5-Hydroxytryptamine increases spontaneous activity of subthalamic neurons in the rat

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Abstract

The effect of 5-hydroxytryptamine on the spontaneous activity of neurons of the subthalamic nucleus was examined by recording the extracellular unitary activity in an in vitro slice preparation. The most frequent response to 5-hydroxytryptamine (84% of 57 neurons tested) was an increase (twofold of basal at 10 μM) of the discharge frequency. The EC₅₀ for the 5-hydroxytryptamine-induced effect was 1.8 ± 0.5 μM (mean ± SEM). The response was dose-dependently blocked by the serotoninergic antagonist mianserin and was not prevented by removal of calcium ions from the perfusing buffer. These results indicate that the serotoninergic input to the rat subthalamic nucleus exerts a postsynaptic excitatory action on most neurons of the nucleus.

Keywords: Basal ganglia; Subthalamic nucleus; Serotonin; Serotoninergic action; Mianserin

The spontaneous firing of subthalamic neurons can be regulated by inputs from several brain nuclei. Thus external globus pallidus, via GABAergic terminals, inhibits such an activity [20] whereas the pedunculo pontine nucleus may enhance it, probably through a cholinergic projection [11]. The cerebral cortex [21] as well as the substantia nigra compacta (via dopaminergic terminals) [5,12] may also regulate the activity of subthalamic neurons.

Autoradiographic [4,6], biochemical [18], immunofluorescence [23] and immunohistochemical studies [15,16] indicate the presence of 5-hydroxytryptamine (5-HT)-containing nerve fibers in the subthalamic nucleus of rats, cats and primates. This serotoninergic input arises from the raphe nuclei [4,10], mainly from the dorsal raphe nucleus [10]. However, the role of such an innervation on the activity of the subthalamic neurons is still unknown. Attempting to clarify this role, we have examined the effect of 5-HT on the extracellular unitary activity of subthalamic neurons in vitro slices of the rat subthalamic nucleus.

Adult, male Wistar rats (190–210 g) were anesthetized with chloral hydrate (300 mg/kg i.p.) and perfused intracardially with 50 mL of cold (4°C) sucrose artificial cerebrospinal fluid (sucrose-ACSF) [1]. Following decapitation, the brain was rapidly removed from the skull and immersed into cold sucrose-ACSF. Sagittal slices (400 μm thick) were obtained in cold sucrose-ACSF using a vibroslicer. The slices were maintained in the oxygenated sucrose medium for 60 min, and then transferred to an immersion recording chamber and superfused with oxygenated (5% CO₂ in O₂) normal artificial cerebrospinal fluid (normal-ACSF) for the rest of the experiment. Extracellular unitary activity was recorded by using glass microelectrodes filled with 2 M NaCl (10–12 MΩ). Signals were processed and analyzed using an on line spike data acquisition program [22]. Concentration-response data for the 5-HT-induced effect were fitted to a Hill (logistic) equation using a non-linear regression program (Prism, GraphPad).

The composition of normal-ACSF for superfusion was as follows (in mM): NaCl, 126; KCl, 5; CaCl₂, 2; MgSO₄, 2; NaHCO₃, 26; NaH₂PO₄, 1.25; L-glucose, 10. For the sucrose-ACSF, NaCl was equimolarly substituted by sucrose [1]. To obtain a calcium-free, high magnesium ACSF, CaCl₂ was removed and MgSO₄ was increased to...
Fig. 1. Effect of 5-HT on the spontaneous firing of rat subthalamic neurons. (a) Unitary recordings from a subthalamic neuron in control, in the presence of 5-HT and in recovery conditions. (b) Time course of the response to 5-HT (black bar). (c) Concentration-response curve for 5-HT, present in the superfusion medium for 5 min. The points are the mean ± SEM of 15–18 determinations. (d) Response to three 5-HT-exposures, and blockade of the response by mianserin, added to the medium 3 min before and during the exposure to 5-HT.
9 mM. 5-Hydroxytryptamine hydrochloride and mianserin hydrochloride were purchased from RBI (Natick, MA). Drugs were dissolved in water and added to the superfusing solution to obtain the desired salt concentrations.

Fifty-seven subthalamic neurons from 31 rats were studied. All neurons showed spontaneous activity (16 ± 2 Hz, mean ± SEM). Three patterns of response to 5-HT were observed. The most frequent response, which occurred in 48 (84%) of the neurons studied, was a simple increase in discharge rate (Fig. 1a,b,d), followed by recovery to basal rates over periods which varied from a few seconds to several minutes after the withdrawal of 5-HT from the medium. The second type of response, observed in only 2 (3.5%) of the neurons, was a mixed response, consisting of an initial increase in discharge rate followed by a delayed depression. The third type of response, exhibited also by only 2 (3.5%) of the neurons studied, was a decrease in discharge firing rate. Five (9%) of the neurons tested did not respond to 5-HT. The increase in spontaneous firing rate induced by 5-HT was concentration-dependent (0.1–100 μM) (Fig. 1c). The minimum concentration required to notice the effect was 0.1 μM and the maximum effect was observed at 10 μM. The EC₅₀ for the increase in firing rate was 1.8 ± 0.5 μM. Mianserin, a non-selective antagonist of 5-HT receptors, blocked the response to 5 μM 5-HT by 20% at 1 μM and by 100% at 3 μM (n = 5). To differentiate presynaptic from postsynaptic effects, we studied the action of 5-HT in a free calcium, high magnesium medium. Although the discharge rate of the subthalamic neurons decreased after 15 min in this medium from 15.1 ± 2.7 Hz to 5.0 ± 1.4 Hz, 5-HT (5 μM) still increased (to 9.7 ± 2.2 Hz; 194% increase, n = 6) the discharge frequency.

These results show that 5-HT predominantly enhances the activity of subthalamic neurons and suggest that under physiological conditions the serotonergic input from the raphe nuclei [4,10] may exert an excitatory command on subthalamic neurons through a receptor-mediated effect, as indicated by the blockade of the response by the 5-HT antagonist mianserin. The 5-HT-induced excitation of subthalamic neurons found here is consistent with the increase in [¹⁴C]2-deoxyglucose labeling produced by 5-HT agonists in the STN reported by Freo et al. [13].

The observation that some neurons were either inhibited or unresponsive to 5-HT (5 μM) suggests the presence of different populations of neurons in the nucleus. Some neurons seem to possess serotonergic receptors different from those that mediate excitation, while some others may have no receptors.

The increase in firing rate produced by 5-HT was still present in a calcium-free, high magnesium medium, a condition that prevents synaptic mediated effects. Accordingly, the receptors that mediated the increase in firing rate most probably are located on the soma and dendrites of the subthalamic neurons. A detailed pharmacological study is required to characterize the type of 5-HT receptors that mediate this response. However, this was not our main objective. We wanted to know the nature of the serotonergic action, whether excitatory or inhibitory, and the relative proportion of cells being either excited or inhibited. The results indicate that most (84%) of the 52 subthalamic neurons tested were excited by 5-HT.

The subthalamic neurons send an excitatory projection to substantia nigra pars reticulata (SNr), which use glutamate as a neurotransmitter [17,19]. Therefore an increase in the serotoninergic input to the STN, increasing the firing rate of the subthalamic neurons, would also increase the firing of SNr neurons, thus enhancing the inhibitory output from SNr to thalamus [2,9]. Inhibition of the thalamic neurons would diminish the facilitatory input from thalamus to the motor cortex, thus reducing the motor output from the cortex to lower motor centers [9]. On the basis of the results presented here, it is predicted that the administration of serotonergic agents to Parkinsonian subjects would increase their motor impairments. Keeping this in mind, antidepressant agents acting by blocking 5-HT reuptake [14] should be used with caution in these patients.

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