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Hyperpolarization-Activated Current I_h in Nucleus of Solitary Tract Neurons: Regional Difference in Serotonergic Modulation

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ABSTRACT—The nucleus of solitary tract (NTS) contains diverse neural circuits responsible for basic vital functions. We examined the effect of serotonin (5-HT) on hyperpolarization-activated current (I_h) in neurons acutely isolated from caudal, medial and rostral parts of the NTS. Caudal and medial NTS neurons showed a large amplitude of I_h compared with rostral neurons. In these neurons, perfusion with 5-HT potentiated I_h amplitude in a concentration-dependent manner. The effect of 5-HT was blocked by NAN-190, a 5-HT_{1A} receptor antagonist. Thus, 5-HT_{1A} receptors may regulate I_h channel activity in caudal and medial NTS neurons.

Keywords: Nucleus of solitary tract, Hyperpolarization-activated current, Serotonin

The nucleus of solitary tract (NTS) is a large, complex neural center to modulate, organize and distribute the sensory information arriving at the central nervous system; and it can be anatomically segregated along structural-functional lines, including rostral, medial and caudal regions (1). The rostral NTS receives primary afferents from taste receptors on the tongue and epiglottis. The medial NTS contains nuclei relaying gastrointestinal, respiratory and cardiovascular information. The caudal NTS contains nuclei that process respiratory and cardiovascular information.

The past few years have seen rapid advances in our understanding of hyperpolarization-activated non-selective cation (I_h) channels (2, 3). Unlike most voltage-sensitive channels, they open in response to negative-going voltage and conduct both K^+ and Na^+, with a threefold greater permeability to K^+, yielding a reversal potential of −30 to −40 mV. Although the first well-characterized role of these channels was the control of pacemaking activity in sinoatrial node cells of the heart, they appear to regulate rhythmic electrical activity and modulate membrane excitability in neuronal cells (2, 3). For instance, I_h channels in the NTS are assumed to control respiratory rhythm (4, 5).

The intracellular C-terminus of this channel contains a region homologous to cAMP- and cGMP-binding domains, and it is the likely site for cAMP regulation of channel opening (2, 3). Indeed, the regulation of I_h by G-protein-coupled receptors, e.g., serotonergic, β-noradrenergic and histaminergic receptors, has been shown in a wide variety of neuronal and non-neuronal cells (2). The NTS is the major recipient site of serotonergic projections from the raphe nuclei (6), the visceral sensoriums (7), and probably the NTS itself (8); and it abundantly expresses mRNAs for diverse subtypes of receptors for serotonin (5-HT) (9). However, there has been no indication for the serotonergic modulation of I_h in the NTS. Therefore, we have focused the present study on the possible contribution of 5-HT to I_h in NTS neurons.

Neurons were acutely dissociated from the NTS of 11- to 13-day-old Wistar rats. Animals were deeply anesthetized by ether and decapitated. The brain was sliced at a thickness of 500 μm with a microslicer (DTK-1500; Dosaka, Kyoto). Following 30 – 60 min of incubation, medulla oblongata slices were treated with 167 μg/ml pronase (Calbiochem, La Jolla, CA, USA) and subsequently with 167 μg /ml thermolysin (Sigma, St. Louis, MO, USA) each at 35°C for 15 min. The NTS was micropunched out under a binocular microscope and mechanically triturated with a glass pipette. Dissociated neurons adhered to the bottom of a 35-mm culture dish coated with poly-L-lysine. The ionic composition of the incubation solution was 124 mM NaCl, 5 mM KCl, 1.2 mM KH_2PO_4, 24 mM $NaHCO_3$, 10 mM glucose, 2.4 mM $CaCl_2$ and 1.3 mM $MgSO_4$, adjusted to pH 7.45, and aerated with 95% O_2 and 5% CO_2. Based on coronal levels, the NTS was divided to three parts, i.e.,

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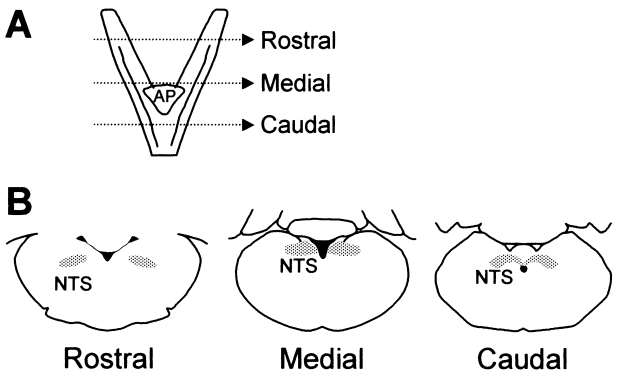

Fig. 1. Schematic illustrations of rostral, medial and caudal parts of the NTS. A: Three parts of the NTS are divided at the caudal end of the fourth ventricle and the obex. Schematic drawings in the panel B show cross sectional diagrams at the levels of the corresponding dotted lines in a top view of the medulla oblongata (A). Gray areas in the section diagrams indicate the NTS. AP, area postrema.

the rostral, medial and caudal NTS, as shown in Fig. 1.

Patch electrode pipettes (5 – 7 MΩ) were pulled from borosilicate glass capillaries (1.5-mm o.d., 0.9-mm i.d., G-1.5; Narishige, Tokyo) in two stages on a vertical micropipette puller (PP-83, Narishige). The current and voltage were measured with a patch-clamp amplifier (CEZ-2300; Nihon Kohden, Tokyo). Standard external solution consisted of 150 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM glucose and 10 mM HEPES, adjusted to pH 7.4. The internal solution consisted of 120 mM KCH_3O_3S, 4 mM NaCl, 1 mM $MgCl_2$, 0.5 mM $CaCl_2$, 10 mM HEPES, 10 mM EGTA, 3 mM Mg-ATP and 0.4 mM Na-GTP (pH 7.2) for conventional whole-cell recording, or 150 mM KCl, 0.5 mM EGTA, and 10 mM HEPES (pH 7.2) for amphotericin B (240 μg/ml)-perforated patch recording.

No morphological difference was observed in neurons derived from the rostral, medial or caudal NTS. Using conventional whole-cell techniques, four fundamental properties, i.e., cell capacitance, resting potential, action potential and fast post-spike afterhyperpolarization, were assessed. The cell capacitance of rostral, medial and caudal NTS neurons was 6.64 ± 0.32 (n = 18, mean ± S.E.M.), 6.68 ± 0.39 (n = 11) and 6.15 ± 0.40 pF (n = 13), respectively. The resting membrane potential of rostral, medial, and caudal NTS neurons was −54.8 ± 5.2, −56.7 ± 5.0 and −55.4 ± 4.8 mV, respectively. Although these acutely isolated neurons showed no spontaneous firing activity, a current injection (150 pA, 10-ms duration) reproducibly elicited an action potential. The spike amplitude was 64.2 ± 6.4 mV (from the resting potential) in rostral neurons, 87.6 ± 8.8 mV in medial neurons and 77.1 ± 7.2 mV in caudal neurons. The amplitude of afterhyperpolarization following a firing was −13.2 ± 1.7 mV (from the resting potential) in rostral neurons, −11.1 ± 2.5 mV in medial neurons, and −10.6 ± 1.5 mV in caudal neurons. None of these parameters showed a significant regional difference [each $P>0.1$, one-way analysis of variance (ANOVA)].

Hyperpolarization-activated current was recorded with amphotericin B-perforated whole-cell mode. A series of 900-ms hyperpolarizations from a holding potential of −45 mV elicited two components of inward currents, an initial instantaneous, rapidly activating current (I_{ins}) and a slowly activating current (Fig. 2A). The latter represents I_h because it was efficiently augmented by increasing $[K^+]_e$, and completely blocked by 2 mM Cs^+ and partially blocked by 5 mM Ba^{2+} (data not shown) (2, 3). In the rostral NTS, 34 of 54 neurons (63.0%) showed I_h and I_{ins}, and the remaining showed I_{ins} only. In the medial NTS, 48 of 58 neurons (82.8%) displayed I_h. Similarly, 54 of 66 caudal neurons (81.8%) possessed it. Thus, the I_h incidence was significantly low in the rostral NTS as compared with the other nuclei ($P<0.05$, χ^2 test).

In the case of neurons bearing both I_h and I_{ins}, the I-V relationship was plotted in Fig. 2B. The amplitude of I_h in rostral NTS neurons was slightly, but significantly, smaller than that in caudal and medial NTS neurons, whereas I_{ins} amplitude was almost uniform in these three subregions.

When the hyperpolarizing step pulse was applied to rostral neurons 5 s after local perfusion with 50 μM 5-HT, no apparent effect on I_h was observed (Fig. 3A). In medial and caudal NTS neurons, however, the same 5-HT application dramatically potentiated I_h (Fig. 3A). The effect showed a concentration-dependency in the range of 10 – 100 μM (Fig. 3B) and was reversible after washout (data not shown). In this figure, the data of the medial and caudal NTS neurons were pooled because there was no significant regional difference in the concentration dependency of the 5-HT effect. Likewise, 8-OH-DPAT, a 5-$HT_{1A/7}$-receptor agonist, augmented I_h in the medial and caudal NTS (Fig. 3B). The 5-HT potentiation was efficiently attenuated by 50 μM NAN-190, a selective antagonist of 5-HT_{1A} receptor (Fig. 3B). The effect of NAN-190 on the 5-HT action was partial even at higher concentrations up to 250 μM, while NAN-190 alone had no effect on I_h. The remaining effect in the presence of NAN-190 was unaffected by the 5-$HT_{1c/2}$-receptor antagonist ritanserin or the 5-$HT_{3/4}$-receptor antagonist tropisetron (data not shown). However, the effect of 8-OH-DPAT was almost completely inhibited by NAN-190 (Fig. 3B). Thus, we believe that some 5-HT receptor subtype other than 5-HT_{1A} receptor is also involved in the 5-HT effect. Incidentally, the serotonergic modulation of I_h could be recorded only when amphotericin B-perforated patch clamp recording was employed; a lack of the 5-HT effect in conventional whole-cell recordings suggests the presence of intracellular soluble factors modulating I_h.

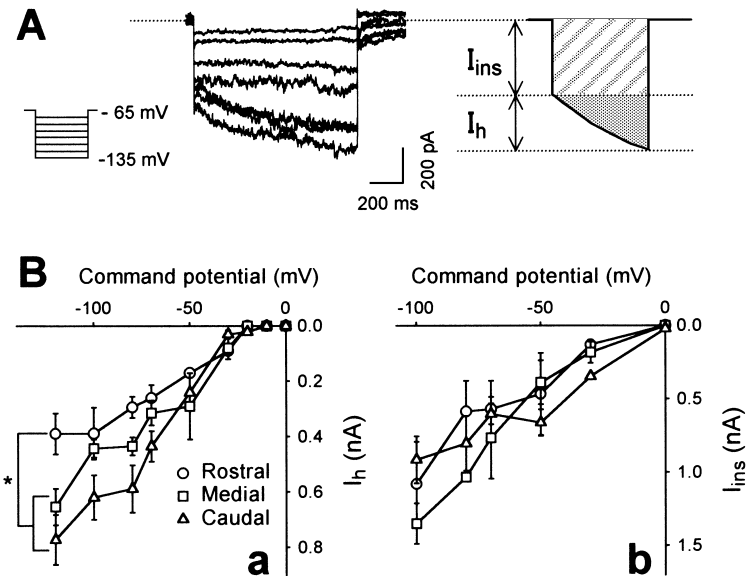

Fig. 2. Regional difference of the distribution of I_h in the NTS. A: Representative traces of hyperpolarization-activated currents recorded from a caudal NTS neuron. To activate I_h, the membrane potential was held at −45 mV, and hyperpolarization current (900-ms duration and 10 mV-step amplitude) was injected through recording electrode. The obtained current consisted of two components: an immediately activating, steady current (I_{ins}) and a delayed, gradually increasing current (I_h). I_{ins} and I_h were measured as shown in the right panel, i.e., the amplitude of the hatched area and the dark area, respectively. B: Plots of I_h (a) and I_{ins} (b) versus amplitude of hyperpolarizing steps in rostral (circles), medial (squares) and caudal (triangles) NTS neurons. Large amplitude of I_h was recoded from the medial or caudal neurons as compared with that in rostral neurons, while there was no regional significant difference in I_{ins}. *P<0.05: two-way ANOVA. Data are means ± S.E.M. of 7 neurons.

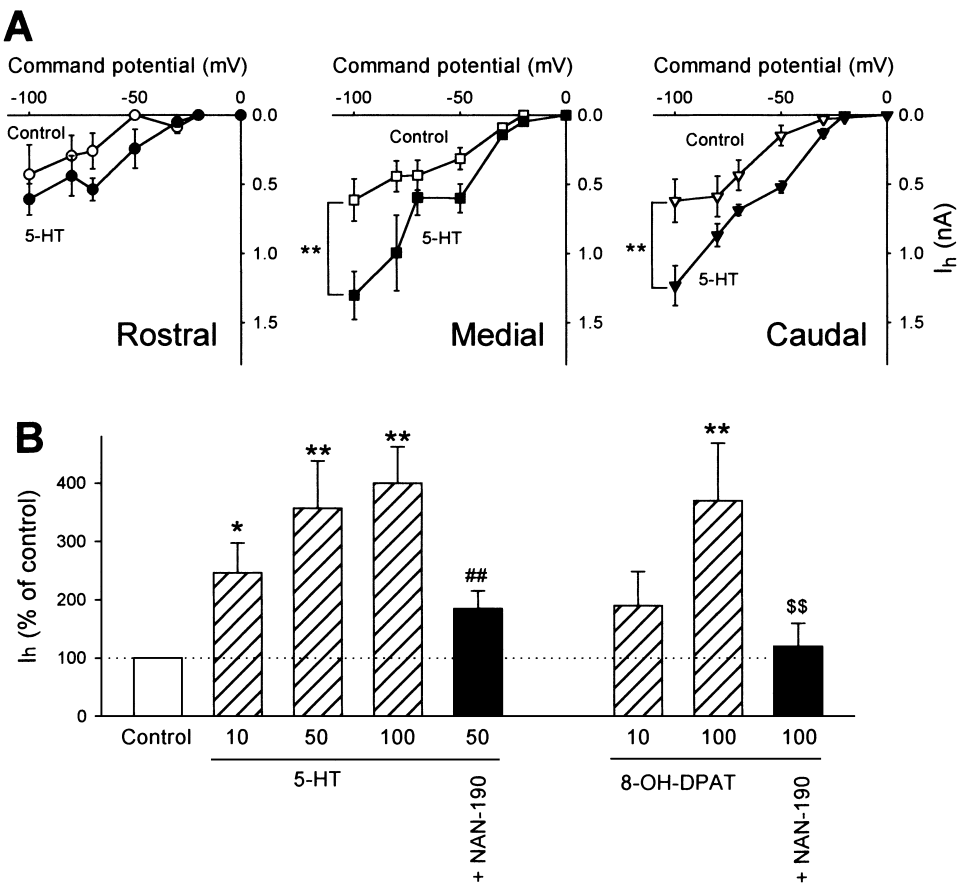

Fig. 3. Serotonergic modulation of I_h in the medial and caudal NTS but not in the rostral NTS. A: I_h in the rostral, medial, and caudal parts of the NTS was evoked by a 900-ms hyperpolarization from a holding potential of −45 mV in the absence (control) or presence (5-HT) of 50 μM 5-HT. **P<0.01: two-way ANOVA. B: Concentration-dependent I_h enhancement by 5-HT. I_h was elicited by a 900-ms hyperpolarization from −45 to −145 mV in the presence of 10, 50 or 100 μM 5-HT; 10 or 100 μM 8-OH-DPAT; or a combination of 100 μM NAN-190 with 50 μM 5-HT or 100 μM 8-OH-DPAT. Data in the medial and caudal NTS were pooled. *P<0.05, **P<0.01 vs control; $^{\#\#}P$<0.01 vs 50 μM 5-HT; $^{\$\$}P$<0.01 vs 100 μM 8-OH-DPAT: Tukey's test following two-way ANOVA. Data represent means ± S.E.M. of 7 to 14 neurons.

This study aimed to establish the regional difference in electrophysiological properties of NTS neurons and has shown for the first time that the density of I_h is not equivalent for rostral, medial and caudal NTS neurons and that 5-HT stimulates I_h via 5-HT_{1A}-receptor activation in medial and caudal neurons.

Because no regional difference was detected in cell capacitance, resting potential, action potential, afterhyperpolarization, or I_{ins} amplitude, NTS neurons seem to be identical in fundamental electrophysiological properties. Therefore, the regional difference in I_h is of particular interesting since the NTS is involved in a wide variety of physiological functions, and the different subregions are associated with their corresponding functions. Considering that the medial and caudal NTS is a pivotal site for respiratory regulation, the large I_h is a reasonable observation because I_h is assumed to regulate respiratory rhythmic activity in the NTS (4, 5). However, smaller but apparent I_h was still recorded in the rostral NTS. This current may regulate neural activity independent of respiratory control.

The serotonergic modulation of I_h also showed a regional difference; 5-HT enhances I_h in the medial and caudal but not rostral NTS. Although this may merely reflect a regional difference in distribution of 5-HT receptor subtypes, it is intriguing to discuss in the context of respiration rhythm. Because the enhancement of neuronal I_h activity results in an increase in rhythm frequency (2, 3), our finding predicts that 5-HT facilitates rhythmic breathing movements. Indeed, Lalley et al. (10, 11) indicated that 5-HT_{1A}-receptor activation stimulates respiratory neurons in the medulla-NTS neural network and thereby can ameliorate apneustic breathing in cats. These phenomena may be explained by 5-HT-induced I_h activation.

In motoneurones (12) and dorsal root ganglion neurons (13), 5-HT is reported to facilitate I_h channel activity probably via activation of the 5-HT_7 receptor, which is coupled to Gs protein that causes a rise in cAMP level. Therefore, it is somewhat surprising that 5-HT- or 8-OH-DPAT-induced I_h channel activation was inhibited by NAN-190, an excellently selective antagonist of 5-HT_{1A} receptor (14), because 5-HT_{1A} receptor is coupled to Gi protein and may reduce the cAMP level. However, receptors coupled to Gi/o protein can also stimulate the type II and IV isoforms of adenylyl cyclase through an action of G$\beta\gamma$ subunits (15). It is, therefore, feasible that 5-HT_{1A}-receptor activation causes an elevation of cAMP level in the medial and caudal NTS, while it is also possible that an unidentified signaling pathway, e.g., mitogen-activated protein kinase cascade through G$\beta\gamma$ and Ras, regulates I_h channels independently of cAMP pathway. Overall, the present study is the first evidence that 5-HT_{1A} receptor stimulates I_h activity. Further investigation would provide a novel insight into I_h regulation.

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