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ORIGINAL PAPER

Regional differences in serotonin content in the nucleus of the solitary tract of male rats after hypovolemia produced by polyethylene glycol

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Abstract Serotonin (5-HT) has been implicated in centrally mediated compensatory responses to volume loss in rats. Accordingly, we hypothesized that slowly developing, non-hypotensive hypovolemia increases serotonin in the hindbrain nucleus of the solitary tract (NTS). We produced volume loss in adult male rats by administering hyperoncotic polyethylene glycol (PEG) and then assessed 5-HT levels in the NTS using measurements of tissue 5-HT content or 5-HT immunohistochemistry. The results show selective increases of 5-HT in the caudal NTS after PEG treatment, but no change in the primary 5-HT metabolite, 5-HIAA. Moreover, the intensity of 5-HT immunolabeled fibers in the caudal NTS was increased after PEG treatment. These findings suggest that, after PEG-induced hypovolemia, 5-HT accumulates in neural elements in the caudal NTS. We propose that this accumulation is attributable to an initial release of 5-HT that then acts at presynaptic autoreceptors to inhibit subsequent 5-HT release.

Keywords: Baroreflex pathway · Hemorrhage · 5-HIAA · Norepinephrine

Introduction

Changes in body fluid volume elicit compensatory behavioral and physiological responses which, collectively, serve to maintain or restore blood volume to optimal levels. Some of these responses, such as water intake, salt intake, and hormonal responses, occur over a comparatively long period of time, whereas changes in autonomic nervous system activity occur in a much shorter time frame. Short-term responses, in particular, involve low pressure baroreceptors in the heart and great veins which signal increased or decreased volume, and subsequently activate hindbrain baroreflex pathways, the connectivity and neurochemistry of which are well defined (for review, see [1]). Activation in these pathways has been reported in response to increased volume [2–4] and, perhaps more familiarly, in response to decreased volume [5–8], such as that produced by experimental hemorrhage. Despite some overlap, however, studies by Badoer and colleagues [9–11] show that subpopulations of neurons within these hindbrain areas that project to specific targets appear to be selectively activated by hypovolemia.

The neurochemical profile of hindbrain neurons activated by hypovolemia is the subject of disagreement and conflicting findings. To some extent, the discrepant findings may be a consequence of the use of hemorrhage to produce volume loss, as severe hemorrhage is typically accompanied by hypotension. In fact, the pattern of activity produced by substantial volume loss [5–8] is remarkably similar to that observed after decreased blood pressure [5–7, 12]. Several investigators have reported that neurons in the A1 and A2 catecholaminergic areas of the hindbrain are activated by severe, hypotensive hemorrhage and by decreased blood pressure, but not by smaller, non-hypotensive hemorrhage [7, 13]. It is possible that differential activation of these neurons by hypotensive and non-hypotensive hemorrhage depends on the degree of hypovolemia, but this idea has been difficult to test, as greater hemorrhage tends to elicit hypotension. In a previous study [14], we circumvented this dilemma by using a hyperoncotic solution of polyethylene glycol (PEG), which produces

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slowly developing [15, 16] volume loss that is isosmotic [16, 17] and non-hypotensive [18, 19]. We combined this approach with immunocytochemical techniques to label hindbrain neurons for the fos protein, a marker of neural activation, and dopamine-β-hydroxylase, the enzyme required to convert dopamine to norepinephrine (NE), and found that A1 and A2 neurons were not activated by PEG despite substantial ($\sim$15–20 %) volume loss. These findings complement reports that A1 and A2 neurons were not activated by non-hypotensive hemorrhage ([7, 13], but see [20]), and, taken together, suggest that NE-containing neurons in the hindbrain are involved in responses to decreased blood *pressure* but not to decreased blood *volume*. Thus, activation in hindbrain pathways in response to volume loss may involve a different neurotransmitter.

Much research has focused on serotonin (5-HT) involvement in the activation of hindbrain baroreflex pathways during hypovolemia. Results to date suggest that 5-HT influences activity in the nucleus of the solitary tract (NTS); however, disagreement exists as to whether 5-HT increases or decreases compensatory blood pressure and/or heart rate responses to hypovolemia. Some studies used severe hemorrhage to investigate 5-HT-mediated compensatory responses (e.g., [21, 22]), while others used pharmacological manipulations to examine 5-HT effects (e.g., [23–27]). Thus, the conflicting findings may be attributable to the hypotension that accompanies severe hemorrhage, to 5-HT receptor subtype specific effects, or to both. The goal of the present study was to assess hypovolemia-induced activation of 5-HT in the NTS while minimizing these confounds. In addressing this goal, two issues were critical: first, separating hypovolemia from hypotension, and thereby eliminating the possibility that hypotension contributes to the findings; and second, reducing the possibility of effects attributable to administration of specific 5-HT agonists or antagonists. Accordingly, we produced volume loss by administering PEG and then examined levels of 5-HT in the NTS using measurements of tissue 5-HT content and 5-HT immunohistochemistry.

Methods

Animals

Adult male Sprague–Dawley rats (Charles River) weighing 400–650 g (i.e., 3.5–6 months of age) at the time of testing were individually housed in a temperature-controlled colony room on a 12:12-h dark:light cycle (lights on at 0700 hours) with ad libitum access to deionized water and standard laboratory chow (Purina #5001) except as noted. Experimental protocols were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals; procedures were approved by the Florida State University (measurement of 5-HT content in brain tissue) and the Oklahoma State University (5-HT immunohistochemistry in brain sections) Animal Care and Use Committees.

PEG-induced hypovolemia

On the morning of the test day (0800–1000 hours), rats were removed from their cages, weighed, and injected s.c. with 5 ml of PEG (8,000 MW, 40 % w/v in 0.15 M NaCl; Fisher Scientific) or 5 ml of the 0.15 M NaCl vehicle (VEH). Rats were then returned to their cages, from which water and chow had been removed. As in our previous study [14], we waited 6 h to ensure that substantial hypovolemia had developed, and then deeply anesthetized rats with sodium pentobarbital (50 mg/rat, ip; Abbot Laboratories). As described in the following, blood was collected for determination of plasma protein concentration and hematocrit; brains were collected for determination of tissue 5-HT content or for 5-HT immunolabeling.

Tissue 5-HT content

Following VEH ($n = 7$) or PEG ($n = 8$) treatment and anesthesia as described, rats were decapitated, brains were rapidly removed, frozen on dry ice, and then transferred to a −80 °C freezer. Trunk blood was collected into microcapillary tubes and centrifuged to evaluate plasma protein concentration using a refractometer, and hematocrit using a hematocrit reader.

Hindbrains were cut into 300-μm sections using a cryostat and transferred to a cold microscope slide. A blunted 18-g needle was used to collect bilateral tissue punches (838 μm diameter) from two levels of the NTS [28]: caudal to calamus scriptorius (−14.30 to −14.60 mm relative to bregma, which we defined as cNTS); and at the level of the Area Postrema between calamus scriptorius and obex (−13.70 to −14.00 mm relative to bregma, which we defined as mNTS). For each animal, the two punches from each level were combined in a single microcentrifuge tube and then transferred to a −80 °C freezer until processed for analysis of tissue content of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA; the primary metabolite of 5-HT), and NE using high performance liquid chromatography. Sections from which punches were taken were examined using darkfield microscopy to verify that punches were located in cNTS and mNTS.

Tissue punches were sonicated in 75 μl of 0.1 N perchloric acid. After centrifugation at 10,000*g* for 10 min, the supernatant was removed and the remaining pellet was retained for total protein assay. The supernatant was further purified by centrifugation through 0.2-μm filters prior to

measurement of 5-HT, 5-HIAA, and NE. Separation of analytes was achieved using a Waters Separation Module (Waters), an HD-150 column (ESA), and mobile phase (0.7 ml/min) consisting of 75 mM sodium dihydrogen phosphate monohydrate (EM Science), 1.7 mM 1-octanesulfonic acid sodium salt (Sigma), 0.01 % triethylamine (Aldrich), 25 μM EDTA (Fisher Scientific), and pH adjusted to 3.0 with 85 % phosphoric acid (Fisher Scientific). Measurement was via electrochemical detection (ESA). For each sample, 10 μl was injected on column. Separated samples then were oxidized at 400 mV, followed by reduction at 350 mV. 5-HT and 5-HIAA were quantified from oxidation peaks, while NE was quantified from a reduction peak. Peak areas were converted to pg of analyte/10 μl supernatant by comparison with a standard curve calculated using standards of known concentration.

Protein content of tissue pellets was measured using a Pierce BCA Protein Assay Kit (Pierce). Tissue pellets were re-suspended in assay buffer and 10 μl of each sample was aliquoted into 96-well plates in duplicate with 200 μl of BCA solution added to each well. Plates were incubated for 30 min at 60 °C. Sample absorbance was then measured at 562 nm using a plate spectrophotometer (Molecular Devices). Protein amount was expressed as equivalents of bovine serum albumen, known amounts of which served as standards against which unknowns were compared. Duplicates were averaged to obtain the protein content for each sample.

To control for possible differences in the size of tissue punches, 5-HT, 5-HIAA, and NE content were expressed as a function of the total protein content of the tissue punch. The relationship between tissue 5-HT and the metabolite was further assessed by examining the ratio between 5-HT and 5-HIAA, calculated as (5-HT/μg protein)/(5-HIAA/μg protein).

5-HT immunolabeling

Following VEH ($n = 5$) or PEG ($n = 6$) treatment and anesthesia as described, blood samples were taken from the heart for later evaluation of plasma protein concentration and hematocrit. Immediately after blood samples were collected, rats were perfused transcardially with ice-cold 0.15 M NaCl followed by cold 4 % paraformaldehyde. Brains were removed, post-fixed in paraformaldehyde overnight at 4 °C, and then transferred to a 30 % sucrose solution until cut into a 1:3 series of 40-μm sections using a cryostat. Sections were stored in a cryoprotectant solution [29] at −20 °C.

One series of free-floating sections through the hindbrain from each rat injected with VEH or PEG was processed for immunolabeling of 5-HT. Briefly, sections were rinsed in 0.5 M Tris-NaCl, incubated for 30 min in a 0.5 % solution of H_2O_2, rinsed again, and then soaked in 10 % normal goat serum (NGS) mixed in 0.5 M Tris-NaCl with 0.5 % Triton-X. Sections were incubated in the 5-HT primary antibody (rabbit anti-5HT; Biogenex, PU068-UP) diluted 1:1,000 in 2 % NGS at 4 °C for 70 h. Sections were rinsed in 2 % NGS and then incubated in the secondary antibody (Cy2; goat anti-rabbit IgG; Jackson Immunoresearch) diluted 1:300 in 2 % NGS at room temperature for 6 h. The reaction was terminated by rinses with 2 % NGS. Sections then were ordered, mounted on slides, and allowed to air-dry. Slides were dehydrated in 70, 95, and 100 % EtOH (3 min/concentration), defatted in xylenes (5 min × 2), and then coverslipped.

An epifluorescent microscope (Nikon Eclipse 80i) equipped with a camera and FITC filter was used to visualize 5-HT immunolabeling in 2–3 representative sections, matched between subjects, from the cNTS (−14.20 to −14.60 mm relative to bregma) and from mNTS (−13.60 to −14.10 mm relative to bregma). Digital photomicrographs of each section were taken, holding exposure time constant. Areas of low 5-HT immunolabeling in each section were identified; these areas, typically in the ventral portions of the sections, were used to subtract background labeling from the images using NIS Elements imaging software (Nikon). Based on landmarks [28], cNTS and mNTS were outlined, and the area of each outlined region and the intensity of the fluorescent labeling within the region were determined using NIS Elements. For each section, intensity was normalized to area (calculated as intensity/μm^2) and the average normalized intensity for each level (cNTS and mNTS) was calculated for each rat. Group mean normalized intensities then were calculated for cNTS and mNTS.

Statistical analyses

Data are shown as group means ± SEM. Differences in plasma protein concentration and hematocrit were assessed separately for the two experiments (tissue 5-HT content and 5-HT immunolabeling) using independent t tests. Hematocrits were used to calculate % change in plasma volume for each experiment (modified from [30]) as $100 \times [(100\text{-hematocrit}_{PEG}) - (100\text{-hematocrit}_{mean\ VEH})]/(100\text{-hematocrit}_{mean\ VEH})$. Independent t tests then were used to compare calculated volume loss between the two experiments.

Differences in tissue 5-HT, 5-HIAA, NE, the 5-HT:5-HIAA ratio, and the intensity of 5-HT immunolabeling were assessed using 1-way ANOVAs (Statistica; StatSoft); statistically significant ($p < 0.05$) effects were further evaluated using Fisher's LSD tests.

Results

In both experiments (tissue 5-HT content, 5-HT immunolabeling), PEG caused significant increases in plasma

Table 1 Body weights, hematocrits, changes in volume, and plasma protein concentrations of male rats from the two experiments (tissue 5-HT content and 5-HT immunolabeling) 6 h after sc injection with 0.15 M NaCl vehicle (*VEH*) or polyethylene glycol (*PEG*)

	Weight (g)	Hematocrit (%)	Change in volume (%)	Plasma protein concentration (g/dL)
Tissue 5HT content				
VEH	461.8 ± 49.4	45.2 ± 0.5		6.0 ± 0.2
PEG	493.7 ± 39.9	54.5 ± 1.8**	−13.19 ± 2.08	8.4 ± 0.2**
5-HT immunolabeling				
VEH	544.4 ± 25.4	45.3 ± 0.7		5.9 ± 0.1
PEG	513.8 ± 20.6	52.3 ± 1.1*	−16.96 ± 3.23	7.6 ± 0.3**

Percent change in plasma volume was calculated as $100 \times [(\text{100-hematocrit}_{\text{PEG}}) - (\text{100-hematocrit}_{\text{mean VEH}})]/(\text{100-hematocrit}_{\text{mean VEH}})$

Within an experiment, ** significantly greater than VEH ($p < 0.001$), * significantly greater than VEH ($p < 0.01$)

protein concentration (both $p < 0.001$; Table 1) and hematocrit ($p < 0.01$, $p < 0.001$, respectively; Table 1) 6 h after treatment, as expected [14, 15, 31]. Importantly, changes in plasma volume after PEG were not different in the two experiments (Table 1). Thus, despite methodological differences including that for blood collection, treatment with PEG significantly and comparably reduced plasma volume. Body weights of VEH-treated and PEG-treated rats were not different in either experiment (Table 1).

Tissue 5-HT content

Figure 1 (top) shows that there was a significant difference in tissue 5-HT content in the NTS ($F_{3,19} = 3.447$, $p < 0.05$). Pairwise comparisons revealed that 5-HT in the cNTS of rats that were treated with PEG was significantly greater than that in the cNTS of rats that were treated with VEH ($p < 0.05$), as well as that in the mNTS of VEH-treated rats ($p < 0.05$). However, 5-HT in the cNTS of rats that were treated with PEG was not different from that in the mNTS of PEG-treated rats. 5-HT levels in the mNTS of PEG-treated rats, the cNTS of VEH-treated rats, and the mNTS of VEH-treated rats were comparable.

Tissue 5-HIAA content (Fig. 1, middle) did not differ among the groups; in contrast, the ratio between 5-HT and 5-HIAA (Fig. 1, bottom) showed significant differences among the groups ($F_{3,14} = 3.838$, $p < 0.05$). Pairwise comparisons revealed that the 5-HT:5-HIAA ratio in the cNTS of PEG-treated rats was significantly greater than that in all other groups (all $p < 0.05$), which, in turn, did not differ from each other. Finally, tissue NE content was not affected by group (Table 2).

5-HT immunolabeling

Figure 2 shows 5-HT immunolabeling in the cNTS of a VEH-treated rat and a PEG-treated rat. As shown in Fig. 3, there was a significant difference in the intensity of 5-HT immunolabeling among the groups ($F_{3,18} = 5.736$, $p < 0.01$). Pairwise comparisons revealed that the intensity of labeling in the cNTS of rats that were treated with PEG was greater than that in the mNTS of rats that were treated with PEG or with VEH (both $p < 0.01$) which, in turn, were not different from each other. The intensity of 5-HT immunolabeling in the cNTS of VEH-treated rats was intermediate and did not differ from any of the other groups. Figure 4 shows digital photomicrographs of the 5-HT fiber labeling at higher magnification.

Discussion

Despite the consensus that 5-HT is involved in centrally mediated responses to volume loss [32, 33], it has been difficult to arrive at a coherent picture of the specific role of 5-HT in hindbrain baroreflex pathways. In part, this difficulty has arisen from the use of hypotensive hemorrhage to produce volume loss (e.g., [34–36]), as it is difficult to separate effects attributable to volume loss from those attributable to hypotension. Another complication involves experimental manipulations that employed selective 5-HT receptor agonists and antagonists [23–26], with the resultant receptor subtype-specific effects. Therefore, in evaluating the role of 5-HT in the NTS, we sought to minimize the hypotension that accompanies rapid, severe hemorrhage by using PEG treatment to produce gradual non-hypotensive, isosmotic volume loss [16–19]. To ensure that substantial hypovolemia had developed, we imposed a 6-h delay after PEG treatment (Table 2; see also [15, 31]). This delay also facilitated comparisons with our previous study [14], in which comparable volume loss was produced.

The major findings in this study were that non-hypotensive hypovolemia produced by PEG increased tissue 5-HT content in the NTS and, more specifically, within the caudal portion of the NTS (cNTS). The intensity of the immunolabeling was also greater in the cNTS of PEG-treated rats compared to that in the mNTS. Surprisingly, however, the intensity of labeling in the cNTS of VEH-treated rats was not different from that in the cNTS after PEG treatment. It is possible that greater levels of

variability in 5-HT immunolabeling in the cNTS of VEH-treated rats prevented the detection of a meaningful difference, or that immunolabeling does not provide the degree of sensitivity obtained with direct measurement of tissue content using HPLC. In either case, we were able to detect differences in 5-HT immunolabeling between the cNTS and mNTS of PEG-treated rats, and the intensity of the immunolabeling was greater in the cNTS. Thus, increased 5-HT in the NTS after PEG-induced hypovolemia was not simply due to a generalized increase in 5-HT. Rather, differences in both tissue 5-HT content and immunolabeling intensity were specific to the cNTS. In addition, there were no changes in tissue NE in the cNTS or the mNTS, suggesting that PEG treatment did not have non-specific effects within the NTS. Thus, non-hypotensive hypovolemia has regionally selective effects to increase 5-HT in the cNTS.

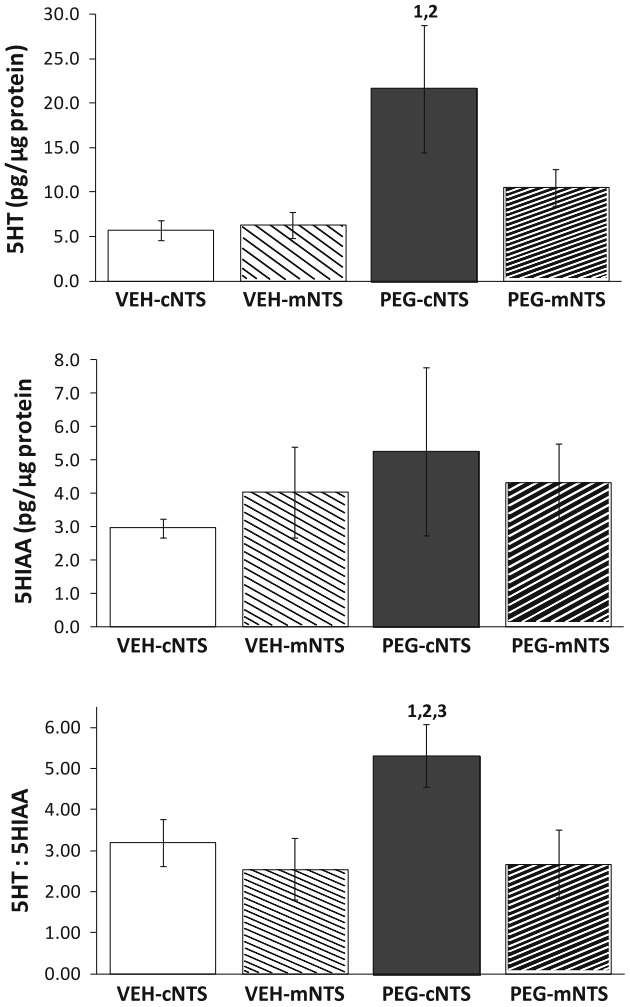

Fig. 1 5-HT and metabolite in the NTS. Tissue content of 5-HT (*top*), normalized to sample protein content, in the caudal NTS (*cNTS*) and in the NTS at the level of the Area Postrema (*mNTS*) after treatment with polyethylene glycol (PEG) or the 0.15 M NaCl vehicle (*VEH*). 5-HT in the cNTS of PEG-treated rats (*PEG-cNTS*) was significantly greater than that in the cNTS of VEH-treated rats (*VEH-cNTS*; *1*; $p < 0.05$) and that in the mNTS of VEH-treated rats (*VEH-mNTS*; *2*; $p < 0.05$). There were no differences in tissue 5HIAA content (*middle*). The 5HT:5HIAA ratio (*bottom*) was significantly greater in the cNTS of PEG-treated rats than that in all other groups (*1* significantly greater than VEH-cNTS, *2* significantly greater than VEH-mNTS, *3* significantly greater than PEG-mNTS, all $p < 0.05$)

Based on previous studies of 5-HT in the NTS [23–27, 37], we expected that PEG treatment would stimulate release of 5-HT in the NTS. However, there was no change in tissue levels of the 5-HT metabolite 5-HIAA (which would be expected to increase), and an increase in the 5-HT to 5-HIAA ratio (which would be expected to decrease) if elevated 5-HT were due to increased release [46]. Thus, the increase in 5-HT may be due to increased accumulation of intracellular 5-HT, rather than to increased 5-HT release. In this regard, closer examination of the 5-HT immunolabeling (Fig. 4) shows the beaded appearance of the labeling—i.e., varicosities—suggestive of terminal boutons and/or en passant synapses. Clearly, immunolabeling alone does not allow conclusive determination of whether 5-HT release was affected; on the other hand, it affords a high degree of spatial resolution that, in conjunction with evidence from HPLC of increased tissue 5-HT without changes in the metabolite, provides important information about the increased 5-HT. Taken together, the tissue content and immunolabeling results are consistent with the accumulation of 5-HT in neural elements and, in particular, in synaptic specializations located on neural fibers that terminate in or pass through the cNTS. Typically, the 5-HT would be thought to originate from one of the brainstem raphe cell groups, and, in fact, the NTS does receive projections from raphe 5-HT cell groups (e.g., [34, 35]). Moreover, severe hypotensive hemorrhage accompanied by hypercapnia activates 5-HT neurons in some raphe cell groups [34–36]. Nonetheless, several findings suggest an additional source. First, vagal afferent input arising from low pressure baroreceptors terminates in the NTS, including in the region caudal to the Area Postrema [38–40]. More importantly,

Table 2 Tissue NE content, normalized to total tissue protein, in the caudal NTS (*cNTS*) and in the NTS at the level of the Area Postrema (*mNTS*) of male rats 6 h after sc injection with VEH or PEG

	VEH-cNTS	VEH-mNTS	PEG-cNTS	PEG-mNTS
Tissue NE (pg/µg tissue protein)	27.85 + 7.38	26.54 + 3.50	31.39 + 7.65	24.79 + 7.84

There were no differences in tissue NE among the groups ($F_{3,17} = 0.174$, $p = 0.9125$)

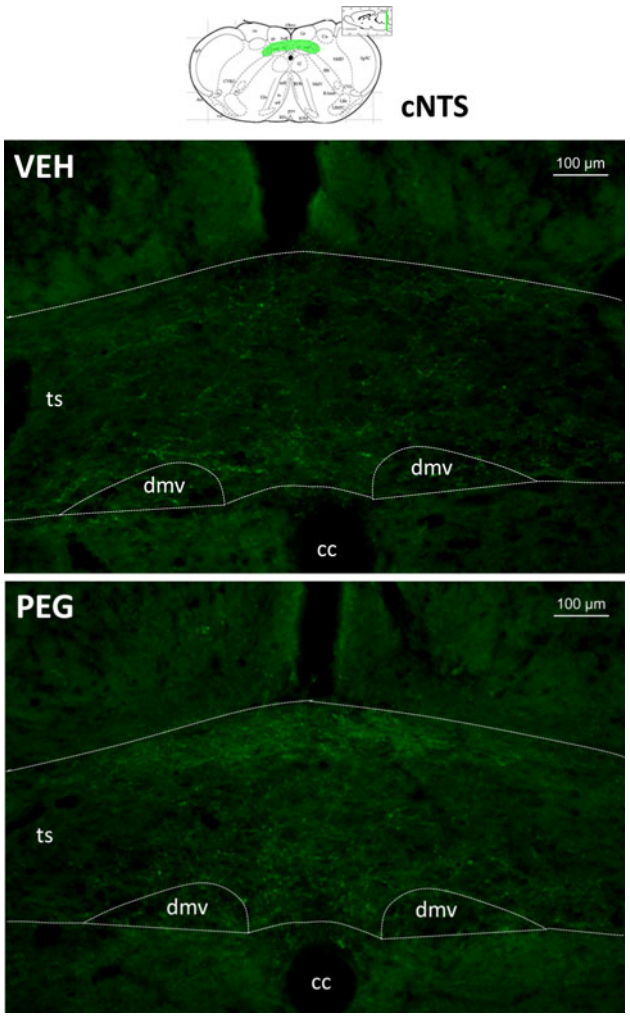

Fig. 2 5-HT immunolabeling intensity in the NTS. Representative digital photomicrographs of 5-HT immunolabeling in the cNTS. *Line drawing* shows cNTS (*green shading*; adapted from [28]. *Top* is 5-HT labeling in the cNTS of a rat treated with VEH; *bottom* is 5-HT labeling in the cNTS of a rat treated with PEG. For purposes of presentation, images were adjusted for brightness and contrast; however, for quantification of intensity, background was subtracted from the images, but no adjustments for brightness or contrast were made. Boundaries of the cNTS are indicated by *dotted white lines*; *scale bars* 100 µm; *cc* central canal; *ts* tractus solitarius; *dmv* dorsal motor nucleus of the vagus

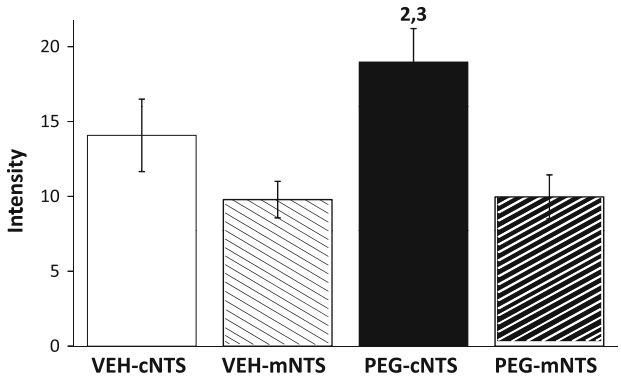

Fig. 3 5-HT immunolabeling intensity in the NTS. Intensity of 5-HT immunolabeling normalized to area. Intensity of labeling in the cNTS of PEG-treated rats (*PEG-cNTS*) was greater than that in the mNTS in both VEH-treated (*VEH-mNTS*; *2*; $p < 0.01$) and PEG-treated (*PEG-mNTS*; *3*; $p < 0.01$) rats. The intensity of labeling in the cNTS of VEH-treated rats (*VEH-cNTS*) was intermediate, and not statistically different from any of the other groups

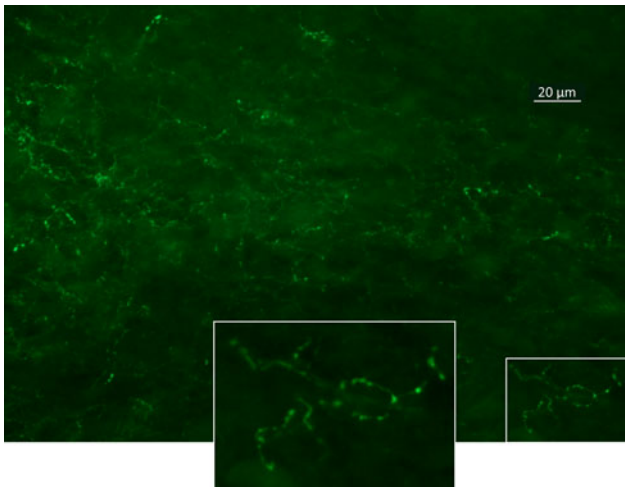

Fig. 4 5-HT fiber labeling in the NTS. High magnification digital micrograph of 5-HT labeling in the cNTS of a rat treated with PEG illustrating 'beaded' appearance of fibers. *Inset* 'digital close-up' showing the varicosities of the fibers in the area indicated by the *box*. *Scale bar* 20 µm

vagal afferents that terminate in the NTS include serotonergic fibers [26, 37], and some NTS neurons that respond to vagal cardiopulmonary input also respond to local application of 5-HT [23, 27]. These observations, in conjunction with the present finding of increased 5-HT specific to the cNTS, suggest that 5-HT of vagal origin may also contribute to compensatory responses to volume loss, and ongoing studies are addressing this possibility.

In the present study, both the immunolabeling and the direct assessment of tissue content clearly demonstrate that 5-HT increased in the cNTS during gradual, non-hypotensive volume loss. Importantly, there was no increase in tissue levels of the metabolite, suggesting that 5-HT accumulated intracellularly. This accumulation could arise if reuptake of 5-HT via the serotonin transporter (SERT) occurs more rapidly than does metabolism to 5-HIAA; however, reports that SERT is downregulated by 5-HT (e.g., [41]) are at odds with this possibility. Instead, we believe our data suggest that accumulation results from the inhibition of 5-HT release. Given the increase in 5-HT levels we observed ($\sim$1.5- to 3.5-fold increase; see Figs. 1, 3), inhibition of basal release seems unlikely to account for the increase, especially without a concomitant increase in synthesis. At the same time, increased synthesis of 5-HT and its subsequent transport to axon terminals in the NTS

would not be expected in the absence of neural activation. These observations lead to the intriguing possibility that, during the early stages of PEG-induced hypovolemia (detailed in, e.g., [15, 16]), 5-HT neurons are activated and 5-HT release is stimulated, but that as the hypovolemia develops and persists (Table 1; [14–17, 31]), further release is inhibited. This phenomenon of activation followed by inhibition has been observed in other 5-HT systems (for review, see [42]). Hence, we suggest that PEG-induced hypovolemia initially stimulates 5-HT release and, with it, an increase in 5-HT synthesis. However, when hypovolemia is prolonged, 5-HT release is inhibited while 5-HT synthesis remains temporarily elevated. Together, these events would be expected to increase intracellular levels of 5-HT but have no affect on (or tend to increase) extracellular levels of 5-HIAA, as we observed.

Inhibition of 5-HT release may seem contradictory given reports of cardiovascular and/or sympathetic effects after site-specific administration of 5-HT agonists into the NTS [24–26]. However, timing may be a critical aspect in understanding these findings. Schadt [33] has proposed that different neural mechanisms underlie different phases of hemorrhage and that 5-HT may be more important during the later-occurring, decompensated phase—the global sympathoinhibition and hypotension that occur after a hemorrhage that reduces blood volume by ~25–30 % (see also [35]). Activation of 5-HT_{1A} receptors prevents or delays the decompensated phase [21, 22], and several lines of evidence suggest that at least some of these receptors are autoreceptors that inhibit subsequent 5-HT release [43, 44]. Therefore, based on the results of these initial studies and in conjunction with previous findings, we propose that the early stage of developing PEG-induced hypovolemia stimulates an initial release of 5-HT that has dual effects. First, 5-HT produces neural activation in the NTS that leads to compensatory cardiovascular and sympathetic responses (e.g., [24–26, 45–47]). Second, 5-HT activates inhibitory presynaptic autoreceptors, thereby reducing subsequent release [43, 44] but without initially affecting ongoing 5-HT synthesis. As a result, 5-HT accumulates in neural fibers 6 h after PEG treatment. It is possible that such a 'shift' in 5-HT release may underlie the shift from the compensated to the decompensated phase of sympathetic responses to hemorrhage. We are not aware of any studies that have specifically addressed this proposed idea, though our findings that PEG increased 5-HT without increasing 5-HIAA are consistent with it. Nonetheless, it is clear that thorough assessment of the role of 5-HT in non-hypotensive hypovolemia will require additional studies to systematically examine the time course of changes in 5-HT and 5-HIAA, and the functional consequences of such changes.

In conclusion, hypovolemia produced by PEG selectively increased 5-HT in the caudal portion of the NTS, but not in the NTS at the level of the Area Postrema. On the surface, these observations would seem to be consistent with increased release of 5-HT within the cNTS in response to non-hypotensive hypovolemia, particularly given reports of serotonergic, but not catecholaminergic, involvement in compensatory responses to volume loss in rats [23, 26, 37, 47, 48]. However, a different conclusion emerges when tissue 5-HT content and 5-HT immunolabeling—two different, though complementary, measures—are considered together. Increased 5-HT immunolabeling and increased tissue 5-HT content, along with the absence of changes in the metabolite, 5-HIAA, raise the possibility that 5-HT release was inhibited by PEG-induced hypovolemia.

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Conflict of interest The authors declare that they have no conflict of interest.

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