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# 5-HT<sub>3</sub> Receptor— Mediated Neural Transmission of Cardiorespiratory Modulation by the Nucleus of the Tractus Solitarius

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## 1. INTRODUCTION

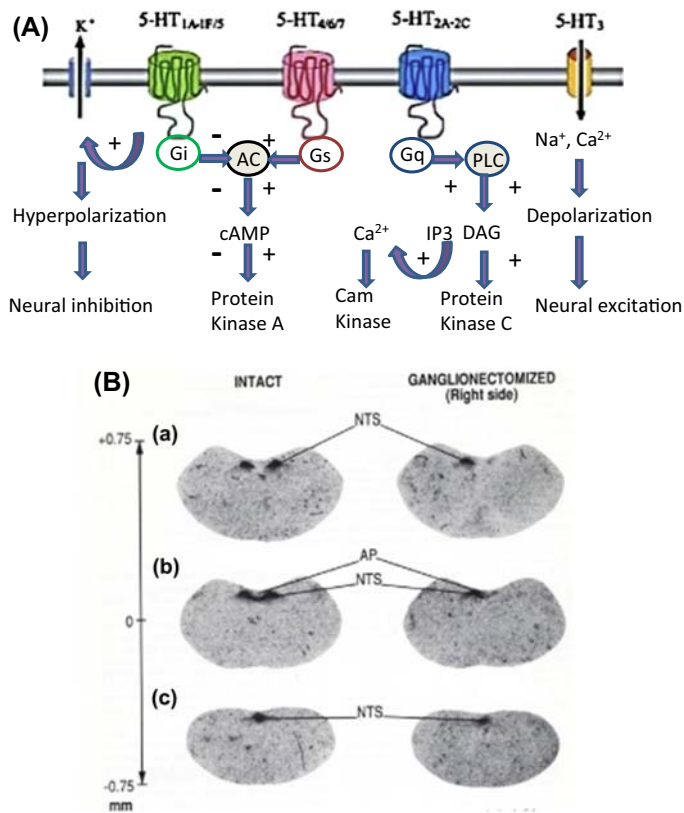
Homeostasis can proceed remarkably normally in mammals without functioning cerebral hemispheres, as demonstrated by a study on decerebrate cats maintained for several months [1]. The cats were not only able to walk, eat, and drink, but basic respiratory and cardiovascular functions were normal. More recently, the Working Heart Brainstem Preparation developed for rats [2] and mice [3,4] by Paton in the 1990s showed that the preparation with a viability of 4–6 h produces stable and consistent cardiorespiratory reflex responses to a wide range of visceral stimuli. The brainstem contains many premotor and motor neurons responsible for homeostasis control and thus has the capacity to regulate the maintenance of bodily homeostasis. Within the brainstem, the nucleus of the tractus solitarius (NTS) is of particular interest. Located in the dorsomedial medulla oblongata, it receives visceral afferent information from the cardiovascular, respiratory, gastrointestinal, and taste systems arising from the sensory components of four cranial nerves: facial, trigeminal, glossopharyngeal, and vagus [5]. With the use of neuroanatomical techniques based on the axonal transport of different tracers, evidence has been provided that facial and trigeminal afferents reach the rostral NTS, whereas both glossopharyngeal and vagus nerves project to the intermediate and caudal subregions of the NTS [6,7].

Nearly all the putative neurotransmitters found in the CNS have been identified in the NTS [8], including serotonin [9]. Serotonin immunoreactivity is present in varicose fibers disseminated throughout the rostral to caudal parts of the NTS [10]. 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) [11–13]. In addition, the NTS also receives serotonergic afferents from the nodose and petrosal ganglia (the site of vagus and glossopharyngeal nerve cell bodies, respectively) [14,15] and the area postrema [13,16]. Numerous pharmacological, electrophysiological, and immunohistochemical studies have provided evidence for the existence of 5-HT<sub>1</sub> [17], 5-HT<sub>2</sub> [18,19], 5-HT<sub>3</sub> [20,21], 5-HT<sub>4</sub> [22], 5-HT<sub>6</sub> [16], and 5-HT<sub>7</sub> [23] receptors in the NTS.

5-HT<sub>3</sub> receptors are members of the Cys-loop superfamily of ligand-gated ion channels [24] (Fig. 17.1A). 5-HT<sub>3</sub> serotonergic receptors exhibit nearly equal permeability to both Na<sup>+</sup> and K<sup>+</sup>. They are composed of a pentameric complex that surrounds a central ion channel with a high homology with nicotinic acetylcholine receptors. Two 5-HT<sub>3</sub> receptor subunits have been cloned to date, the A subunit (5-HT<sub>3A</sub>), which is expressed by both central and peripheral neurons, and the B subunit (5-HT<sub>3B</sub>) that seems to be restricted to peripheral neurons [20]. When expressed alone, the 5-HT<sub>3B</sub> subunit fails to form functional 5-HT<sub>3</sub> receptors. However, when it is coexpressed with the 5-HT<sub>3A</sub> subunit, the resulting heteromeric 5-HT<sub>3</sub> receptor complex fully replicates the biophysical characteristics of native neuronal 5-HT<sub>3</sub> receptors [25]. Only minimal pharmacological differences have been identified between homomeric 5-HT<sub>3A</sub> receptor and heteromeric 5-HT<sub>3A/3B</sub> receptors. In 2003, three human genes encoding novel 5-HT<sub>3C</sub>, 5-HT<sub>3D</sub>, and 5-HT<sub>3E</sub> receptor subunits were reported [26]. The genes for these subunits are located on human chromosome 3q27, whereas those for 5-HT<sub>3A</sub> and 5-HT<sub>3B</sub> are on chromosome 11q23. Further, the reported mRNA expression of the 5-HT<sub>3C–E</sub> subunits tended to show a peripherally restricted pattern, including high levels in the gastrointestinal tract [26]. Intriguingly, their orthologues seem to be absent in rodents [27]. These data suggest that 5-HT<sub>3C–E</sub> subunits are formed for a specific purpose within the peripheral nervous systems in some species, including humans, which is both distinct from the more general functions of receptors formed by 5-HT<sub>3A</sub> and 5-HT<sub>3B</sub>.

In the central nervous system, low levels of 5-HT<sub>3</sub> receptor expression have been demonstrated in the forebrain. Nevertheless, in the majority of species investigated to date, 5-HT<sub>3</sub> receptors were expressed notably in the amygdala and hippocampus [28], but the highest levels of 5-HT<sub>3</sub> receptor-binding sites were found in the NTS [21,29]. The nodose ganglion gives rise to the sensory fibers of the vagus nerve [30]; the majority of NTS 5-HT<sub>3</sub> receptors disappears in nodose ganglionectomized animals, suggesting a presynaptic location of these receptors on vagal afferents [21] (Fig. 17.1B). These afferents are likely to originate from the gastrointestinal tract because subdiaphragmatic vagotomy that removes abdominal visceral afferents of gastric origin abolished binding of [<sup>3</sup>H]granisetron in the NTS [31].



**FIGURE 17.1** The  $5-HT_3$  receptor.

(A)  $5-HT$  receptors and their effector systems.  $5-HT_{1/5}$ ,  $5-HT_2$ ,  $5-HT_{4/6/7}$  corresponds to the members of the G protein–coupled receptors and share a putative seven transmembrane structure. The  $5-HT_{1/5}$  and  $5-HT_{4/6/7}$  receptors are negatively and positively coupled to adenylyl cyclase (AC) to increase or reduce the formation of cAMP, respectively. The  $5-HT_2$  receptor is coupled positively to phospholipase C (PLC) and is at the origin of diacyl glycerol (DAG) and inositol triphosphate (IP<sub>3</sub>) synthesis. The  $5-HT_3$  receptor is a ligand-gated ion channel. (B) Autoradiographic localization of [125I]iodozacopride binding to brain sections at the level of the nucleus of the tractus solitarius (NTS), in intact and unilateral ganglionectomized rats.

Adapted from Merahi N, Orer HS, Laporte AM, Gozlan H, Hamon M, Laguzzi R. Baroreceptor reflex inhibition induced by the stimulation of serotonin<sub>3</sub> receptors in the nucleus tractus solitarius of the rat. *Neuroscience* 1992;46:91–100.

## 2. ROLE OF NTS 5-HT<sub>3</sub> RECEPTORS IN THE CARDIOVASCULAR REGULATION

### 2.1 MODULATION OF BLOOD PRESSURE

Whereas microinjection of picomolar doses of 5-HT into the NTS elicits hypotension and bradycardia [32,33], nanomolar doses evoke a dose-dependent increase in blood pressure without any significant change in heart rate [32]. This effect is consequent on the activation of 5-HT<sub>3</sub> receptors because it can be mimicked by local bilateral microinjections of 5-HT<sub>3</sub> receptor agonists, such as 1-(m-chlorophenyl)-biguanide (CPBG), phenylbiguanide (PBG), and 2-methyl-serotonin; conversely it is prevented by prior local administration of selective 5-HT<sub>3</sub> receptor antagonists such as granisetron, ondansetron, and zacopride [32,34,35] but not antagonists at other serotonergic receptor types, such as methysergide and ketanserin [35].

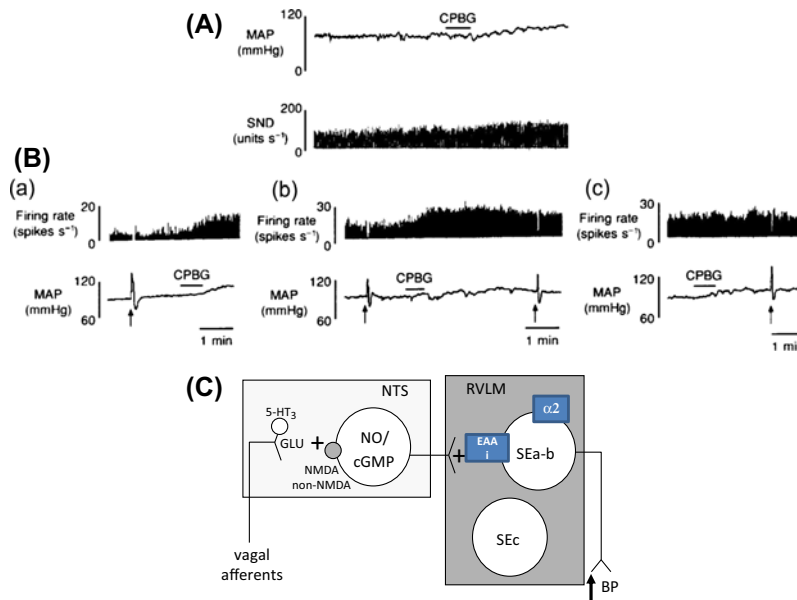
#### 2.1.1 At the Level of the NTS

Administration by reverse microdialysis of a 5-HT<sub>3</sub> receptor agonist into the NTS increased the local release of endogenous glutamate from vagal afferents [36]. The ability of granisetron to reduce glutamatergic mEPSCs [37] confirms the observations of Wan and Browning that the spontaneous release of 5-HT in rat brainstem slice preparations treated with TTX and gabazine acts via 5-HT<sub>3</sub> receptors on vagal afferent terminals to release glutamate in the NTS via a presynaptic mechanism [38]. Whereas intra-NTS administration of  $\alpha$ -amino-4-carboxyphenylglycine, a metabotropic glutamate receptor antagonist, did not affect the pressor response to intra-NTS CPBG, this response was suppressed when NTS ionotropic excitatory amino acid (EAA) receptors were blocked by local administration of kynurenic acid or a mixture of 2-amino-5-phosphonopentanoic acid and 6,7-dinitroquinoxaline-2,3-dione, an NMDA- and a non-NMDA—receptor antagonist, respectively [39].

Nitric oxide synthase (NOS) and ionotropic glutamate receptors appear to coexist in NTS neurons [40], and NO is produced downstream of the stimulation of ionotropic glutamate receptors [41]. Moreover, data in the literature support the idea that a functional link exists between the pressor response elicited by microinjection of glutamate in some NTS subnuclei and the NO/cGMP-transducing system [42]. For these reasons, we analyzed the effects of prior intra NTS microinjection of 7-nitroindazole and 1-H-(1,2,4)-oxadiazol-(4,3-A)-quinoxalin, which inhibit NOS and the soluble guanylyl cyclase, respectively, on the pressor response to NTS 5-HT<sub>3</sub>—receptor stimulation in anaesthetized rats [39]. Both pretreatments markedly reduced this response, indicating that the release of glutamate and the resulting activation of ionotropic glutamate receptors and the associated NO/cGMP transduction mechanism, contribute downstream to the pressor effect elicited by 5-HT<sub>3</sub>-receptor stimulation in the NTS.

#### 2.1.2 At the Level of the Ventral Medulla

Activation of 5-HT<sub>3</sub> receptors in the NTS by high (nmol) doses of serotonin increases blood pressure and sympathetic nerve discharge [43,44] (Fig. 17.2A). The



**FIGURE 17.2 Cardiovascular responses to nucleus of the tractus solitarius (NTS) 5-HT<sub>3</sub>-receptor activation.**

(A) Activation of NTS 5-HT<sub>3</sub> receptors by 1-(m-chlorophenyl)-biguanide (CPBG) microinjection increased the sympathetic lumbar nerve discharge (SND) and the mean arterial pressure (MAP). (B) CPBG increased the firing rate of slowest (a) and intermediate (b) clonidine-sensitive, but not fastest clonidine-insensitive (c) cardiovascular sympathoexcitatory cells of the rostroventrolateral medulla (RVLM).

Arrow: aortic occlusion. (C) Putative mechanism responsible for the pressor response to 5-HT<sub>3</sub> receptor stimulation in the NTS. NTS 5-HT<sub>3</sub> receptors are located on glutamatergic vagal (gastrointestinal) afferents. Their stimulation promotes the release of glutamate (GLU), which in turn activates ionotropic EAA receptors (NMDA and non-NMDA) and the downstream NO/cGMP transduction system. NTS neurons activated by glutamate send excitatory projections to clonidine-sensitive (but noncatecholaminergic) sympathoexcitatory cells (SE<sub>a</sub> and SE<sub>b</sub>) located in the RVLM. The resulting sympathoexcitation produces an increase in blood pressure (BP). SE<sub>c</sub>: clonidine-insensitive sympathoexcitatory neurons in the RVLM.

(A and B) Adapted from Sévoz-Couche C, Nosjean A, Franc B, Hamon M, Laguzzi R. Dorsal medullary 5-HT<sub>3</sub> receptors and sympathetic premotor neurones in the rat. *J Physiol* 1998;508(Pt. 3):747–62.

ventrolateral medulla contains different populations of medulla-spinal neurons that influence sympathetic nerve activity [45,46]. In particular, the rostroventrolateral part of the medulla (RVLM), known as the “pressor” area, contains sympathoexcitatory cells in contact with spinal preganglionic neurons. Intra-NTS administration of CPBG, but not saline, produces a dramatic increase in the density of c-Fos immunoreactive neurons in the RVLM [47]. As expected, this effect was prevented by

prior intra-NTS microinjection of ondansetron [47]. We observed that kynurenic acid administration into the RVLM blocked the pressor response to NTS 5-HT<sub>3</sub>–receptor stimulation by CPBG [48], thereby indicating that ionotropic EAA receptors in the RVLM are crucially involved in this response. The pressor effect of NTS 5-HT<sub>3</sub>–receptor activation by CPBG is actually the consequence of the excitation of two different pools of medullo-spinal sympathoexcitatory neurons, i.e., those with the slowest and the intermediate axonal conduction velocity, without affecting those with the highest conduction velocity [44] (Fig. 17.2B). Despite the fact that RVLM neurons sensitive to CPBG were inhibited by i.v. administration of the  $\alpha_2$ -adrenergic receptor agonist clonidine [44], the possibility that a monosynaptic pathway from the NTS to the sympathoexcitatory adrenergic neurons of the RVLM might convey the pressor message triggered by NTS 5-HT<sub>3</sub>–receptor stimulation is unlikely, because if an increased number of c-fos immunoreactive cells in the RVLM was observed after intra-NTS microinjection of CPBG, these cells were not double-stained for c-fos and tyrosine hydroxylase [49]. Thus the pressor response to NTS 5-HT<sub>3</sub>–receptor stimulation is associated with the excitation of noncatecholaminergic neurons within the RVLM. A summary of the NTS–RVLM pathway involved in the pressor effect of 5-HT<sub>3</sub>–receptor agonist is presented in Fig. 17.2C.

## 2.2 MODULATION OF REFLEX BRADYCARDIA

Vagal NTS second-order neurons afferents from aortic and carotid baroreceptors (sensitive to blood pressure modifications), atrial cardiopulmonary receptors (sensitive to diverse chemical substances), and carotid chemoreceptors (sensitive to changes in blood gas concentrations) contact cardiac premotor neurons located within the nucleus ambiguus to switch cardiovagal (bradycardia) reflex responses [50]. Intra-NTS administration of nanomolar doses of 5-HT or 5-HT<sub>3</sub>–receptor agonists, in anaesthetized as well as unanaesthetized rats, inhibits the cardiac component of the three aforementioned cardiovascular reflexes. This effect is specific because it can be blocked by prior intra-NTS administration of 5-HT<sub>3</sub>–receptor antagonists but remains unchanged after local administration of antagonists acting at other receptor types [32,35,51,52]. These data clearly show that NTS 5-HT<sub>3</sub> receptors mediate a general 5-HT inhibitory influence on the parasympathetic component of the reflex control of heart rate. Specific activation of 5-HT<sub>3</sub> receptors results in clear-cut excitation of most NTS cells [17]. Therefore, an inhibitory effect on the reflex cardiac responses could have been the consequence, at least in part, of a presynaptic inhibition. However, microinjections of EAA-receptor antagonists into the NTS block both the baroreflex- and Bezold–Jarisch-evoked reflex bradycardia [32,53]. Another possibility was that the EAA released in the NTS under 5-HT<sub>3</sub>–receptor activation could, directly or indirectly, activate GABAergic interneurons. Accordingly, the inhibitory effect of intra-NTS microinjection of

5-HT<sub>3</sub>—receptor agonists on the bradycardic responses to baroreflex, chemoreflex, or Bezold—Jarisch reflex activation could be prevented by prior local microinjection of low doses of bicuculline [32,35,51].

### 2.3 ROLE OF THE NTS 5-HT<sub>3</sub> RECEPTOR IN THE MODULATION OF STRESS-INDUCED CARDIOVASCULAR RESPONSES

Hypertension and reduced baroreflex cardiac response is also observed during acute stressful conditions such as the defense reaction. Various hypothalamic areas were originally defined as defense areas (HDA) because electrical or chemical stimulation produced characteristic behavioral and physiological defense responses [54–56]. Among the various HDA, the dorsomedial nucleus of the hypothalamus (DMH) appears to be of particular interest [57,58]. Other key regions involved in the defense reaction were subsequently described, such as the dorsolateral periaqueductal gray (dIPAG) in the midbrain [59,60]. In previous studies, we confirmed that intense dIPAG and DMH activation induced a marked increase in mean blood pressure and a decrease in parasympathetic activity [61,62]. To establish whether endogenous 5-HT actually occurs in animals expressing this type of behavioral reaction, we analyzed the effects of dIPAG stimulation on the baroreflex bradycardia response in rats pretreated with p-chlorophenylalanine (PCPA), a serotonin synthesis inhibitor. We showed that inhibition of 5-HT synthesis almost totally prevented the inhibitory effects of dIPAG stimulation on the baroreflex bradycardia [63]. Moreover, the role of 5-HT in the inhibitory influence of dIPAG 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. The involvement of NTS 5-HT<sub>3</sub> receptors during acute stress was assessed by the fact that intra-NTS microinjection of granisetron drastically reduced the inhibitory effects of dIPAG and DMH stimulation on baroreceptor sensitivity [62,64]. Similarly, a study by Gau et al. showed that thermal and mechanical nociceptions induced inhibition of the baroreflex bradycardia via activation of 5-HT<sub>3</sub> receptors in the NTS [65]. Interestingly, data indicated that:

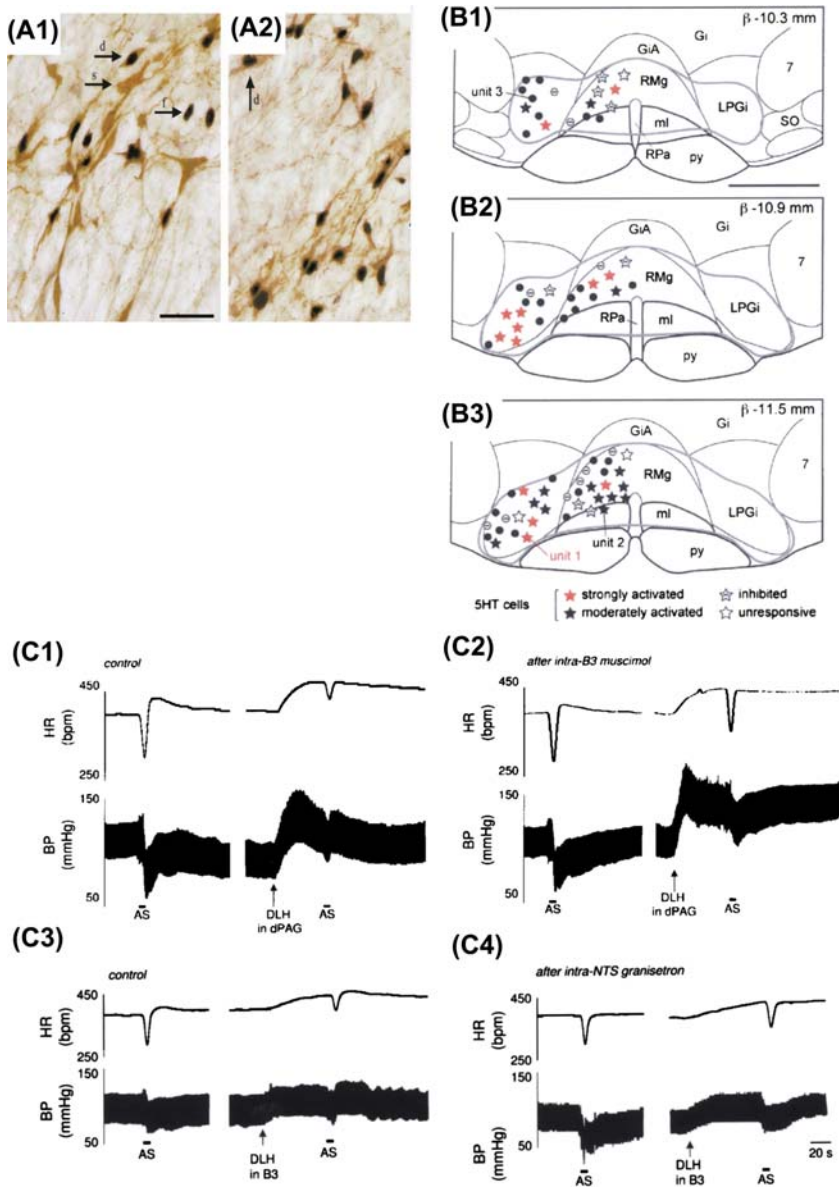
1. intra-NTS GR205171, a selective NK<sub>1</sub>-receptor antagonist, reversed the cardiac baroreflex (and cardiopulmonary, unpublished data) inhibition produced by dIPAG activation or local administration of PBG [64], and
2. intra-NTS bicuculline reversed the baroreflex (and cardiopulmonary, personal data) bradycardia inhibition produced by local administration of substance P [64]. These data indicate that NK<sub>1</sub> and GABA<sub>A</sub> receptors may contribute downstream to the 5-HT<sub>3</sub> receptor—mediated inhibition of the reflex bradycardia. It is noteworthy that neither microinjection of granisetron into the NTS nor PCPA treatment affected the sympathetically mediated increase in blood pressure induced by DMH and dIPAG stimulation [62–64]. Taken together, the data indicate that another mechanism is at the origin of this hypertensive stress response. Thus the physiological basis for the pressor effect of NTS 5-HT<sub>3</sub>—receptor activation remains unknown.



The origin of serotonin released into the NTS during the defense reaction is likely to 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 [22,66]. However, we found that a large majority of serotonergic cells in the B3 raphe (raphe magnus and lateral paragangliocellular nuclei) but not in other raphe nuclei were excited (as confirmed by the presence of c-fos protein) following DMH as well as dPAG stimulation [61,67] (Fig. 17.3A), or after application of nociceptive stimuli [65] (Fig. 17.3B). In addition,

1. the reduction in baroreflex bradycardia induced by dPAG activation was reduced by muscimol injection into the B3 region [67], and
2. the activation of the B3 region induced an inhibitory effect on aortic baroreflex bradycardia, which was prevented by intra-NTS granisetron [67] (Fig. 17.3C).

Decades of clinical, epidemiological, and experimental research in animals and humans has provided strong evidence in support of a close correlation between psychosocial factors and cardiovascular abnormalities [68–70]. 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 [71]. The increased arrhythmogenic feature in stress-related disorders was commonly linked to exaggerated sympathetic stimulation, as reflected by increased blood pressure, heart rate, and plasma catecholamines. Recently, the prospective study Autonomic Tone and Reflexes After Myocardial Infarction demonstrated that markers of reduced vagal activity, such as depressed baroreflex bradycardic response and high-frequency domain (vagal mediated) of the heart rate variability (HRV), are strong predictors of cardiac mortality after myocardial infarction [72,73]. A large proportion of sudden cardiac death is correlated with a low baroreflex response in patients taking, or not taking, beta blockers [74]. Cardiac sympathetic excitation is associated with low vagal tone and reciprocal vagal baroreflex sensitivity impairment in many stress disorders, including depression [75] and anxiety [69,76]. Interestingly, unmedicated females are especially affected by depression and present a lower reduction in baroreflex sensitivity (BRS) than males [77,78]. Therefore the prevention of reduced vagal cardiac response may also be a target of choice to reduce arrhythmias, but the origin of this dysfunction is still unclear. However, it was possible that similar neurocircuitry to that underlying cardiac alteration during the defense reaction may occur during chronic stress. Using a social defeat paradigm triggering arrhythmias [79], we showed that vagally mediated high-frequency band (short-term regulation) of the HRV [80] and baroreflex bradycardia were lower in defeated animals [81]. Blockade of the DMH by muscimol or NTS 5-HT<sub>3</sub> receptors by local microinjection of granisetron prevented both modifications. It is interesting that granisetron did not block the long-term increase in blood pressure. Taken together, the results suggest that the reduction of the cardiac baroreflex (but not the hypertension) in animals with social stress–induced anxiety is produced by chronic activation of 5-HT<sub>3</sub> receptors in



**FIGURE 17.3** The source of serotonin release in the nucleus of the tractus solitarius (NTS) after stress induction.

(A) Color-digitized photomicrographs showing the increase of neurons labeled in the lateral paraventricular nucleus for c-Fos+ 5-HT (d) after dorsolateral periaqueductal gray (A2) compared to sham (A1). (f) neurons labeled for c-Fos, (s) neurons labeled for 5-HT alone. (B) Schematic drawing of coronal sections through the B3 region, showing

the NTS by serotonin from the medullary caudal raphe, from the DMH and the dIPAG. Interestingly, systemic granisetron (a drug that crosses the blood–brain barrier and has a high safety profile) also prevented the low BRS in defeated rats, suggesting a potential role for this substance as a preventive or curative treatment against reduced vagal activity (and therefore possibly against arrhythmias) in chronic stress but also in all pathologies linked to dysautonomia.

### 3. ROLE OF NTS 5-HT<sub>3</sub> RECEPTORS IN THE RESPIRATORY REGULATION DURING STRESS

There is an important reciprocal link between breathing and anxiety [82]. Thus, respiratory distress and asphyxia cause sensation of dread. On the other hand, fear and anxiety can have profound effects on breathing. Clinical studies show that panic disorder, which is characterized by acute and unexpected anxiety attacks and substantial anxiety over the possibility of experiencing further attacks, is associated with symptoms that include palpitations, shortness of breath, sweating, and hyperventilation [83]. In addition, high levels of anxiety-related behavior in rats are associated with an elevation of the resting respiratory rate [84]. Comparatively less is known about the long-term effects of emotional stress on breathing. In adult rats, a strong neonatal emotional stress such as maternal separation leads to a decrease in breathing rate during non-REM sleep [85]. A lower breathing rate was also noted under anesthesia in the Flinder-Sensitive rat, a well-validated animal model of depression [86]. However, in patients with mood-depression disorders, breathing rate does not seem to be altered, although cardiovascular changes do occur [87]. A study on the long-term effects of social defeat on breathing was conducted recently. Peripheral and central respiratory and cardiovascular regulatory mechanisms are tightly coupled. An element of this interaction is the respiratory sinus arrhythmia (RSA),

that neurons activated during noxious application, recorded and juxtacellularly filled, are extensively found in the lateral paragigantocellular nucleus. (C) Representative tracings showing that the baroreflex bradycardia evoked by aortic stimulation (AS) was reduced (control, left) by chemical (DLH, 0.3 M) activation of dPAG (C1) or B3 (C3). Pretreatment with muscimol (5 mM) into the B3 region (C2) or granisetron (2.5 mM) into the NTS (C4) prevented these inhibitory effects, respectively.

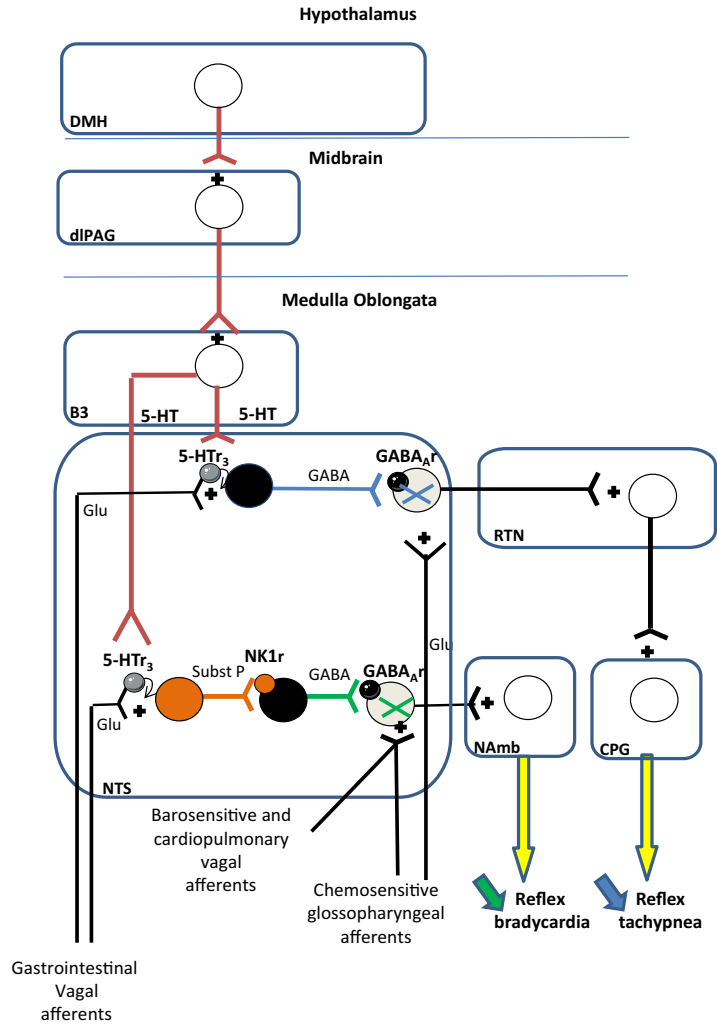
(A) Adapted from Bernard J-F, Netzer F, Gau R, Hamon M, Laguzzi R, Sévoz-Couche C. Critical role of B3 serotonergic cells in baroreflex inhibition during the defense reaction triggered by dorsal periaqueductal gray stimulation. *J Comp Neurol* 2008;506:108–21. (B) Adapted from Gau R, Sévoz-Couche C, Hamon M, Bernard J-F. Noxious stimulation excites serotonergic neurons: a comparison between the lateral paragigantocellular reticular and the raphe magnus nuclei. *Pain* 2013;154:647–59. (C) Adapted from Bernard J-F, Netzer F, Gau R, Hamon M, Laguzzi R, Sévoz-Couche C. Critical role of B3 serotonergic cells in baroreflex inhibition during the defense reaction triggered by dorsal periaqueductal gray stimulation. *J Comp Neurol* 2008;506:108–21.

which reflects the regular increase in heart rate during inspiration and its decrease during expiration [88]. RSA appears in the high frequency range at which the parasympathetic but not the sympathetic branch of the autonomic nervous system can respond to respiration and influence heart rate. As such, RSA peak frequency seen in HRV analysis (fast Fourier transformation of the cardiac pulse interval signal) is a reliable method for extracting breathing frequency in both the anaesthetized and conscious animal, as it is comparable to tracheal and pleural respiratory rates in both conditions, during low or high activity [89]. Telemetric recordings in social defeat demonstrated a lower RSA frequency (i.e., a lower respiratory rate) in stressed rats, during and after (that effect lasted at least 10 days) the stress procedure [90]. To find out whether the DMH and NTS 5-HT<sub>3</sub> receptors were not only involved in the reduction of the BRS but also in the bradypnea evoked by social defeat, we blocked the DMH with bilateral microinjections of muscimol and antagonized 5-HT<sub>3</sub> NTS receptors with bilateral microinjections of granisetron. As with cardiac vagal inhibition, local blockade of DMH and 5-HT<sub>3</sub> NTS receptors abolished the bradypnea of defeated rats. This bradypnea does not appear to be associated with increased tidal volume because pleural pressure seems to be unaffected in defeated rats (unpublished observation).

A complex network of lower brainstem neurons, termed the respiratory central pattern generator (CPG), generates the respiratory rhythm and defines the onset and offset of the various respiratory motor outflows and regulates the frequency and intensity of these periodic outflows [91]. It receives central inputs, and in particular participates in the hyperventilatory response seen during acute stress and intense activation of the DMH [92]. The chemical drive to breathe also relies on central chemoreceptors that detect brain extracellular fluid  $PCO_2$  via pH and on carotid body chemoreceptors that respond to low arterial  $PO_2$ -dependent manner [93]. The retrotrapezoid nucleus (RTN) contains very superficial propriobulbar neurons that have properties consistent with such specialized chemoreceptors [94] and is an important site of integration between central and peripheral chemoreception [95]. The RTN is a key source of tonic excitatory drive to both rhythm-generating and pattern-generating components of the CPG [96,97] and as such is responsible for hyperventilatory responses to central and peripheral chemoreflexes. Interestingly, the commissural NTS neurons that are activated by carotid body (peripheral chemoreceptor) stimulation innervate the RTN neurons and this projection is predominantly glutamatergic [95], as:

1. chemodenervation reduces baseline breathing rate [98], and
2. 5-HT<sub>3</sub> receptor—activation blocks the carotid chemoreflex bradycardia and induces stress-induced long-term bradypnea [90].

It is possible that DMH activation provokes long-term respiratory reduction through NTS 5-HT<sub>3</sub> activation—induced inhibition of the peripheral chemoreflex hyperventilation. In support of this hypothesis, minimal subthreshold activation of the DMH negatively modulates this reflex respiratory response [99,100]. We suggest that in acute stress, when respiratory values are already high because of intense



**FIGURE 17.4 Putative neurocircuitry underlying the stress-induced long-term cardiorespiratory alteration.**

NTS second-order aortic baroreflex and atrial cardiopulmonary neurons activate preganglionic cells in the nucleus ambiguus (Namb) to induce a reflex bradycardia. Carotid chemoreflex neurons project massively to the NTS, to produce a reflex bradycardia via Namb activation, and a reflex tachypnea through activation of the retrotrapezoid nucleus (RTN) in close relationship with the central pattern generator (CPG) of the respiration. Activation of the dorsomedial nucleus of the hypothalamus (DMH) activate efferents projecting to the dIPAG that may be at the origin of serotonin release into the NTS (presumably from the B3 region) to ultimately activate presynaptic 5-HT<sub>3</sub> receptors on vagal afferents; activation of these receptors induces the presynaptic release of glutamate that stimulates: (1) substance P interneurons, which activate GABAergic interneurons (black cell) endowed with NK<sub>1</sub> receptors. GABA will act on GABA<sub>A</sub> receptors present on NTS second-order neurons to inhibit the production of reflex bradycardia (green way); (2) GABAergic interneurons (black cell), and GABA will act on GABA<sub>A</sub> receptors present on second-order neurons to inhibit the production of the carotid chemoreflex tachypnea (blue way).

activation of neurons in the DMH, peripheral chemoreflex inhibition by NTS 5-HT<sub>3</sub>—receptor activation helps to prevent an additional increase in ventilation. In the longer term, the defense zone remains only mildly activated and is not able to increase the respiration by its direct action on CPG, and bradypnea appears due to chronic chemoreflex respiratory reflex response inhibition.

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#### 4. CONCLUSION

Activation of NTS 5-HT<sub>3</sub> receptors produces activation of three modulatory circuits:

1. the first is at the origin of an increase in blood pressure through an NO/GMPc activation of NTS second-order neurons in contact with clonidine-sensitive sympathoexcitatory cells located in the RVLM,
2. the second is at the origin of a reduction of reflex bradycardia, through a substance P-GABAergic inhibition of NTS second-order neurons in contact with cardiac premotor neurons in the nucleus ambiguus,
3. the third is at the origin of a bradypnea, presumably through a GABAergic inhibition of NTS second-order neurons in contact with chemosensitive cells in the RTN.

The second and third pathways are activated by a hypothalamo-midbrain circuitry after stress induction. A suggested neurocircuitry underlying stress-induced cardiorespiratory alteration is shown in Fig. 17.4. We showed that systemic treatment with granisetron was able to prevent these alterations [81,90]. Thus, in patients with high anxiety scores and in patients with induced autonomic dysfunction, as observed after ischemic stroke for example [101], systemic treatment with the 5-HT<sub>3</sub> receptor antagonist granisetron—a potent antiemetic [102] with a highly safe profile [103]—could be used to restore parasympathetic activity and, thus, reduce the likelihood of adverse cardiac events. In addition, this treatment may also help to prevent alveolar hypoventilation.

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