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Distinct modulatory effects of 5-HT on excitatory synaptic transmissions in the nucleus tractus solitarius of the rat

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ABSTRACT

The second-order relay neurons in the nucleus tractus solitarius (NTS) receive numerous peripheral afferent inputs mainly from the vagus nerve. Their activity is modified by several neuromodulators and hence autonomic responses are properly regulated. Serotonin (5-HT) is an important candidate for such neuromodulators, since serotonergic inputs and distribution of 5-HT receptors are provided in the NTS. However, its mechanism of action remains unclear. To evaluate the serotonergic modulation of synaptic transmission, we examined the effects of 5-HT (1.0–10.0 μM) on the solitary tract-evoked excitatory postsynaptic currents (eEPSCs) and spontaneously occurring EPSCs (sEPSCs) in the preselected second-order neurons of the rat NTS. 5-HT concentration-dependently decreased the amplitude of eEPSCs, which was accompanied by an increase in paired-pulse ratio. The inhibitory effect of 5-HT was mimicked by α-methylserotonin and blocked by ketanserin. 5-HT had no effect on the inward current induced in the NTS neurons by topically applied α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA). On the other hand, 5-HT increased the frequency of sEPCSs without effect on their amplitude. This excitatory effect of 5-HT was mimicked by 2-methylserotonin and antagonized by ondansetron. The results suggest a dual modulation of the excitatory synaptic transmission by 5-HT in the NTS; presynaptic inhibition of the peripheral inputs synapsing to the relay neurons via 5-HT_2 receptors and presynaptic excitation of inputs from the intrinsic local network via 5-HT_3 receptors. These effects of 5-HT may provide important means of optimizing the autonomic responses mediated by the NTS network.

1. Introduction

The nucleus tractus solitarius (NTS), situated in the rostral dorsomedial medulla oblongata, is the principal sensory nucleus that receives cardiovascular, respiratory and gastrointestinal information (Andresen and Kunze, 1994). Afferent fibers traveling mainly in the vagus and glossopharyngeal nerves primarily synapse to the second-order relay neurons in the NTS (Beckstead and Norgrenm, 1979). In addition to the peripheral afferent inputs, the second-order neurons receive projections from the intrinsic local network that contributes to maintain and/or modulate the neuronal excitability (Champagnat et al., 1986; Fortin and Champagnat, 1993; Kawai and Senba, 1996). The excitatory synaptic transmission in second-order neurons is carried principally by glutamate via ionotropic receptors (Andresen and Yang, 1990). This signal processing is influenced by inputs from several regions of the brain (Sofroniew, 1983; Thor and Helke, 1987), and subsequently various autonomic regulatory systems and behaviors are adjusted. Serotonin (5-HT) is one of the important candidates for such modulations. The NTS neurons are densely innervated by serotonergic terminals (Steinbusch, 1981), of which the majority originate from the raphe nuclei (Schaffar et al., 1988; Thor and Helke, 1987, 1989) and some from the vagal afferents (Raul, 2003; Sykes et al., 1994). Many subtypes of 5-HT receptors are expressed in the NTS. Immunohistochemical and mRNA analyses revealed the existence of 5-HT_{1A} (Manaker and Verderame, 1990; Thor et al., 1992), 5-HT_2 (Pompeiano et al., 1994), 5-HT_3 (Steward et al., 1993), 5-HT_{5A} (Oliver et al., 2000) and 5-HT_7 receptors (Gustafson et al., 1996) in the NTS.

Several lines of evidence suggest involvement of 5-HT in the cardiorespiratory functions including related reflex responses (Comet et al., 2007; Dando et al., 1998; Hodges and Richerson, 2008; Llewellyn-Smith et al., 2004). Activation of 5-HT_3 receptors within the NTS results in a rise in blood pressure (Jeggo et al., 2005) and inhibition of bradycardia evoked by chemoreceptor activation (Jordan, 2005) as well as Bezold–Jarisch reflex (Sévoz et al., 1996). Activation of 5-HT_2 and 5-HT_3 receptors causes apnea (Yoshioka et al., 1992) and that of 5-HT_{1A} receptors inhibits cough reflex in animal models (Kamei et al., 1991; Stone et al., 1997). Developmental abnormality in the medullary serotonergic system has been implicated in sudden infant death syndrome (Kinney et al., 2009). Furthermore, there are some reports concerning the effects of 5-HT on the NTS neuron activity or synaptic transmission (Glaum et al., 1992; Jeggo et al., 2007;

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Oskutyte et al., 2009; Sévoz-Couche et al., 2000; Wan and Browning, 2008). However, since the results are inconsistent and controversial, the mechanism of 5-HT action is less understood.

To clarify the serotonergic modulation of excitatory synaptic transmission in the second-order NTS neuron, the effects of 5-HT on the afferent-evoked and spontaneously occurring excitatory postsynaptic currents (eEPSCs and sEPCSs, respectively) and its mechanisms were investigated using in vitro brainstem slice preparations of the rat.

2. Materials and methods

2.1. Slice preparation

This study was approved by the Animal Care Committee at the Aichi Gakuin University and conducted in accordance with Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society.

Slice preparations were performed as described previously (Haji and Ohi, 2010; Ohi et al., 2007). Briefly, 63 male Wistar rats (3–5 w, 50–100 g) were deeply anesthetized with inhalation of halothane and decapitated. The brainstem was excised and submerged in ice-cold low-calcium artificial cerebrospinal fluid (aCSF) containing (mM): NaCl, 125; KCl, 2.5; $CaCl_2$, 0.1; $MgCl_2$, 5; NaH_2PO_4, 1.25; D-glucose, 12.5; L-ascorbic acid, 0.4; $NaHCO_3$, 25. The pH was adjusted to 7.4 when continuously bubbled with 95% O_2–5% CO_2. The brainstem was glued to the cutting stage of a vibratome (Liner Slicer Pro 7, Dosaka, Kyoto, Japan) with the caudal side up. Two to three transverse slices of a 400-μm thickness including the NTS region, extending from 0.5 mm caudal to 1.2 mm rostral to the obex, were made from one animal. The slices were incubated in standard aCSF (mM): NaCl, 125; KCl, 2.5; $CaCl_2$, 2; $MgCl_2$, 1.3; NaH_2PO_4, 1.25; D-glucose, 12.5; L-ascorbic acid, 0.4; $NaHCO_3$, 25; saturated with 95% O_2–5% CO_2 for 30–40 min at 37 °C, and then kept at room temperature (23 ± 2 °C) until the recording. The slice was fixed in a recording chamber (ca. 0.4 ml volume, RC-26GLP, Warner Instruments, Hamden, CT, USA) under nylon mesh attached stainless anchor, then submerged in and continuously perfused with the standard aCSF at a flow rate of 1–2 ml/min. The neurons with small diameters (<15 μm) which may receive predominantly excitatory synaptic inputs (Champagnat et al., 1986; Kawai and Senba, 1996) were visually preselected in the medial and dorsal regions of NTS with an infrared-differential interference contrast videomicroscope (BX-51WI, Olympus, Tokyo and C2741, Hamamatsu Photonics, Hamamatsu, Japan) and their images were stored on a hard disk.

2.2. Whole-cell transmembrane current recording

Recordings were made at room temperature. The composition of the pipette solution was (mM): potassium gluconate, 120; NaCl, 6; $CaCl_2$, 5; $MgCl_2$, 2; MgATP, 2; NaGTP, 0.3; EGTA, 10; HEPES, 10; pH 7.2 with KOH. The tip resistance of the electrodes ranged from 4 to 6 MΩ in the standard aCSF. After establishing the cell-attached configuration with a seal resistance of 1–10 GΩ, the whole-cell mode was established with a brief negative current and pressure pulse. The series resistance (<30 MΩ) and membrane capacitance were compensated and checked regularly during the recording. At a holding potential of −60 mV, the transmembrane current was recorded with a patch-clamp amplifier (Axopatch 200B, Axon Instruments, Foster City, CA, USA) with a high-cut filter at 2 kHz. The membrane current was sampled on-line at 4 kHz (PowerLab, AD Instruments, Castle Hill, Australia) and stored on a hard disk for later analysis.

A stainless concentric bipolar electrode was placed on the tractus solitarius (TS) ipsilateral to the recorded neuron. The distance between the two poles was 100 μm. The stimulus intensity with a 0.1-ms pulse width was set to a minimal voltage with which every pulse of the TS stimulation constantly induced a clear peak of monosynaptic EPSC without failure. Usually, the intensity was 10–20 V and the stimulation was given every 10 s. The eEPSC with a latency of less than 7.5 ms with little variation was judged to be monosynaptic (Champagnat et al., 1986). For calculation of paired-pulse ratio (PPR), two serial eEPSCs were evoked by double stimulus pulses at an inter-pulse interval of 20–30 ms to the TS (Debanne et al., 1996). The PPR was defined as the peak amplitude of the second eEPSC divided by that of the first eEPSC.

2.3. Drugs

The following drugs were dissolved in aCSF: AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid hydrobromide, 100 μM, AMPA receptor agonist, Research Biochemicals International, Natick, MA, USA), α-Me-5-HT (α-methylserotonin maleate salt, 1.0 μM, 5-HT_2 receptor agonist, Sigma, St. Louis, MO, USA), CGS-12066A (CGS-12066A maleate, 1.0 μM, 5-HT_{1B} receptor agonist, Sigma), cisapride (cisapride monohydrate, 10.0 μM, 5-HT_4 receptor agonist, Sigma), CNQX (6-cyano-7-nitroquinoxaline-2, 3-dione disodium, 10.0 μM, AMPA receptor antagonist, Sigma), dizocilpine ((−)-MK-801 hydrogen maleate, 10.0 μM, NMDA receptor antagonist, RBI), 8-OH-DPAT ((±)-8-hydroxy-2-(di-n-propylamino)tetralin hydrobromide, 1.0 μM, 5-HT_{1A} receptor agonist, Sigma), 5-HT (serotonin creatinine sulfate complex, 1.0–10.0 μM, Sigma), ketanserin (ketanserin tartrate salt, 10.0 μM, 5-HT_2 receptor antagonist, Sigma), ondansetron (ondansetron, 10.0 μM, 5-HT_3 receptor antagonist, Enzo, Farmingdale, New York, USA) and 2-Me-5-HT (2-methylserotonin hydrochloride, 1.0 μM, 5-HT_3 receptor agonist, Enzo). The concentrations of these 5-HT receptor agonists and antagonists were used in the range of general use referring to the previous in vitro reports (Dergacheva et al., 2009; Glaum et al., 1992; Kamendi et al., 2008; Rainnie, 1999).

Application of all drugs except AMPA was delivered for 5 min by gravity feed from 60 ml reservoirs bubbled with 95% O_2–5% CO_2. The neuronal recording during the first 60 s was not included in the data analysis to compensate for dead space of tubing between bath and reservoirs. The aCSF containing AMPA (500 μM) was applied locally to the slice with a glass pipette for 5 s, whose tip was placed at the most upstream of the recording chamber and never moved throughout the recording. The final concentration of AMPA in the chamber was 100 μM. Bath perfusion with aCSF was discontinued during application of AMPA and restarted after application.

2.4. Data analysis

The recorded membrane currents were analyzed off-line with Chart 5 and Scope 4 (AD Instruments). The amplitude of eEPSC was calculated as the difference between the post-stimulus through current and the pre-stimulus mean current over 10 ms. Averaged traces of eEPSCs were made by adding 5 to 10 sampled data using stimulus pulses as a trigger. The onset latency, rise time and 50% decay time of eEPSCs were calculated as well. The sEPSCs were detected by ORIGIN software (Origin Lab, Northampton, MA, USA) where the threshold for detection was set just above baseline noises of the recordings, which was 8–10 pA. The inter-event interval (IEI) and peak amplitude of sEPSCs occurring for 3 min were measured. For the group analysis, collected data were compared before (control), during drug application (2–3 min after onset of the drug perfusion) and during washout (3 min after washout). Group values are expressed as the mean ± S.D. Derived parameters were compared using a one-way analysis of variance (ANOVA) followed by multiple comparisons (Bonferroni's corrected multiple *t*-test) or Kolmogorov–Smirnov test with the level of significance set at $P<0.05$.