Key role of 5-HT3 receptors in the nucleus tractus solitarii in cardiovagal stress reactivity

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Highlights

- The nucleus tractus solitarii (NTS) is the primary target site for reflex cardiovascular afferents.
- Many neurotransmitters are present in the NTS, including GABA, substance P and serotonin.
- A functional interaction between 5-HT_3, $GABA_A$ and NK_1 receptors blocks the reflex parasympathetic component.
- This functional interaction is involved in both acute and chronic stress-induced reduction of parasympathetic tone, downstream to activation of the dorsolateral nucleus of the hypothalamus.

Abstract

Serotonin plays a modulatory role in central control of the autonomic nervous system (ANS). The nucleus tractus solitarii (NTS) in the medulla is an area of viscerosomatic integration innervated by both central and peripheral serotonergic fibers. Influences from different origins therefore trigger the release of serotonin into the NTS and exert multiple influences on the ANS. This major influence on the ANS is also mediated by activation of several receptors in the NTS. In particular, the NTS is the central zone with the highest density of serotonin$_3$ (5-HT_3) receptors. In this review, we present evidence that 5-HT_3 receptors in the NTS play a key role in one of the crucial homeostatic responses to acute and chronic stress: inhibitory modulation of the parasympathetic component of the ANS. The possible

functional interactions of 5-HT_3 receptors with $GABA_A$ and NK_1 receptors in the NTS are also discussed.

1. Introduction

There is now a large body of evidence indicating that the autonomic nervous system (ANS) plays a crucial role in the pathogenesis and progression of cardiovascular disease. The ANS, with its two divisions – the sympathetic and parasympathetic systems exerting opposing effects – ensures adaptation of the cardiovascular system to the various activities of daily life. While the sympathetic system increases heart rate, myocardial contractility and peripheral resistance, the parasympathetic system slows heart rate with a limited effect on cardiac contractility. The antagonism between sympathetic and parasympathetic control of the heart is mediated via the effects of their specific neurotransmitters (catecholamines and acetylcholine). It is noteworthy that the electrophysiological properties, not only of the sinus node, but of the whole heart, are modulated by these antagonistic influences (151).

The autonomic outflow to the heart and the peripheral circulation, and therefore the ANS, is regulated by various cardiovascular reflexes, such as the baroreflex and the carotid chemoreflex. The primary relay for afferents mediating these two reflexes (i.e. aortic depressor and carotid sinus nerves, traveling in the vagus and glossopharyngeal nerves, respectively) is the nucleus tractus solitarii (NTS) (Fig. 1A). A wide range of neurotransmitters and receptors are present in subnuclear regions of the NTS, and some participate in ANS modulation. In particular, we have shown that serotonin, acting on 5-HT_3 receptors in the NTS, modulates both divisions of the ANS (137, 139, 144).

This article will review the functional organization of neural cardiac control by the NTS and discuss the evidence that NTS and serotonin, together with excitatory amino acids and peptides, play a key role in modulation of the ANS during acute and chronic stress.

2. Neurotransmitters and neuropeptides in the NTS associated with modulation of the ANS

The NTS is located in the dorsal part of the medulla and receives visceral afferent information from the cardiovascular, respiratory, gastrointestinal and taste systems traveling in four cranial nerves: facial, glossopharyngeal, vagus, and trigeminal (32).

In the horizontal plane, the NTS has a V-shaped appearance (Fig 1A). The most rostral part of the NTS is entirely devoted to taste (57) (A in Fig 1A). The termination of the lingual-tonsillar branch of the glossopharyngeal nerve, situated more caudally and extending into the rostral part of the area postrema (B in Fig 1A), conveys somatosensory information along with gustatory information and this region of the NTS is referred to as the "intermediate NTS" (9). Vagus nerves also project onto this part of the NTS. The "caudal NTS" situated caudally to the intermediate NTS, is considered to be the general viscероceptive part of the nucleus. The anterior part of the caudal NTS, ending caudally at the posterior tip of the area postrema, is referred to as the "subpostremal NTS" (C in Fig 1A), and the portion extending to the caudal end of the nucleus is referred to as the "commissural NTS" (D in Fig 1A) (9). Terminals of afferent fibers derived from various viscera are distributed somewhat topographically in the nucleus. Sensory information from esophageal and gastric mechanoreceptors, duodenal glucoreceptors, and hepatic chemoreceptors and osmoreceptors project via the vagus nerve to sites in the subpostremal NTS. The cecum is represented in the caudal tip of the AP and in the commissural NTS (150). Aortic baroreceptors (activated by changes in blood pressure) and carotid chemoreceptors (sensitive to blood gas composition) project onto the subpostremal NTS and to more caudal sites in the commissural NTS (72, 98, 112). Baroreceptor afferents are located more rostrally in the intermediate and commissural NTS, while chemoreceptor afferents mostly terminate in the commissural NTS (74, 131). The NTS therefore plays a key role in the transmission of reflex messages initiated by activation of these receptors and plays a critical role in the control of sympathetic and parasympathetic activity.

Nearly all of the putative neurotransmitters found in the central nervous system have been identified in the rostral to caudal parts of the NTS. Excitatory and inhibitory amino acids and several neuropeptides and amines might be involved in the processing of messages for the control of cardiovascular function. It is now widely accepted that glutamate, or at least an excitatory amino acid, is the main neurotransmitter present in vagus and glossopharyngeal afferent nerves released into the NTS. For instance, NTS

microinjections of kynurenic acid (a glutamate receptor antagonist) that antagonizes both NMDA and non-NMDA ionotropic receptors completely blocked baro- and chemoreflexes (56, 87, 162). However, adaptive changes in reflex gains (facilitation or inhibition) are observed under various physiological conditions (33), as expected from a certain degree of modulation of glutamatergic neurotransmission in the NTS by other neuroactive substances, especially GABA, substance P and 5-HT.

2.1 GABA

The inhibitory amino acid, GABA, is present at a relatively high density in puncta and neurons throughout the NTS (38, 90). The parvocellular subdivision contains the fewest GABA-immunoreactive varicosities, but the greatest number of GABA-immunoreactive neurons. GABA-immunoreactive puncta form what appear to be pericellular arborizations around both GABA-immunoreactive neurons and non-immunoreactive neurons in many subdivisions of the NTS (90). Central structures such as the paraventricular hypothalamus (PVN) send GABAergic projections to the NTS (3) (Fig. 1B). Intra-NTS microinjections of GABA produce an increase in blood pressure and heart rate, which can also be induced by microinjections of $GABA_A$ as well as $GABA_B$ receptor agonists into this nucleus (156, 176). Electrophysiological studies have shown that all NTS neurons activated by aortic depressor nerve or carotid sinus nerve stimulation (second-order neurons) can be inhibited by GABA application (71). This inhibition appears to be physiologically relevant because pharmacological stimulation of $GABA_A$ and $GABA_B$ receptors in the NTS leads to inhibition of maximum reflex bradycardia (95, 137) in anesthetized rats. In awake rats, Callera *et al.* (20) also reported that activation of $GABA_A$ or $GABA_B$ receptors blocked baroreflex bradycardia. However, the bradycardic response to chemoreflex activation can only be inhibited by intra-NTS $GABA_A$ but not $GABA_B$ receptor agonists (19). These GABA-mediated cardiovascular effects appear to be mediated by postsynaptic inhibition of glutamatergic excitatory inputs to NTS neurons (128).

2.2 Substance P

Substance P (SP) is present in both fibers and cell bodies throughout the entire rostral to caudal parts of the NTS (88, 89). However, SP immunoreactivity is considerably reduced in rostral parts of the nucleus, although a few immunoreactive fibers are present in the medial and ventral subdivisions. The same regions that contain SP fibers also possess scattered SP-containing neurons (59, 88). The majority of SP is thought to be of vagal origin (83). However, some SP innervation is also derived from glossopharyngeal afferents (159) and caudal raphe nuclei including B1 to B3 group cells (158). It has also been suggested that direct afferents containing SP may be derived from the dorsolateral part of the periaqueductal gray (dlPAG) (17) (Fig. 1B). The presence of SP interneurons in the NTS as well as SP afferents from the nodose ganglia and raphe nuclei is compatible with the idea that different SP-ergic pathways in the NTS may modulate the reflex control of cardiovascular parameters (83). Indeed, data in the relevant literature seem to indicate that SP in the NTS exerts a facilitatory as well as an inhibitory influence on the neuronal circuit responsible for the cardiovagal component of the baroreceptor reflex. In particular, i) administration through reverse microdialysis or microinjections of SP into the NTS elicits an increase in maximum baroreflex cardiac response (26, 136), ii) activation of baroreceptor afferents elicits the release of SP within the NTS (118), and iii) ablation of NK_1 receptors in the rat NTS blocks the cardiac component of the baroreceptor reflex (125). Consequently, SP released within the NTS, presumably from peripheral afferents, seems to play a tonic facilitatory role in the cardiac baroreceptor reflex. However, more recently, microinjection of SP into the intermediate NTS of rat brain stem preparations dramatically decreased pharmacological cardiac baroreflex sensitivity (BRS, determined by matching variations of mean blood pressure induced by nitroprusside and phenylephrine administration with heart rate responses) via a GABAergic link (116). In addition, an elegant study by Chen et al. in 2009 demonstrated an interaction between NK_1 receptors and GABA neurons, and showed that exercise-induced internalization of NK_1 receptors located on GABAergic interneurons in the NTS resulted in reduced intrinsic inhibitory input to the neurons of the baroreflex pathway in spontaneously hypertensive rats (27).

2.3 Serotonin

Serotonin immunoreactivity is present in varicose fibers disseminated throughout the rostral to caudal parts of the NTS (88). There is a general consensus on the essentially extrinsic origin of NTS serotonergic innervation. Serotonin-immunoreactive neurons projecting onto the lateral and medial NTS are mostly found in the caudal raphe group of cells, namely the nucleus raphe magnus and lateral paragigantocellular nucleus (B3), nucleus raphe pallidus (B2) and nucleus raphe obscurus (B1) (115, 133, 158). In addition, the NTS also receives serotonergic afferents from the nodose and petrosal ganglia (the site of vagus and glossopharyngeal nerve cell bodies) (58, 108), and the area postrema (44, 153) (Fig 1B). Numerous pharmacological, electrophysiological and immunohistochemical studies have provided evidence for the existence of 5-HT_1 (91, 168), 5-HT_2 (29, 66, 94, 114, 145), 5-HT_3 (50, 95, 119), 5-HT_4 (42), 5-HT_6 (45) and 5-HT_7 (109) receptors in the NTS.

A study on brain stem slices concerning the implication of these various receptors in cardiovascular modulation has recently shown that activation of 5-HT_{1A} receptors (located somatodendritically) by the 5-HT_{1A} receptor agonist 8-OH-DPAT decreased the amplitude of glutamatergic tractus solitarii-evoked excitatory postsynaptic currents, and reduced overall spontaneous excitatory NTS network activity (110). However, in anesthetized rats, microinjection of 8-OH-DPAT into the caudal NTS had minimal effects on arterial pressure and heart rate (110). Neurons in the NTS are affected in different ways by 5-HT_2 receptor ligands, in relation to their vagal postsynaptic location, the type of afferents they receive and the various subtypes activated (146). Activation of 5-HT_{2A}, but not 5-HT_{2B} or 5-HT_{2C}, receptors, located on NTS neurons, elicited depressor and bradycardic responses. In addition, NTS 5-HT_{2A} receptor activation facilitated the cardiac but not the sympathetic baroreflex response (29). Stimulation of 5-HT_4 receptors within the NTS had no effect *per se* on baseline cardiorespiratory values, but depressed the reflex bradycardia components of the cardiopulmonary reflex via a cAMP-dependent PKA pathway (42). The non-selective 5-HT_7 receptor agonist, 5-carboxamidotryptamine maleate (5-CT), applied to 78 neurons in the NTS also increased the firing rate by 59% in 18 neurons and decreased the activity by 47% in 38 neurons (109). The effect of intracisternal application of SB-269970, a selective 5-HT_7 receptor antagonist, dose-dependently attenuated the fall in heart rate induced by stimulating arterial baroreceptors (76). Similarly, topical application of SB-269970 significantly reduced vagus-induced NTS activity with a time-course similar to the reduction

in reflex bradycardia. As 5-HT_7 receptor mRNA has only been localized in the NTS (55), this nucleus is presumably the target site for this effect.

On the other hand, specific activation of 5-HT_3 receptors resulted in clear-cut excitation of most NTS cells (168). 5-HT_3 receptors are members of the Cys-loop superfamily of ligand-gated ion channels (79). These serotonergic receptors, which exhibit nearly equal permeability to both Na^+ and K^+, form a pentameric complex, that surrounds a central ion channel with a high homology with nicotinic acetylcholine receptors. They are the only monoamine receptor type to be associated with fast synaptic neurotransmission and are likely to be composed of multiple subunits, like other members of this superfamily (8). Two 5-HT_3 receptor subunits have been cloned to date, the A subunit (5-HT_{3A}), which is expressed by both central and peripheral neurons, and the B subunit (5-HT_{3B}) which seems to be restricted to peripheral neurons (99). When expressed alone, the 5-HT_{3B} subunit fails to form functional 5-HT_3 receptors. However, when it is co-expressed with the 5-HT_{3A} subunit, the resulting heteromeric 5-HT_3 receptor complex fully replicates the biophysical characteristics of native neuronal 5-HT_3 receptors (8). Only minimal pharmacological differences have been identified between homomeric 5-HT_{3A} receptor and heteromeric 5-$HT_{3A/3B}$ receptors. In the central nervous system, low levels of 5-HT_3 receptor expression have been demonstrated in the forebrain. Nevertheless, in the majority of species investigated to date, 5-HT_3 receptors a-were expressed notably in the amygdala and hippocampus (113), but the highest levels of 5-HT_3 receptor binding sites were found in the NTS (80, 119).

Most studies have shown that the majority of NTS 5-HT_3 receptors are located presynaptically on vagal sensory afferents (40, 95, 119). Central 5-HT_3 receptors are known to mediate an excitatory action on target cells (77). Accordingly, administration of a 5-HT_3 receptor agonist into the NTS increases the local release of endogenous glutamate from vagal afferents (5), resulting in increased activity in second-order neurons (69) (Fig 2). We have shown that stimulation of NTS 5-HT_3 receptors activates a pressor efferent pathway via indirect facilitation of NO and EAA receptor-mediated mechanisms (143), without modifying heart rate. In addition, only 5-HT_3 receptor antagonists (but not other serotonin subtype receptor antagonists) prevented reduction of the baroreflex and chemoreflex bradycardia induced by bilateral microinjection of serotonin into the commissural NTS of awake or anesthetized rats. Interestingly, cardiovascular 5-HT_3 receptor inhibitory effects on cardiac

reflex responses were linked to local GABAergic system activation, and more specifically to activation of $GABA_A$ receptors (21, 95, 137, 138).

Considering that i/ $GABA_A$ receptors are linked with both 5-HT_3 receptors and NK1 receptors to produce inhibition of the parasympathetic component of cardiovascular reflexes, and that ii/ the existence of a functional link between 5-HT_3 and NK_1 receptors has been proposed in the gastrointestinal tract (121), the existence of functional interactions between 5-HT_3 $GABA_A$, and NK_1 receptors in the NTS to induce an alteration of reflex cardiac responses can be postulated.

This pathway has been essentially demonstrated during acute and/or chronic stress, as discussed below.

3. NTS pathway involving 5-HT_3, $GABA_A$ and NK_1 receptor activation: evidence for a role in cardiovascular responses to arousal stress

3.1 Defense reaction

During acute stressful conditions, animals often react with behaviors, such as the defense reaction, which requires the activity of skeletal muscles and a concomitant increase in blood flow in these muscles. Characteristic components of the defense reaction in animals are mydriasis, vibrissae movements, rise in blood pressure, tachycardia, and hyperventilation (49). These stress responses can be observed, for example, after application of air-jet (2), painful stimuli (130), and also when the animal is placed in a novel and/or noisy environment (73). Similar cardiovascular and respiratory stress responses are observed in humans after application of combined physical and evaluative stressors (18), during mental stress (123), or in response to racially noxious images (28) or fearful faces (46). Changes in muscle blood flow induced during the defense reaction are mediated *via* a concomitant decrease in visceral blood flow caused by major sympathetic vasoconstriction in the vascular beds of internal organs (106). This visceral vasoconstriction induces elevation of heart rate and blood pressure. This response therefore inevitably activates the arterial baroreceptor reflex. However, the resulting baroreflex sympathoinhibitory and bradycardic responses would interact with the blood flow to the skeletal muscles and counteract the animal's

behavioral performance. Consequently, the combination of a concomitant increase in arterial pressure and skeletal muscle blood flow would not be possible if baroreceptor reflex sensitivity was not reduced. Indeed, reference studies in rats have confirmed that the parasympathetic pharmacological BRS is reduced during the defense reaction (63, 107).

Various hypothalamic areas were originally defined as hypothalamic defense areas (HDA) because electrical or chemical stimulation of these regions produced characteristic behavioral and physiological defense responses (34, 39, 49, 62, 106). Other key regions involved in the defense reaction were subsequently described, such as the PAG (7, 24). Among the various HDA, more than the PVN (usually considered to be the origin of the hypothalamo-pituitary-adrenal or "stress" axis), the dorsomedial nucleus of the hypothalamus (DMH) appears to be of particular interest because i) activation of the DMH induced maximal cardiovascular changes (39, 155), and ii) blockade of the DMH but not PVN suppressed the cardiovascular effects of stress (155). In previous studies, we have confirmed that intense DMH activation induced a marked increase in mean blood pressure and heart rate and a decrease in parasympathetic activity and that the cuneiform nucleus is an intermediate structure that mediates DMH defense responses to the dlPAG (102). The implication of these different regions in stress responses has been confirmed in various human studies (70, 132).

Studies in cats and rats have indicated that the NTS is a key structure in brain mechanisms inducing inhibition of the parasympathetic component of the baroreflex associated with the defense reaction (71, 96), although contradictory results have led some authors to conclude that the major site of this inhibition is actually the preganglionic parasympathetic cells of the nucleus ambiguus (65, 68). In addition, numerous projections from the dlPAG and hypothalamus to different raphe nuclei have also been described and, in particular, the caudal raphe projects onto the NTS (12, 61, 86, 124, 158). As the cardiovascular changes associated with the defense reaction are similar to those elicited by 5-HT_3 receptor stimulation in the NTS, i.e. inhibition of the cardiac component of the baroreflex and a rise in blood pressure due to sympathoexcitation, mediated by $GABA_A$ receptors (71), it could be inferred that these receptors play a key role in the sympathetic and parasympathetic defense responses induced by dlPAG and DMH activation.

In support of this hypothesis, pharmacological blockade of these receptors by intra-NTS microinjection of granisetron, a specific 5-HT_3 receptor antagonist, drastically reduced the inhibitory effects of dlPAG and DMH stimulation on baroreceptor (31, 141) and chemoreceptor reflex bradycardia(103). Similarly, a study by Gau et al. showed that thermal and mechanical nociception induced inhibition of the baroreflex bradycardia via activation of 5-HT_3 receptors in the NTS (48). In order to clearly establish that NTS 5-HT_3 receptors mediate the defense reaction-induced inhibition of the cardiac response of the baroreflex, it was also necessary to demonstrate that stimulation of these receptors by endogenous 5-HT actually occurs in animals expressing this type of behavioral reaction. We therefore analyzed the effects of dlPAG stimulation on the baroreflex bradycardia response in rats pretreated with p-chlorophenylalanine (PCPA), a serotonin synthesis inhibitor. The results of this study (31) showed that inhibition of 5-HT synthesis almost totally prevented the inhibitory effects of dlPAG stimulation on the baroreflex bradycardia. Moreover, the role of 5-HT in the inhibitory influence of dlPAG stimulation was confirmed by the fact that 5-hydroxytryptophan administration, designed to restore 5-HT synthesis in PCPA-pretreated rats, allowed recovery of this negative control of the cardiac reflex response to nearly the same level as that observed in naive rats (31). The most likely origin of serotonin released into the NTS during the defense reaction would be one of the B1-B3 cell groups. Two studies have shown that the nucleus raphe obscurus and the nucleus raphe pallidus (B1 and B2, respectively) are both involved in inhibition of baroreflex bradycardia (42, 172). However, we found that only serotonergic cells in the raphe magnus and lateral paragigantocellular nucleus, part of the B3 raphe, were excited (as confirmed by the presence of c-fos protein) following DMH or cuneiform (personal data) as well as dlPAG stimulation (13), or after application of nociceptive stimuli (47).

In addition, NTS NK_1 receptors contribute downstream to GABA-mediated inhibition of the cardiac component of the baroreflex in response to another stressful condition, somatic nociception (16). SP receptors therefore play a role in the defense reaction-induced inhibition. In a study in which the defense reaction was triggered by dlPAG stimulation (30), we found that i/ intra-NTS microinjections of SP significantly reduced the inhibitory effect of dlPAG stimulation on aortic (but not carotid) baroreflex bradycardia (30), and ii/ intra-NTS GR205171, a NK1 receptor antagonist, reversed the blockade of aortic baroreflex

bradycardia only after dlPAG stimulation or after local microinjections of phenylbiguanide, a 5-HT_3 receptor agonist. These and previous results strongly suggest that NK_1 (and $GABA_A$) receptors contribute downstream to 5-HT_3 receptor-mediated inhibition of the aortic but not the carotid cardiac baroreflex response occurring during the defense reaction elicited by DMH and dlPAG activation.

A hypothetical complex mechanism involving interactions in the NTS leading to inhibition of the cardiac response of major reflexes is proposed in Fig. 2.

It is noteworthy that neither the microinjection of granisetron or GR205171 into the NTS, nor PCPA treatment affected the sympathetically-mediated increase in blood pressure and heart rate induced by DMH and dlPAG stimulation (30, 31, 141). A serotonergic system nevertheless appears to be involved in both types of acute stress-induced sympathoexcitatory responses. Systemic administration of 8-OH-DPAT (a selective 5-HT_{1A} receptor agonist) dose-dependently suppressed the tachycardic and pressor responses to psychological stressors—air-jet stress in rabbits and restraint stress in rats (101, 105). These results were confirmed by Vianna et al. in restrained rats (165). Local administration of 8-OH-DPAT into the caudal raphe mimicked the anti-tachycardic effects of the systemically injected drug (105), supporting a role of this region in acceleration of heart rate. In contrast, intra-raphe microinjection of the drug did not modify the stress-induced rise in blood pressure (101) supporting the hypothesis that the antipressor action of 8-OH-DPAT may be mediated by 5-HT_{1A} receptors in another region, i.e. the rostroventrolateral medulla (RVLM) vasopressor region, that receives direct projections from the DMH and dlPAG (35, 43, 64, 67). Accordingly, increases in both blood pressure and renal sympathetic nerve activity, but not heart rate, induced by DMH stimulation are markedly reduced by microinjections of the $GABA_A$ receptor agonist muscimol into the RVLM (43). RVLM therefore appears to play a key in the pressor, but not the tachycardic response to acute stress.

3.2 Chronic stress

Decades of clinical, epidemiological and experimental research in animals and humans have provided strong evidence in support of a close correlation between psychosocial factors and cardiovascular abnormalities (92, 104, 167, 171). These psychosocial variables appear to be independent risk factors, as important as traditional risk factors (serum cholesterol, body mass index and poor physical activity), for the onset and progression of hypertension and arrhythmias (60).

In the 1990s, Lespérance et al. suggested an association between negative emotions and coronary heart disease (CHD, (81)). In addition, a history of depression is associated with an increased risk of myocardial infarction (MI) (82). A subsequent study demonstrated that a history of dysphoria and major depressive episode increases the risk of MI (120). This link between mental illness and cardiovascular comorbidity results in poorer prognosis. Depressive symptoms are associated with CHD events, and the strongest relationship is observed for fatal CHD; in addition, a proxy variable for clinical depression is most often associated with sudden cardiac death in multivariable models (173). In a clinical study performed in 2006 on mortality among patients with serious mental illness, heart disease was the leading cause of death (97), essentially due to ventricular arrhythmia (84, 164). In line with this idea, a series of clinical studies designed to investigate the processes underlying cardiac vulnerability and influences of various factors on this vulnerability showed that psychological stress produces significant reductions in the vulnerable-period threshold for repetitive extrasystole (163). In animals, social challenge seems to be the most appropriate model to induce arrhythmia (100, 148), compared to maternal separation (160) or open-field test exposure (149).

The increased arrhythmogenic feature in stress-related disorders is commonly linked to exaggerated sympathetic stimulation, as reflected by increased blood pressure, heart rate and plasma catecholamines (127, 174). This result was confirmed by analysis of heart rate variability (HRV). In addition to the commonly used temporal analysis of HRV (square root of the variance of beat intervals or SDNN), power spectral density analysis allows decomposition of the total variance (“power”) of a continuous series of beats into its frequency components (i.e., how the power is distributed as a function of frequency) (14, 37, 177). The spectral power for a given frequency can then be quantified by determining the

area under the curve within a specified frequency range. Low frequency (LF) power is strongly affected by the oscillatory rhythm of the baroreceptor system (75) and has been shown to be reduced by selective parasympathectomy and eliminated when denervation is combined with beta-adrenoceptor blockade (122). The LF component of HRV consequently reflects the activity of both the sympathetic and parasympathetic divisions of the ANS. On the other hand, a large proportion of changes in heart rate occurs synchronously with respiration [heart rate increases (R–R interval shortens) during inspiration and decreases (R–R interval prolongs) during expiration] and are therefore referred to as respiratory sinus arrhythmia (RSA) (37), also called HF HRV, with reference to the relatively high frequency (HF) range in HRV at which the parasympathetic but not the sympathetic division of the autonomic nervous system can respond to respiration and influence heart rate (37, 117). By taking into account the involvement of each division of the autonomic system in these frequency domains, the ratio of LF to HF (LF/HF) is commonly used as an index of the sympathetic/parasympathetic balance (111, 157), although this concept has been challenged (41).

Many studies have demonstrated an elevated LF/HF ratio in patients with depression (175), anxiety (152), panic attack (93), and bipolar disorder (25). Various experimental models of chronic stress-induced anxiety or depression have been used. For example, Grippo et al. showed that social isolation in prairie-voles increased susceptibility to sympathetic hyperactivity (52, 53). An aggressive/active coping strategy among male members of a wild-type strain of rats differing in terms of their level of aggression was also shown to be associated with high sympathetic-adrenomedullary activation (23, 147). However, the origin of this sympathetic imbalance in social stress had not been studied until recently. In addition, cardiac sympathetic excitation is associated with low vagal tone and reciprocal vagal baroreflex sensitivity impairment in many stress disorders (36, 78, 129, 135), including anxiety (169, 170). Interestingly, unmedicated females are especially impacted by depression and present a lower reduction in pharmacological BRS than males (166). However, experimental studies have failed to demonstrate any effect of chronic stress-induced depressive state on BRS, as cardiac responses to spontaneous variations in mean blood pressure (spontaneous BRS) or pharmacological BRS induced by administration of vasoactive agents remained unaltered by stress (4, 54).

To demonstrate the effect of anxiety on parasympathetic tone and investigate the origin of sympathetic hyperactivity in an experimental model of stress, we have recently been using a social defeat paradigm based on anticipation (10). This procedure includes four daily conditioning sessions with the same pairs of residents and intruders. During the first part of the session, intruders (defeated animals) are placed individually in a protective cage inside the resident home cage, allowing unrestricted visual, auditory and olfactory contact with the resident but precluding close physical contact. The protective cage is then removed during the second part with the resident present, allowing physical contact. This social-defeat procedure induces physiological and behavioral changes (HPA axis hyperactivity, anhedonia, absence of body weight gain), as well as drastic sevenfold increases in serum corticosterone levels 5 days after completion of the procedure (11). Defeated animals also display anxiety-like behavior (126). For the first time, we have found that chronic social defeat, at a distance from stressor application, induces global cardiovascular modifications resembling those observed in anxiety (140). Defeated animals had a higher heart rate and lower HRV than controls. The LF/HF ratio, providing an indirect estimate of sympathovagal balance, increased, as did LF. Vagally mediated HF power (117, 134, 154) was decreased. These results indicate a shift towards sympathetic predominance over parasympathetic activity after social defeat. With our paradigm, spontaneous BRS was also much lower in defeated than in non-defeated animals. In addition, pharmacological BRS was lower in animals presenting an anxiety-like profile. Interestingly, chlordiazepoxide treatment, which prevented increases in adrenal gland weight and modifications in the open-field test, also prevented the reduction of HRV and baroreflex responses (140), supporting the hypothesis that all modifications in the autonomic balance induced by social defeat are linked to anxiety.

DMH activation during the defense reaction induces a sympathetically mediated increase in heart rate and mean blood pressure, as well as a reduction of pharmacological BRS (102, 141). In view of these data, it was reasonable to propose that this nucleus could be involved in cardiovascular alterations induced by chronic stress. Local microinjections of muscimol were performed to explore this possibility. This procedure decreased heart rate and blood pressure, and restored HRV and spontaneous as well as pharmacological BRS (140). These results suggest that the DMH may be overactivated and involved in all of the

cardiovascular modifications observed in animals subjected to social challenge, and may therefore represent a key component of the central pathway activated during chronic stress-induced anxiety. As mentioned above, it is highly likely that the vasomotor and cardiac components of the response evoked from the DMH may be mediated by pathways that are dependent and independent of neurons of the RVLM, respectively. In particular, the tachycardic response to chronic stress may be mediated by 5-HT_{1A} receptors in the caudal raphe (43, 100). It is noteworthy that, in some experiments, when muscimol was injected into the ventromedial hypothalamic nucleus, baseline parameters and HRV were not affected, while when muscimol was injected into structures adjacent to the DMH, such as the perifornical nucleus, HRV and BRS were restored to a lesser degree (personal data). Muscimol may have reached the DMH due to the proximity of these structures; another possibility is that the perifornical area may also be part (upstream or downstream to the DMH) of the neurocircuitry involved in the cardiovascular modifications induced by stress. Further experiments are needed to explore this possibility.

As mentioned above, during acute stress, the DMH acts on the rostral cuneiform nucleus, that, in turn, activates the dorsolateral periaqueductal gray (102). Downstream to this structure, serotonin is released from the B3 region in the NTS to activate local 5-HT_3 receptors and induce baroreflex inhibition (142)(141)(140)(139)(141). We investigated whether 5-HT_3 receptor blockade also could prevent the baroreflex reduction induced by chronic stress. We targeted the NTS receptors by local microinjection of granisetron into the NTS (140). This study showed that granisetron prevented the reduction in spontaneous and pharmacological baroreflex parameters in defeated animals. Vagally mediated HF power, which is normally low in animals subjected to chronic stress, was similar to that observed in controls after both treatments. Taken together, these results suggest that the reduction of the cardiac baroreflex in animals with social stress-induced anxiety is produced by chronic activation of 5-HT_3 receptors in the NTS by serotonin from the medullary caudal raphe. The circuitry involving NTS 5-HT_3/NK_1/$GABA_A$ receptors to modulate BRS during acute stress (30) may also play a key role in the dysautonomia observed after social challenge. Further experiments are needed to explore this possibility.

4. Conclusion

In conclusion, high anxiety-related behavior is characterized by low vagally mediated HRV coupled with increased vulnerability to pharmacologically-induced arrhythmias (23). Specific treatment for mood disorders only partially restores HRV (22). It is noteworthy that 4 weeks of fluoxetine treatment, that can inhibit binding of 5-HT_3 receptor antagonists (85, 161), can prevent behavioral responses and can partially prevent cardiovascular changes associated with chronic mild stress (51). Thus, in patients with high anxiety scores and in patients with induced dysautonomia, systemic treatment with granisetron —a potent anti-emetic used to treat patients undergoing cancer chemotherapy (6) with a very good safety profile (1)— could be used to restore parasympathetic activity and could therefore represent a specific target to reduce the likelihood of adverse cardiac events. In support of this hypothesis, it is noteworthy that systemic administration of granisetron is able to prevent the reduction in vagal activity and BRS induced by social defeat (140).

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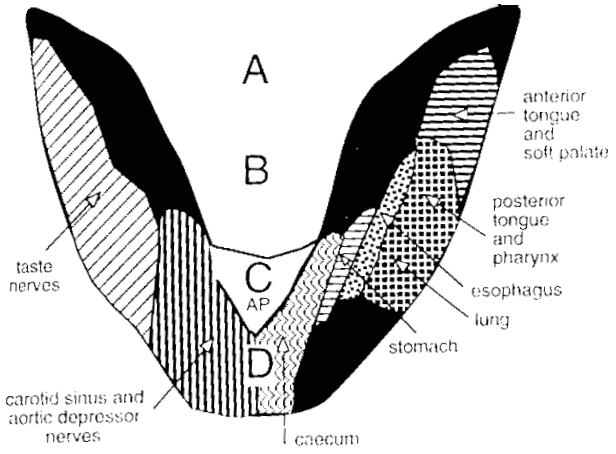
Legends of Figures

Figure 1

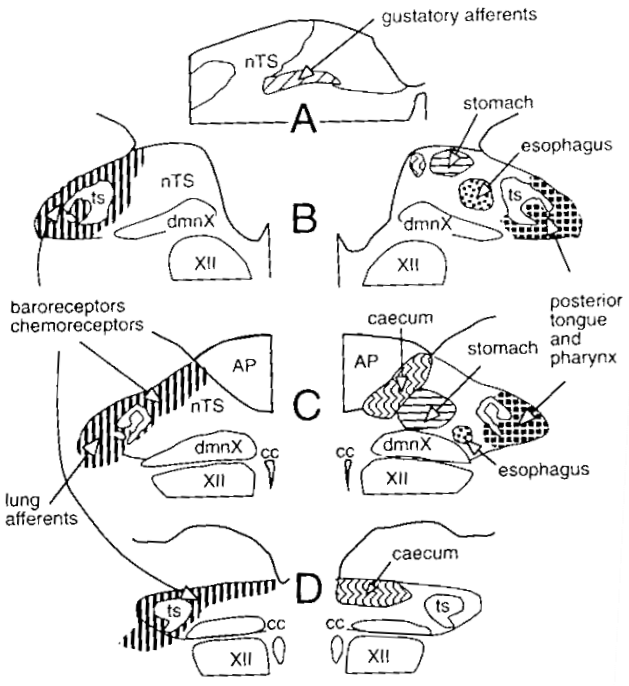
A. Projection sites within the Rostral (A and B), Intermediate (C) and Commissural (D) NTS from peripheral target organs and glossopharyngeal or vagal afferents. *Modified from* (15).

B. Principal central and peripheral afferents to the NTS containing GABA, 5-HT and/or Substance P.

A



horizontal section



transverse sections

B

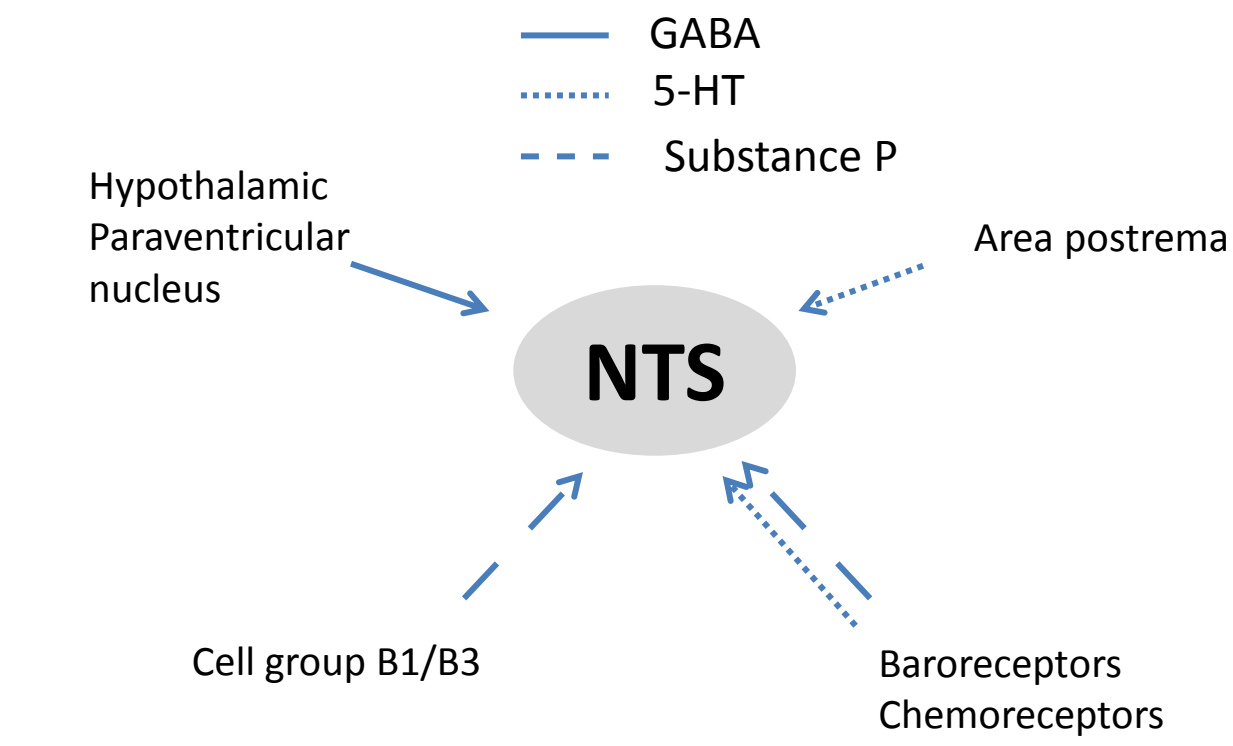
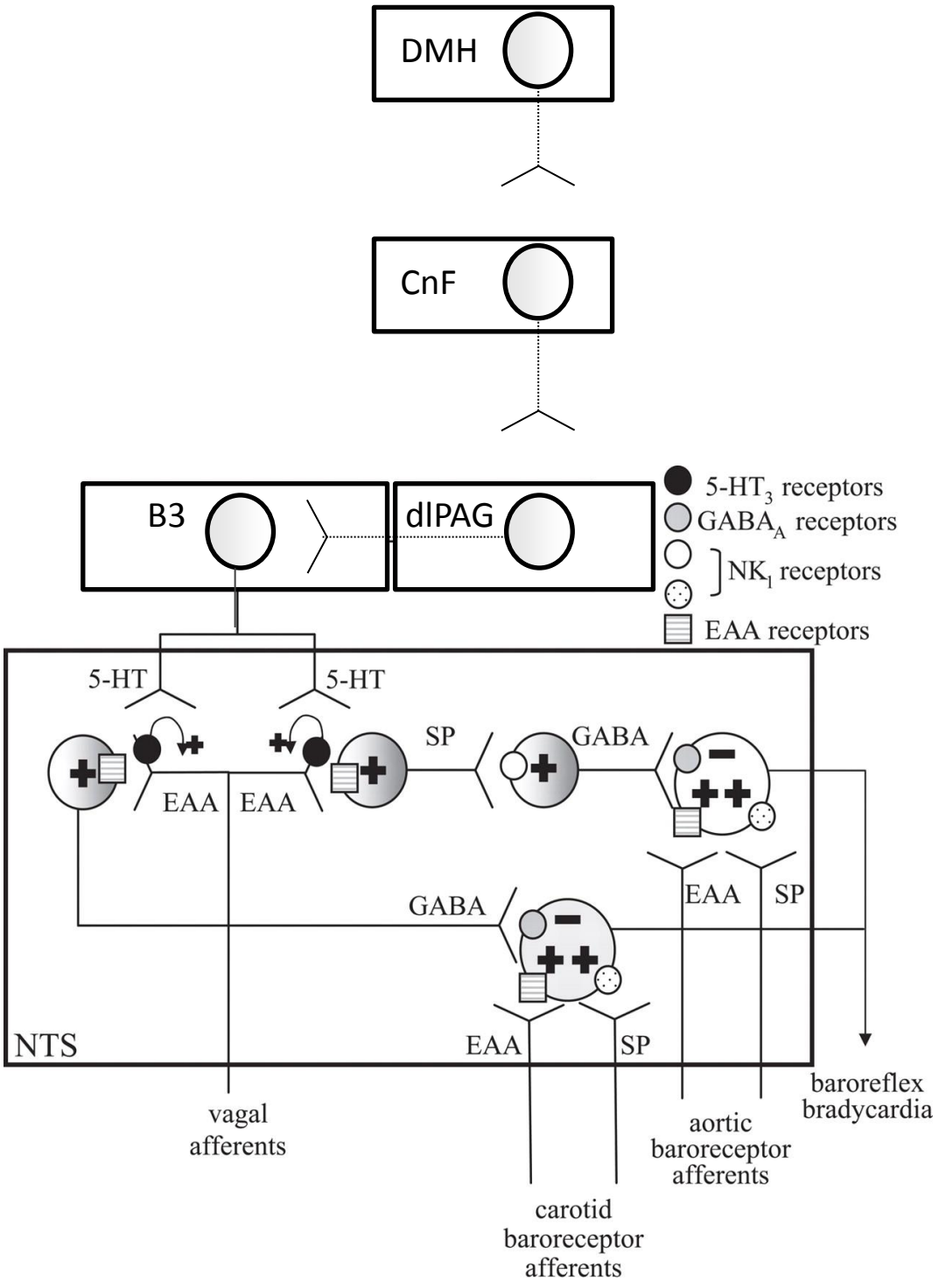


Figure 2

Hypothetical neurocircuitry involved in the baroreflex inhibition during stress. Activation of the DMH produces indirect excitation of the Cuneiform nucleus (CnF) then dorsolateral periaqueductal (dlPAG) neurons. Secondarily, following serotonin release into the NTS, presumably from neurons originating in the B3 region (containing the nucleus raphe magnus associated with the lateral paragigantocellular nucleus), 5-HT_3 receptors (small black circle), localized presynaptically on vagal glutamatergic afferents in the NTS, are activated. Depending on the origin (i.e., aortic or carotid sinus) of baroafferents, second-order baroreceptor cells may be modulated by different pathways. Hence, in the pathway involving modulation of NTS cells receiving aortic afferents (white circle), the local release of glutamate, induced by stimulation of 5-HT_3 receptors, excites substance P interneurons and in turn GABAergic interneurons expressing NK_1 receptors (small white circle). In a second pathway involving modulation of NTS cells receiving carotid sinus afferents (pale grey circle), the abovementioned glutamate release directly excites GABAergic interneurons. In both pathways, the resulting GABAergic activation finally produces inhibition of baroreflex bradycardia via stimulation of $GABA_A$ receptors (small grey circle) present on each type of NTS baroreceptor cell. *Modified from* (30)


DMH
CnF
B3
dlPAG
5-HT3 receptors
GABAA receptors
NK1 receptors
EAA receptors
5-HT
5-HT
SP
GABA
EAA
EAA
GABA
EAA
SP
EAA
SP
NTS
baroreflex bradycardia
vagal afferents
aortic baroreceptor afferents
carotid baroreceptor afferents