Histamine receptor H₁ in the nucleus tractus solitarii regulates arterial pressure and heart rate in rats

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Bhuiyan ME, Waki H, Gouraud SS, Takagishi M, Kohsaka A, Maeda M. Histamine receptor H₁ in the nucleus tractus solitarii regulates arterial pressure and heart rate in rats. Am J Physiol Heart Circ Physiol 301: H523–H529, 2011. First published May 27, 2011; doi:10.1152/ajpheart.00263.2011.—Axons of histamine (HA)-containing neurons are known to project from the posterior hypothalamus to many areas of the brain, including the nucleus tractus solitarius (NTS), a central brain structure that plays an important role in regulating arterial pressure. However, the functional significance of NTS HA is still not fully established. In this study, we microinjected HA or 2-pyridylethylamine, a HA-receptor H₁-specific agonist, into the NTS of urethane-anesthetized Wister rats to identify the potential functions of NTS HA on cardiovascular regulation. When HA or H₁-receptor-specific agonist was bilaterally microinjected into the NTS, mean arterial pressure (MAP) and heart rate (HR) were significantly increased, whereas pretreatment with the H₁-receptor-specific antagonist cetirizine into the NTS significantly inhibited the cardiovascular responses. The maximal responses of MAP and HR changes induced by HA or H₁-receptor-specific agonist were dose dependent. We also confirmed gene expression of HA receptors in the NTS and that the expression level of H₁ mRNA was higher than that of the other subtypes. In addition, we found that H₁ receptors are mainly expressed in neurons of the NTS. These findings suggested that HA within the NTS may play a role in regulating cardiovascular homeostasis via activation of H₁ receptors expressed in the neurons.

Central nervous system; Cardiovascular regulation; 2-pyridylethylamine; Cetirizine

Histamine (HA) is a biogenic amine that is found in and released by mast cells and that can induce local immune responses in peripheral organs. Besides these roles, HA is also contained in neurons in the central nervous system (CNS; Refs. 22, 26, 28, 29, 30) and central HA possesses neurotransmitter properties (2, 13, 23). In the rat CNS, HA-immunoreactive neuronal cell bodies are found exclusively in the tuberomammillary nucleus of the posterior hypothalamus (14, 22, 26) but immunoreactive fibers are observed throughout the cerebral cortex and in parts of other brain regions including the olfactory bulb and tubercle, amygdala, substantia nigra, parabrachial nucleus, and the nucleus of the solitary tract (NTS; Refs. 28, 29). With regard to HA receptors, four distinct subtypes, designated H₁, H₂, H₃, and H₄, have been identified and all of them are known to be expressed in the CNS (7, 8, 17, 22).

Information regarding the physiological role of HA within the CNS is still emerging. It is an important regulator of the sleep-wake cycle, arousal level, learning, pain sensation, stress responses, fluid balance, food intake, and body temperature (7, 15, 17, 22). In addition to these functions, efforts have been devoted to identifying brain regions innervated by histaminergic fibers that could also be involved in cardiovascular regulation. In previous studies (2), microinjection of HA into the posterior hypothalamic area or the anterior hypothalamic area produced pressor and bradycardia responses similar to those observed after intracerebroventricular administration of HA. In contrast, local administration of HA into the paraventricular nucleus (PVN) increased both arterial pressure (AP) and heart rate (HR; Ref. 4). Those responses appear to have been mediated predominantly by stimulation of H₁ receptors, because they were produced by central administration of either HA or an H₁-receptor-specific agonist and were abolished by prior administration of H₁-receptor antagonists (2, 21). Furthermore, significant increases in plasma catecholamines were measured after injections of HA into the PVN, indicating an increase in sympathetic tone (1, 10). In contrast with the effects of HA injection into forebrain areas, bilateral administration of HA into the rostral ventrolateral medulla (RVLM) in anesthetized rats produced dose-dependent hypotension and bradycardia and this response was prevented by pretreatment with H₂ antagonist (2, 12). These results suggest that the cardiovascular effects of the central histaminergic system are likely to be brain region specific (2).

Although these previous findings (11, 25) indicate that multiple central areas may have functions in regulating the cardiovascular system, some of the primary cardiovascular centers including the NTS have not yet been studied. The NTS is one of the medullary structures that plays an important role in regulating AP. Since it has been reported that both HA-containing fibers and H₁ receptors are abundant in the NTS (19), it is conceivable that the NTS may be the site where central HA regulates the cardiovascular system via activation of H₁ receptors.

In the present study, we attempted to characterize the functions of HA within the NTS in cardiovascular regulation by examining AP and HR responses induced by NTS microinjections of HA or H₁-receptor-specific agonist. In addition to these functional studies, gene expression levels of HA-receptor subtypes and the cellular localization of H₁ receptors in the NTS were also studied. Our findings demonstrated that HA within the NTS increases both AP and HR and these responses are predominantly mediated through activation of H₁ receptors. The physiological conditions that stimulate HA release and consequently H₁ receptors in the NTS remain to be elucidated.
MATERIALS AND METHODS

Animals and animal care. Male Wistar rats (280–350 g), obtained from Kiwa Laboratory Animals (Wakayama, Japan) ≥1 wk before the experiments, were used in this study. The animals were housed in a temperature-controlled room with a fixed 12:12-h light-dark cycle (8:00 AM to 8:00 PM and 8:00 PM to 8:00 AM). Food and tap water were provided ad libitum. All experiments were approved by the Ethics Committee for Animal Experiments at Wakayama Medical University and complied with the guidelines of the Physiological Society of Japan.

General procedures. The animals were anesthetized with urethane (1.45 g/kg; Tokyo Kogyo, Tokyo, Japan) given intraperitoneally. The level of anesthesia was monitored regularly by assessing the limb withdrawal response to a noxious pinch, and an additional dose of urethane (0.145 g/kg ip) was administered when necessary. Rectal temperature was monitored and maintained at 37°C using a heating pad (BWT-100; Bio Research Center, Nagoya, Japan). The trachea was cannulated to facilitate artificial breathing using a ventilator (SN-480–7 Shinano Respirator; Shinano Manufacturing, Tokyo, Japan). A polyethylene catheter (PE-50 tubing filled with heparinized saline) was inserted into the right femoral artery to record pulsatile AP (AP-601G and P23ID; Nihon Kohden, Tokyo, Japan). A polygraph system (RM-7000; Nihon Kohden) was used for continuous recording of mean AP (MAP) and HR, which was derived from the pulsatile pressure signal using a cardiotachometer (AT-601G; Nihon Kohden). These parameters were monitored and recorded using the MacLab system (PowerLab/8s; ADInstruments, Nagoya, Japan). The femoral vein was also cannulated with a polyethylene tube (PE-50) for continuous infusion of physiological saline [0.8 ml·100 g⁻¹·h⁻¹ containing the muscle relaxant pancuronium bromide (0.08 mg·kg⁻¹·h⁻¹)]. When muscle relaxant was used, the adequacy of anesthesia was assessed periodically throughout the experiment by observing the AP response to noxious stimuli (i.e., toe pinch) and supplemental urethane (0.145 g/kg ip) was given as necessary.

NTS microinjections. The experimental procedures were modified from those reported in previous studies (5, 24). The rats were placed in a stereotaxic head holder (SR-5; Narishige Scientific Instrument Lab, Tokyo, Japan), and the caudal dorsal medulla was exposed through a midline incision in the dorsal neck. Microinjections of drug solution or vehicle were made into the NTS using either single- or multibarreled glass micropipettes (GC200F-10; Harvard Apparatus, Edenbridge, UK) with an outside diameter of 20–30 μm. For bilateral microinjections into the NTS, the tip of the micropipette was positioned 0.5 mm rostral to the calamus scriptorius, 0.5 mm lateral from the midline, and 0.5 mm deep from the dorsal surface of the brain stem. A Hamilton microsyringe and a syringe pump (CVF 3200, Nihon Kohden) were used for microinjections.

Experimental protocol. In the first set of experiments, l-glutamate (0.56 nmol in a total volume of 50 nl) was bilaterally microinjected into the NTS to verify the microinjection site. Following the recovery of MAP and HR to baseline levels after l-glutamate microinjection, normal saline vehicle (50 nl) was bilaterally microinjected. Approximately 30 min after the saline injection, a single dose of HA or 2-pyridylethylamine dihydrochloride, a specific H1-receptor agonist, was microinjected into the same site. Bilateral injections in the NTS were made within 2 min of each other. The doses of HA and H1-receptor-specific agonist used for microinjection were 12.5, 25, and 50 nmol each and were delivered in a volume of 50 nl (n = 6 for each concentration). These doses of HA are similar to those that have previously been used to induce cardiovascular responses (hypotension and bradycardia) by RVLH microinjection of HA (12). The precise physiological concentration of extracellular HA, specifically in the rat NTS, is not currently known.

In the second set of experiments (n = 11), bilateral microinjections of HA (25 nmol in a volume of 50 nl) into the NTS were performed. After complete recovery from the initial microinjections, either cetirizine dihydrochloride (0.1 nmol in a volume of 50 nl; n = 6), or saline as a control (n = 5) was bilaterally injected at the same sites. We chose this dose of H1-receptor-specific agonist because a higher dose caused unstable cardiovascular effects, mostly gradual increase of both arterial pressure and HR. We also confirmed that this dose of H1-receptor-specific agonist can inhibit HA- and H1-receptor-specific agonist-induced cardiovascular effects ≥30 min after its injection into the NTS. Within 2 min after H1-receptor-specific agonist/saline injections, HA (the same dosage as the initial injections) was again bilaterally administered to examine whether the HA-induced responses were mediated by H1 receptors.

In the final set of experiments (n = 11), the same protocol as that for HA was followed, but the H1-receptor-specific agonist (25 nmol in a volume of 50 nl) and either H1-receptor-specific agonist (1 nmol in a volume of 50 nl; n = 6) or saline as a control (n = 5) were bilaterally microinjected into the NTS.

Drugs. The following drugs were used: HA (Sigma-Aldrich, St Louis, MO), 2-pyridylethylamine dihydrochloride and cetirizine dihydrochloride (Tocris Bioscience, Ellisville, MO), and l-glutamic acid monosodium salt (Sigma, St. Louis, MO). All drugs were dissolved in normal saline (0.9% NaCl solution) and delivered in a volume of 50 nl.

Histological verification of injection sites. After the experiment was completed, the microinjection sites were marked using 50 nl of India ink for histological verification. The rats were then perfused transcranially with heparinized saline followed by 4% paraformaldehyde. The brains were then removed and stored in 4% paraformaldehyde for 48 h, followed by 1X PBS with 30% sucrose for an additional 24 h. The brains were then frozen and serial coronal sections (40 μm thick) of the medulla oblongata were obtained using a freezing microtome (RM-710; Yamato Kohki Industrial, Saitama, Japan). The sections were mounted on slides and counterstained with 1% neutral red. Microinjection sites were identified under a microscope by referring to the Paxinos and Watson atlas (20). Only those rats with microinjection sites wholly within the NTS were used for analysis.

Immunohistochemistry for HA receptors in the NTS. Animals (n = 6) were perfused as described above. The brain stem was then removed, fixed with 4% paraformaldehyde for ≥24 h, and transferred to PBS containing 30% sucrose for an additional 24 h. Serial sections (40 μm thick) through the NTS were obtained using a freezing microtome (REM-710). The sections were stained with biotinylated (10% serum, 1% sodium chloride and 0.3% Triton X-100) for 15 min at room temperature, rinsed again, and then incubated with H1-receptor antibody (sc-20633; Santa Cruz Biotechnology, Santa Cruz, CA; dilution 1:100–200 in PBS with 1% serum and 0.3% Triton X-100). After overnight incubation at 4°C, the sections were rinsed in PBS and incubated with biotinylated goat anti-rabbit immunoglobulin G (Vector Laboratories, Burlingame, CA; dilution 1:500) for 1 h. The sections were rinsed and then incubated in streptavidin-conjugated Alexa-Fluor 594 (Molecular Probes; dilution 1:500) for 1 h. Finally, sections were washed in PBS before mounting in Vectashield (Vector Laboratories). Sections were imaged using a laser scanning confocal microscope (LSM 5 Pascal; Carl Zeiss, Jena, Germany). To determine the cell types expressing these receptors, we used double-labeling fluorescence immunohistochemistry with either a glial cell marker (anti-glial fibrillary acidic protein; Invitrogen, Carlsbad, CA) or neuron marker (anti-NeuN; Millipore, Temecula, CA).

Gene expression measurement of H1, H2, H3, and H4 receptors in the NTS. The rats (300–325 g; n = 5) were humanely killed by cervical dislocation. The NTS was dissected rapidly from each animal and homogenized in 400 μl TRIzol reagent (Invitrogen). To avoid contamination with genomic DNA, the RNA samples were treated with RNase-free DNase I (Roche Diagnostics, Mannheim, Germany). Quantitative RT-PCR targeting rat β-actin, H1, H2, H3, and H4 receptors genes was performed in this study. For all primer sets,
we used the QuantiTect primer assay from Qiagen (for β-actin, QT00193473; for H1 receptor, QT00419993; for H2 receptor, QT00459578; for H3 receptor, QT00176127; for H4 receptor, QT00192388; Valencia, CA), which provides highly specific and sensitive results using SYBR Green-based real-time RT-PCR. The sequences of these primers are held with the company. Real-time RT-PCR reactions were carried out using a Thermal Cycler Dice real-time system (Takara, Japan), and the PrimeScript RT reagent kit and SYBR Premix EX Taq (Perfect Real Time, Takara) were used according to the manufacturer’s protocol (27). Correct PCR products were confirmed by melting curve analysis and by agarose gel electrophoresis. Expression of target genes was assessed relative to a housekeeping gene (β-actin) using the comparative (ΔΔCt) method (18) for each sample. Fold differences from average values for H1 receptors were 97.0 ± 2.0. The baseline measurements for MAP and HR in urethane-anesthetized rats were 166 ± 3.3, 33.2 ± 4.4, and 46.4 ± 5.9 mmHg, respectively (n = 6 for each concentration; Fig. 1B), and the increases in HR with the same concentrations were 15.6 ± 3.7, 34.4 ± 7.8, and 50.6 ± 6.6 beats/min, respectively (Fig. 1C). Unilateral microinjections of HA (50 nmol) into the same site also produced pressor responses (Fig. 1A), but the magnitude and duration of these were smaller than those with bilateral injections. In a small number of animals, in addition to the pressor and tachycardic responses, HA also induced initial minor depressor and bradycardic responses (very short duration; Fig. 1A), but this observation was not further explored. We also confirmed that microinjection of normal saline into the NTS elicited no responses, while L-glutamate (0.56 nmol in a volume of 50 nl) injection into this area immediately decreased MAP (−Δ33.6 ± 2.0 mmHg; n = 15) and HR (−Δ95.0 ± 3.4 beats/min; n = 15). To examine whether the pressor and tachycardic responses to intra-NTS injection of HA were due to leakage of drug from the central injection site to the peripheral circulation, we administered HA (50 nmol in a volume of 0.05 ml) intravenously to four rats. In contrast to the effects of NTS microinjection of HA, intravenous administration of HA resulted in a decrease in MAP (Δ35.6 ± 1.4 mmHg) and an increase in HR (Δ41.4 ± 6.5 beats/min). Both responses occurred immediately upon drug administration and returned to normal values within 1 min. The peripheral depressor effects of HA were opposite to the central effects, suggesting that the central effects originated from the central nervous system and were not due to leakage of HA into the periphery.

RESULTS

Effects of HA microinjection into the NTS on cardiovascular parameters. The baseline measurements for MAP and HR in urethane-anesthetized rats were 97.0 ± 2.1 mmHg and 409 ± 8 beats/min, respectively (n = 24). Bilateral administration of HA into the NTS of anesthetized rats significantly increased both the MAP and HR (Fig. 1A), whereas microinjection of vehicle (saline) had no significant effect (Fig. 1, B and C). The onset and duration of cardiovascular responses to the HA injections were within 30 s, and 15–20 min, respectively, and the maximum BP and HR responses were dose dependent. The increases in MAP elicited by the three different doses of HA (12.5, 25, and 50 nmol) were 16.6 ± 3.3, 33.2 ± 4.4, and 46.4 ± 5.9 mmHg, respectively (n = 6 for each concentration; Fig. 1B), and the increases in HR with the same concentrations were 15.6 ± 3.7, 34.4 ± 7.8, and 50.6 ± 6.6 beats/min, respectively (Fig. 1C). Unilateral microinjections of HA (50 nmol) into the same site also produced pressor responses (Fig. 1A), but the magnitude and duration of these were smaller than those with bilateral injections. In a small number of animals, in addition to the pressor and tachycardic responses, HA also induced initial minor depressor and bradycardic responses (very short duration; Fig. 1A), but this observation was not further explored. We also confirmed that microinjection of normal saline into the NTS elicited no responses, while L-glutamate (0.56 nmol in a volume of 50 nl) injection into this area immediately decreased MAP (−Δ33.6 ± 2.0 mmHg; n = 15) and HR (−Δ95.0 ± 3.4 beats/min; n = 15). To examine whether the pressor and tachycardic responses to intra-NTS injection of HA were due to leakage of drug from the central injection site to the peripheral circulation, we administered HA (50 nmol in a volume of 0.05 ml) intravenously to four rats. In contrast to the effects of NTS microinjection of HA, intravenous administration of HA resulted in a decrease in MAP (Δ35.6 ± 1.4 mmHg) and an increase in HR (Δ41.4 ± 6.5 beats/min). Both responses occurred immediately upon drug administration and returned to normal values within 1 min. The peripheral depressor effects of HA were opposite to the central effects, suggesting that the central effects originated from the central nervous system and were not due to leakage of HA into the periphery.

Fig. 1. Cardiovascular changes induced by histamine microinjected into the nucleus tractus solitarii (NTS). A: representative recordings illustrating the cardiovascular changes induced by histamine (50 nmol) microinjected into the NTS. Bilateral microinjection of histamine elicited increases in pulsatile arterial pressure (AP), mean arterial pressure (MAP), and heart rate (HR). Group data show the dose-dependent increase in MAP (Δ35.6 ± 1.4 mmHg) and an increase in HR (Δ41.4 ± 6.5 beats/min). Both responses occurred immediately upon drug administration and returned to normal values within 1 min. The peripheral depressor effects of HA were opposite to the central effects, suggesting that the central effects originated from the central nervous system and were not due to leakage of HA into the periphery.
Effects of 2-pyridylethylamine dihydrochloride (H1-receptor-specific agonist) microinjection into the NTS on cardiovascular parameters. Baseline values of MAP and HR were 93.9 ± 1.8 mmHg and 395 ± 6 beats/min, respectively (n = 24). As found in studies on bilateral administration of HA (a nonspecific HA-receptor agonist), a H1-receptor-specific agonist, 2-pyridylethylamine dihydrochloride, microinjected bilaterally into the NTS, also produced a significant increase in both MAP and HR (Fig. 2A). The onset and duration of H1-receptor agonist-induced cardiovascular responses were within 30 s and 20–25 min, respectively, and the maximum AP and HR responses were dose dependent. The maximum increases in MAP elicited by the three different doses of H1-receptor-specific agonist (i.e., 12.5, 25, and 50 nmol) were 20.6 ± 2.2, 38.8 ± 4.6, and 54.3 ± 4.2 mmHg, respectively (n = 6 for each concentration; Fig. 2B), and the increases in HR with the same concentrations were 17.7 ± 4.4, 43.0 ± 7.7, and 61.3 ± 4.6 beats/min, respectively (Fig. 2C). To examine whether the pressor and tachycardic responses to intra-NTS injection of the H1-receptor agonist were due to leakage of drug from the central injection site to the peripheral circulation, we administered the H1 agonist intravenously (50 nmol in a volume of 0.05 ml) to four rats. No significant changes in baseline MAP (4.9 ± 1.3 mmHg) and HR (Δ4.6 ± 1.1 beats/min) were seen after systemic administration of the H1-receptor agonist.

Blockade of HA- and H1-receptor agonist-induced cardiovascular effects by cetirizine (H1-receptor-specific antagonist). To further explore the pharmacological mechanisms underlying the effect of HA or H1-receptor-specific agonist administered into the NTS, we examined the effect of pretreatment with an H1-receptor-specific antagonist (cetirizine hydrochloride) on both HA- and H1-agonist-induced cardiovascular responses. Pretreatment with 1 nmol of H1 antagonist significantly inhibited the hypertensive and tachycardic effects of bilateral HA microinjections into the NTS (Fig. 3A). In addition, pretreatment with 1 nmol of H1-receptor-specific antagonist also significantly attenuated the effect of H1-receptor-specific agonist on both MAP and HR (Fig. 3B). These results suggest that the cardiovascular effects of exogenous administration of HA into the NTS were dominantly mediated by the H1-receptor subtype. Microinjection of vehicle (saline) had no significant...
effects on cardiovascular changes in response to both HA and H1-receptor-specific agonist (e.g., MAP changes in response to 25 nmol of H1-receptor-specific agonist: before saline, Δ39.6 ± 5.5 mmHg, and after saline, Δ36.5 ± 4.7 mmHg, n = 5, NS; HR changes in response to 25 nmol of H1-receptor-specific agonist: before saline, Δ44.7 ± 7.7 beats/min, and after saline, Δ36.8 ± 3.6 beats/min, n = 5, NS). Bilateral injections of H1-receptor-specific antagonist (1 nmol in a volume of 50 nl) alone into the NTS induced only transient pressor (6.2 ± 1.9 mmHg) and tachycardic (3.3 ± 4.0 beats/min) responses.

Histological verification of injection sites. Figure 4A shows a photomicrograph of a coronal section of the brain stem of a rat representative of the group that received NTS microinjection of drugs. We confirmed that microinjection sites were wholly within the restricted area of the NTS in all rats used for data analysis (see Fig. 4B; only data for H1 agonist are shown). We also bilaterally microinjected the H1 agonist (25 nmol) into the gracile nucleus (n = 3; Fig. 4B) and confirmed that it did not affect MAP and HR levels.

Gene expression profiles of HA receptors in the NTS. To confirm that transcripts encoding HA-receptor subtype proteins are expressed in the NTS, we performed real-time RT-PCR analysis on mRNA extracted from the rat NTS. We found that mRNAs for H1-, H2-, and H3-, but not H4-, receptor subtypes are expressed in the NTS, we performed real-time RT-PCR to confirm that transcripts encoding HA-receptor subtype proteins were expressed in the NTS (Fig. 5, A and B). The level of H1-receptor mRNA was also shown to be significantly higher than that of other subtypes of histamine-receptor mRNA in the NTS. mRNA expression of H4 receptor was negligible in the NTS. These gene expression profiles were also found in the medulla oblongata without NTS; however, the level of H1-receptor mRNA was significantly higher in the NTS compared with the medulla oblongata (C).***P < 0.001 vs. H1 receptor in the NTS. SS P < 0.01 vs. H1 receptor in the medulla oblongata. ##P < 0.01 comparison between the NTS and medulla oblongata. R, receptor.

H1-receptor mRNA was also shown to be significantly higher than that of other subtypes of HA-receptor mRNA in the NTS (fold differences: H1, 1.03 ± 0.14; H2, 0.29 ± 0.04; H3, 0.23 ± 0.04; P < 0.001; Fig. 5C). These gene expression profiles were also found in the medulla oblongata without NTS; however, it should be noted that the level of H1-receptor mRNA was significantly higher in the NTS compared with the medulla oblongata (fold differences: NTS, 1.03 ± 0.14; medulla oblongata, 0.53 ± 0.05; P < 0.01; Fig. 5C).

H1-receptor localization within the NTS. The expression of H1-receptor protein in the brain stem was characterized immunohistochemically. We found that H1 receptors were widely distributed in the brain stem area, including the dorsal vagal nucleus, hypoglossal nucleus, and NTS. In addition, H1 receptors were extensively colocalized with NeuN, a neuronal marker, but not with glial fibrillary acidic protein, a glial cell marker (data not shown), indicating that this receptor is expressed in neurons predominantly (Fig. 6).

**DISCUSSION**

In this study, a series of experiments was conducted to identify whether the histaminergic system in the NTS can...
regulate peripheral cardiovascular activities. We found that pharmacological activation of HA receptors in the NTS by direct microinjection of HA or H1-receptor-specific agonist into this region elicited hypertension and tachycardic responses in anesthetized rats. This was presumably mediated by an increased sympathetic nerve activity and reduced cardiac vagal activity, since the cardiovascular responses were rapidly induced following microinjections, although this awaits confirmation. The HA-responsive sites are histologically in good accordance with the classical cardiovascular area within the intermediate NTS, as shown by typical hypotension and bradycardic responses to microinjection of an excitatory amino acid L-glutamate. The HA- and H1-specific agonist-induced cardiovascular activity was largely inhibited by a H1-receptor-specific antagonist, cetirizine, indicating the significance of the H1-receptor subtype in mediating the cardiovascular effects of exogenous administration of HA. We also confirmed gene expression of HA receptors in the NTS and that the expression level of H1 mRNA was higher than that of the other subtypes. In addition, we found that H1 receptors are mainly expressed in neurons of the NTS. These findings suggested that HA within the NTS may play a role in regulating cardiovascular homeostasis via activation of H1 receptors expressed in the NTS neurons.

As previously reported (19, 28, 29), in the medulla, the NTS is heavily innervated by histaminergic projections and this nucleus contains a high density of H1 receptors. We have also confirmed that H1 receptors are mainly expressed in neurons in the NTS. Bilateral injections of HA or H1-specific agonist into the NTS might postsynaptically inhibit NTS neurons surrounding the injection site. This inhibition could reduce responsiveness or excitability of NTS neurons to baroreceptor inputs, which could lead to activation of vasomotor/cardiac sympathetic outflow and inhibition of cardiac vagal outflow and subsequently lead to an increase in peripheral vascular resistance and tachycardia. This speculation is supported by our findings that prior injection of HA into the NTS significantly inhibited the cardiovascular effects induced by L-glutamate (unpublished data). In addition, intra-NTS HA might also affect local transmitter release through presynaptically located HA receptors. For example, HA could directly inhibit release of excitatory transmitters, such as glutamate, a proposed transmitter of primary baroreceptor afferents at the level of the NTS. Reduction of glutamate release can result in reduction of neuronal activity and thus increases in AP and HR. This hypothesis is supported by Brown and Hass (6) who found that central HA inhibited glutamate release in the rat dentate gyrus. Moreover, previous electrophysiological and neurochemical evidence suggested that activation of HA receptors inhibited depolarization-evoked, Ca2+-dependent glutamate release (7, 9).

Previous studies have also shown a functional relationship between HA and adrenergic receptors in the CNS. HA can induce catecholamine release from hypothalamic brain slices and isolated synaptosomes, as well as selected brain sites in vivo (2). In the anesthetized rat, for example, central pretreatment of the animal with an α-adrenergic receptor antagonist or destruction of central noradrenergic nerve terminals can prevent or greatly reduce the pressor response to intracerebroventricular HA injections (2). In addition, previous findings (4) have demonstrated that the pressor response evoked by HA administration in the region of the PVN/anterior hypothalamus was mediated by stimulation of central α-adrenergic receptors. Moreover, it has also been shown that HA induces an enhancement of the release of adrenergic neurotransmitter from nerve endings through activation of H1 receptors in this brain region (3). In our previous study (5), we found that pharmacological activation of α-adrenergic receptors in the same area of the NTS with a direct microinjection of phenylephrine, an α-adrenergic receptor agonist, produced hypertension and tachycar-
dia. In conjunction with previous findings, results from the present studies suggest that the NTS may be a brain site where histaminergic and catecholaminergic systems could interact to regulate AP and HR via modulation of peripheral sympathetic outflow.

In the present study, we have also identified gene expression of both H2 and H3 receptors in the NTS. Previous reports (2, 12) demonstrated that H3 receptors expressed in the RVLM were involved in hypotension and bradycardiac responses. Similarly, central H3 receptors are known to induce hypotensive effects (2). This may due to the autoregulation of HA release stimulated by presynaptically expressed H3 receptors (2). Whether H2 and H3 receptors can exert functions in regulating cardiovascular control at the level of the NTS needs to be elucidated. However, considering our results obtained by real-time RT-PCR analysis, we speculate that these effects are minor compared with H1-receptor-induced cardiovascular effects.

In summary, our findings indicate that HA within the NTS may play a role in regulating the cardiovascular system via activation of H1 receptors in neurons. However, there are some limitations of this study. Since high concentrations of HA and H1-receptor-specific agonist were tested in this study, whether our findings demonstrate normal physiological or pathological condition remains unknown. To this end, the precise physiological concentration of extracellular HA, specifically in the rat NTS, needs to be elucidated. Moreover, the brain contains receptors that are not innervated, demonstrating mismatches between neurotransmitters and receptors localizations (16). As an inherent limitation of this type of study, the effect produced by microinjection of an agonist may not be mimic the effect produced by releasing HA from the histaminergic neurons. Nevertheless, our findings of the present study provide the first step for future consideration on the role of HA within the NTS in regulating the cardiovascular homeostasis. Since the activity of histaminergic neurons is low or absent in the sleeping states, whereas a high level of activity of histaminergic neurons throughput H1 and H2 receptors is seen in the arousal states (e.g., 14, 15), it would be important to know whether AP and HR responses during sleeping-arousal states are mediated by H1 receptors in the NTS as well as other cardiovascular centers such as the PVN.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

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