Modulation of synaptic transmission by serotonin in second-order neurons of the rat nucleus tractus solitarius

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Serotonin (5-HT) influences the cardiorespiratory regulatory functions by pheripheral and central mechanisms. Serotonergic inputs and distribution of 5-HT receptors are provided in the nucleus tractus solitarius (NTS) of the brainstem at which the peripheral afferents form the first synapses and a part of reflex arc of the network is constituted. To evaluate modulation of synaptic transmission by 5-HT, we examined the effects of 5-HT (1.0-10.0 μM) on the solitary tract-induced excitatory postsynaptic currents (evoked EPSCs; eEPSCs) and spontaneously occurring EPSCs (spontaneous EPCSs; sEPSCs) in the second-order neurons of the rat NTS. Perfusion of 5-HT decreased the amplitude of eEPSCs in a concentration-dependent manner with little change in the basal current. 5-HT increased the frequency of sEPCSs without effect on their amplitude. These results suggest the dual modulation of 5-HT, an inhibitory effect on the peripheral inputs synapsing to the relay neurons and an excitatory effect on the NTS local network. The detected actions of 5-HT may be involved in the regulation of the NTS neuronal activity.

Keywords: EPSC, NTS, Serotonin, Glutamatergic transmission, Rat

INTRODUCTION

The nucleus tractus solitarus (NTS) includes parts of brainstem neuronal networks regulating cardiovascular, respiratory and gastrointestinal functions[1-4]. It also contains the terminal arborizations of those primary sensory fibers of vagal and glossopharyngeal nerves[1]. Within the NTS, excitatory amino acid (glutamate) and its receptors (AMPA and NMDA receptors) are directly involved in the transmission of those information in the second-order NTS neurons[5-7]. In addition, the NTS is extremely rich in other receptors for many endogenous neuroactive substances, activation of which can modify the signal processing in the NTS and subsequently influence various regulatory systems and behaviors. Among them, serotonin (5-HT) seems to be the most important candidate for such modulators. Indeed, the NTS is densely innervated by 5-HT terminals, the majority of them coming from the raphe nuclei[8] and some of them from the vagal afferents[9]. Several subtypes of 5-HT receptor exist in the NTS[10]. The mRNA and immunochemical localization has identified 5-HT_{1A}[11], 5-HT_{2A} and 5-HT_{2C}[12], 5-HT_3[13], 5-ht_{5A}[14], and 5-HT_7[15] receptors. These results provide the possibility that 5-HT exerts fine-tuning of synaptic transmission in the NTS. However, the 5-HT's action and underlying receptors remain controversial. For example, application of 5-HT to the NTS neurons had a variety of effects including excitatory, inhibitory, biphasic or no effects in previous reports[16-21]. The present study was performed to evaluate the effects of 5-HT on the excitatory synaptic transmission in the second-order NTS neurons.

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MATERIALS AND METHODS

This study was 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 [22, 23]. Briefly, male Wister rats (3-5 w, 50-100 g) were deeply anesthetized with inhalation of ether 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 7.4 when continuously bubbled with 95% O_2-5% CO_2. Two to three transverse brainstem slices (400 μm thickness) including the NTS region were made by a slice cutter (Linear Slicer Pro 7, Dosaka, Kyoto, Japan). 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℃, and then kept at room temperature (25 ± 1℃) until the recording. The slice was fixed in a recording chamber (0.4 ml volume, RC-26GLP, Warner Instruments, Hamden, CT, USA) 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 [3, 24] were visually preselected in the medial and dorsal regions of NTS with an infrared-differential interference contrast videomicroscope (BX-51WI, Olympus; C2741, Hamamatsu, Japan). The composition of 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 electrode ranged from 4 to 6 MΩ when filled with the pipette solution. 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 and membrane capacitance were compensated and checked regularly during the recording. At a holding potential of -60 mV, the membrane 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 hard disk of a computer. Recordings were made at room temperature.

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 intensity of stimulation was set to a minimal voltage with which every pulse of the TS stimulation constantly induced a clear peak of monosynaptic excitatory postsynaptic currents (evoked EPCSs; eEPSCs) without failure. Usually, the stimulation intensity was 2.0-30 V with a 0.1-ms pulse width. 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 [3]. The spontaneously occurring EPSCs (spontaneous EPCSs; sEPSCs) were also recorded from the second-order NTS neurons. The following drugs were dissolved in aCSF: 6-cyano-7-nitroquinoxaline-2, 3-dione disodium (CNQX; 10.0 μM, Sigma, St Louis, MO, USA), dizocilpine (10.0 μM, Research Biochemicals International, Natick, MA, USA), picrotoxin (0.1 mM, Sigma) and 5-HT (1.0-10.0 μM, Sigma). Application of all drugs was delivered for 5-6 min by gravity feed from 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 recorded membrane currents were analyzed off-line with Chart 5 and Scope 4 (AD Instruments). Averaged traces of eEPSCs were made by adding 10 sampled data using stimulus pulses as a trigger. The amplitude of eEPSC was calculated as the difference between the post-stimulus through current and the pre-stimulus mean current over 10 ms. 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 3-5 pA. The inter-event interval and amplitude of sEPSCs occurring for 3 min were measured. For the group analysis, data were compared before, during 5-HT (2 min after the onset of 5-HT perfusion) and during washout (3 min after washout). Group values are expressed as the mean ± SEM. Derived parameters were compared using paired t-test with the level of significance set at $p<0.05$.

RESULTS

Characteristics of eEPSCs and effects of 5-HT

Membrane currents were recorded from 32 second-order NTS neurons. Out of these, 24 neurons responded constantly by eEPSCs with a short latency in response to electrical stimulation of the TS. The mean amplitude of

eEPSCs was 168.3 ± 11.1 pA (n=19) at a holding potential of -60 mV. The onset latency was 3.4 ± 0.13 pA (n=19), suggestive of monosynaptic [3]. To investigate the characteristics of evoked membrane currents, two protocols were employed. First, dependency of the eEPSC amplitude on the stimulus intensity was tested. Increasing the intensity increased the peak amplitude of eEPSC and gave rise to a plateau EPSC amplitude at which the EPSC amplitude became almost maximal and was not detectably affected with small changes in the intensity (Fig. 1A). The fact might suggest that the stimulation activated mostly the TS fibers and contribution of releases from local excitatory neurons distributed around the TS fiber tract is small. Second, dependency of the eEPSCs amplitude on the holding potential was tested. The eEPSC amplitude increased when the holding potential was shifted to a negative direction, vise versa, it decreased when shifted to a positive direction (Fig. 1B). These eEPSCs were completely abolished by CNQX (10.0 μM) application, but not by dizocilpine (10.0 μM, n=4, Fig. 1C). Polysynaptic inhibitory currents were not induced with or without picrotoxin (0.1 mM). Fig. 1D illustrates typical changes in eEPSCs during the perfusion of 5-HT (3.0 μM). Perfusion of 5-HT significantly decreased the eEPSC amplitude without any detectable effect on the basal current. This effect of 5-HT was concentration-dependent. The onset latency was not changed during 5-HT application. The eEPCS amplitude recovered completely to the control level within 3 min after washout.

Characteristics of sEPSCs and effects of 5-HT

The sEPSCs were recorded from 26 out of 32 second-order neurons while voltage-clamping the membrane at -60 mV. The mean frequency was 2.1 ± 0.38 events/s and the amplitude was 22.0 ± 1.71 pA (n=10). The sEPCS amplitude increased when the holding potential was shifted to negative values, while it decreased when shifted to positive values (Fig. 2A). The sEPSCs were completely abolished by perfusion of CNQX, but not by dizocilpine (n=4, Fig. 2B). No spontaneous inhibitory postsynaptic

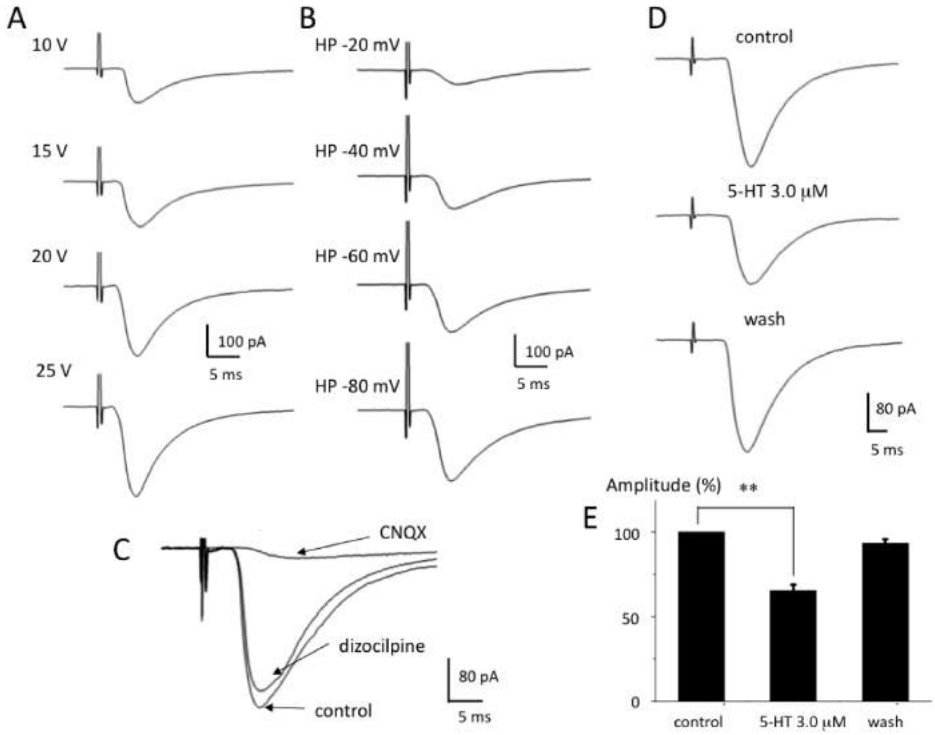

Fig.1 (A) Membrane current responses to the TS stimulation with various intensities (indicated in V) in a second-order NTS neuron. (B) TS-evoked current responses at various holding potentials (indicated in mV) with a constant stimulus intensity. (C) Pharmacological characteristics of the TS-evoked current response. Traces are taken before (control), after CNQX (10.0 μM) and after dizocilpine (10.0 μM). (D) Effects of 5-HT on the TS-evoked current response (eEPSC). Traces were taken before (control), 3 min after the onset of 5-HT (5-HT 3.0 μM) and 3 min after washout (Wash). (E) Percent of inhibition of the eEPSC amplitude by 5-HT. Data are represented as the mean ± SEM (n=19). ** $p<0.01$ vs. control (paired t-test).

currents were recorded in all cases with or without perfusion of picrotoxin. Fig. 2C illustrates a typical example of 5-HT effect on sEPSCs. Perfusion of 5-HT (3.0 μM) significantly increased the sEPCS frequency, but had no effect on their amplitude. This effect of 5-HT was concentration-dependent. The frequency of sEPSCs returned to the baseline value within 3 min after washout of 5-HT.

DISCUSSION

It has been shown that the fast synaptic transmission from the axon terminal of TS fibers to second-order neurons is mainly mediated by glutamate and ionotropic glutamate receptors [1, 5, 6]. Furthermore, small diameter NTS neurons receive exclusively glutamatergic synaptic inputs from intrinsic NTS neuron network [3, 7, 24]. Both eEPSCs and sEPCSs were blocked by CNQX but not by dizocilpine [3, 7, 24]. These characteristics are consistent with those analyzed in the present study. This indicates that the neurons analyzed here receive predominantly glutamatergic synaptic inputs through activation of the non-NMDA or AMPA receptors in the second-order neurons. Therefore, the neurons described in this study are likely to be members of local circuit neurons and relay neurons of the NTS, because of excitatory synaptic inputs, insensitive to $GABA_A$ receptor antagonists, small soma diameters and their distribution. It is proposed that the NTS is not simple 'relay' nucleus of afferent information. Rather, it controls the downstream autonomic networks through two types of intrinsic activities. First, the NTS network generates a tonic background excitatory activity through re-excitatory interconnections between intrinsic neurons, providing a tonic excitatory and inhibitory influence over other nuclei [7, 24]. Second, this tonic activity is modulated by incoming neural and humoral information resulting in a rapid phasic adaptation of outputs.

The most striking finding of this study is that the decrease of eEPSC amplitude and enhancement of sEPSC

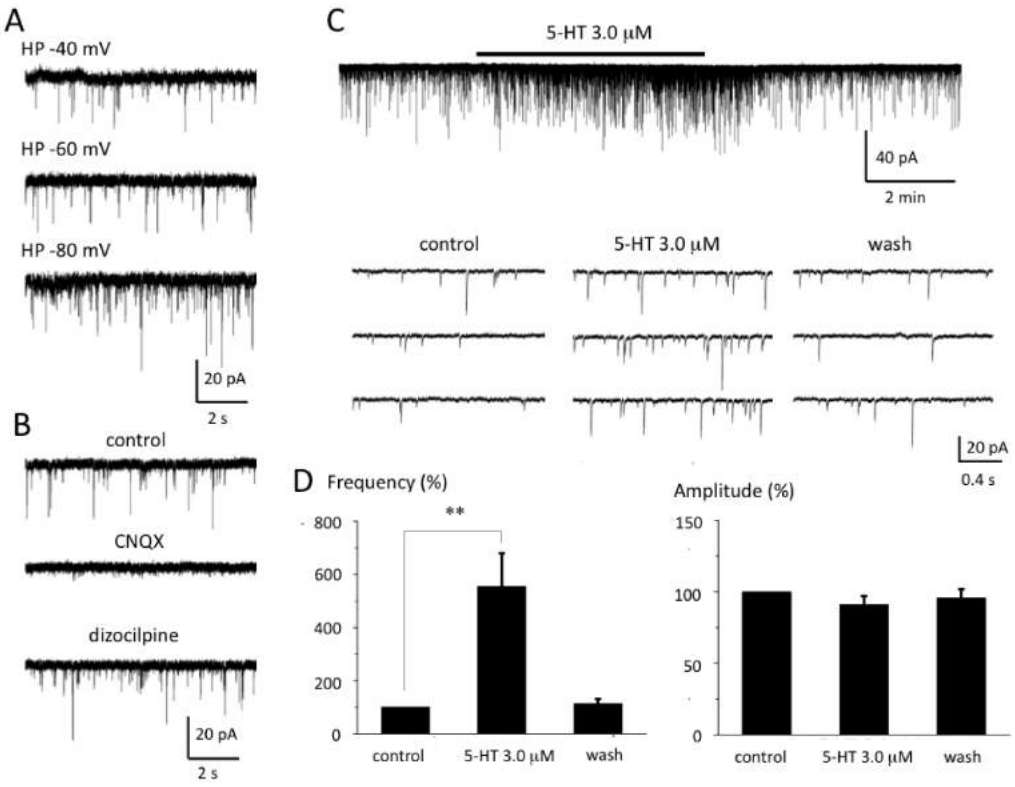

Fig.2 (A) Spontaneously occurring current responses (sEPSCs) at various holding potentials (indicated in mV) in a second-order NTS neuron. (B) Pharmacological characteristics of sEPCSs. Traces are taken control, after CNQX (10.0 μM) and after dizocilpine (10.0 μM). (C-upper) Effects of 5-HT on sEPCSs. A raw trace of sEPSCs at a slow sweep-speed. 5-HT (3.0 μM) was perfused during a horizontal bar. (C-lower) Traces were taken before (control), 3 min after the onset of 5-HT (5-HT 3.0 μM) and 3 min after washout (Wash) at a fast sweep-speed. (D) Percent of inhibition of the mean frequency and amplitude of sEPSCs by 5-HT. Data are represented as the mean ± SEM (n=10). ** $p<0.01$ vs. control (paired t-test).

frequency by 5-HT were simultaneously observed in single cells in a large population of NTS neurons. The present results suggest dual modulation of 5-HT, an inhibitory effect on the peripheral inputs to the relay neuron and an excitatory effect on the NTS local network. Two possibilities are proposed underlying this differential regulation; the receptor subtype and receptor distribution responsible for each response may be different. This implies that the axon terminals of different origins with distinct 5-HT mechanisms make synaptic connections on the second-order NTS neuron. Therefore, the present results indicate that 5-HT enhances the tonic activity maintained by the local network excitation, while it suppresses the phasic reflex gain through attenuation of the afferent inputs.

Although numerous reports concerning modulation of 5-HT in the synaptic transmission have been accumulated in various neurons, its effects and underlying receptors are still controversial[10, 16, 25-27]. Interestingly, Glaum et al.[18] have already demonstrated using in the rat NTS neurons that 5-HT depolarized the membrane and increased the amplitude and frequency of spontaneous excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs). On the contrary, 5-HT decreased the amplitude of TS-evoked EPSPs and IPSPs. The neurons recorded by Glaum and coworkers[18] are thought to be different from ours, because the second-order neurons in the present study did not show any IPSC. However, their results could be, at least partly, comparable with our findings. The relative role of 5-HT and its receptor mechanisms in the regulation of the synaptic transmission await further experiments.

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