrine causes peripheral vasodilation via activation of β_2-adrenergic receptors. Differential regional effects of circulating epinephrine depend on differential distribution of β_2-adrenergic receptors in different vascular beds[90]. These receptors are preferentially expressed in the muscle vascular bed which contributes to active vasodilation of the muscle vasculature, for example during stress or the hypothalamic defense response[93].

The other important humoral vasoactive factor controlled by the baroreflex mechanisms is vasopressin which is a potent vasoconstrictor operating via peripheral V_1 receptors[17]. Vasopressin is synthesized in the magnocellular portion of hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei[17; 20]. From these hypothalamic nuclei vasopressin is delivered via descending axons to the posterior part

of the pituitary gland and released into the circulation. Under normal, resting conditions vasopressin is tonically inhibited/restrained by the baroreflex mechanism shown in Figure 2. Therefore normal, resting levels of vasopressin remain low, usually between 1 - 4 pg/ml[17; 32; 53; 54]. The tonic baroreflex inhibition of vasopressin release is provided mainly via a multisynaptic, glutamatergic pathway which begins in the NTS neurons and then travels via the locus ceruleus and diagonal band of Broca reaching GABA-ergic neurons located in the periphery of the hypothalamic paraventricular nucleus (Figure 2)[20]. It was also postulated that inhibition of vasopressin release may involve inhibition of noradrenergic CVLM neurons by NTS GABA-ergic interneurons activated by afferent baroreflex traffic. These CVLM neurons are believed to tonically facilitate vasopressin release under normal conditions[20]. Decreases in arterial blood pressure unload baroreceptors and decrease baroreceptor inhibition of vasopressin release. This results in disinhibition of magnocellular PVN and SON neurons which release vasopressin into the posterior pituitary gland and from there into the circulation.

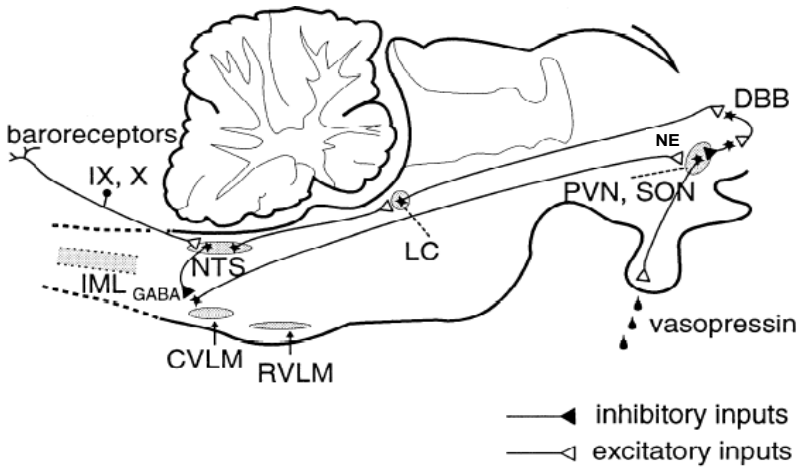

Figure 2. Baroreflex control of vasopressin release. GABA, γ-amino-butyric acid; NE, norepinephrine; NTS, nucleus of the solitary tract; CVLM, caudal ventral lateral medulla; RVLM, rostral ventral lateral medulla; IML, intermediolateral cell column; LC, locus ceruleus; DBB, diagonal band of Broca; PVN, paraventricular nucleus; SON supraoptic nucleus; IX, glossopharyngeal nerve; vagal nerve. Modified from Dampney RAL 1994 (20).

The activity of basic medullary cardiovascular centers is modulated by descending (mostly hypothalamic) and ascending (spinal) projections[58; 65]. Descending

fibers, mostly from hypothalamic paraventricular and dorsomedial nuclei, or ascending spinal afferents may inhibit baroreflex activity at the level of the NTS. Descending hypothalamic projections to RVLM and IML may also directly modulate regional sympathetic drive. Baroreflex mechanisms together with the descending and ascending (afferent) projections create specific patterns of autonomic responses. For example, during stimulation of the hypothalamic defense area (which mimics a stress response) the descending hypothalamic projections activate intrinsic NTS GABA-ergic neurons which inhibit glutamatergic transmission in the baroreflex arc[40; 49]. Similar modulation/resetting of baroreflex mechanisms occurs during exercise via both descending (central command) and ascending projections from muscle receptors[58; 62; 63]. This allows for a simultaneous increase of arterial pressure and heart rate and contractility which is a key cardiovascular adjustment which increases cardiac output and skeletal muscle perfusion in these situations. The other crucial adjustment increasing muscle blood flow during stress is the redistribution of blood from the visceral to the muscle vascular bed. This is achieved via simultaneous vasoconstriction of visceral vascular beds and active muscle vasodilation, usually measured as hindlimb vasodilation. The active hindlimb vasodilation is mediated mostly via β_2-adrenergic receptors preferentially located in the muscle vascular beds[90], which are activated by epinephrine released from the adrenal medulla. This mechanism is crucial for stress related hindlimb vasodilation in rats[2; 93] whereas in dogs, cats and humans sympathetic cholinergic vasodilation may also contribute to this response[39; 46; 60]. Epinephrine may be also released into the hindlimb vasculature from sympathetic terminals which previously absorbed circulating epinephrine[10; 22]. It has been also reported that sympathetic nerves supplying the hindlimb vasculature may release nitric oxide[21; 57].

Similar mechanisms as those described above may create other differential patterns of regional cardiovascular responses; for example, during hemorrhage, thermoregulatory adjustments and other physiological and pathological situations[50; 78; 91].

Adenosine as a neuromodulator in central cardiovascular control

Numerous studies from our laboratory and by others showed that purinergic mechanisms (operated by adenosine and its nucleotides) are involved in cardiovascular control at the level of the NTS[4; 5; 7; 8; 15; 37; 51; 52; 55; 72; 77; 82; 85]. Adenosine operating in the NTS originates from the breakdown of ATP, both intra- and extracellularly, and is involved under both physiological and pathological conditions. Under physiological conditions neuronally released ATP is broken to adenosine via ectonucleotidases[94]. This may occur during stress or the hypothalamic defense response[82]. ATP may be also neuronally released as a co-transmitter with glutamate in the baroreflex arc at the level of the NTS, as suggested by previous studies from our laboratory[70; 74]. Under pathological conditions, such as severe hemorrhage, ischemia, and hypoxia, intracellular ATP is catabolized to adenosine in hypoxic cells and adenosine is directly released into the extracellular space without the contribution of nucleoside transporters [56; 84; 89; 92]. Therefore, adenosine is a neuromodulator activated in life threatening situations to regain homeostasis. A recent paper from our laboratory showed that adenosine operating via both A_1 and A_{2a} receptors located in the NTS contribute to the paradoxical renal sympathoinhibition and cardiac slowing observed in the hypotensive stage of severe hemorrhage[78]. In addition, the same study showed that both A_1 and A_{2a} adenosine receptor subtypes may exert tonic effects on hemodynamic and sympathetic variables under control conditions. Thus adenosine operating in the NTS may play an important role as a neuromodulator of reflex control of the circulation under

normal, physiological conditions. In a recent study from our laboratory we found that A_1 adenosine receptor stimulation in the NTS resets baroreflex functions towards higher mean arterial pressure (MAP)[71].

A_1 adenosine receptor activation in the central nervous system, including the NTS, stimulates an inhibitory G protein resulting in decreased cAMP formation, lower intracellular Ca^{2+} levels and thus decreased neurotransmitter release from synaptic terminals[59]. A_{2a} receptor activation operates in a reciprocal fashion, activating a stimulatory G protein, increasing cAMP and Ca^{2+} influx and consequently facilitating neurotransmission[59]. Postsynaptic A_1 and A_{2a} adenosine receptors may also inhibit and activate neurons, respectively[59]. Therefore selective activation of adenosine A_1 and A_{2a} receptor subtypes in the NTS often, although not always, evokes reciprocal hemodynamic and sympathetic responses.

Selective activation of NTS A_1 adenosine receptors typically evokes pressor and sympathoexcitory responses consistent with the inhibition of glutamatergic transmission at the baroreflex arc[4; 8; 76]. For example, Scislo and O'Leary showed that sino-aortic denervation plus vagotomy as well as blockade of glutamatergic transmission in the NTS eliminated the pressor and sympathoactivatory responses[76]. A recent study from our laboratory directly confirmed that activation of NTS A_1 adenosine receptors resets regional sympathetic and heart rate baroreflex functions toward higher MAP which is consistent with presynaptic inhibition of glutamate release from baroreceptor terminals[71].

In contrast, selective activation of NTS A_{2a} adenosine receptors, as well as nonselective activation of these receptors with adenosine evokes baroreflex-like responses, i.e. decreases in MAP and HR. Numerous data suggested that presynaptic

A_{2a} adenosine receptors may facilitate release of glutamate from baroreceptor afferents terminating in the NTS[4; 7; 8; 15; 51; 52; 85; 88]. However, analysis of regional sympathetic responses to selective stimulation of NTS A_{2a} adenosine receptors leads to the opposite conclusions. Although the stimulation inhibited renal sympathetic nerve activity (RSNA), which is consistent with activation of baroreflex pathway, lumbar sympathetic nerve activity (LSNA) did not change significantly, whereas preganglionic adrenal sympathetic nerve activity (pre-ASNA) increased, which is inconsistent with the effects evoked by activation of the baroreflex pathway[73; 74]. It should be stressed that all these sympathetic outputs were uniformly inhibited when arterial baroreceptors were stimulated with increases in MAP[68]. In addition, bilateral sinoaortic denervation plus vagotomy, essentially the denervation of baroreceptor input to the NTS, did not affect the depressor and renal sympathoinhibitory responses mediated via NTS A_{2a} adenosine receptors[74]. Also, blockade of glutamatergic transmission in the NTS did not abolish the baroreflex-like response to stimulation of NTS A_{2a} adenosine receptors[75]. Finally, a recent study from our laboratory directly confirmed that selective stimulation of NTS A_{2a} adenosine receptors did not reset baroreflex functions towards lower MAP, which would be expected if A_{2a} adenosine receptors facilitated baroreflex transmission[37]. This study strongly suggested that hemodynamic and regional sympathetic responses to stimulation of NTS A_{2a} adenosine receptors are mediated via non-baroreflex mechanisms.

Typically, NTS A_1 and A_{2a} adenosine receptors exert counteracting hemodynamic effects: increases and decreases in MAP and RSNA as well as vasoconstriction and vasodilation, respectively[4; 8; 47; 73; 74; 76]. Interestingly, despite of those reciprocal effects both receptor subtypes preferentially increase sympathetic

nerve activity directed to the adrenal medulla[74-76]. This suggests that adenosine operating in the NTS via both receptor subtypes may preferentially activate the adrenal medulla, release epinephrine into the circulation and exert vasodilation in the muscle vascular bed via activation of β_2-adrenergic receptors. Yardley and Hilton suggested that in the rat epinephrine release is a major vasodilator of the hindlimb vasculature in response to stress or stimulation of the hypothalamic defense area[93]. These data taken together suggest that both A_1 and A_{2a} adenosine receptors may be involved in mechanisms mediating stress and the defense response.

Adenosine and autonomic response to stress

The hypothalamic defense response (HDR) is an experimental model which allows one to study stress induced patterns of autonomic responses in anesthetized animals. These patterns may be evoked by precisely controlled electrical or chemical stimulation of forebrain, diencephalic and midbrain stress related structures: central amygdala, posterior/dorsomedial hypothalamus and periaqueductal grey, respectively[14; 20; 24; 26; 79; 93]. The hypothalamic defense response consists of increases in MAP, HR, efferent sympathetic nerve activity, pulmonary ventilation and is accompanied with powerful vasodilation in the muscle vascular bed, especially in the hindlimb vasculature[93]. These responses are at least partially mediated via inhibition of baroreflex mechanisms at the level of the NTS[40; 49]. Several studies from Spyer's laboratory showed that adenosine contributes to the pressor component of HDR. For example, blockade of A_1 adenosine receptors via microinjections of the selective antagonist, 8-Cyclopentyl-1,3-dipropylxanthine, into the IV ventricle, in close proximity to the NTS, attenuated the pressor component of HDR[80]. The blockade of ectonucleotidases, which prevents the conversion of neuronally released ATP to

adenosine in the NTS, also attenuated the pressor component of HDR[82]. This study strongly suggested that during HDR, ATP is neuronally released into the NTS and becomes a source of adenosine which subsequently facilitates the pressor response. Finally, the most recent paper from this group directly confirmed that adenosine levels in the NTS increase during HDR[19]. However, the contribution of NTS adenosine receptor subtypes to hindlimb vasodilation has not been studied in respect to HDR. Taking into consideration that the activation of both A_1 and A_{2a} adenosine receptors located in the NTS preferentially increases sympathetic efferent activation of the adrenal medulla[73; 74; 76], and that β_2-adrenergic mechanism is the crucial mechanism of hindlimb vasodilation in rats[93] it is likely that adenosine operating in the NTS may significantly contribute to the stress-related redistribution of blood from the viscera to the skeletal muscle.

Previous studies from our laboratory indicated that activation of NTS A_{2a} adenosine receptors elicits preferential vasodilation in the iliac vascular bed directed mostly to the hindlimb in the rat[7]. The subsequent study showed that the hindlimb vasodilation was mediated by both neural and humoral mechanisms[42]. After β-adrenergic blockade, NTS A_{2a} receptor mediated responses turned from depressor to pressor and from hindlimb vasodilation to hindlimb vasoconstriction. Lumbar sympathectomy as well as bilateral adrenalectomy attenuated the hindlimb vasodilation. Combined lumbar sympathectomy and bilateral adrenalectomy abolished the response. In addition, a recent study from our laboratory showed that activation of NTS A_1 adenosine receptors inhibits baroreflex mechanisms[71], similarly as it occurs during HDR[49]. Taken together the results of Spyer's group and those from our laboratory, these data suggest that adenosine acting via A_1 receptors in the NTS may contribute to the pressor component of HDR, whereas adenosine A_{2a} (and to some extent A_1)

receptors located in the NTS may contribute to HDR related hindlimb vasodilation via a β_2-adrenergic mechanism[7; 19; 42; 49; 71; 80; 82]. However, these hypotheses have yet to be directly proven.

NTS A_1 adenosine receptors and counteracting vascular effects

Previous studies from our laboratory suggested that adenosine A_1 receptors may also contribute to HDR related hindlimb vasodilation, as selective stimulation of these receptors preferentially activates sympathetic output directed to the adrenal medulla[76]. This may result in the release of epinephrine and β-adrenergic vasodilation in the hindlimb vasculature similar to that observed following selective stimulation of NTS A_{2a} adenosine receptors[42]. However, the cardiovascular actions of NTS A_1 adenosine

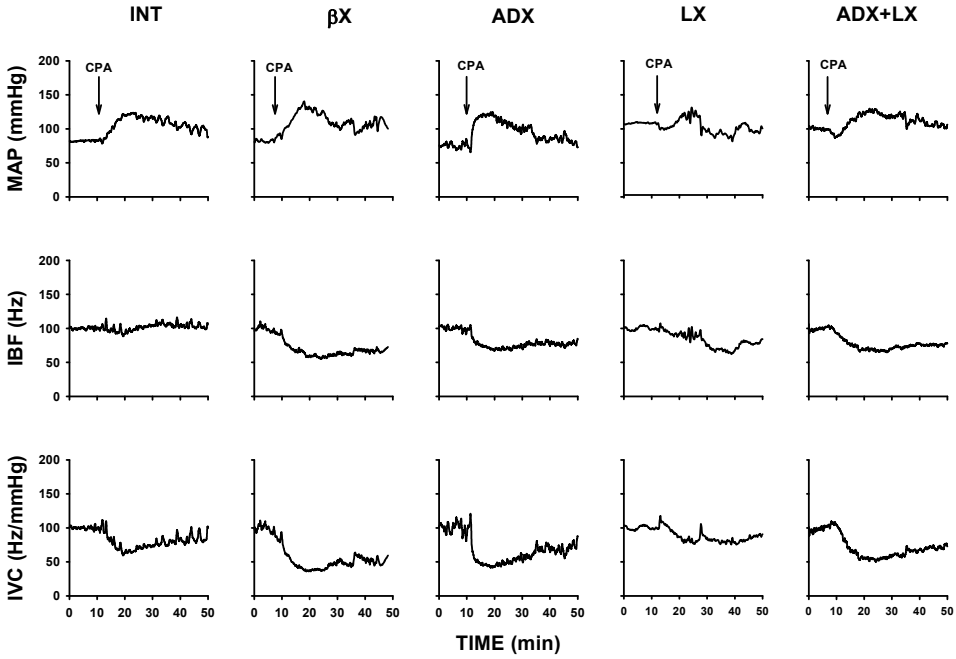

Figure 3. Mean arterial pressure (MAP), Iliac blood flow (IBF), and iliac vascular conductance (IVC) responses to microinjection of CPA (330 pmol/50nL), an A_1 adenosine receptor agonist, into the subpostremal NTS. Intact (INT), β-adrenergic blockade (βX), bilateral adrenalectomy (ADX), lumbar sympathectomy (LX), and combined adrenalectomy plus lumbar sympathectomy (ADX + LX). Microinjections of CPA denoted by vertical arrow. Reprinted from McClure et al. 2005 (47).

receptors seem to be more complex than that elicited by NTS A_{2a} adenosine receptor

subtypes. NTS A_1 adenosine receptors, in addition to the aforementioned potential β_2-adrenergic vasodilation, exert mostly pressor and vasoconstrictor effects; selective activation of these receptors elicits uniform, although differential, regional sympathoactivation. These counteracting mechanisms are most likely responsible for the relatively high variability of MAP responses triggered by selective activation of NTS A_1 adenosine receptors: pressor, but also biphasic and depressor responses were observed[76]. The competition between neural vasoconstriction and epinephrine mediated vasodilation may be especially powerful in the hindlimb vascular bed where expression of β_2-adrenergic receptors is relatively greater than in other vascular beds [90]. In the recent study from our laboratory I confirmed this hypothesis showing that selective stimulation of NTS A_1 adenosine receptors evokes simultaneous β_2-adrenergic vasodilation and sympathetic/humoral vasoconstriction in the iliac vascular bed with prevailing pressor and vasoconstrictor responses[47]. The variability of the responses (pressor vs. depressor and iliac vasoconstriction vs. vasodilation) were relatively high in intact animals: ~70% pressor and ~65% iliac vasoconstrictor responses were observed. These results were similar to those previously observed in our laboratory[76]. The initial variability of the responses observed in intact animals (INT) was completely eliminated following β-adrenergic blockade (βX) as well as following bilateral adrenalectomy (ADX); under these experimental conditions only pronounced increases in MAP and decreases in iliac vascular conductance (IVC) were observed (Figures 3 & 4). Bilateral lumbar sympathectomy attenuated the pressor and vasoconstrictor responses observed in the intact group; however, combined bilateral adrenalectomy plus sympathectomy (ADX+LX) did not abolish the responses. Instead, relatively large increases in MAP and decreases in iliac vascular conductance were observed under these conditions. The

increases in MAP in the ADX+LX group were not different from those observed in intact animals, whereas iliac vasoconstriction was approximately 4 fold greater in comparison to the intact group and similar to that observed following βX and ADX (Figure 4). Since a powerful vasoconstriction persisted after the removal of sympathetic innervation combined with bilateral adrenalectomy this response must be mediated via unknown humoral factors released upon stimulation of NTS A_1 adenosine receptors. Three major vasoconstrictor substances may be potentially released into the circulation under these conditions: vasopressin, angiotensin II and norepinephrine. Since the release of vasopressin is tonically inhibited via baroreflex mechanisms[20], the attenuation/resetting of the baroreflex may remove this restraint and trigger the release of vasopressin into the circulation. In support of this hypothesis, a recent study from our laboratory showed that selective stimulation of A_1 adenosine receptors in the NTS resets the baroreflex control of the circulation to a higher MAP[71]. As previously mentioned the stimulation of NTS A_1 adenosine receptors increases RSNA[76]. The increase in RSNA facilitates the release of renin into the circulation, which is subsequently transformed into angiotensin II[23]. The circulating

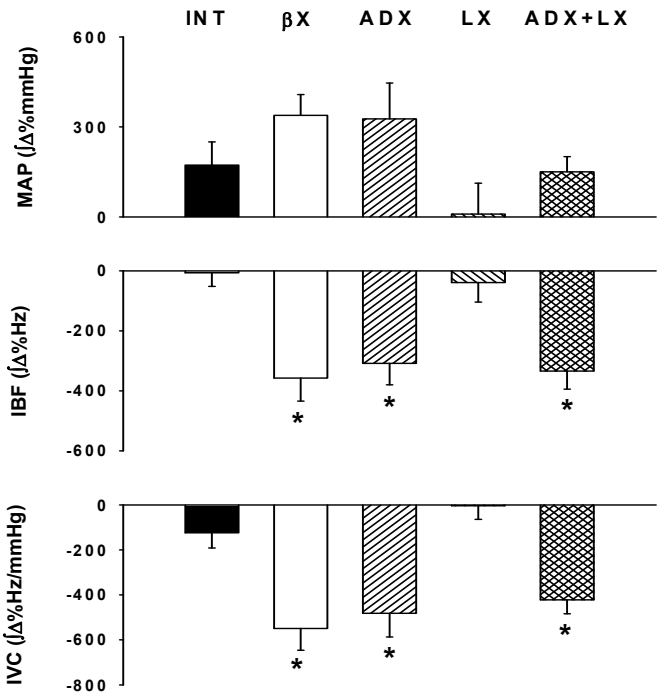

Figure 4. Integral responses of MAP, IBF, and IVC evoked by microinjection CPA (330 pmol) into the NTS. Abbreviations the same as in Figure 3. *Different versus intact (INT) group ($P < 0.05$). Reprinted from McClure et al. 2005 (47).

angiotensin II may contribute to the iliac vasoconstriction via AT_1 angiotensin receptors. Finally, since the stimulation of NTS A_1 adenosine receptors increases activity of various sympathetic outputs it is possible that norepinephrine released from synaptic terminals, other than those directed to the hindlimb, may reach the iliac vascular bed via circulating blood and contribute to the iliac vasoconstriction. Taking into consideration that β_2-adrenergic receptors, V_1 vasopressin receptors, and AT_1 angiotensin II receptors may be differentially expressed in various vascular beds and that regional vascular beds differentially respond to stimulation/inhibition of those receptors[1; 16; 27-29; 34; 36; 45; 90], the contribution of neural and humoral factors to regional vascular responses (iliac vs. mesenteric vs. renal) may likely be different. Since NTS A_1 adenosine receptors contribute to the pattern of autonomic response to stress/HDR it is especially interesting if these receptors also contribute to the redistribution of blood from the visceral to muscle vasculature, which is a key component of stress/HDR-related cardiovascular adjustments[80-82; 93]. All the above hypotheses are addressed in this dissertation.

Experimental plan

During life threatening situations such as stress, hypoxia, ischemia, or severe hemorrhage, adenosine is released into the central nervous system including the nucleus tractus solitarii (NTS), a primary integrative center for cardiovascular and other autonomic reflexes. Selective activation of A_1 adenosine receptors in the NTS evokes inhibition of baroreflex transmission, differential regional sympathoactivation (adrenal>renal≥lumbar) and variable hemodynamic responses with prevailing pressor and vasoconstrictor responses. These receptors facilitate the hypothalamic defense response, and therefore may contribute to the redistribution of blood from the visceral to the muscle vascular beds. My previous study showed that stimulation of A_1 adenosine

receptors in the NTS triggers in iliac vascular bed β_2-adrenergic vasodilation opposed by vasoconstriction mediated by efferent sympathetic nerves and an unknown vasoconstrictor factor(s). Therefore, the goal of this dissertation was to identify these humoral vasoconstrictor(s) and to evaluate the relative role of all vasoactive factors triggered by NTS A_1 adenosine receptors in regional vascular beds.

Aims of dissertation:

1. To determine which humoral vasoconstrictor(s) (vasopressin, angiotensin II, and /or norepinephrine) oppose β_2-adrenergic vasodilation in the iliac vascular bed evoked by selective stimulation of A_1 adenosine receptors in the NTS.
2. To determine if A_1 adenosine receptor stimulation contributes to the redistribution of blood flow from visceral (mesenteric and renal) to somatic (iliac) vascular beds; specifically, if vasoconstrictor factors prevail over β_2-adrenergic vasodilation in visceral vs. somatic vascular beds.

CHAPTER 1

Vasopressin is a major vasoconstrictor involved in hindlimb vascular responses to stimulation of adenosine A_1 receptors in the nucleus of the solitary tract

ABSTRACT

Our previous study showed that stimulation of adenosine A_1 receptors located in the nucleus of the solitary tract (NTS) exerts counteracting effects on the iliac vascular bed: activation of the adrenal medulla and β-adrenergic vasodilation versus vasoconstriction mediated by neural and unknown humoral factors. In the present study we investigated the relative contribution of three major potential humoral vasoconstrictors: vasopressin, angiotensin II and norepinephrine in this response. In urethane/chloralose anesthetized rats we compared the integral changes in iliac vascular conductance evoked by microinjections into the NTS of the selective A_1 receptor agonist, N^6-cyclopentlyadenosine (CPA, 330 pmol in 50 nl) in intact (INT) animals and following: V_1 vasopressin receptor blockade (VX), angiotensin II AT_1 receptor blockade (ATX), bilateral adrenalectomy + ganglionic blockade (ADX+GX, which eliminated the potential increases in circulating norepinephrine and epinephrine), ADX+GX+VX and ADX+GX+VX+ATX. In INT animals, stimulation of NTS A_1 adenosine receptors evoked typical variable responses with prevailing pressor and vasoconstrictor effects. VX reversed the responses to depressor ones. ATX did not significantly alter the responses. ADX+GX accentuated pressor and vasoconstrictor responses whereas ADX+GX+VX and ADX+GX+VX+ATX virtually abolished the responses. Stimulation of NTS A_1 adenosine receptors increased circulating vasopressin over 4-fold (26.4±10.4 vs. 117.0±19 pg/ml). These data strongly suggest that vasopressin is a major vasoconstrictor factor opposing β-adrenergic vasodilation in iliac vascular responses

triggered by stimulation of NTS A_1 adenosine receptors whereas angiotensin II and norepinephrine do not contribute significantly to the vasoconstrictor responses.

INTRODUCTION

It is now widely accepted that adenosine operating via A_1 and A_{2a} receptors modulates neural cardiovascular control at the level of the *nucleus tractus solitarii* (NTS) and other brainstem cardiovascular centers[4; 7; 8; 44; 72; 77; 82; 86]. Under normal, physiological conditions a natural source of adenosine is ATP released synaptically from neurons as well as from glial cells activated by neighboring neurons[9; 13; 19; 33; 82]. This extracellular ATP is catabolized via ectonucleotidases to adenosine which further acts more broadly as a neuromodulator operating via pre or postsynaptic A_1 and A_{2a} adenosine receptor subtypes[13; 59; 82; 94]. Under pathological conditions such as ischemia, hypoxia, and severe hemorrhage a global release of adenosine from many cell types occurs via the breakdown of intracellular ATP[56; 89; 92]. Thus, adenosine which is generated in or released into the extracellular space under physiological or pathological conditions acts not via specific synapses in specific neuronal pathways but rather spatially, reaching all adenosine receptor subtypes in the vicinity; this may resemble signaling via diffusive neurotransmitters/neuromodulators like nitric oxide or carbon monoxide. This spatial aspect of the action of naturally released adenosine seems especially important for the NTS where groups of functionally different neurons usually overlap allowing for "adenosine crosstalk". Specific physiological effects exerted via nonselective, spatial spread of adenosine in the NTS may depend on differential expression of adenosine receptor subtypes on functionally distinct NTS neurons/terminals, as we suggested previously[72; 77].

In the central nervous system adenosine may inhibit or facilitate release of

neurotransmitters from synaptic terminals as well as directly inhibit or activate neurons via pre- and postsynaptic A_1 or A_{2a} receptors, respectively[13; 59; 72; 77]. Since A_1 vs. A_{2a} adenosine receptors exert contrasting effects on central neurons/terminals reciprocal effects are usually, although not always, observed in response to selective stimulation of the two receptor subtypes. NTS A_1 adenosine receptor stimulation predominately yields differential regional sympathoactivation (adrenal>renal≥lumbar) and pressor responses[4; 8; 71; 76]. In contrast, NTS A_{2a} receptor stimulation typically evokes depressor responses accompanied by contrasting regional sympathetic responses: decreases in renal (RSNA), no changes in lumbar (LSNA) and increases in preganglionic adrenal (pre-ASNA) sympathetic nerve activity[4; 8; 37; 73-75]. Note that whereas stimulation of NTS A_1 and A_{2a} receptors evoke contrasting changes in RSNA and LSNA, both adenosine receptor subtypes activate the sympathetic output to the adrenal medulla[74;76].

Recent studies from our laboratory showed that the pressor and sympathoexcitatory responses evoked by stimulation of NTS A_1 adenosine receptors are mediated mostly via inhibition of baroreflex mechanisms at the level of the NTS whereas hemodynamic and differential sympathetic responses evoked by stimulation of NTS A_{2a} adenosine receptors are mediated mostly via activation of non-baroreflex mechanisms[37; 71; 74-76]. It should be stressed that A_1 adenosine receptors may also modulate non-glutamatergic, non-baroreflex mechanisms operating in the NTS. For example, sinoaortic denervation or ionotropic glutamatergic blockade abolished A_1-adenosine-receptor-mediated increases in RSNA and LSNA whereas this attenuated, but did not abolish, the increases in pre-ASNA[76]. The activation of pre-ASNA which persisted after sinoaortic and the glutamatergic blockade was most likely mediated via

A_1 adenosine receptor modulation of non-glutamatergic pathways descending into the NTS from higher structures, such as from hypothalamic paraventricular and/or dorsomedial nuclei[26; 65; 87]. A_1 adenosine receptors located on these descending nonglutamatergic pathways and/or NTS interneurons could selectively activate the sympathetic output to the adrenal medulla (but not RSNA and LSNA) via disinhibition of direct NTS-RVLM pathways[20]. NTS A_1 adenosine receptors may also modulate the control of heart rate (HR) via both baroreflex and non-baroreflex mechanisms[71; 76]. Taken together these observations strongly suggest that A_1 adenosine receptors are differentially located on functionally different NTS neurons/terminals which are mostly, but not exclusively glutamatergic and involved in the baroreflex arch.

Although selective stimulation of NTS A_1 adenosine receptors evokes predominantly pressor responses[4; 8], occasionally biphasic or even depressor responses are observed[76]. This variability of the responses is a natural consequence of simultaneous activation of at least two counteracting mechanisms: sympathetic vasoconstriction and β-adrenergic vasodilation mediated via epinephrine released from the activated adrenal medulla. Since β-adrenergic receptors are preferentially located in the muscle vascular[90] bed and both pre-ASNA and LSNA increase upon stimulation of NTS A_1 adenosine receptors it was likely that these two counteracting factors may significantly contribute to the variability of the iliac vascular responses. We confirmed this hypothesis in our recent study by showing that removal of the vasodilatory mechanism (via bilateral adrenalectomy as well as peripheral blockade of β-adrenergic receptors) abolished the variability of the responses normally observed in intact animals and markedly increased the pressor and hindlimb vasoconstrictor responses[47]. In

contrast, bilateral lumbar sympathectomy tended to increase the vasodilatory component of the responses although the variability still persisted. To our surprise, following combined adrenalectomy plus lumbar sympathectomy a marked, consistent vasoconstrictor component still persisted suggesting that some unknown humoral vasoconstrictor factor(s) are involved[47].

The most likely humoral candidates contributing to the iliac vasoconstriction are vasopressin, angiotensin II and norepinephrine. Since activation of A_1 adenosine receptors may inhibit glutamate release in baroreflex pathway at the level of the NTS[71; 76], and given that the NTS is a crucial, primary relay station for tonic baroreflex inhibition of vasopressin release[20; 66; 83] it is likely that the activation of A_1 adenosine receptors may inhibit the baroreflex restraint of vasopressin release. When released, vasopressin could evoke powerful peripheral vasoconstriction. However, whether A_1 adenosine receptors are present on those NTS baroreflex neurons/terminals which inhibit the release of vasopressin is unknown. The present study was designed to test this hypothesis.

Since renal sympathetic nerve activity (RSNA) directed to the kidney has been shown to increase following stimulation of NTS A_1 adenosine receptors[76], this may facilitate the renin/angiotensin mechanism leading to humoral vasoconstriction of the hindlimb vasculature mediated via AT_1 angiotensin II receptors[23]. In addition, since stimulation of NTS A_1 adenosine receptors increases the activity of various sympathetic outputs[76] it is also possible that circulating norepinephrine released from other synaptic terminals may reach the iliac vasculature and cause vasoconstriction. Therefore, in the present study we investigated the extent to which these three potential humoral vasoconstrictors (vasopressin, angiotensin II and/or norepinephrine) contribute to NTS-

A_1-adenosine-receptor-elicited iliac vasoconstriction[47].

MATERIALS AND METHODS

All protocols and surgical procedures employed in this study were reviewed and approved by the institutional Animal Care and Use Committee and were performed in accordance with the *Guiding Principles in the Care and Use of Animals* endorsed by the American Physiological Society and published by the National Institutes of Health.

Design

This study investigates further the mechanisms responsible for the consistent variability of hemodynamic responses elicited by activation of adenosine A_1 receptors in the NTS[47; 76]. Previously we showed that the variability of the pressor/depressor and iliac vasoconstrictor/vasodilator responses is not a simple effect of competitive interactions between sympathetic vasoconstriction vs. β-adrenergic vasodilation but some unknown, powerful, humoral vasoconstrictor factor(s) are also involved[47]. Therefore, the present study assessed the relative contribution of potential humoral vasoconstricting factors to the iliac vascular responses evoked by selective stimulation of NTS A_1 adenosine receptors. Experiments were performed on a total of 102 male Sprague Dawley rats. In 63 rats we compared the relative vasoconstrictor effects potentially mediated via vasopressin, angiotensin II, norepinephrine and sympathetic innervation of the hindquarters, the effects normally opposed by simultaneous β-adrenergic vasodilation mediated via activation of the adrenal medulla. In an additional 20 rats, respective time controls were performed and in 6 rats the effectiveness of vasopressin and angiotensin II receptor blockades was assessed. These functional experiments strongly suggested that the major vasoconstrictor factor triggered by activation of adenosine A_1 receptors in the NTS may be vasopressin. Therefore, in an

additional group of 13 animals the levels of circulating vasopressin were evaluated before and following microinjections into the NTS of the selective A_1 adenosine receptor agonist, N^6-cyclopentlyadenosine (CPA) or vehicle control.

Instrumentation and measurements

All the procedures were described in detail previously[7; 42; 47; 73-76]. Briefly, male Sprague-Dawley rats (350-400 g, Charles River) were anesthetized with a mixture of α-chloralose (80 mg/kg) and urethane (500 mg/kg, ip), tracheotomized, connected to a small animal respirator (SAR-830, CWE, Ardmore, PA) and artificially ventilated with 40% oxygen 60% nitrogen mixture. Catheterization of the right femoral artery and vein were performed to monitor arterial blood pressure and infuse drugs, respectively. Arterial blood gases were tested occasionally for appropriate experimental values (Radiometer, ABL500, OSM3). Averaged values measured at the end of each experiment were the following: pH = 7.38±0.01, Po_2 = 140.1±3.8 mmHg, and Pco_2 = 36.2±0.7 mmHg.

From a mid-abdominal incision, the left iliac artery was exposed. A pulse Doppler blood flow velocity transducer was placed around the artery and connected to the flowmeter (Baylor Electronics). From the same mid-abdominal incision in some animals, bilateral adrenalectomy or lumbar sympathectomy (L1-L6) was performed. The intermesenteric nerves were also severed in sympathectomized animals.

Arterial blood pressure and iliac flow signals were digitized and recorded with an analog-digital converter (Modular Instruments) interfaced to a laboratory computer. The signals were recorded continuously using Biowindows software (Modular Instruments), averaged over 5 second intervals and stored on hard disk for subsequent analysis.

Microinjections into the NTS

Animals were placed in stereotaxic frame with head tilted down at 45º. After the exposure of the brainstem via dissected atlantoccipital membrane, the animals were allowed to stabilize for at least 30 min before microinjections. Unilateral microinjections of the selective A_1 adenosine receptor agonist CPA (Tocris, 330 pmol) dissolved in 50 nl of artificial cerebrospinal fluid (ACF) were made through multibarrel, glass micropipettes into the medial region of the caudal subpostremal NTS as described previously[7; 42; 73-76]. This dose of CPA produced the most consistent, predominantly pressor responses in our previous study[76]. The CPA was dissolved in ACF and the pH adjusted to 7.2. In several previous studies we

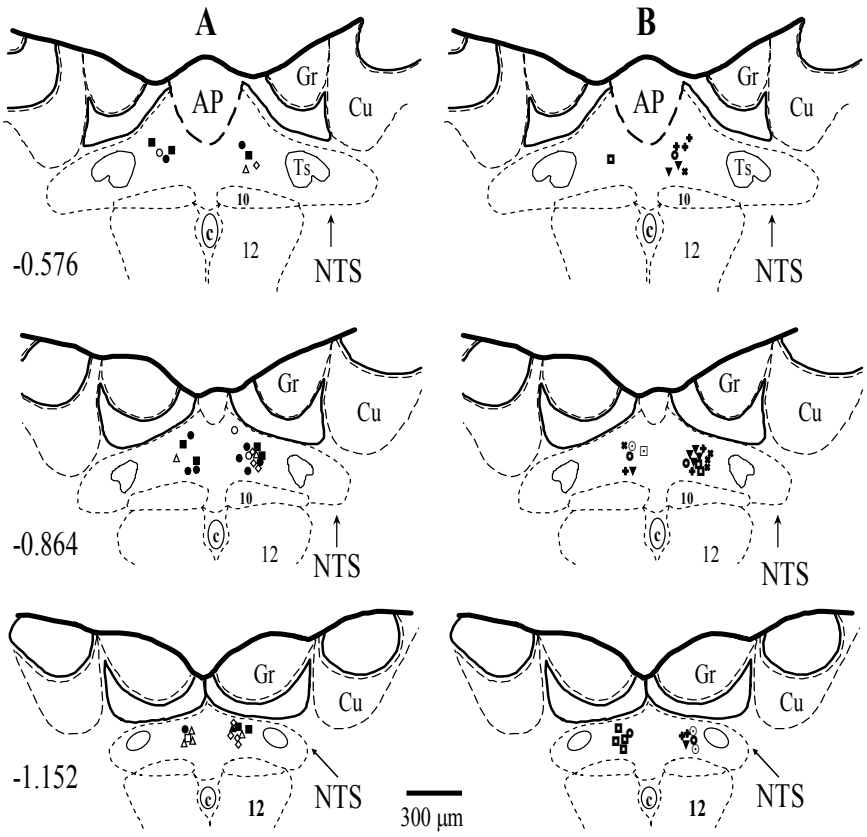

Figure 5. Microinjection sites in the caudal subpostremal NTS for all experiments. Schematic diagrams of transverse sections of the medulla oblongata from a rat brain. NTS, nucleus tractus solitarii; AP, area postrema; c, central canal; 10, dorsal motor nucleus of the vagus nerve; 12, nucleus of the hypoglossal nerve; ts, tractus solitarius; Gr, gracile nucleus; Cu, cuneate nucleus. Scale is shown at the bottom; the number on the left side of the schematic diagram denotes the rostro-caudal position in millimeters of the section relative to the obex according to the atlas of the rat subpostremal NTS by Barraco et al. (3). Microinjection sites were marked with fluorescent dye and are denoted with filled symbols for the pressor responses to CPA and corresponding open symbols for the depressor responses. *A*: microinjections of CPA in intact animals (●,○), after vasopressin V_1 receptor blockade (VX) (◇), lumbar sympathectomy plus VX (▲,△), and after angiotensin II AT_1 receptor blockade (ATX) (■,□). *B:* microinjections of CPA after bilateral adrenalectomy (ADX) plus ganglionic blockade (GX) (▼), following ADX+GX+VX (⦿⊙), after ADX+GX+VX+ATX (◘,⊡). In experiments where vasopressin assay was performed the microinjection sites were denoted: **+** and **x** for CPA and ACF microinjections, respectively.

have shown that microinjections of the same amount of ACF into the same site of the NTS did not markedly affect mean arterial pressure (MAP), HR, RSNA, LSNA and pre-ganglionic adrenal (pre-ASNA) sympathetic nerve activity and vascular flows in iliac, renal and mesenteric arteries[7; 73-76]. The changes in all these variables were either not different from zero or smaller than natural, random fluctuations of these variables over the time of measurements. To avoid the effect of desensitization of A_1 adenosine receptors, in all experiments only one dose of the agonist was microinjected into left or right side of the NTS. All microinjection sites were marked with fluorescent dye (DiI, Molecular Probes) and verified histologically (Figure 5) as described previously[7; 42;73-76].

We believe that the microinjection technique mimics natural, spatial (not strictly synaptic) action of adenosine in the central nervous system as adenosine is naturally produced in the intracellular space by ectonucleotidases from extracellular ATP (released from neurons and glial cells under physiological conditions)[9; 13; 33; 82; 94] or it is directly released into the intracellular space from ischemic/hypoxic neurons and glial cells under pathological conditions[56; 89; 92].

Experimental protocols

In a previous study from our laboratory we showed that in addition to sympathetic iliac vasoconstriction and β-adrenergic vasodilation some unknown humoral vasoconstrictor(s) contribute to the consistent variability of hemodynamic responses evoked by selective stimulation of adenosine A_1 receptors located in the NTS[47]. This conclusion was based on comparing of the responses observed in intact animals and following four experimental protocols: 1) β-adrenergic blockade, 2) adrenalectomy, 3) lumbar sympathectomy and 4) combined adrenalectomy plus lumbar sympathectomy. In the last experimental condition a powerful iliac vasoconstriction was observed

indicating that non-sympathetic, humoral vasoconstrictors are involved[47]. The present study is a direct extension of our previous findings and focuses on the relative contribution to the responses of three potential humoral vasoconstrictors: vasopressin, angiotensin II and norepinephrine. Six experimental protocols were designed, according to the diagrams presented in Figure 6. Data collected in each protocol were compared with responses observed in intact group. In Protocols 1 and 2 the contribution of vasopressin and angiotensin II was assessed by comparing hemodynamic responses elicited by stimulation of NTS A_1 adenosine receptors in intact animals with those obtained following selective blockade of vasopressin V_1 receptors and angiotensin II AT_1 receptors via iv. injections of selective antagonists: [β-mercapto-β,β-cyclopentylmethylenepropionyl,1-O-Me-Tyr^2,Arg^8]-vasopressin, (20 μg/kg, Sigma) and losartan (5 mg/kg, Merck Inc.), respectively. To evaluate the potential contribution of circulating norepinephrine to the responses ganglionic blockade (hexamethonium bromide, 25 mg/kg iv, Sigma) was combined with adrenalectomy (Protocol 3) and these data and the responses observed previously

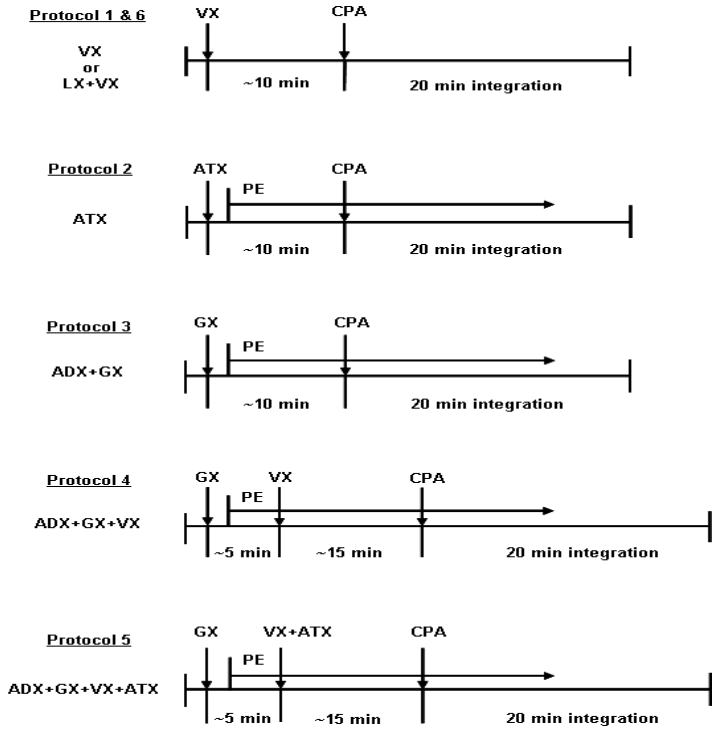

Figure 6. Time line of how experimental protocols are executed: vasopressin V_1 receptor blockade (VX), lumbar sympathectomy + V_1 receptor blockade (LX+VX), angiotensin II AT_1 receptor blockade (ATX), bilateral adrenalectomy + ganglionic blockade (ADX+GX), adrenalectomy + ganglionic blockade + V_1 receptor blockade (ADX+GX+VX) and adrenalectomy + ganglionic blockade + V_1 receptor blockade + AT_1 receptor blockade (ADX+GX+VX+ATX). Time control experiments were performed for protocols including ATX and/or GX (protocols 2-5) according to the respective diagrams; however microinjections of CPA were omitted.

following adrenalectomy alone[47] are discussed together. This indirect evaluation of norepinephrine contribution to the responses was necessary because total sympathetic denervation is impossible and ganglionic blockade, which prevents secretion of norepinephrine from sympathetic terminals, also impairs/abolishes the effects of activation of the adrenal medulla; thus ganglionic blockade removes β-adrenergic vasodilation simultaneously. Therefore, the appropriate reference point for the responses obtained following the ganglionic blockade (Protocol 3) were the responses obtained following adrenalectomy alone which has been already performed in our previous study[47]. Protocol 4 removed the combined contribution of norepinephrine and vasopressin to the responses (ganglionic blockade + vasopressin V_1 receptor blockade) whereas Protocol 5 removed the combined effect off all three potential vasoconstrictors considered via ganglionic blockade + vasopressin V_1 receptor blockade + angiotensin AT_1 receptor blockade. Protocols 3-5 were performed in adrenalectomized animals to clarify the experimental conditions by removing any residual adrenal responses which may potentially persist following the ganglionic blockade. Since preliminary results of the above five experimental protocols strongly suggested that only vasopressin has a marked contribution to the responses, in Protocol 6 the magnitude of β-adrenergic vasodilation alone, not opposed by major vasoconstrictor factors (sympathetic vasoconstriction and vasopressin) was assessed. In this protocol bilateral lumbar sympathectomy was combined with blockade of V_1 vasopressin receptors and these data were compared with data following V_1 vasopressin receptor blockade alone (Protocol 1) and discussed together with previous data obtained following bilateral lumbar sympathectomy alone[47].

The effectiveness of vasopressin V_1 and angiotensin AT_1 receptor blockades were tested in separate groups of animals (*n*=3 for each blockade) with iv injections of arginine-vasopressin (50 mU/kg, Sigma) and angiotensin II (300 ng/kg, Sigma), respectively, before and after the blockade. Both blockades remained effective for over

Table 1. Maximal hemodynamic responses evoked by blockade of V_1 vasopressin receptors (VX), AT_1 angiotensin II receptors (ATX) and ganglionic blockade (GX).

Experimental groups	*n*	Δ% Mean Arterial Pressure, mmHg	Δ% Heart Rate, beats/min	Δ% Iliac Blood Flow	Δ% Iliac vascular Conductance
VX	16	-4.7±1.8#	1.2±0.9	10.2±1.6#	15.3±2.7#
GX	39	-39.7±1.9#	3.1±1.9	16.6±3.3#	99.9±9.9#
ATX	15	-49.2±2.8#	-13.9±3.5#	8.6±6.1	125.1±18.2#

Data are means ± SE. # P<0.05 vs. zero. Numbers of responses to VX, GX and ATX were combined from those protocols where these blockades were applied as a first pharmacological manipulation: $\boldsymbol{n}_{VX} = \boldsymbol{n}_{VX} + \boldsymbol{n}_{LX+VX}$; $\boldsymbol{n}_{GX} = \boldsymbol{n}_{ADX+GX} + \boldsymbol{n}_{ADX+GX+VX} + \boldsymbol{n}_{ADX+GX+VX+ATX} + \boldsymbol{n}$ of respective controls; $\boldsymbol{n}_{ATX} = \boldsymbol{n}_{ATX} + \boldsymbol{n}$ of respective control (see **Table 2**). The small changes in mean arterial pressure and iliac vascular conductance caused by VX were allowed to return spontaneously toward resting levels, whereas the large, sustained changes in these variables caused by ATX and GX were compensated via iv infusion of phenylephrine (see **Table 1**).

1 hour. Blockade of V_1 vasopressin receptors caused relatively small decreases in MAP and increases in IVC (Table 1) which spontaneously returned toward resting values in approximately 10 min; therefore approximately 10 min after V_1

Table 2. Infusion rates of phenylephrine used to maintain mean arterial pressure and iliac vascular conductance at pre-blockade levels in experimental and respective time control groups in which angiotensin AT_1 receptor blockade (ATX) and/or ganglionic blockade (GX) were performed.

Protocol number	Experimental procedure	*n*	Infusion rate ml/h/kg	Time controls	*n*	Infusion rate ml/h/kg
2	ATX	10	3.18±0.12*	ATX	5	2.39±0.25*
3	ADX + GX	8	3.02±0.24*	ADX + GX	5	3.20±0.33*
4	ADX + GX + VX	8	3.45±0.41*	ADX + GX + VX	5	3.94±0.62
5	ADX + GX + VX + ATX	8	6.33±0.24	ADX + GX + VX + ATX	5	5.29±0.62

Data are means ± SE. # P<0.05 vs. zero. Numbers of responses to VX, GX and ATX were combined from those protocols where these blockades were applied as a first pharmacological manipulation: $\boldsymbol{n}_{VX} = \boldsymbol{n}_{VX} + \boldsymbol{n}_{LX+VX}$; $\boldsymbol{n}_{GX} = \boldsymbol{n}_{ADX+GX} + \boldsymbol{n}_{ADX+GX+VX} + \boldsymbol{n}_{ADX+GX+VX+ATX} + \boldsymbol{n}$ of respective controls; $\boldsymbol{n}_{ATX} = \boldsymbol{n}_{ATX} + \boldsymbol{n}$ of respective control (see **Table 2**). The small changes in mean arterial pressure and iliac vascular conductance caused by VX were allowed to return spontaneously toward resting levels, whereas the large, sustained changes in these variables caused by ATX and GX were compensated via iv infusion of phenylephrine (see **Table 1**).

vasopressinergic blockade the microinjection of CPA was performed in Protocols 1 and 6 (Figure 6). However, following blockade of angiotensin AT_1 receptors and ganglionic blockade marked and sustained decreases in MAP and increases in IVC were observed (Table 1). Therefore, in Protocols 2-5, where these blockades were performed iv infusions of phenylephrine (PE, Sigma, 200 μg/ml) were used to return the hemodynamic parameters toward baseline, pre-blockade values. Table 2 presents the rates of PE infusion needed for the compensation. No differences were observed in PE infusion rates between Protocols 2-4. However, significantly greater PE infusion rates were required when angiotensin AT_1 receptor antagonists, losartan, were combined with ganglionic and V_1 vasopressin receptor blockades in Protocol 5 (Table 2). During the responses to stimulation of NTS A_1 adenosine receptors PE infusion was continued at the same rates as needed to compensate for the altered hemodynamic values in Protocols 2-5. The effect of PE infusion on baseline hemodynamic values was estimated in respective time controls for Protocols 2-5 (Table 2). In the time-control experiments all procedures except microinjections of CPA were performed in the same time-pattern as in

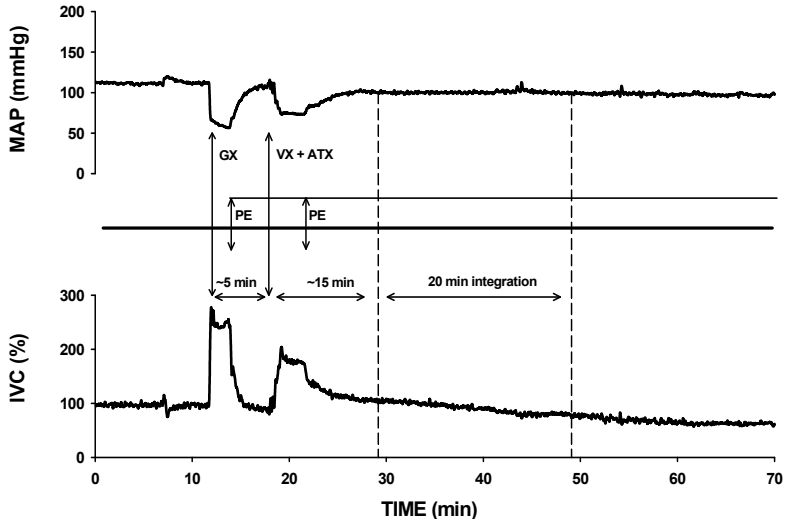

Figure 7. An example of time control experiment following adrenalectomy plus ganglionic blockade (GX) plus vasopressin blockade (VX) plus angiotensin II AT_1 receptor blockade (ATX); no CPA was microinjected in this experiment. The dashed arrow denotes a potential microinjection of CPA and the subsequent 20 min integration of the response. Note that although the infusion of phenylephrine (PE) did not fully compensated for the decrease in mean arterial pressure (MAP) the iliac vascular conductance (IVC) gradually declined constricting iliac

experimental Protocols 2-5. Figure 7 shows an example of a time control for the most complex experimental Protocol 5. PE infusion rates were similar in the experimental protocols and respective time controls (Table 2).

Vasopressin assay

Since the hemodynamic experiments (Protocols 1-6) suggested that vasopressin plays a dominant role in the iliac vascular responses to stimulation of NTS A_1 adenosine receptors, in an additional group of animals the effect of microinjections into the NTS of CPA (*n*=8) or respective volume control (50 nl of ACF, *n*=5) on circulating vasopressin were evaluated. We compared the levels of plasma vasopressin measured 30 min before and ~8 min after the microinjections (the average time when maximal hemodynamic responses to stimulation of NTS A_1 receptors occur). Arterial blood samples (~1 ml) were slowly withdrawn from the femoral artery into prechilled, heparinized tubes. Blood volume was kept unchanged via simultaneous infusion of the same volume of donor blood into the femoral vein. The samples were immediately placed on ice and centrifuged at 5000 g for 10 min at 4º C. Plasma was collected and stored at -70º C. Plasma vasopressin concentration was assessed via standard radioimmunoassay procedures in our laboratory as described previously[32; 54; 61]. The sensitivity of the vasopressin assay was 0.1 pg/ml and 50% displacement was 4.1 pg/tube. Intra- and inter-assay variability was 7.0% and 13.4%, respectively.

Data analysis

Hemodynamic responses were analyzed over a 20 min period following the microinjections, similar to our previous study[47]. The responses were quantified as an integration of the differences between the baseline and response values averaged in 1 min periods and summed for 20 min of the response, i.e. when the majority of the

responses occur. The integral reflects the predominant trend of the responses despite transient, sometimes large, bidirectional fluctuations in each variable. Because hemodynamic effects evoked by stimulation of NTS A_1 adenosine receptors were variable, often biphasic, or even polyphasic, as we previously reported[47; 76], we used the integral values for the comparison between the experimental groups. The absolute values of blood flow depend to some extent on positioning of the probe around the iliac artery; therefore the comparison between the relative changes in MAP, IBF, and IVC were more reliable. The HR responses, calculated from pulse intervals through the flow probe, were expressed in absolute values (beats/min). Iliac vascular conductance (IVC) was calculated by dividing iliac blood flow (IBF), expressed as a Doppler shift (in Hz) by MAP (in mmHg). In experimental Protocols 2-5, where PE was infused to compensate for the decreased MAP and increased IVC the direct effect of PE on baseline hemodynamic variables was evaluated in respective time control experiments and subtracted from experimental data. Specifically, changes occurring in each variable during time-control experiments were integrated for 20 min and subtracted from the respective 20 min integral values obtained in each animal of the experimental groups (Protocols 2-5).

One-way ANOVA for independent measures was used to compare hemodynamic responses versus experimental conditions. Differences observed were further evaluated by *t*-test with Bonferroni adjustment for independent measures. Differences between circulating vasopressin levels measured before and after microinjections of ACF or CPA were evaluated using paired t-test; the differences in vasopressin levels between the groups (ACF vs. CPA) were evaluated using unpaired t-test. The changes in all recorded variables were also compared with zero by means of SYSTAT univariate

F test. An α level of $P < 0.05$ was used to determine statistical significance.

Table 3. Resting values of hemodynamic parameters in each experimental group.

Protocol number	Experimental procedure	*n*	Mean Arterial Pressure, mmHg	Heart Rate, beats/min	Iliac Blood Flow, Hz	Iliac Vascular Conductance, Hz/mmHg
	INTACT	13	98.1±.1	353.9±4.7	1030.2±3.6	10.9±3.3
1	VX	8	105.3±4.9	359.7±11.2	1091.0±204.2	10.6±2.0
2	ATX	10	95.3±4.9	342.2±8.2	992.8±122.5	10.8±1.5
3	ADX + GX	8	93.1±5.1	383.6±11.4	1190.4±167.2	13.2±2.1
4	ADX + GX + VX	8	88.7±1.3	383.3±9.0	1394.0±222.7	15.8±2.7
5	ADX + GX + VX + ATX	8	88.1±2.1	405.0±10.9*	1271.0±102.9	14.4±1.1
6	LX + VX	8	95.8±4.1	340.6±7.0	1336.4±132.8*	14.3±1.7
(2)	Control (ATX)	5	99.3±2.8	330.0±11.8	973.7±173.2	10.0±2.0
(3)	Control (ADX + GX)	5	84.2±2.8	365.8±17.2	1073.1±144.7	12.8±1.8
(4)	Control (ADX + GX + VX)	5	88.1±3.5	381.6±7.8	1121.6±141.1	12.7±1.4
(5)	Control (ADX + GX + VX + ATX)	5	90.2±4.7	398.9±13.3	1144.6±302.0	12.2±2.4

Data are means ± SE; *n*=number of rats. Numbers in parentheses show time controls for respective protocols. Resting values for intact animals and following: vasopressin V_1 receptor blockade (VX), angiotensin II AT_1 receptor blockade (ATX), bilateral adrenalectomy + ganglionic blockade (ADX+GX), adrenalectomy + ganglionic blockade + V_1 receptor blockade (ADX+GX+VX), adrenalectomy + ganglionic blockade + V_1 receptor blockade + AT_1 receptor blockade (ADX+GX+VX+ATX) and lumbar sympathectomy + V_1 receptor blockade (LX+VX). . * P<0.05 vs. Intact

RESULTS

Resting values of MAP, HR, IBF and IVC for each experimental group, measured just before stimulation of NTS A_1 adenosine receptors, are presented in Table 3. The resting MAP and IVC for all the groups where blockades were performed were not different from those for intact animals; this provided reliable comparison between the experimental protocols. The direct effects of ganglionic blockade, and blockade of V_1 vasopressin and AT_1 angiotensin II receptors on all hemodynamic variables are presented in Table 1. The large decreases in MAP and increases in IVC evoked by blockade of AT_1 angiotensin II receptors and/or ganglionic blockade required additional compensation with PE, whereas the small decreases in MAP and increases in IVC evoked by V_1 vasopressin receptor blockade were allowed to partially recover without compensation.

Effects of V_1, AT_1 and ganglionic blockades on responses to stimulation of NTS A_1 adenosine receptors

Figure 8 presents examples of the responses evoked by selective stimulation of NTS A_1 adenosine receptors which were observed most often under each experimental condition. The average integral responses for each experimental group are presented

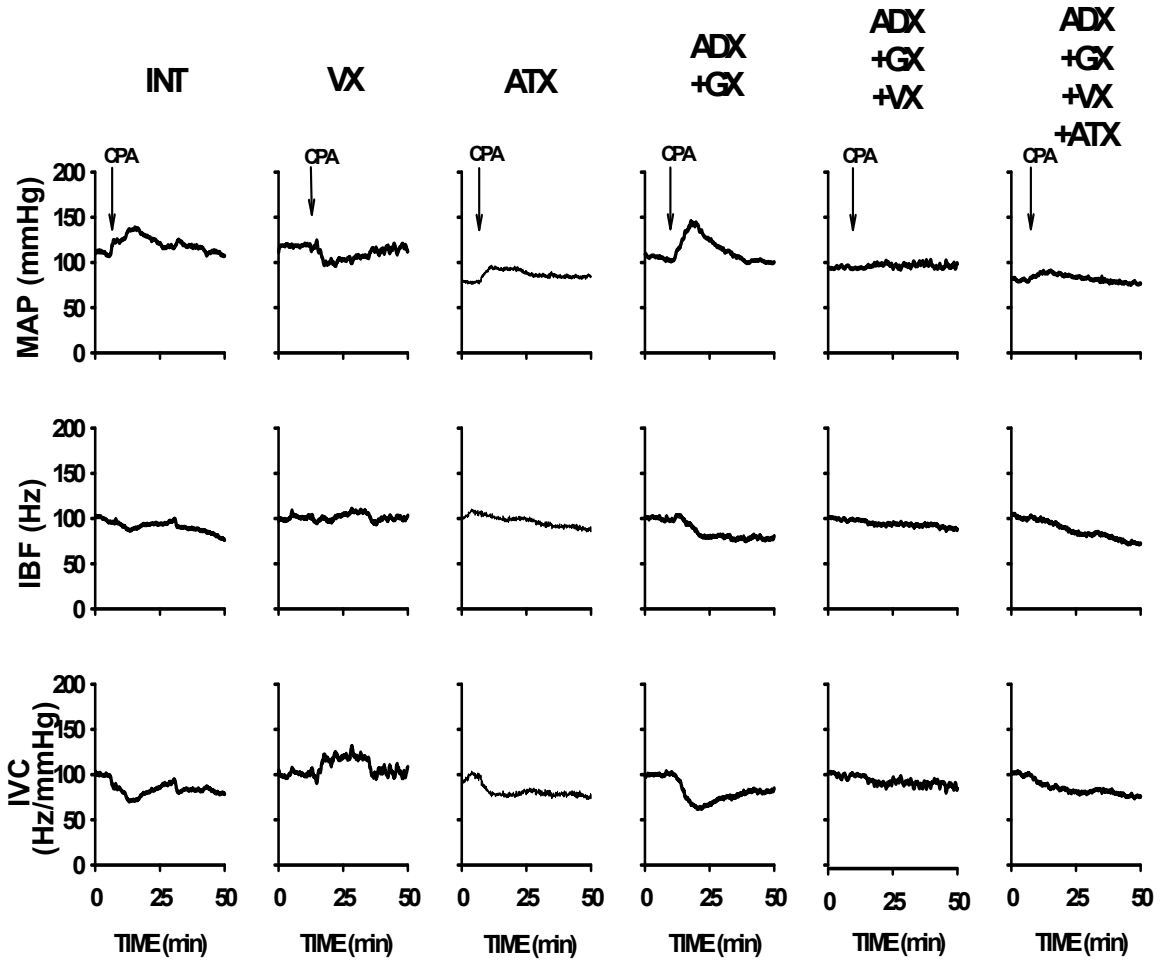

Figure 8. Mean arterial pressure (MAP), iliac blood flow (IBF) and iliac vascular conductance (IVC) responses to microinjection of adenosine A_1 receptor agonist (CPA, 330 pmol/50 nl) into the subpostremal NTS in intact rats (INT) and following: vasopressin V_1 receptor blockade (VX), angiotensin II AT_1 receptor blockade (ATX), bilateral adrenalectomy + ganglionic blockade (ADX+GX), adrenalectomy + ganglionic blockade + V_1 vasopressin receptor blockade (ADX+GX+VX) and adrenalectomy + ganglionic blockade + V_1 vasopressin receptor blockade + AT_1 angiotensin II receptor blockade (ADX+GX+VX+ATX). Microinjections of CPA, marked by vertical arrows, were applied ~10 -20 min after the blockades, when baseline levels of all variables stabilized (**see Figure 6**). Note that VX reversed pressor and vasoconstrictor responses most often observed in INT group into depressor and vasodilatory responses. ADX+GX exaggerated the pressor and vasoconstrictor response. These exaggerated vasoconstrictor responses were virtually abolished following ADX+GX+VX and ADX+GX+VX+ATX.

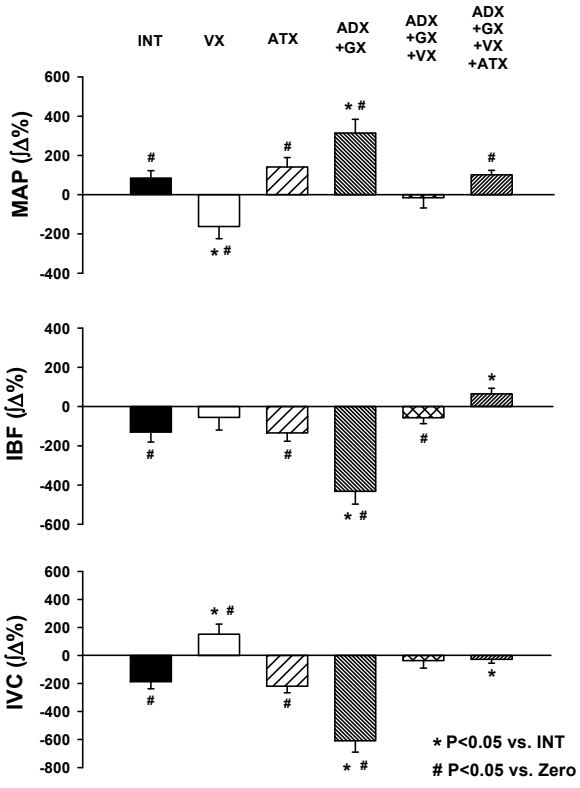

Figure 9. Integral responses of MAP, IBF and IVC evoked by microinjections of CPA (330 pmol /50 nl) into the caudal subpostremal NTS. Abbreviations as in Figure 8. Data are means ± SE. In groups ATX, ADX+GX, ADX+GX+VX, ADX+GX+VX+ATX respective time control values were subtracted. *, different versus intact group (P<0.05). #, different versus zero (P<0.05). VX reversed iliac vasoconstrictor responses observed in intact group into vasodilation and virtually abolished exaggerated vasoconstrictor responses observed following ADX+GX.

in Figure 9. In intact animals the typical variability in the responses to stimulation of NTS A_1 adenosine receptors was observed: the pressor and vasoconstrictor responses prevailed (Table 4, Figures 8 and 9), although biphasic, polyphasic or, more rarely, depressor and vasodilatory responses were also observed. As we previously demonstrated, these patterns and variability of the responses reflected counteracting effects of β-adrenergic vasodilation vs. sympathetic and humoral vasoconstriction[47; 76]. V_1 vasopressin receptor blockade reversed iliac vasoconstrictor responses observed in the

Table 4. Number of individual experiments where overall increments or decrements were observed for each recorded hemodynamic parameter based on its integral values

Protocol number	Experimental procedure	*n*	Mean Arterial Pressure, mmHg		Heart Rate, beats/min		Iliac Blood Flow, Hz		Iliac Vascular Conductance, Hz/mmHg	
			Incr	Decr	Incr	Decr	Incr	Decr	Incr	Decr
	INTACT	13	10	3	1	12	1	12	1	12
1	VX	8	0	8	0	8	3	5	7	1
2	ATX	10	9	1	4	6	2	8	0	10
3	ADX + GX	8	8	0	1	7	0	8	0	8
4	ADX + GX + VX	8	5	3	2	6	0	8	1	7
5	ADX + GX + VX + ATX	8	7	1	2	6	1	7	0	8
6	LX + VX	8	1	7	1	7	5	3	8	0

Number of increments (Incr) and decrements (Decr) observed in intact animals and following: vasopressin V_1 receptor blockade (VX), angiotensin II AT_1 receptor blockade (ATX), bilateral adrenalectomy + ganglionic blockade (ADX+GX), adrenalectomy + ganglionic blockade + V_1 receptor blockade (ADX+GX+VX), adrenalectomy + ganglionic blockade + V_1 receptor blockade + AT_1 receptor blockade (ADX+GX+VX+ATX), and lumbar sympathectomy + V_1 receptor blockade (LX+VX).

intact group into slight iliac vasodilation (P=0.0001 vs. intact). Blockade of angiotensin II AT_1 receptors alone did not significantly alter the responses in comparison to the intact group (P>0.05 for all variables). Elimination of adrenal and sympathetic neural effects on the iliac vasculature (Protocol 3) increased the iliac vasoconstrictor responses almost 4-fold in comparison to the intact group indicating that other humoral factor(s) different than circulating norepinephrine play a crucial role in the iliac vasoconstrictor responses. Subsequent blockade of vasopressin V_1 receptors (Protocol 4) virtually abolished the exaggerated iliac vasoconstriction observed following adrenalectomy plus ganglionic blockade alone (Protocol 3) (Figures 8 and 9) indicating that the humoral iliac vasoconstriction evoked by stimulation of NTS A_1 adenosine receptors is mediated mostly via the release of vasopressin. Combined blockade of neural and all considered humoral factors (adrenalectomy + ganglionic + V_1 vasopressinergic + AT_1 angiotensinergic blockades, Protocol 5) had very similar effects on the responses to that observed in Protocol 4 (adrenalectomy + ganglionic + V_1 vasopressinergic blockades); there were no significant differences between these two groups with respect to MAP, HR and IVC responses (P>0.05 for all comparisons). The lack of differences between responses observed in Protocols 4 and 5 additionally confirmed that vasopressin is the dominant humoral vasoconstrictor factor triggered by stimulation of NTS A_1 adenosine receptors. The analysis of absolute values of the integral responses presented in Table 5 leads to the same conclusions as those based on the relative responses (Figure 9). The absolute values of the responses, presented in Table 5, show residual iliac vasoconstrictor effects in Protocols 4 and 5. However, this vasoconstriction was abolished when the respective time control values were subtracted from the direct experimental values presented in Table 5.

Table 5. Absolute values of integral changes in MAP, HR, IBF and IVC evoked by microinjections of CPA (330 pmol in 50 nl) into the NTS in each experimental group.

Protocol number	Experimental procedure	*n*	Mean Arterial Pressure, mmHg	Heart Rate, beats/min	Iliac Blood Flow, Hz	Iliac Vascular Conductance, Hz/mmHg
	INTACT	13	86.0±37.7$^{\#}$	-379.3±96.4$^{\#}$	-1480.3±588.7$^{\#}$	-23.1±7.1$^{\#}$
1	VX	8	-195.2±63.9$^{*\#}$	-411.9±99.3$^{\#}$	59.6±784.5	20.8±9.3$^{*\#}$
2	ATX	10	198.4±47.3$^{\#}$	-70.6±126.0	-1585.8±445.4$^{\#}$	-34.8±6.4$^{\#}$
3	ADX + GX	8	312.3±67.1$^{*\#}$	-139.0±48.6$^{\#}$	-5758.2±1284.4$^{*\#}$	-91.6±20.2$^{*\#}$
4	ADX + GX + VX	8	7.1±46.3	-237.0±137.5	-2752.3±715.7$^{\#}$	-31.3±11.5$^{\#}$
5	ADX + GX + VX + ATX	8	74.9±19.0$^{\#}$	-249.8±134.8	-1784.9±447.1$^{\#}$	-30.9±5.5$^{\#}$
6	LX + VX	8	-162.0±71.5$^{*\#}$	-502.0±177.3$^{\#}$	2127.6±1489.5*	57.5±11.4$^{*\#}$
(2)	Control (ATX)	5	80.0±37.6	504.1±91.8$^{*\#}$	-303.2±573.6	-9.8±3.6$^{\#}$
(3)	Control (ADX + GX)	5	22.3±36.2	54.5±104.8*	-166.1±364.4	-5.9±3.8
(4)	Control (ADX + GX + VX)	5	15.6±39.7	163.3±54.5$^{*\#}$	-1224.1±380.7$^{\#}$	-13.8±5.1$^{\#}$
(5)	Control (ADX + GX + VX + ATX)	5	-11.4±23.7	206.5±61.6$^{*\#}$	-2694.9±1428.2	-24.7±13.0

Data are means ± SE. * P<0.05 vs. Intact; # P<0.05 vs. zero. Numbers in parentheses show time controls for respective protocols. Integral changes for intact animals and following: vasopressin V_1 receptor blockade (VX), angiotensin II AT_1 receptor blockade (ATX), bilateral adrenalectomy + ganglionic blockade (ADX+GX), adrenalectomy + ganglionic blockade + V_1 receptor blockade (ADX+GX+VX), adrenalectomy + ganglionic blockade + V_1 receptor blockade + AT_1 receptor blockade (ADX+GX+VX+ATX), and lumbar sympathectomy + V_1 receptor blockade (LX+VX).

Data collected in Protocols 1-5 (Figures 4 and 5) strongly suggested that the only significant humoral vasoconstrictor contributing to iliac vascular responses evoked by selective stimulation of NTS A_1 adenosine receptors is vasopressin. Therefore, in Protocol 6 vasopressin and lumbar sympathetic vasoconstrictor components of the responses were eliminated to unmask β-adrenergic vasodilation alone, not opposed by neural and humoral vasoconstriction. Following V_1 vasopressin receptor blockade combined with bilateral lumbar sympathectomy (Protocol 6) the iliac vasodilation response doubled in comparison to that observed following V_1 vasopressin receptor blockade alone (405.1± 58.4∫Δ% vs. 195.8±51.2∫Δ%, P=0.0175).

The above observations are supported by comparison of the frequency of which we observed increases vs. decreases in hemodynamic variables in each experiment for all experimental groups (Table 4). In intact animals, decreases in IVC prevailed

whereas following V_1 vasopressinergic blockade the increases in IVC prevailed. Combined V_1 vasopressinergic blockade and lumbar sympathectomy completely eliminated iliac vasoconstrictor responses (Protocol 6). AT_1 angiotensinergic blockade increased the number of pressor and vasoconstrictor events in comparison to the INT group (Table 4), although there were no significant differences between the averaged responses observed in this vs. intact groups (Figure 9). Adrenalectomy plus ganglionic blockade completely eliminated the depressor and iliac vasodilatory responses as expected (Protocol 3). When V_1 vasopressinergic blockade was added to adrenalectomy and ganglionic blockade (Protocol 4) or all the blockades were performed in adrenalectomized animals (Protocol 5) again variable vasoconstrictor/vasodilatory responses were observed; however, this variability reflected rather random variations of IVC as vascular responses were virtually abolished in these groups (changes in IVC were not different from zero, Figure 9). Prevailing pressor and depressor responses were consistent with prevailing vasoconstrictor and vasodilatory responses in each experimental group. However, the decreases in HR dominated in all experimental groups despite the different experimental conditions.

Release of vasopressin in response to stimulation of NTS A_1 adenosine receptors

The above results, obtained via pharmacological blockade approaches, strongly suggested that vasopressin is a dominant vasoconstrictor factor released into the circulation following the selective stimulation of NTS A_1 adenosine receptors. Therefore, in an additional two groups of animals we tested this hypothesis more directly by measuring whether the plasma levels of vasopressin indeed increase as a result of stimulation of NTS A_1 adenosine receptors (Figure 10). The resting levels of vasopressin were similar in both groups (P=0.931) and they were moderately elevated

in comparison to vasopressin levels normally observed in intact conscious animals[12; 32; 35; 53; 54] consistent with levels associated with anesthesia and surgical stress[12; 35]. Stimulation of NTS A_1 adenosine receptors increased circulating vasopressin levels over 4-fold (P=0.0006). Microinjection of ACF into the NTS also tended to increase the level of circulating vasopressin; however the increase did not reach statistical significance (P=0.083). Notably the difference between vasopressin levels measured across the groups, i.e. following microinjections of CPA vs. ACF was also significant (P=0.041).

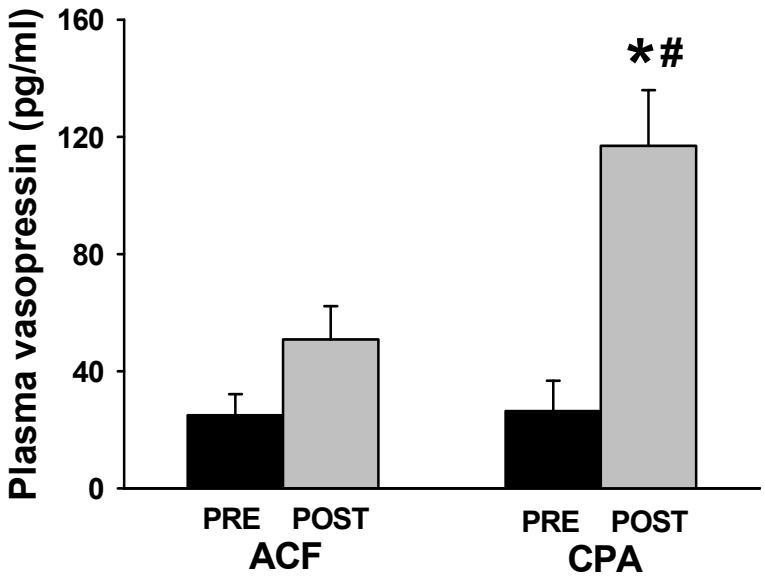

Figure 10. Comparison of plasma vasopressin levels measured before (Pre) and after (Post) microinjections into the NTS of vehicle (ACF, *n*=5) or selective A_1 adenosine receptor agonist (CPA, *n*=8). *, differences between pre- vs. post-microinjections (P<0.05); #, differences between the groups (P<0.05). CPA increased circulating vasopressin levels 4-fold whereas ACF did not significantly increase the level of circulating vasopressin.

DISCUSSION

The present study assessed the relative roles of three major humoral vasoconstricting factors in mediating the responses to stimulation of A_1 adenosine receptors located in the NTS. The release of humoral vasoconstrictor factor(s) had been implied by our previous study which showed that the large iliac vasoconstriction, triggered by stimulation of NTS A_1 adenosine receptors, persisted after bilateral lumbar sympathectomy and adrenalectomy[47]. The major finding of the present study is that vasopressin is released into the circulation upon stimulation of NTS A_1 adenosine receptors and that it is the primary humoral factor contributing to the iliac

vasoconstriction. The potential release of norepinephrine and angiotensin II did not contribute significantly to the iliac vascular responses.

NTS A_1 adenosine receptors are involved in control of vasopressin release

Our previous studies strongly suggested that A_1 adenosine receptors may modulate vasopressin release at the level of the NTS similarly as they modulate baroreflex control of regional sympathetic outputs and HR[71; 76]. However, considering differential localization/expression of adenosine receptor subtypes on functionally different NTS neurons[72; 77], it remained unknown if A_1 adenosine receptors are indeed located on these baroreflex neurons controlling vasopressin release. The present study showed that activation of A_1 adenosine receptors in the NTS disinhibits vasopressin release. Therefore, pre- and/or postsynaptic A_1 adenosine receptors are likely located on those NTS baroreflex neurons/terminals which control vasopressin release. This hypothesis based on integrative, systemic data may be confirmed in future studies at the cellular level.

The most likely mechanism responsible for the release of vasopressin into the circulation in response to stimulation of NTS A_1 adenosine receptors is the inhibition of baroreflex transmission in the NTS resulting in the disinhibition of vasopressin release from hypothalamic nuclei (paraventricular and supraoptic)[20]. The role of NTS in baroreflex control of vasopressin release has been well established[66; 83]. Bilateral inhibition, anesthetization or lesioning of the NTS, which removes baroreflex mechanisms, results in 10-fold increases of circulating vasopressin levels and vasopressin-mediated hypertension[83]. Sinoaortic baroreceptor denervation prevents this response[66]. In the present study the unilateral inhibition of NTS baroreflex mechanisms via unilateral activation of A_1 adenosine receptors resulted in 5-fold

increases in circulating vasopressin.

Relative roles of vasoactive factors triggered by activation of A_1 adenosine receptors in the NTS

Our previous study showed that activation of A_1 adenosine receptors in the NTS evokes neural and humoral iliac vasoconstriction opposed by β-adrenergic vasodilation[47]. The counteracting vascular effects contributed to the variability of the overall pressor/depressor responses with prevailing vasoconstriction and increases in MAP. These conclusions were based on comparison of iliac vascular responses observed in intact animals and following β-adrenergic blockade, bilateral adrenalectomy, lumbar sympathectomy and combined adrenalectomy plus lumbar sympathectomy. The present study investigated potential humoral vasoconstrictors (vasopressin, angiotensin II and norepinephrine) by assessing the effects of vasopressin V_1 receptor and angiotensin AT_1 receptor blockade as well as ganglionic blockade performed in adrenalectomized animals. Perhaps the most compelling results were that the blockade of peripheral V_1 vasopressin receptors reversed the normal iliac vasoconstriction into marked vasodilation. This vasodilation was further enhanced by adding lumbar sympathectomy to the V_1 receptor blockade. It is likely that sympathetic nerves have a smaller role than vasopressin in mediating this iliac vasoconstriction as lumbar sympathectomy alone, performed in our previous study[47], had a relatively smaller effect on the IVC responses then V_1 vasopressinergic blockade alone, performed in the present study. Collectively, these data indicate that vasopressin alone can override epinephrine induced iliac vasodilation and induce vasoconstriction, but that sympathetic nerves alone do not. The roles of vasopressin and sympathetic nerves appear additive as the sum of the individual effects approximate that of the combined

effects. Vasopressin together with increases in LSNA can induce significant vasoconstriction without which a powerful β adrenergic vasodilation is revealed. We conclude that the variability often seen in the arterial pressure response to A_1 receptor stimulation is the direct result of these "push-pull" counteracting mechanisms.

Circulating norepinephrine potentially released upon stimulation of NTS A_1 adenosine receptors likely has little role as ganglionic blockade did not lessen the intense iliac vasoconstriction seen after bilateral adrenalectomy[47]. Probably the amount of norepinephrine released from sympathetic terminals was too small to exert measureable effects beyond that of circulating norepinephrine already likely to be elevated as a result of anesthesia and surgical stress. A previous study from our laboratory showed that pre-ASNA increased much more than RSNA and LSNA in response to stimulation of NTS A_1 adenosine receptors[76]. It is possible that other sympathetic outputs responded even less or might even be inhibited with the stimulation. This could cause the increase of circulating norepinephrine in the present study to be functionally irrelevant.

Angiotensin II blockade alone markedly decreased baseline MAP and increased IVC. However the normal pressor and vasoconstrictor responses to microinjections of CPA did not decrease but rather tended to increase. This indicates that angiotensin II, similarly as circulating norepinephrine, does not contribute to the iliac vasoconstriction elicited by stimulation of NTS A_1 adenosine receptors. Although increases of RSNA may cause an increase in the release of renin from the kidney[23], the total time from activation of the renal nerve to the subsequent release of renin and conversion of angiotensinogen to angiotensin I and then to angiotensin II may have exceeded the time of the analyzed responses. In addition, losartan used to block AT_1 receptors in this

study most likely crossed the blood-brain barrier and might evoke central effects counteracting the peripheral actions. Nevertheless, our data show that circulating angiotensin II had no functional effect as a potential humoral iliac vasoconstrictor triggered by stimulation of NTS A_1 adenosine receptors.

Vasoactive effects of NTS A_1 vs. A_{2a} adenosine receptor subtypes

The predominately pressor but often variable responses to selective stimulation of NTS A_1 adenosine receptors are mediated most likely via inhibition of baroreflex glutamatergic transmission in the NTS and resetting of baroreflex functions toward higher MAP[71; 76]. In contrast, selective stimulation of NTS A_{2a} adenosine receptors evokes decreases in MAP and preferential iliac vasodilation mediated via non-baroreflex, nonglutamatergic mechanism(s)[37; 74; 75]. These responses were mediated mostly via activation of the adrenal medulla and β-adrenergic mechanism and to a lesser extent via lumbar sympathetic nerves[42]. However, no other humoral mechanisms were involved as bilateral adrenalectomy and lumbar sympathectomy abolished the responses[42]. How do these two adenosine receptor subtypes contribute to the responses mediated by adenosine operating in the NTS under physiological[19; 82] or pathological[56; 78; 89; 92] conditions? It is well known that microinjections of adenosine into the NTS results in depressor responses similar to those observed following selective activation of A_{2a} adenosine receptors[6; 51; 85]. However, both adenosine receptor subtypes may contribute to the depressor responses as they both activate the adrenal medulla and facilitate β-adrenergic vasodilation via baroreflex and unknown, non-baroreflex mechanisms[74; 76]. Other components of the responses elicited by NTS A_{2a} receptors (decreases in RSNA and HR) may further contribute to the depressor responses, whereas A_1 receptor-mediated sympathoactivation and vasopressin release

would oppose these responses. Under physiological conditions adenosine may contribute to the pressor effects evoked by stimulation of the hypothalamic defense area[19; 82] via A_1 receptor-mediated inhibition of baroreflex mechanisms and resulting sympathoactivation and vasopressin release[71; 76]. In contrast, under pathological conditions naturally released adenosine, acting via both A_1 and A_{2a} adenosine receptor subtypes, may contribute to the paradoxical sympathoinhibition and cardiac slowing observed during hypovolemic phase of hemorrhagic shock[78].

Limitations of the method

In the central nervous system adenosine is not released synaptically, but rather produced in the extracellular space via the degradation of ATP which is released at synaptic terminals from neurons as well as released from glial cells activated by extracellular glutamate diffused from nearby active nerve terminals[9; 13; 33; 94]. In addition, under pathological conditions adenosine is released into the extracellular space in a global, non-synaptic manner from hypoxic/hypoperfused neurons and glial cells[56; 56; 89; 92]. Therefore the natural, spatial (not strictly synaptic) action of adenosine in the NTS, may be simulated well via microinjections of selective agonists of adenosine receptor subtypes. Importantly, although adenosine is released non-selectively in the NTS, it does produce specific differential responses in regional sympathetic outputs and vascular beds as shown by numerous studies from our laboratory[7; 71; 73-76]. We believe that these specific, differential effects evoked by nonspecific, spatial activation of adenosine receptors result from differential location/expression of adenosine receptor subtypes on NTS neurons and synaptic terminals controlling different sympathetic outputs and involved in different mechanisms integrated in the NTS[72; 77]. To confirm this hypothesis, based on systemic, integrative approaches, further studies at the

cellular level are required.

The experiments were performed in anesthetized animals and these conditions most likely attenuated baroreflex mechanisms which may have contributed to the increased vasopressin levels. In the conscious rat circulating vasopressin level is ~ 2 pg/ml[12; 32; 35; 53; 54; 61]. With even limited surgery under anesthesia baseline vasopressin rises to ~6-10 pg/ml[12; 35; 66; 83]. In our study after extensive surgery under anesthesia baseline vasopressin levels were ~25 pg/ml. However, despite the increased baseline vasopressin levels, activation of NTS A_1 adenosine receptors triggered marked release of vasopressin into the circulation (Figure 10) and a powerful V_1 receptor mediated vasoconstrictor effect in the hindlimb vasculature was apparent (Figure 9). In contrast, no functional effects of potential, A_1-adenosine-receptor-mediated increases in circulating norepinephrine or angiotensin II were observed.

Glucocorticoid deficiency, which may be observed following chronic adrenalectomy, increases circulating levels of vasopressin[41]. However, this effect seems to be mediated at the level of transcription of the vasopressin gene and not an immediate release[41]. Therefore, in the animals in which the adrenal glands were removed acutely, as in the present study, increased resting levels of vasopressin were most likely due to anesthesia and surgical stress.

The large and sustained changes in resting hemodynamic variables following AT_1 angiotensinergic and ganglionic blockades required compensation with iv infusions of phenylephrine to make the relative responses comparable across the experimental groups. It was extremely difficult to return both MAP and IVC to the pre-blockade levels. Since we tried to compensate most accurately for IVC, the resting MAP, preceding the microinjections into the NTS in the groups where ganglionic blockade was

combined with V_1 vasopressinergic and with AT_1 angiotensinergic blockades (Protocols 4 and 5), was slightly lower in comparison to other experimental groups. The long lasting compensatory infusions of phenylephrine in Protocols 2-5 could facilitate central baroreflex mechanisms[38]. Therefore, the inhibition of enhanced baroreflex activity via stimulation of NTS A_1 adenosine receptors could result in relatively greater pressor and vasoconstrictor responses compared to those observed in intact animals and following V_1 vasopressinergic blockade alone (Protocol 1) and V_1 vasopressinergic blockade combined with lumbar sympathectomy (Protocol 6). In fact, pressor and vasoconstrictor responses tended to increase following AT_1 receptor blockade although this tendency did not reach statistical significance. The pressor and vasoconstrictor responses observed after adrenalectomy combined with ganglionic blockade (Protocol 3) were not different from those observed following adrenalectomy alone in our previous study[47]. Finally, these responses (even if slightly exaggerated) were virtually abolished by the vasopressinergic blockade (Protocols 4 and 5). Therefore, the potential central effects of infusions of phenylephrine on responses to stimulation of NTS A_1 adenosine receptors were likely negligible and did not affect the primary conclusions of the present study. The infused phenylephrine activated α_1 adrenergic receptors located on vascular smooth muscles, including those in the iliac vascular bed. The occupation of α_1 adrenergic receptors by an exogenous agonist could mask the effect of circulating endogenous norepinephrine which may be potentially increased following stimulation of NTS A_1 adenosine receptors. The peripheral interactions between simultaneously activated V_1, α_1 and AT_1 receptors could further complicate the comparison of relative roles of humoral vasoconstrictor factors obscuring the smaller ones. Nevertheless, the

marked effects of vasopressin V_1 receptor blockade were evident in experimental groups with and without phenylephrine compensation (Protocols 1 and 6 vs. Protocols 4 and 5). This indicates that vasopressin contribution to the iliac vasoconstriction was much larger than the contribution (if any) of other potential vasoconstrictors (norepinephrine and/or angiotensin II).

Conclusion

Selective stimulation of NTS A_1 adenosine receptors triggers the release of vasopressin into the circulation, strongly suggesting that A_1 adenosine receptors are likely located on afferent terminals and/or NTS interneurons mediating baroreflex control of vasopressin release. Vasopressin is a major humoral vasoconstrictor factor contributing to the iliac vascular responses. The natural variability of MAP and IVC responses to stimulation of NTS A_1 adenosine receptors observed in intact animals is a result of the simultaneous triggering of sympathetic and vasopressinergic vasoconstriction counteracted by β-adrenergic vasodilation resulting mainly via epinephrine release in response to the large increase in adrenal pre-ganglionic sympathetic activity.

CHAPTER 2

Activation of NTS A_1 adenosine receptors provides differential neural and humoral control of regional vascular beds

ABSTRACT

Our previous studies showed that stimulation of adenosine A_1 receptors located in the nucleus of the solitary tract (NTS) exerts counteracting effects on the iliac vascular bed: activation of the adrenal medulla and β-adrenergic vasodilation versus sympathetic and vasopressinergic vasoconstriction. Since NTS A_1 adenosine receptors inhibit baroreflex transmission in the NTS and contribute to the pressor component of the hypothalamic defense response, we hypothesized that these receptors also contribute to the redistribution of blood from the visceral to the muscle vasculature via prevailing sympathetic and vasopressinergic vasoconstriction in the visceral (renal and mesenteric) vascular beds and prevailing β-adrenergic vasodilation in the somatic (iliac) vasculature. To test this hypothesis we compared the A_1 adenosine-receptor-mediated effects of each vasoactive factor triggered by NTS A_1 adenosine receptor stimulation (N^6-cyclopentyladenosine, CPA, 330 pmol in 50 nl) on the regional vascular responses in urethane/chloralose anesthetized rats. The single factor effects were separated using adrenalectomy, β-adrenergic blockade, V_1 vasopressinergic blockade and sinoaortic denervation. In intact animals initial vasodilation was followed by large, sustained vasoconstriction with smaller responses observed in renal vs. mesenteric and iliac vascular beds. The initial β-adrenergic vasodilation prevailed in the iliac vs. mesenteric and renal vasculature. The large and sustained vasopressinergic vasoconstriction was similar in all vascular beds. Small sympathetic vasoconstriction was observed only in the iliac vasculature in this setting. We conclude that although A_1 adenosine-receptor-

mediated β-adrenergic vasodilation may contribute to the redistribution of blood from the visceral to the muscle vasculature, this effect is overridden by sympathetic and vasopressinergic vasoconstriction.

INTRODUCTION

Recent studies have firmly established that adenosine operating as a neuromodulator in the nucleus of the solitary tract (NTS) modifies cardiovascular control [30; 37; 47; 48; 71; 77; 78; 80-82]. The NTS is a major integrative center for visceral and autonomic reflexes and contains the greatest density of adenosine uptake sites within the central nervous system [11]. Adenosine operates in the NTS in both physiological and pathological situations. Under normal, physiological conditions a natural source of adenosine is ATP released from neurons and glial cells[13]. This occurs, for example during the stress or hypothalamic defense response[80-82]. Extracellular ATP is catabolized via ectonucleotidases to adenosine which acts globally on pre or post-synaptic A_1 or A_{2a} adenosine receptors located in the NTS[13; 94]. Adenosine is also released under pathological conditions such as ischemia, hypoxia and severe hemorrhage via the breakdown of intracellular ATP[56; 89; 92]. Therefore, adenosine is an important neuromodulator helping to regain homeostasis in these life threatening situations via specific modulation of central mechanisms of cardiovascular control. Although adenosine is released globally, it may however, differentially modulate autonomic reflexes integrated in the NTS[37; 71]. The differential action of adenosine is most likely due to differential localization of adenosine receptor subtypes on NTS terminals/interneurons involved in different reflexes which finally target different sympathetic outputs and vascular beds as we suggested previously[72; 77].

Adenosine may either inhibit or facilitate neurotransmission via A_1 or A_{2a}

receptors, respectively. Selective stimulation of A_1 versus A_{2a} receptors often, but not always, exerts counteracting hemodynamic effects. Stimulation of A_1 adenosine receptors in the NTS yields predominately pressor and vasoconstrictor effects in the hindlimb accompanied with differential regional sympathoactivation (adrenal>renal≥lumbar)[76]. In contrast, NTS A_{2a} adenosine receptor stimulation yields depressor responses, decreases in renal sympathetic nerve activity (RSNA), no changes in lumbar sympathetic nerve activity (LSNA) and activation of pre-ganglionic adrenal sympathetic nerve activity (pre-ASNA)[73; 74]. Interestingly, both receptor subtypes preferentially activate the adrenal medulla. We have previously shown that both A_1 and A_{2a} adenosine receptors evoke β-adrenergic vasodilation in the iliac vascular bed, as β-adrenergic receptors are predominantly located in the skeletal muscle vasculature[90]. However, stimulation of A_1 adenosine receptors alone in the NTS often yields variable responses, although predominantly pressor and iliac vasoconstrictor responses prevail[76]. Recent studies have attributed this variability to the activation of the adrenal medulla, the subsequent release of epinephrine eliciting activation of β-adrenergic vasodilation which is simultaneously opposed by sympathetic and vasopressinergic vasoconstriction[47; 48]. The increased vasoconstrictor drive and simultaneously triggered β-adrenergic vasodilation were most likely mediated via inhibition of NTS baroreflex mechanisms[68; 71; 76].

Stimulation of NTS A_1 adenosine receptors differentially affects baroreflex control and baseline levels of regional sympathetic outputs, and baroreflex control of vascular beds is regionally different[25; 64; 71]. Furthermore, there is differential regional distribution of α_1 and β_2-adrenergic receptors[36; 90] as well as differential reactivity of

regional vascular beds to vasopressin and α_1-adrenergic activation[28; 29; 34; 45]. Therefore it was likely that the contribution of vasoconstrictor and vasodilatory effects may be different between somatic (iliac) vs. visceral vascular beds (mesenteric and renal). In support of this hypothesis it has been shown that adenosine, operating mostly via A_1 receptors located in the NTS and rostral ventrolateral medulla, participates in the pressor component of the hypothalamic defense response[80-82] and may possibly contribute to the redistribution of blood flow from the viscera to skeletal muscle which is a key element of this response[93]. We hypothesized that NTS A_1 adenosine receptor induced sympathetic and vasopressinergic vasoconstriction would prevail over β-adrenergic vasodilation to a greater extent in the visceral vascular beds compared to muscle. To address this hypothesis we compared the simultaneously recorded vascular responses of iliac, mesenteric and renal vascular beds evoked by selective activation of NTS A_1 adenosine receptors in intact animals and after selective elimination of each major vasodilating and vasoconstricting factor.

MATERIALS AND METHODS

All protocols and surgical procedures employed in this study were reviewed and approved by the institutional Animal Care and Use Committee and were performed in accordance with the *Guiding Principles in the Care and Use of Animals* endorsed by the American Physiological Society and published by the National Institutes of Health.

Design

The relative contribution of three neural and humoral vasoactive factors (sympathetic and vasopressinergic vasoconstriction opposed by β-adrenergic vasodilation) to the regional vascular responses evoked by selective stimulation of NTS

A_1 adenosine receptors were studied in 53 male Sprague Dawley rats. The relative changes in iliac (IVC), mesenteric (MVC) and renal (RVC) vascular conductance evoked by microinjections into the NTS by the selective A_1 adenosine receptor agonist N^6-cyclopentyladenosine (CPA, Tocris) observed in intact animals (*n*=8) were compared with the responses evoked after the following six experimental procedures:

Protocol 1, bilateral adrenalectomy (ADX, *n*=8)

Protocol 2, blockade of β-adrenergic receptors (βX, *n*=7)

Protocol 3, selective vasopressin V_1 receptor blockade (VX, *n*=7)

Protocol 4, sinoaortic denervation (SAD, *n*=7)

Protocol 5, bilateral adrenalectomy combined with selective vasopressin V_1 receptor blockade (ADX+VX, n=8)

Protocol 6, sinoaortic denervation combined with bilateral adrenalectomy (SAD+ADX, *n*=8).

Blockade of β-adrenergic receptors was performed as in our previous studies via injection of propranolol (2 mg/kg i.v.)[42; 47]. Vasopressin V_1 receptors, mediating vascular constriction, were blocked by i.v. injections of [β-Mercapto-β,β-cyclopentylmethylenepropionyl,1-O-Me-Tyr,2Arg8]-Vasopressin, selective V_1 antagonist, (20 μg/kg, Sigma) in doses which completely blocked vasoconstriction evoked by i.v. injection of vasopressin (50 mU/kg, Sigma). The blockades were applied ~10 min before the microinjection into the NTS[47; 48]. Sinoaortic denervation was accomplished at the beginning of surgery, as described previously[69; 76]. The completeness of the denervation was tested ~2 hr later before the start of the protocol. The procedure was considered complete if intravenous phenylephrine-induced

increases in MAP > 30 mmHg did not decrease HR more than 5 beats/min.

Instrumentation and measurements

All procedures were described in detail previously[7; 42; 47; 48; 68; 74-76]. Briefly, male Sprague-Dawley rats (350-400 g, Charles River) were anesthetized with a mixture of α-chloralose (80 mg/kg) and urethane (500 mg/kg) i.p., tracheotomized, connected to a small animal respirator (SAR-830, CWE, Ardmore, PA) and artificially ventilated with 40% oxygen 60% nitrogen mixture. Catheterizations of the right femoral artery and vein were performed to monitor arterial blood pressure and infuse drugs. Arterial blood gases were tested occasionally for appropriate experimental values (Radiometer, ABL500, OSM3). Averaged values measured at the end of each experiment were the following: pH =7.37±0.01, Po_2 =144.2±5.0 mmHg, and Pco_2 = 36.2±0.9 mmHg.

From a mid-abdominal incision, the left iliac artery, superior mesenteric artery and the left renal artery were exposed. Pulsed Doppler blood flow velocity transducers (Baylor Electronics) were placed around the arteries and connected to the flowmeter. From the same mid-abdominal incision, in some animals, bilateral adrenalectomy was performed.

Arterial blood pressure and iliac, mesenteric and renal and flow signals were digitized and recorded with an analog-digital converter (Modular Instruments) interfaced to a laboratory computer. The signals were recorded continuously using Biowindows software (Modular Instruments), averaged over 5 second intervals and stored on hard disk for subsequent analysis.

Microinjections into the NTS

After the exposure of the brainstem via dissection of the atlantooccipial the animals were allowed to stabilize for at least 30 minutes before microinjections.

Unilateral microinjections of CPA (330 pmol in 50 nl of artificial cerebrospinal fluid, ACF) were made through multibarrel glass micropipettes into the medial region of the caudal subpostremal NTS as described previously[7; 68; 74-76]. This dose of CPA produced most

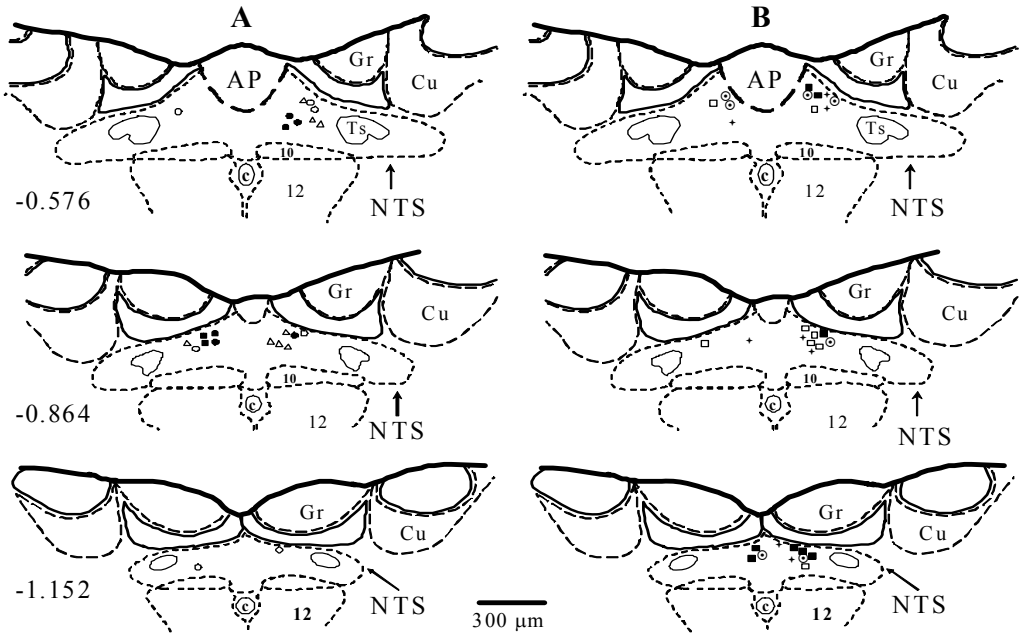

Figure 11. Microinjection sites in the caudal subpostremal NTS for all experiments. Schematic diagrams of transverse sections of the medulla oblongata from a rat brain. NTS, nucleus tractus solitarii; AP, area postrema; c, central canal; 10, dorsal motor nucleus of the vagus nerve; 12, nucleus of the hypoglossal nerve; Ts, tractus solitarius; Gr, gracile nucleus; Cu, cuneate nucleus. Scale is shown at the bottom; the number on the left side of the schematic diagram denotes the rostro-caudal position in millimeters of the section relative to the obex according to the atlas of the rat subpostremal NTS by Barraco et al. (3). Microinjection sites were marked with fluorescent dye and are denoted with the following symbols *A*: microinjections of CPA in intact animals (●), after bilateral adrenalectomy (△), and following β-adrenergic blockade (○). *B:* microinjections of CPA after V_1 vasopressin receptor blockade (□), following bilateral sinoaortic denervation (⊙), after combined adrenalectomy and V_1 vasopressinergic blockade (■) and following combined sinoaortic denervation and adrenalectomy (✦).

consistent, predominantly pressor responses in our previous study[76]. The CPA was dissolved in ACF and the pH adjusted to 7.2. In several previous studies we have shown that microinjections of the same amount of vehicle (ACF) into the same site of the NTS did not markedly affect MAP, HR, RSNA, LSNA and pre-ganglionic adrenal sympathetic nerve activity (pre-ASNA) and regional vascular blood flows[7; 67; 71; 73-76]. The changes in all these variables were either not different from zero or smaller than

natural, random fluctuations of these variables over the time of measurements. To avoid the effect of desensitization of A_1 adenosine receptors, in all experiments only one dose of the agonist was microinjected into left or right side of the NTS. All microinjection sites were verified histologically (Figure 11).

Experimental protocols

Comparing regional vascular responses obtained in intact vs. specific experimental conditions, we evaluated the specific contribution of three major vasoactive factors (β-adrenergic vasodilation vs. sympathetic and vasopressinergic vasoconstriction) to the responses of somatic (iliac) vs. visceral (mesenteric and renal) vascular beds evoked by selective activation of A_1 adenosine receptors in the NTS. Our previous studies showed that only these three vasoactive factors significantly contributed to iliac vascular responses evoked by stimulation of NTS A_1 adenosine receptors.

Basic experimental protocols allowed for comparisons of how elimination of one vasoactive factor affects the responses observed in intact animals. However, selective denervation of each vascular bed, which would allow for selective removal of the sympathetic component of the responses, was extremely difficult and in a few successful experiments arterial pressure was markedly lower than in other experimental groups (<70 mmHg). Therefore we evaluated the contribution of sympathetic vasoconstriction alone to the regional vascular responses indirectly by removing the adrenal and vasopressinergic components of the responses via adrenalectomy combined with systemic V_1 vasopressinergic blockade (Protocol 5). Adrenalectomy and β-adrenergic blockade (Protocols 1 and 2, respectively) removed β-adrenergic vasodilation; therefore, these protocols showed how sympathetic and vasopressinergic

vasoconstriction triggered by activation of NTS A_1 adenosine receptors affected regional vascular beds. Blockade of V_1 vasopressinergic receptors (Protocol 3) removed the vascular action of vasopressin; therefore, showed how regional vascular beds respond to β-adrenergic vasodilation opposed by sympathetic vasoconstriction. The contribution of the vasopressinergic component alone to the regional vascular responses was evaluated by subtracting sympathetic vasoconstriction (evaluated in Protocol 5) from combined vasopressinergic and sympathetic vasoconstriction (Protocol 1). The contribution of β-adrenergic vasodilation alone to the regional vascular beds responses was evaluated by subtracting sympathetic vasoconstriction (Protocol 5) from combined sympathetic neural and adrenergic responses (evaluated in Protocol 3). To evaluate separate effects of each humoral factor we subtracted the sympathetic component of the responses (averaged values obtained in Protocol 5) from each single animal response in Protocols 1 and 3 and then averaged the data in each experimental group.

In addition, sinoaortic denervation (Protocol 4), which removed baroreflex vasopressinergic and sympathetic vasoconstriction and the baroreflex dependent part of the activation of the adrenal medulla, evaluated the relative regional vasodilation mediated via the non-baroreflex component of the activation of the adrenal medulla[76]. Finally, sinoaortic denervation combined with bilateral adrenalectomy removed all three major vasoactive factors triggered by NTS A_1 adenosine receptor stimulation. This protocol tested if the three vasoactive factors (sympathetic and vasopressinergic vasoconstriction opposed by β-adrenergic vasodilation), known to affect the somatic (iliac) vascular bed[47; 48], are the only major factors affecting visceral vascular beds (mesenteric and/or renal) in response to stimulation of NTS A_1 adenosine receptors.

Data Analysis

The regional vascular conductance was calculated similarly as in our previous studies by dividing the regional blood flows, expressed as Doppler shift (in Hz), by MAP (in mmHg)[47; 48]. The absolute values of vascular conductance depend to some extent on positioning of the probe around the arteries; therefore comparisons between the relative changes were more reliable. In all experimental groups the hemodynamic data were averaged in 1 min intervals over a 20 min period of the response. Since the responses were biphasic: initial decrease in MAP and vasodilation (~ first 5 min of the response) followed by the pressor and vasoconstrictor phase of the response (last ~15 min). In addition we calculated the overall responses for each vascular bed calculating the integral of the response for first 5 min of the responses, where vasodilation prevailed, and the last 15 min of the responses, where vasoconstriction prevailed. To make the integral values comparable between the phases of the responses we normalized the integral values to one minute by dividing the total integral for the first and second phase of the response by 5 and 15, respectively.

Two way ANOVA was used to evaluate the relative contribution of the three vasoactive factors (sympathetic and vasopressinergic vasoconstriction opposed by β-adrenergic vasodilation) to the responses of the three vascular beds (iliac, mesenteric and renal) in respect to the integral responses. Two way ANOVA was also used for comparing the dynamics of the response in each vascular bed observed under each experimental condition, and for each single vasoactive factor (20 time points of the responses vs. 3 vascular beds). A t-test with Bonferroni adjustments was used for the calculation of specific differences between the groups for those comparisons where interactions of the vasoactive factors or the time vs. the vascular beds were significant.

RESULTS

Resting values of MAP, HR, iliac, mesenteric and renal blood flow and conductance for each experimental group, measured just before stimulation of NTS A_1

Table 6. Resting values of hemodynamic parameters in each experimental group.

Experimental procedure	*n*	Mean Arterial Pressure, mmHg	Heart Rate, beats/min	Blood Flow, Hz			Vascular Conductance, Hz/mmHg		
				Iliac	Mesenteric	Renal	Iliac	Mesenteric	Renal
INTACT	8	91.4±4.0	323.5±9.4	826.6±63.1†‡	1228.4±72.3	1771.9±213.7	9.3±1.0†‡	13.7±1.2	19.3±2.2
ADX	8	82.3±1.8	336.1±16.3	834.4±68.7†‡	1344.0±169.7•	2283.6±215.2	10.3±0.9†‡	16.4±2.0•	27.8±2.5*
ßX	7	92.1±3.8	300.0±7.5	697.0±71.3‡	1424.8±138.8	1761.4±307.3	7.6±0.7‡	15.5±1.3	19.3±3.6
VX	7	81.2±2.6	351.7±22.3	997.0±194.4‡	1184.6±129.8•	2685.2±123.5*	12.5±2.6‡	14.6±1.5•	33.1±1.3*
SAD	7	86.1±5.7	386.7±16.3*	732.4±86.8‡	1150.7±163.7	1609.9±200.2	8.7±1.2†‡	13.6±1.9	18.7±2.1
ADX+VX	8	76.5±2.7*	341.8±10.1	1554.6±149.1*	1384.8±154.4	1550.9±153.3	20.6±2.2*	18.6±2.4	20.3±1.9
SAD+ADX	8	78.8±2.3*	414.4±21.9*	1076.3±192.2‡	969.1±71.7*•	2115.6±231.5	13.7±2.4‡	12.4±1.0•	27.1±3.2

Data are means ±SE; *n*=number of rats. Resting values for intact (INT) animals and following: bilateral adrenalectomy (ADX), β-adrenergic blockade (βX), vasopressin V_1 receptor blockade (VX), adrenalectomy +V_1 receptor blockade (ADX+VX), sinoaortic denervation (SAD) and sinaortic denervation + adrenalectomy (SAD+ADX). *P<0.05 VS INTACT. P<0.05: † Iliac vs. mesenteric, ‡ Iliac vs. renal, • Mesenteric vs. renal.

adenosine receptors, are presented in Table 6. In most cases vascular conductance observed in specific beds in all experimental groups was not different from that observed in intact animals; the only exemptions were the increase in iliac vascular conductance following bilateral adrenalectomy combined with V_1 vasopressinergic blockade and a smaller increase in renal conductance following V_1 adrenalectomy alone and vasopressinergic blockade alone. Therefore changes in the relative vascular responses evoked by activation of NTS A_1 adenosine receptors were comparable across the experimental groups with only small limitations. HR was significantly higher in the two groups where sinoaortic denervation was performed, as expected. MAP was lower compared to intact animals only in two experimental groups: following bilateral

adrenalectomy combined with V_1 vasopressinergic blockade or adrenalectomy combined with sinoaortic denervation.

The maximal effects evoked by β-adrenergic and V_1 vasopressinergic blockades in the regional vascular beds and MAP are presented in Table 7. Note that iliac vasoconstriction in response to β-adrenergic blockade was approximately twice as large as that observed in the mesenteric and renal vascular beds. This suggests larger tonic β-adrenergic vasodilation in the iliac vs. both visceral vascular beds. In contrast, no significant differences between the regional vascular beds were observed in response to V_1 vasopressinergic blockade.

Table 7. Average maximum hemodynamic responses evoked by β-adrenergic and V_1 vasopressinergic blockades.

Experimental procedure	*n*	Δ%MAP	Δ%HR	Δ% Iliac	Δ% Mesenteric Vascular Conductance	Δ% Renal
βX	7	19.2±4.0	-8.6±1.6	-30.3±4.2†‡	-16.2±4.0	-13.0±3.3
VX	8	-16.6±4.7	6.1±4.0	38.4±9.9	26.7±6.3	25.9±7.4
ADX + VX	8	-30.6±5.2	12.9±5.0	55.6±11.7†‡	38.4±7.4	27.9±8.6

Data are means ± SE; *n* = number of rats. Beta adrenergic blockade (βX), Vasopressin V_1 receptor blockade (VX). P<0.05:† Iliac vs. mesenteric, ‡ Iliac vs. renal. No significant differences between mesenteric vs. renal vascular beds were observed.

Comparison of hemodynamic responses observed in intact vs. experimental groups

Figure 12 presents comparisons of averaged responses evoked by selective stimulation of NTS A_1 adenosine receptors in intact animals and following specific experimental procedures. Two way ANOVA showed significant differences between all experimental vs. intact conditions except for MVC in adrenalectomized animals and RVC following combined adrenalectomy plus vasopressinergic blockade, which did not reach statistical significance (P=0.195 and P=0112, respectively) (Figure 12). In intact animals the typical variability of the responses to stimulation of NTS A_1 adenosine receptors was observed, similar as that reported in previous studies from our

laboratory[47; 48; 76]. In the initial phase of the responses (approximately first 5 minutes) depressor and vasodilatory responses prevailed. In the subsequent 15 minutes (or

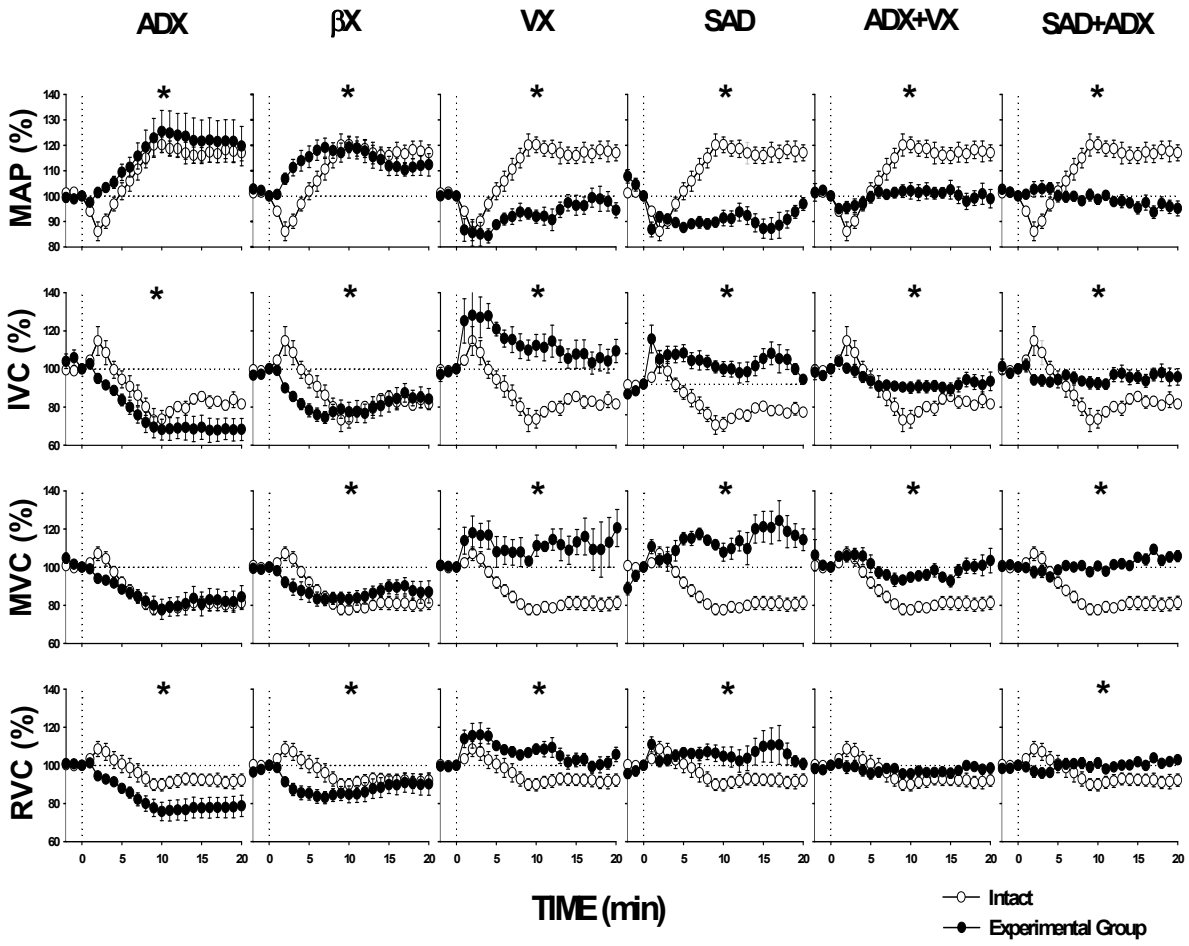

Figure 12. Mean arterial pressure (MAP), iliac (IVC), mesenteric (MVC) and renal (RVC) vascular conductance responses to microinjection of adenosine A_1 receptor agonist (CPA, 330 pmol/50 nl) into the subpostremal NTS in intact rats (-○-, open circles) compared to the responses evoked following different experimental procedures (-●-, filled circles). Data are means ± SE. *P<0.05 vs. intact animals. Abbreviations: bilateral adrenalectomy (ADX), β-adrenergic blockade (βX), vasopressin V_1 receptor blockade (VX), bilateral sinoaortic denervation (SAD), bilateral adrenalectomy + vasopressin V_1 receptor blockade (ADX+VX) and bilateral sinoaortic denervation + bilateral adrenalectomy (SAD+ADX).

more) of the responses pressor and vasoconstrictor responses prevailed. Overall, the increases in mean arterial pressure (MAP) and vasoconstriction in all vascular beds dominated over 20 minutes of the responses in the intact group. Adrenalectomy and β-adrenergic blockade eliminated initial vasodilation thus sympathetic and

vasopressinergic vasoconstriction dominated in these groups. Blockade of V1 vasopressinergic receptors virtually eliminated the vasoconstrictor component of the responses; adrenergic vasodilation prevailed over sympathetic vasoconstriction in this setting in all vascular beds. Sinoaortic denervation eliminated reflex sympathetic vasoconstriction and vasopressin release(48; 76). In this situation non-baroreflex activation of the adrenal medulla(76) was smaller than the activation mediated via combined baro- and non-baroreflex mechanisms; however, in this setting the vasodilatory factor was not opposed by any vasoconstricting factors (sympathetic nerves and vasopressin). Therefore, vasodilation observed in this group did not differ substantially compared to that observed after blockade of V1 vasopressin receptors alone. Combined adrenalectomy and V1 vasopressinergic blockade eliminated both major humoral factors, revealing that the contribution of sympathetic vasoconstriction to the responses was very small, especially in the mesenteric and renal vascular beds. Finally, combined sinoaortic denervation and bilateral adrenalectomy eliminated all three primary vasoactive factors triggered by stimulation of NTS A1 adenosine receptors and these residual responses were not different from those where only sympathetic nerves were active. Since in most of the experimental groups more than one vasoactive factor was at play, the relative comparisons of the effect of the vasoactive factors on regional vascular beds was rather complex, especially that these factors may differentially interact with each other in a specific vascular bed. To simplify the picture we further compared regional vascular responses due to the separate effects of sympathetic nerves, vasopressin and epinephrine; these single factor effects were calculated from basic experimental groups as described in the *experimental protocols* section.

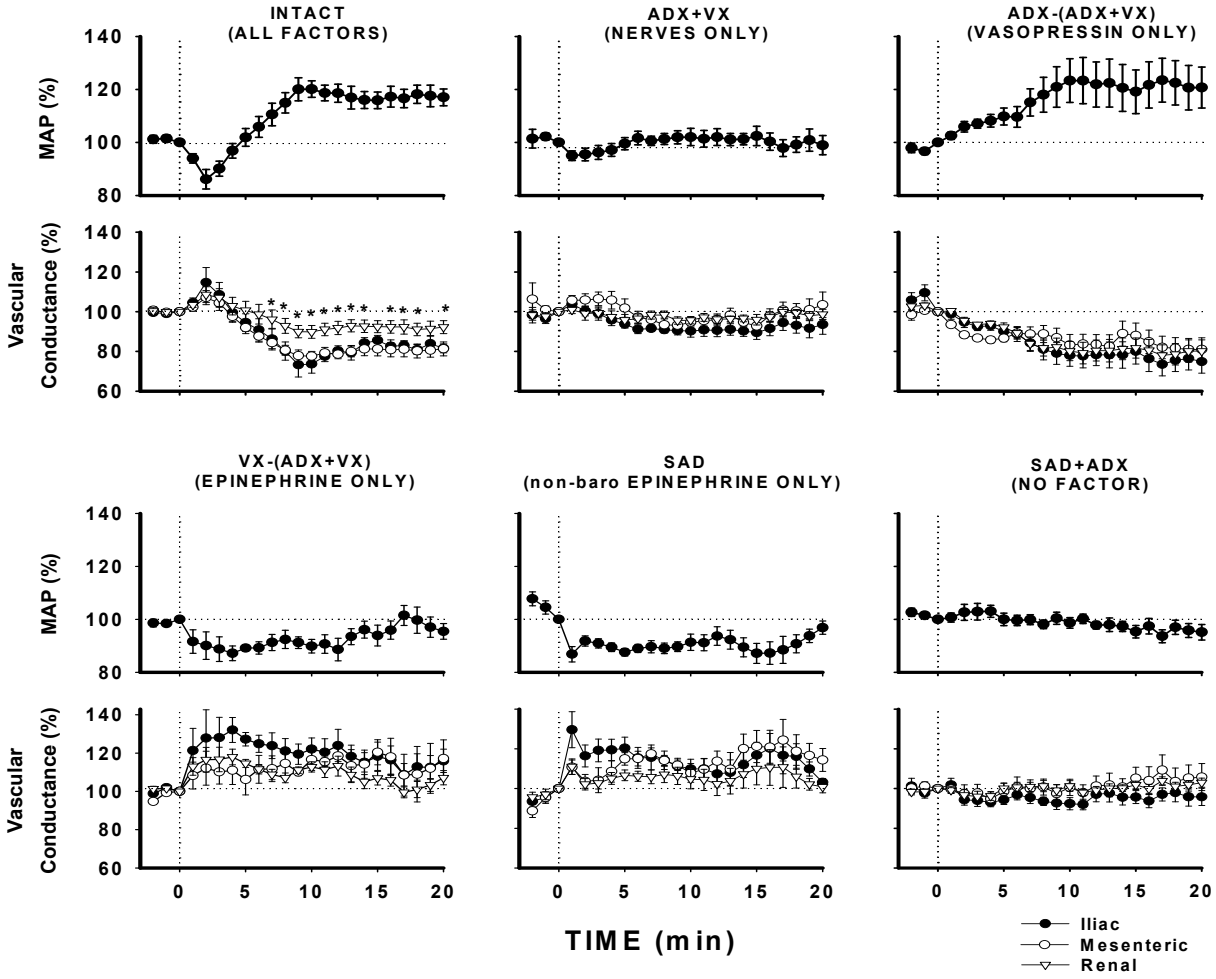

Figure 13. Comparison of regional hemodynamic responses (iliac, mesenteric and renal) mediated via three major vasoactive factors (sympathetic nerves, vasopressin and epinephrine) triggered by selective stimulation of A_1 adenosine receptors located in the NTS (microinjections of CPA, 330 pmol/50 nl). Data are means ± SE. *, P<0.05 renal vs. iliac and mesenteric vascular beds. Abbreviations as in Figure 2. Single factor effects were calculated from basic experimental groups: sympathetic nerve effect (ADX+VX), vasopressin ((ADX-(ADX+VX)), epinephrine ((VX-(ADX+VX)), and epinephrine released via non-baroreflex mechanism following sinoaortic denervation (SAD). These effects are compared to combined effect of all vasoactive factors operating in intact animals and after elimination of all major vasoactive factors in

Differential regional vascular responses to single vasoactive factors triggered by stimulation of NTS A_1 adenosine receptors

Figure 13 compares the averaged responses of three vascular beds (iliac vs. mesenteric vs. renal) mediated via the three major vasoactive factors (sympathetic nerves, vasopressin and epinephrine) activated by the selective stimulation of A_1 adenosine receptors located in the NTS. The impact of each factor on each vascular

bed was compared with the combined impact of all these factors in intact animals and with a "negative control" group where activation of all these factors was prevented via combined bilateral sinoaortic denervation and adrenalectomy (SAD+ADX). Two way ANOVA did not show significant differences between time-courses of regional vascular responses evoked by each vasoactive factor alone ($P>0.05$ for vascular bed x time interactions). Nevertheless, all these factors acting together in intact animals revealed significantly smaller vasoconstriction in renal vs. both the iliac and mesenteric vascular beds, especially so in the later phase of the responses ($P=0.024$ for vascular beds x time interaction) (Figure 13, first upper panel). The lack of differences between time-courses of whole vascular responses to single vasoactive factors were not surprising taking into consideration the biphasic dynamics of the responses: β-adrenergic vasodilation prevailed in first 5 min of the responses whereas neural and vasopressinergic vasoconstriction prevailed in the remaining portion of responses. The differences between regional vascular responses became significant when both phases of the responses were analyzed separately as an integral of the first 5 minutes (mostly vasodilation) and the last 15 minutes (mostly vasoconstriction) of the responses (Figure 14 A and B). In the initial phase of the responses (first 5 minutes) two way ANOVA showed significant vascular bed vs. experimental condition interaction ($P=0.026$), whereas in the later phase the regional vascular responses were more uniform ($P= 0.441$ and $P= 0.205$ for vascular beds and vascular bed x experimental conditions interaction, respectively).

Interestingly, in the initial phase of the responses virtually no neural vasoconstriction was observed; the mesenteric vascular bed even tended to dilate ($P=0.114$ vs. zero). In the later phase of the response iliac vasoconstriction was

significant (P=0.010 vs. zero) whereas renal and mesenteric vasoconstrictions remained very small and not different from zero. Vasopressin initially evoked relatively small responses with significantly greater vasoconstriction in the mesenteric vs. the iliac and renal vascular beds (Figure 14A); however, in the later phase of the responses vasopressinergic vasoconstriction increased systematically and large, sustained

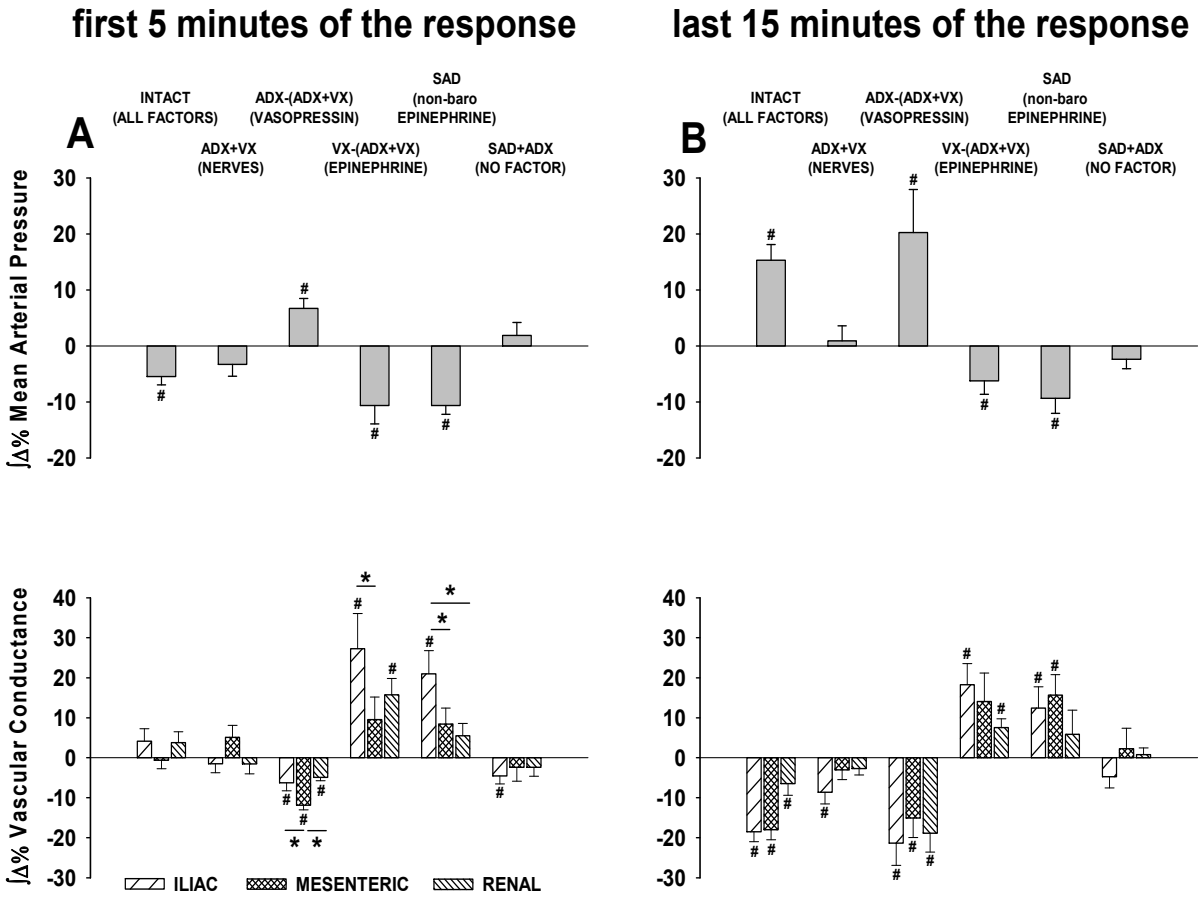

Figure 14. Integral hemodynamic responses (iliac, mesenteric and renal) evoked by selective stimulation of A_1 adenosine receptors located in the NTS. Data were calculated from traces presented in Figure 3. Panel A, integrals measured over the first 5 min of the responses, when decreases in MAP and vasodilation prevailed. Panel B, integrals measured over the last 15 min of the responses, when increases in MAP and vasoconstriction prevailed. Data are means ± SE. *, P<0.05 between vascular beds linked with the horizontal lines. #P<0.05 vs. zero. Abbreviations as in Figure 2.

vasoconstriction with no differences between the vascular beds was observed (Figure 13, left upper panel). In the initial phase of the responses iliac β-adrenergic vasodilation was significantly greater than that observed in the mesenteric vascular bed (P=0.020)

and tended to be greater than that observed in the renal vasculature (P=0.113) (Figure 14A). In the later phase of the responses these regional differences persisted to some degree (Figure 14B). The regional effects of epinephrine released via non-baroreflex mechanisms (in sinoaortic denervated animals) were similar to those observed when the adrenal medulla was activated by both baro- and non-baroreflex mechanisms (Figures 13 and 14 A and B). Finally, the elimination of all three vasoactive factors by means of combined bilateral sinoaortic denervation and adrenalectomy virtually abolished the responses in all vascular beds.

DISCUSSION

Activation of A_1 adenosine receptors in the NTS contributes to cardiovascular component of the hypothalamic defense response (HDR); specifically, it contributes to the inhibition of baroreflex transmission in the NTS and to the pressor component of HDR[71; 76; 80-82]. The present study tested the hypothesis that activation of NTS A_1 adenosine receptors may also contribute to the crucial component of HDR which is the redistribution of blood from the viscera to the muscle[93]. Therefore in the present study for the first time regional vascular responses evoked by selective stimulation of NTS A_1 adenosine receptors were compared. Furthermore, we investigated the mechanisms mediating the complex hemodynamic responses and their regional variability. The major finding of the present study is that three major vasoactive factors naturally triggered in response to the stimulation, i.e. sympathetic and vasopressinergic vasoconstriction counteracted by β-adrenergic vasodilation[47; 48], had differential effects on somatic (iliac) vs. visceral (mesenteric and renal) vasculatures. Sympathetic vasoconstriction was observed in the iliac vasculature only. The greatest vasodilation mediated via released epinephrine and β-adrenergic mechanism was observed in the

iliac compared to the two other vascular beds. The released vasopressin initially evoked small preferential mesenteric vasoconstriction, and then large, steady vasoconstriction, which was similar in all vascular beds. Taken together these results suggest that A_1 adenosine receptors operating in the NTS may contribute to the redistribution of blood from the visceral to the somatic (muscle) vasculature via preferential iliac vasodilation; however, two vasoconstricting factors simultaneously triggered by the stimulation diminished this effect by limiting skeletal muscle vasodilation. When all vascular factors act simultaneously (intact group) vasoconstriction prevailed in the iliac and mesenteric vs. the renal vascular bed. These data combined with previous studies from our laboratory and by others show that although the A_1 adenosine receptors located in the NTS contribute to the pressor component of the stress/hypothalamic defense response, the activation of these receptors may have a small if any effect on the redistribution of blood from the visceral to the muscle vasculature[71; 76; 80-82].

NTS adenosine receptors and differential control of regional vascular beds by neural and humoral factors: Sympathetic nerves

It has been reported that stimulation of the superior laryngeal nerve, which conducts also the aortic depressor nerve fibers in rats, evokes much greater iliac than renal and mesenteric vasodilation[25]. Also stimulation of the aortic depressor nerve evoked preferential iliac vasodilation in rats[64]. The activation of α_1 adrenergic receptors (a major mechanism mediating sympathetic baroreflex responses) with metoxamine in mongrel dogs led to a greater vasoconstriction in iliac compared to renal and mesenteric vascular beds[34]. Similarly, iliac capacity for baroreflex vasoconstriction and vasodilation in greyhounds was greater in iliac than mesenteric an renal vascular

beds[18]. These reports suggested that baroreflex sympathetic responses may be greater in iliac than in renal and mesenteric vascular beds. Therefore we expected that A_1 adenosine receptor mediated inhibition of NTS baroreflex mechanisms[71; 76] will result in a greater sympathetic iliac vasoconstriction compared to that observed in mesenteric and renal vascular beds. The present study generally confirmed this hypothesis. However, the lack of neural vasoconstrictor responses in renal and mesenteric vascular beds was unexpected (Figure 14A and B). The neural vasoconstrictor component of the responses was relatively small in all vascular beds compared to the relatively large effects evoked by the increases in circulating epinephrine and vasopressin. No significant vasoconstriction was observed in first 5 min of the responses whereas in the later phase of the response (the last 15 min) only iliac vasoconstriction was significantly different from zero as well as from both other vascular beds, consistent with our previous reports[47; 48].

Interestingly, the mesenteric vascular bed tended to vasodilate and vasoconstrict in the initial and the later phase of the response, respectively, although the differences did not reach statistical significance vs. zero (P= 0.114 and P= 0.249, respectively). This may suggest that in the early phase of the responses sympathetic vasoconstriction was counteracted by neurogenic vasodilation and that this vasodilation weakened with time. The most likely vasodilator factor responsible for this counteraction could be pre-absorbed epinephrine released from sympathetic terminals, as suggested previously by Berecek and Brody[10]. The action of pre-absorbed epinephrine would decrease with time over the response in adrenalectomized animals. Consistently sympathetic (α_1-adrenergic) vasoconstriction would increase with time as was observed. This is also consistent with an initially smaller and then greater neural vasoconstriction in the iliac

vascular bed (Figure 14 A and B). Among other vasodilatory neurotransmitters potentially released from sympathetic nerve terminals in the mesenteric and iliac vasculature, for example, nitric oxide (NO), calcitonin gene related peptide (CGRP) and adenosine as a catabolite of neuronally released ATP should be considered[13; 21; 43; 57].

Vasopressin

In the present study we found that vasopressin triggered by selective activation of NTS A_1 adenosine receptors initially constricts the mesenteric vascular bed to a greater extent than the renal and iliac vasculature, whereas in the later phase of the response no regional differences between vasopressinergic vasoconstriction were observed. Our data are consistent with regional vascular responses observed following i.v. infusion of exogenous vasopressin in rats where small doses of vasopressin evoked preferential vasoconstriction in the mesenteric compared to the renal and iliac vasculature, whereas with larger doses the regional differences diminished or disappeared[28; 29]. It should be mentioned that regional effects of vasopressin in rats are different from those observed in dogs where vasopressinergic vasoconstriction dominates in the iliac vasculature[34; 45]. Most likely in the present study the initial release of vasopressin was small and it increased with the time of the response (Figures 13 and 14). In our previous study we found that the level of circulating vasopressin measured ~ 8 min after microinjection of A_1 receptor agonist (CPA) into the NTS increased 4-fold compared to the resting level. Note that in the present study vasopressinergic vasoconstriction increased systematically up to ~ 8 min of the response and then was maintained at this high, steady level throughout the observed response (Figure 13 last upper panel). Circulating vasopressin is quickly catabolized at normal body temperature[17]; therefore, the large, sustained vasopressinergic

vasoconstriction observed in the present study suggests that vasopressin was continuously released into the circulation as long as the baroreflex mechanism was inhibited by stimulation of A_1 adenosine receptors, which can last over an hour at this dose of A_1 agonist (CPA, 330 pmol)[71].

In contrast to activation of NTS A_1 adenosine receptors the activation of A_{2a} adenosine receptors do not affect vasopressin release as A_{2a} receptors do not inhibit baroreflex transmission in the NTS[37; 76]. In addition, previous studies from our laboratory showed that preferential iliac vasodilation observed after selective activation of NTS A_{2a} adenosine receptors was completely abolished following bilateral adrenalectomy and lumbar sympathectomy and no vasoconstrictor component of the response persisted following these procedures[7; 42].

Epinephrine

The preferential iliac vs. mesenteric and renal vasodilation dominated in the initial phase of the responses (Figures 13 and 14). This vasodilation was mediated via activation of the adrenal medulla, release of epinephrine and β-adrenergic vasodilation. The initial vasodilation was abolished in all vascular beds after bilateral adrenalectomy or β-adrenergic blockade (Figure 12). The greatest β-adrenergic vasodilation was observed in the iliac vascular bed, which supplies mostly skeletal muscles, compared to the vasodilation of both visceral vascular beds. This is consistent with preferential expression of β-adrenergic receptors in the skeletal muscle vasculature[90] as well as with the greater tonic β-adrenergic vasodilation in iliac compared to mesenteric and renal vascular beds as observed in the present study (Table 7). The preferential iliac vasodilation was observed in the group of animals where the whole epinephrine effect

was calculated from basic experimental groups ((VX-(ADX+VX)) as well as in the group where sinoaortic denervation was performed and non-baroreflex epinephrine effects were directly measured (Figures 13 and 14). The consistent results obtained using the two different experimental approaches attested that the arithmetical separation of single vasoactive factors in the present study was accurate.

According to our previous reports sinoaortic denervation or blockade of glutamatergic transmission in the NTS abolishes pressor and regional sympathoexcitatory responses[76]. However, A_1 receptor mediated activation of the adrenal medulla is only attenuated but not abolished in this setting. Therefore, following sinoaortic denervation the adrenal medulla was still activated by stimulation of NTS A_1 adenosine receptors, although to a lesser extent than in intact animals where both baro- and non-baroreflex components of the activation of the adrenal medulla were present. In fact, in the present study regional vasodilatory responses evoked by combined baro- and non-baroreflex mechanisms tended to be greater compared to those where only non-baroreflex mechanism was active (Figure 14), although these differences did not reach statistical significance.

Preferential β-adrenergic iliac vasodilation, triggered by selective stimulation of NTS A_1 adenosine receptors, was masked by simultaneous neural and vasopressinergic vasoconstriction. However, combined activation of both A_1 and A_{2a} adenosine receptor subtypes in the NTS may significantly contribute to the preferential iliac vasodilation and to the redistribution of blood from visceral to somatic vascular beds. Previous studies from our laboratory showed that selective stimulation of NTS A_{2a} adenosine receptors evoked much greater iliac vs. renal and mesenteric vasodilation[7]; the β-adrenergic vasodilation contributed to ~80% of this preferential

iliac vasodilation[42].

Potential Mechanisms

The present study showed that selective activation of NTS A_1 adenosine receptors preferentially disinhibited vasopressin release and activated sympathetic outputs to the adrenal medulla, whereas other sympathetic outputs were activated to a much lesser extent. What possible mechanism(s) may be responsible for this difference? Our previous studies strongly suggested that A_1 adenosine receptors act mainly via inhibition of baroreflex transmission in the NTS[71; 76]. This inhibition is responsible for all pressor and sympathoexcitatory responses including vasopressin release[48]. Since the vasopressinergic vasoconstriction observed in the present study was several fold greater than the regional sympathetic vasoconstriction, it seems that A_1 adenosine receptors may be located preferentially on those NTS baroreflex terminals/interneurons which are responsible for the tonic baroreflex restraint of vasopressin release and to a much lesser extent on NTS neurons responsible for tonic baroreflex restraint of regional sympathetic outputs. In fact, the increases in lumbar and renal sympathetic nerve activity, observed in a previous study from our laboratory[76], were 2-3-fold smaller than that observed in the adrenal nerve.

One other reason for much smaller neural than humoral vasoconstriction observed in the present study may be the non-homogenous character of efferent sympathetic fibers selectively activated/disinhibited by A_1 adenosine receptors in the NTS. Efferent sympathetic terminals, in addition to the major neurotransmitter, norepinephrine, may secrete also several vasodilatory neurotransmitters including NO, CGRP, and ATP which after degradation to adenosine via ectonucleotidases may cause vasodilation via both A_1 and A_{2a} adenosine receptor subtypes[13; 20; 21; 43; 57; 94]. A_1

adenosine receptors may disinhibit both vasoconstrictor and vasodilatory efferent fibers and these effects may cancel each other. The net effect may be relatively small vasoconstriction (as observed in the iliac vasculature), no significant response (as observed in renal vasculature) or even a tendency to vasodilation (as observed in the mesenteric vascular bed). In a previous study from our laboratory we observed a similar phenomenon revealed by selective stimulation of NTS A_{2a} adenosine receptors. This stimulation triggered preferential iliac vasodilation, compared to the mesenteric and renal vasculatures, whereas sympathetic activity directed to the iliac vascular bed did not change in this setting[7; 73]. Taken together these observations could suggest that LSNA may not contribute to the response. However, although the total LSNA did not alter, the lumbar sympathectomy did contribute to over 20% of the preferential iliac vasodilation suggesting that both vasoconstricting and vasodilatory fibers (most likely releasing NO) might have been simultaneously activated[21; 42; 57]. Which specific neurotransmitters are released from these regional sympathetic nerves in response to stimulation of NTS adenosine receptor subtypes awaits further investigation.

In absolute terms the regional β-adrenergic vasodilatory responses were much greater than the sympathetic vasoconstrictor responses, as blockade of V_1 vasopressinergic receptors reversed vasoconstriction (which dominated in intact animals) into marked vasodilation in all three vascular beds (Figure 12). This indicated that β-adrenergic vasodilation markedly prevailed over sympathetic vasoconstriction in all vascular beds. It should be stressed that NTS A_1 adenosine receptors activate the adrenal medulla via both baroreflex and non-baroreflex mechanisms which is consistent with the greater overall activation of ASNA compared to other sympathetic outputs[76]. The baroreflex component of this response was relatively weak (similarly as it was

observed for baroreflex-mediated regional vasoconstriction discussed above) as there were no significant differences between total vs. non-baroreflex β-adrenergic vasoconstriction observed in each vascular bed in these two situations (Figure 14). The non-baroreflex component of the activation of the adrenal medulla is most likely mediated via descending pathways from hypothalamic nuclei which utilize nonglutamatergic neurotransmitters as we proposed previously[77]. However, specific pathways and neurotransmitters responsible for non-baroreflex activation of the adrenal medulla remain unknown. All the above hypotheses should be addressed in future studies.

Limitations of the method

Anesthesia and recent surgical stress most likely elevated resting levels of sympathetic activity, circulating epinephrine and vasopressin. This could attenuate further increases of these vasoactive factors due to stimulation of NTS A_1 adenosine receptors. In fact, in our previous study, which was performed under similar experimental conditions, the resting vasopressin levels were moderately elevated compared with the levels measured in conscious animals[12; 32; 35; 48; 53; 54]. Nevertheless, the activation of NTS A_1 adenosine receptors evoked large (over 4-fold) increase of circulating vasopressin compared to the elevated baseline[48]. Also regional sympathetic outputs, especially that directed to the adrenal medulla, were significantly activated in response to stimulation of NTS A_1 adenosine receptors[76]. This attests that despite of the increased resting levels of humoral and neural factors the stimulation of NTS A_1 receptors is potent to evoke marked responses in all these factors. In addition, the potential effects of anesthesia and recent surgery presumably affected all vascular beds similarly; therefore we believe that the relative regional differences in

responsiveness to the vasoactive factors were preserved under these experimental conditions.

In this and our previous study, MAP decreased following V_1 vasopressinergic blockade and was allowed to return spontaneously toward resting levels. We did not compensate for the decrease of MAP with phenylephrine infusion to avoid potentially different α_1 adrenergic vasoconstriction of different vascular beds due to differential regional expression of these receptors[34; 36]; such a compensation could distort the responses to the experimental factors. The baroreflex compensation of the depressor response evoked by V_1 vasopresinergic blockade probably elevated baseline sympathetic activity which may have attenuated the sympathoactivation in response to stimulation of NTS A_1 adenosine receptors. Therefore, the neural sympathetic component of the responses could be underestimated in this study. Nevertheless, we believe that regional differences in sympathetic responses were preserved, as vasoconstriction in the iliac vascular bed significantly increased whereas the responses of the mesenteric and renal vascular beds were not different from zero.

Adrenalectomy and β-adrenergic blockade most likely diminished cardiac output regulatory capacity. Therefore, following combined adrenalectomy and V_1 vasopressinergic blockade (Protocol 5) MAP and IVC did not fully recover, whereas following V_1 vasopressinergic blockade alone (Protocol 3) full recovery of these parameters was observed (Table 6).

Renal auto-regulation was most likely responsible for much smaller responses evoked in this vascular bed in intact animals and under most of the experimental conditions (Figure 14). Nevertheless, vasopressin had a relatively large effect on the renal vasculature, not different from that observed in the iliac and mesenteric

vasculature beds during the whole time-course of the responses (Figure 13). Consistently, following V_1 vasopressinergic blockade significantly increased renal vascular conductance remained significantly elevated compared to that observed in intact animals whereas MAP, IVC and MVC completely recovered following the blockade (Table 6).

Although we used a standard method of recording regional vascular conductance, which is well established in our laboratory, the relatively small neural vs. humoral regional vascular responses could raise a question if sympathetic nerves were potentially damaged during the procedure. This was rather unlikely, because electrical stimulation of the aortic depressor nerve (8V, 0.1 ms, 8-64 Hz) and activation of cardiopulmonary receptors with i.v. phenylbiguanide (1-8 μg/kg) applied in some intact animals evoked large sympathetic vasodilation in all vascular beds with patterns similar to that described previously by Faber and Brody[25].

Conclusions

Three major vasoactive factors triggered by stimulation of NTS A_1 adenosine receptors in the NTS (sympathetic and vasopressinergic vasoconstriction opposed by β-adrenergic vasodilation) differentially affect regional vascular beds. The β-adrenergic vasodilation, which dominates in the initial phase of the response, was significantly greater in the iliac than the mesenteric and renal vasculatures. Significant sympathetic vasoconstriction was observed in the iliac but not in the mesenteric and renal vascular beds. In contrast, vasopressin exerted marked, sustained vasoconstriction similar in all vascular beds, except for a small, initial preferential mesenteric vasoconstriction. This pattern of regional vascular responses suggests that activation of A_1 adenosine

receptors in the NTS has minor, if any, effect on redistribution of blood from the visceral to somatic (muscle) vasculature.

Perspectives

Previous studies from our laboratory and by others strongly suggested that adenosine operating via A_1 adenosine receptors in the NTS may contribute to the pressor component of hypothalamic defense response, most likely via the inhibition of baroreflex transmission in the NTS which disinhibits efferent sympathetic vasoconstriction and vasopressin release[48; 71; 76; 80-82]. Adenosine operating in the NTS via both A_1 and A_{2a} receptor subtypes preferentially activates the adrenal medulla leading to preferential iliac vasodilation[7; 42], which is the major mechanism mediating increases in blood supply to skeletal muscles during stress/HDR in the rat[93]. However, the present study showed that although activation of NTS A_1 adenosine receptors preferentially increases iliac vascular conductance in the early phase of the response, this effect is overridden by the powerful V_1 vasopressinergic vasoconstriction in later phase of the response, which diminished the contribution of NTS A_1 adenosine receptors to the redistribution of blood. To what extent do these potential mechanisms, triggered by stimulation of NTS adenosine receptor subtypes, contribute to the pattern of autonomic responses evoked by the stress or HDR requires further investigation. Interestingly, some components of the autonomic responses evoked by selective activation of adenosine receptor subtypes in the NTS remain inconsistent with the pattern of HDR. For example, the decreases in HR due to A_1 and A_{2a} adenosine receptor stimulation, the depressor responses evoked by A_{2a} receptor stimulation, and the lack of contribution of NTS A_{2a} receptors to the baroreflex inhibition are most likely components of other autonomic mechanisms, not related to the HDR. Alternatively, the

responses opposite to the HDR pattern may contribute to fine tuning of the HDR autonomic pattern. Further studies are necessary to specify the autonomic mechanisms to which these non-HDR effects mediated by NTS adenosine receptor subtypes may contribute.

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ABSTRACT

NEURAL AND HUMORAL CONTROL OF REGIONAL VASCULAR BEDS VIA A_1 ADENOSINE RECEPTORS LOCATED IN THE NUCLEUS OF THE SOLITARY TRACT

by

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Previous studies from our laboratory showed that activation of NTS A_1 adenosine receptors yields variable hemodynamic responses with prevailing pressor and iliac vasoconstrictor responses. These responses are accompanied with differential activation of regional sympathetic activity (adrenal>>renal≥lumbar) and inhibition of baroreflex mechanisms at the level of the NTS. The variability of the hemodynamic responses was a result of simultaneous β_2-adrenergic vasodilation counteracted with sympathetic and unknown humoral vasoconstriction. Among many potential vasoconstrictors vasopressin, angiotensin II and circulating norepinephrine were considered. Therefore, blood pressure and iliac vascular responses evoked by selective stimulation of NTS A_1 adenosine receptors (CPA 330 pmol/ 50 nl) in intact anesthetized (urethane/chloralose) Sprague Dawley rats were compared with the responses evoked following the blockade of each potential vasoconstrictor mechanism. I found that vasopressin is the major vasoconstrictor released into the circulation most likely as a result of A_1-adenosine-receptor-mediated inhibition of baroreflex mechanism and disinhibition of tonic restraint of vasopressin release. Angiotensin II and circulating

norepinephrine had virtually no contribution to the responses. The direct evaluation confirmed that the levels of circulating vasopressin increased over 4-fold in response to stimulation of NTS A_1 adenosine receptors.

Since NTS A_1 adenosine receptors contribute to the pressor component of the stress/hypothalamic defense (HDR) response it was interesting if these receptors contribute to the redistribution of blood from visceral (mesenteric and renal) to somatic (iliac) vascular beds, which is and integral part of HDR. Therefore, regional vascular effects of three major vasoactive factors triggered by stimulation of NTS A_1 adenosine receptors (β_2-adrenergic vasodilation opposed by sympathetic and vasopressinergic vasoconstriction) were compared; these vasoactive factors differentially affected the regional vascular beds. The β_2-adrenergic vasodilation, which dominates in the initial phase of the response, was significantly greater in the iliac than the mesenteric and renal vasculatures. Significant sympathetic vasoconstriction was observed in the iliac but not in the mesenteric and renal vascular beds. In contrast, vasopressin exerted a marked, sustained vasoconstriction similar in all vascular beds. This pattern of regional vascular responses suggests that activation of A_1 adenosine receptors in the NTS has minor, if any, effect on the redistribution of blood from the visceral to the somatic vasculature.

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1. **McClure JM**, Rossi NF, Chen H, O'Leary DS, Scislo TJ. Vasopressin is a major vasoconstrictor involved in hindlimb vascular responses to stimulation of adenosine A(1) receptors in the nucleus of the solitary tract. *Am J Physiol Heart Circ Physiol*. 2009 Nov;297(5):H1661-72. Epub 2009 Sep 11.
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