Modulation of Spontaneous Firing in Rat Subthalamic Neurons by 5-HT Receptor Subtypes

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Xiang, Zixiu, Lie Wang, and Stephen T. Kitai. Modulation of spontaneous firing in rat subthalamic neurons by 5-HT receptor subtypes. J Neurophysiol 93: 1145–1157, 2005 doi:10.1152/jn.00561.2004. The subthalamic nucleus (STN) is considered to be one of the driving forces in the basal ganglia circuit. The STN is innervated by serotonergic afferents from the raphe nucleus and expresses a variety of 5-HT receptor subtypes. We investigated the effects of 5-HT and 5-HT receptor subtype agonists and antagonists on the firing properties of STN neurons in rat brain slices. We used cell-attached, perforated-patch, and whole cell recording techniques to detect changes in firing frequency and pattern and electrical membrane properties. Due to the depolarization of membrane potential caused by reduced potassium conductance, 5-HT (10 μM) increased the firing frequency of STN neurons without changing their firing pattern. Cadmium failed to occlude the effect of 5-HT on firing frequency. 5-HT had no effect on afterhyperpolarization current. These results indicated that the 5-HT action was not mediated by high-voltage-activated calcium channel currents and calcium-dependent potassium currents. 5-HT had no effect on hyperpolarization-activated cation current (Ih) amplitude and voltage-dependence of Ih activation, suggesting that Ih was not involved in 5-HT-induced excitation. The increased firing by 5-HT was mimicked by 5-HT2a receptor agonist α-methyl-5-HT and was partially mimicked by 5-HT1a receptor agonist DOI or 5-HT4 receptor agonist cisapride. The 5-HT action was partially reversed by 5-HT1a receptor antagonist SB 23597-190, 5-HT2 receptor antagonist ketanserin, and 5-HT2c receptor antagonist RS 102211. Our data indicate that 5-HT has significant ability to modulate membrane excitability in STN neurons; modulation is accomplished by decreasing potassium conductance by activating 5-HT2a and 5-HT2c receptors.

INTRODUCTION

The subthalamic nucleus (STN) is considered to be a major driving force in the basal ganglia circuit (Albin et al. 1989; Kitai and Kita 1987; Robledo and Feger 1990; Smith and Parent 1988) and is involved in motor functions (DeLong 1983). The action of serotonin is mediated by a variety of 5-HT receptors, including G protein–coupled subtypes (5-HT1, 5-HT2, 5-HT4), and a ligand-gated ion channel (5-HT3) (Barnes and Sharp 1999; Derkach et al. 1989; Gerhardt and van Heerikhuizen 1997; Hoyer et al. 1994, 2002). Several subtypes of 5-HT receptors are expressed in the STN. Histochemical studies by in situ hybridization have shown that 5-HT2c and 5-HT4 receptor mRNA is present at very high levels in the STN, whereas 5-HT1A and 5-HT2A receptor mRNA appears to be expressed at lower levels (Pompeiano et al. 1994).

The effects of 5-HT on membrane excitability of hippocampal and cerebral cortical neurons have been studied extensively. For instance, activation of 5-HT2, 5-HT3, or 5-HT4 receptors resulted in depolarization of membrane potential and increased neuronal excitability (Davies et al. 1987; McCormick et al. 1993; McMahon and Kauer 1997; Ropert and Guy 1991; Xiang and Prince 2003), whereas activation of 5-HT1A receptors caused hyperpolarization and decreased excitability (Andrade and Nicoll 1987; Beck et al. 1992; Davies et al. 1987; Tanaka and North 1993; Xiang and Prince 2003). The 5-HT2 or 5-HT4 receptor-mediated excitation is associated with Ia decrease in potassium conductances (Bockaert et al. 1992; Davies et al. 1987; Zhang 2003), 2) reduction in high-voltage activated (HVA) calcium channel currents (Bayliss et al. 1997; Foehring 1996) and calcium-activated afterhyperpolarization (AHP) (Andrade and Chaput 1991; Bockaert et al. 1992; Torres et al. 1994), and 3) facilitation in hyperpolarization-activated nonselective cation current [Ih or hyperpolarization-activated cyclic nucleotide-gated channels (HCN)] (Bickmeyer et al. 2002; Zhang 2003). For 5-HT2 receptor activation, evidence suggests that in some cases the excitatory responses involve the 5-HT2a receptor subtype while others are mediated by the 5-HT2c receptor (Aghajanian 1995). The excitatory responses to 5-HT3 activation, primarily found in interneurons of the cerebral cortex and hippocampus, result from an increase in a mixed cationic conductance (Kawa 1994; McMahon and Kauer 1997; Roerig and Katz 1997; Ropert and Guy 1991; Xiang and Prince 2003; Zhou and Hablitz 1999). On the other hand, neuronal hyperpolarization induced by 5-HT1A activa-
tion is due to opening of potassium channels (Andrade and Nicoll 1987; Davies et al. 1987; Xiang and Prince 2003).

Electrophysiological investigation of serotonin in STN neurons is limited to only one extracellular recording study (Flores et al. 1995), which reported an increase in firing by 5-HT. Our study was designed to analyze in detail the effect of 5-HT on firing properties of STN neurons and to determine underlying ionic mechanisms and the receptor subtypes involved.

METHODS

All procedures were performed according to protocols approved by the University of Tennessee Institutional Animal Care and Use Committee and in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Slice preparation

Sprague-Dawley rats (P16–P23) were deeply anesthetized with pentobarbital sodium and transcardially perfused with ~20 ml of ice-cold oxygenated (95% O2-5% CO2) “cutting solution,” which was composed of (in mM) 230 sucrose, 2.5 KCl, 0.5 CaCl2, 10 MgSO4, 1.25 NaH2PO4, 26 NaHCO3, and 10 d-glucose. The brain was rapidly removed from the skull, blocked in the sagittal plane, and glued to the stage of a D.S.K. microslicer (Dosaka EM). Sagittal slices containing the STN were cut at a thickness of 300 μm. The slices were transferred to a recording chamber. The recording chamber was continuously perfused with oxygenated ACSF at 32°C. The ACSF contained 126 NaCl, 2.5 KCl, 2 CaCl2, 2 MgSO4, 1.25 NaH2PO4, 26 NaHCO3, and 10 d-glucose. In the experiments where CdCl2 or BaCl2 was used, MgSO4 and NaH2PO4 were replaced by MgCl2 and KCl, respectively.

Electrophysiology

Whole cell, cell-attached, or perforated-patch recordings were made from visually identified STN neurons under a Nikon microscope (Olympus, Lake Success, NY). A low-power objective (4×) was used to identify the STN, and a 40× water immersion objective coupled with Hoffman optics and infrared video was employed to visualize individual STN neurons. An Axopatch 1D amplifier (Axon Instruments, Union City, CA) was used for the recordings. Patch pipettes were prepared from borosilicate glass (Sutter Instruments, Novato, CA) using a Flaming-Brown micropipette puller (Model P-97, Sutter Instruments). The pipette solution contained (in mM) 106 K-MesO4, 25 KCl, 1 MgCl2, 0.025 CaCl2, 10 HEPES, 0.1 EGTA, 2 ATP, and 0.2 GTP. The pH of the pipette solution was adjusted to 7.3 with 1 M KOH, and osmolarity was adjusted to 290–295 mOsm. For perforated-patch recordings, gramicidin was added to the pipette solution to a final concentration of 18–40 μg/ml immediately before use. When filled with the above solution, patch pipettes had resistances of 2.5–4 MΩ.

Drug application

GABAzine (SR-95531), α-methyl-5-hydroxytryptamine (α-m-5-HT), and 1-(m-chlorophenyl)-biguanide (mCPBG), RS 23597-190, and cisa-pride were obtained from Tocris (Ballwin, MO). All the other drugs including 5-HT, ketanserin, 2.5-dimethoxy-4-iodoamphetamine (DOI), DNQX, and APV were obtained from Sigma (St. Louis, MO). They were made up as concentrated stock solutions in water or DMSO and stored at −20°C. Stocks were thawed and diluted to final concentrations in ACSF immediately before use and either bath-applied or applied through a multibarreled microperfusion pipette (inner diameter ~200 μm) placed within 0.5 mm from the recorded neuron.

Statistical analysis

Statistical comparison was performed using the nonparametric Wilcoxon’s matched pairs test or Mann-Whitney U test. P value <0.05 was considered to be statistically significant. Data are presented as means ± SE.

RESULTS

Effect of 5-HT on spontaneous firing and action potential properties of STN neurons

Perforated-patch recording technique was used to examine the effect of 5-HT on the firing frequency, firing pattern, and action potential properties of STN neurons. This and the following experiments were carried out in the presence of ionotropic glutamate receptor antagonists, DNQX (20 μM) and APV (50 μM), and GABA_A receptor antagonist GABAzine (SR-95531, 10 μM) to rule out a possible presynaptic effect of 5-HT. Subthalamic neurons exhibited spontaneous, rhythmic firing in unstimulated slices. Blockade of glutamatergic and GABAergic synaptic transmission had no significant effect on the mean values of the interspike interval (ISI) and CV of ISI (158.1 ± 20.2 vs. 144.4 ± 18.8 ms for ISI before and after applying the antagonists, respectively, P = 0.08; 0.068 ± 0.004 vs. 0.068 ± 0.006 for CV of ISI, P = 0.5, n = 15). These observations suggest that a rhythmic firing of STN neurons is governed mainly by their intrinsic membrane properties and that spontaneous synaptic activity in slice preparation plays an insignificant role in the firing patterns of STN neurons. These findings are consistent with the previous experiment on firing pattern of STN neurons with ionotropic glutamate receptor antagonists by Bevan and Wilson (Bevan and Wilson 1999).

An application of 5-HT (10 μM) caused a robust increase in firing frequency, evidenced by a reduction in ISI (Fig. 1, A–C). This effect was reversed following washout of 5-HT (Fig. 1, A–C). In eight STN cells, 5-HT decreased ISI by 15–69% with a mean value of 38.5 ± 6.9% (158.6 ± 19.2 ms in control and 94.0 ± 17.6 ms after 5-HT application, P = 0.01; Fig. 1D, left). 5-HT did not alter the pattern of rhythmic firing (Fig. 1B). The ISI of STN neurons exhibited a normal distribution in controls and shifted to the left without changing its “normalness” after application of 5-HT (Fig. 1C). Quantitative analysis revealed that the CV of ISI did not change following 5-HT application (0.061 ± 0.007 in control and 0.065 ± 0.010 in 5-HT, P = 0.4, n = 8; Fig. 1D, right), suggesting that 5-HT did not modulate the firing pattern of STN neurons. A similar result of 5-HT action on firing properties of STN neurons was obtained with cell-attached recording technique, which minimized a possible influence of intracellular dialysis on neuronal firing properties (data not shown).

The 5-HT–induced increase in firing frequency was associated with a depolarization of the interspike voltage (Fig. 1D), with a change in the peak of AHP from −64.5 ± 1.3 to −61.5 ± 1.4 mV (P = 0.01, n = 8). However, 5-HT had no significant effect on the threshold and the width of action potential (44.8 ± 1.4 mV in control and −44.3 ± 1.6 mV in 5-HT for the threshold, P = 0.3, n = 8; 1.96 ± 0.11 ms in control and 2.03 ± 0.10 ms in 5-HT for the width, P = 0.07, n = 8; cf. Fig. 1D). Furthermore, the slow depolarizing membrane potential trajectory immediately preceding the onset of action potential was essentially identical in the control and with 5-HT (Fig. 1D), suggesting that the persistent
sodium conductance (Bevan and Wilson 1999; Hallworth et al. 2003) was not affected by 5-HT.

**5-HT had no effect on calcium-dependent K**⁺** channel-mediated AHP current, but directly depolarized the membrane potential**

It has been reported that HVA calcium channel currents and calcium-dependent potassium currents play important roles in spontaneous rhythmic firing of STN neurons (Bevan and Wilson 1999). To determine whether 5-HT–induced increase in firing frequency of STN neurons is through its action on HVA calcium channel currents and/or calcium-dependent potassium channels, we examined the effect of 5-HT in the presence of HVA calcium channel blocker cadmium to assess if cadmium could occlude the action of 5-HT. As shown in Fig. 2, applying cadmium (600 μM) gave rise to an increase in firing frequency of STN neurons, reflected by a decrease in ISI; adding 5-HT (10 μM) to the cadmium-containing perfusate caused a further increase in firing frequency (Fig. 2, A, B, D, and E). In six STN neurons, cadmium caused a significant decrease in ISI by 12–28%, with a mean value of 22.1 ± 3.0% (141.5 ± 18.2 ms in control and 109.5 ± 13.8 ms in cadmium, *P* = 0.03, *n* = 6; Fig. 2E). Adding 5-HT (100 μM) in the presence of cadmium resulted in a further decrease in ISI by 15–69%, with a mean value of 39.0 ± 8.1% (109.5 ± 13.8 ms in cadmium and 65.2 ± 10.2 ms in 5-HT and cadmium, *P* = 0.03, *n* = 6; Fig. 2E). It should be noted that the CV of ISI increased following application of cadmium (0.074 ± 0.018 in control and 0.139 ± 0.032 in cadmium, *P* = 0.03, *n* = 6) but did not change after adding 5-HT (0.163 ± 0.034 in 5-HT and cadmium compared with 0.139 ± 0.032 in cadmium alone, *P* = 0.1, *n* = 6; Fig. 2E). These data indicate that 5-HT action in STN neurons did not involve modulation of HVA calcium channels and calcium-dependent potassium channels. It is interesting to note that applying cadmium also caused a depolarization of the interspike voltage with a decrease in amplitude of AHP (Fig. 2C), similar to the action of 5-HT (cf. Fig. 1E).

However, the underlying mechanisms for the actions of 5-HT and cadmium are probably different. The cadmium-induced depolarization of interspike voltage is likely due to a decrease in AHP current as a result of blockade of HVA calcium channels, whereas the less hyperpolarization of “apparent” AHP produced by 5-HT has little to do with AHP current. To test this hypothesis, whole cell voltage-clamp experiments were conducted to examine the effects of cadmium and 5-HT on AHP current. As anticipated, applying cadmium significantly decreased AHP current (37.2 ± 4.9 pA in control and 13.3 ± 3.4 pA in cadmium, *P* = 0.02, *n* = 7; Fig. 3, A and B), whereas 5-HT had no effect on the AHP current (28.5 ± 4.4 pA in control and 28.3 ± 4.4 pA in 5-HT, *P* = 0.6, *n* = 7; Fig. 3C). These results suggested that the 5-HT–induced depolarization of interspike voltage with de-
crease in AHP amplitude was most likely due to an overall membrane potential depolarization rather than a direct effect on the AHP current. This notion was supported by the observation that an increase in firing by intracellular injection of depolarizing current was accompanied by a reduction of “apparent” AHP amplitude (Fig. 3, D and E).

To further test whether 5-HT caused depolarization of membrane potential or inward current in STN neurons, we applied 5-HT in the presence of TTX (1 μM), GABA/zine (10 μM), DNQX (20 μM), and APV (50 μM) under the whole cell current-clamp or voltage-clamp condition. As shown in Fig. 3F1, 5-HT reversibly depolarized the membrane potential by 3.7 ± 0.4 mV (−60.6 ± 2.5 mV in control and −56.9 ± 2.4 mV in 5-HT, P = 0.03, n = 6). Under voltage-clamp condition, 5-HT elicited an inward current at a holding potential of −60 mV (Fig. 3F2), with a mean value of 19.6 ± 2.9 pA (n = 8).

These data indicate that the mechanisms underlying a modulation of firing properties by cadmium and 5-HT were different. That is, 5-HT–induced increase in firing frequency is due to depolarization of membrane potential, which subsequently leads to a reduction of AHP amplitude. In contrast, cadmium–induced increase in firing frequency is likely to be a direct consequence of a decrease in AHP current. We therefore could conclude that HVA calcium channel currents and calcium–dependent potassium channel-mediated AHP were not involved in the serotonergic modulation of firing frequency in STN neurons.

**Decrease in potassium conductance underlies 5-HT–induced membrane potential depolarization**

To determine the ionic mechanism underlying the 5-HT–induced depolarization, we conducted whole cell voltage-clamp experiments in which 1-s voltage ramp commands from −60 to −120 mV were applied every 10 s. As shown in Fig. 4A, 5-HT–induced inward current was associated with a decrease in the amplitude of current in response to the voltage ramp command, indicating a decrease in membrane conductance. To estimate the reversal potential (E_r) of the 5-HT–induced conductance change, we obtained 5-HT–induced current (∆I) by subtracting the current in control from the current in the present of 5-HT (I_{5-HT}−I_{control}) and plotted it as a function of the voltage ramp command (Fig. 4B). The linear portion of the current-voltage relationship was fitted with a linear regression line (Fig. 4B). The voltage corresponding to the crossing point of the linear regression line and the voltage abscissa at ∆I = 0 was taken as a measurement of E_r (Fig. 4B).

The mean value of E_r for the 5-HT–induced current was −100.4 ± 4.1 mV (n = 5), which is close to the calculated K^+
equilibrium potential of −104 mV. The involvement of K+ conductance in the 5-HT action was further confirmed by using K+ channel blocker barium. Similar to the 5-HT action, the application of BaCl$_2$ (500 μM) caused an inward shift of the holding current, a reduction in membrane conductance, and a suppression of the response of STN neurons to 5-HT (Fig. 4C; n = 5). These results strongly suggest that a reduction of K+ conductance is involved in the 5-HT–induced excitation in STN neurons.

5-HT had no effect on $I_{H}$

It is well known that STN neurons express prominent $I_{H}$ current. To determine whether an enhancement or a change in the voltage dependence of $I_{H}$ activation is contributing to 5-HT–induced excitability in STN neurons, we have examined the effect of 5-HT on $I_{H}$ using a whole cell voltage-clamp recording technique. An application of a series of hyperpolarizing voltage steps (0.8–1.5 s) elicited voltage- and time-dependent inward currents that resembled the characteristics of $I_{H}$. These responses were blocked by $I_{H}$ blocker ZD 7288 (50 μM; Fig. 5A). Figure 5B shows the records of hyperpolarization-activated currents in control and following application of 5-HT (10 μM). An apparent effect of 5-HT was reduction of membrane conductance. This is shown in Fig. 5C1, where current-voltage relationships for instantaneous current ($I_{inst}$) and steady-state current ($I_{ss}$) were plotted. The reduction of membrane conductance was more apparent when the current differences for $I_{inst}$ and $I_{ss}$ in control and 5-HT were plotted as a function of membrane potential (Fig. 5C2). The reversal potential of the 5-HT–induced conductance change was about −90 mV. This is consistent with the involvement of potassium conductance indicated by the ramp-voltage-clamp experiment (Fig. 4, B and C). The subtraction of the steady-state current and the instantaneous current ($I_{H} = I_{ss} - I_{inst}$) was taken as the measurement of $I_{H}$. As showed in Fig. 5C3, 5-HT did not enhance $I_{H}$ at the membrane potential ranging from −50 to −140 mV. A quantitative analysis on seven STN neurons revealed that 5-HT had no significant effect on $I_{H}$ measured at −60, −80, or −120 mV (0.7 ± 1.7 pA in control and 0.9 ± 1.1 pA in 5-HT at −60 mV, $P = 1$; 11.3 ± 3.4 pA in control and 8.7 ± 1.7 pA in 5-HT at −80 mV, $P = 0.3$; 94.9 ± 19.6 pA in control and 83.8 ± 16.6 pA in 5-HT at −120 mV, $P = 0.2$).

To determine whether 5-HT modulated the voltage dependence of $I_{H}$ activation, we measured the tail current amplitude at a fixed membrane potential (−140 mV or −120 mV) with hyperpolarizing voltage at various test potentials (Fig. 5B1, $I_{tail}$). The normalized $I_{H}$ was determined from a tail current relative to that obtained at −140 or −120 mV. The activation curve was generated by plotting the normalized $I_{H}$ as a function of voltage.

**FIG. 3.** Cadmium reduced AHP current and 5-HT depolarized membrane potential. A: averaged AHP currents (I-AHP) in control, during application of cadmium, and washout for a STN neuron recorded with whole cell voltage-clamp technique. B: bar graph summarizes the effect of cadmium on I-AHP for 7 cells, $*P = 0.02$. C: left and middle: averaged AHP currents in control and during application of 5-HT for another STN neuron recorded with whole cell voltage-clamp technique. Right: 2 traces were superimposed, showing no change in amplitude and decay kinetics of I-AHP in 5-HT. In A and C, I-AHP was elicited by a brief voltage step (4 ms, 80 mV) from holding potential of −60 mV. Each trace was an average of 10 trials. D: spontaneous firing (left) and current injection induced-firing (right) recorded from a STN neuron with perforated-patch recording technique. E: individual action potentials (left) obtained from the same neuron as in D during spontaneous firing (thick traces) and depolarization-induced firing (thin traces) and their average (right) were aligned to the peak and superimposed, respectively. Note that a decrease in amplitude of AHP is associated with depolarization of interspike voltage in response to current injection. F: 5-HT depolarized membrane potential under current clamp (F1) and elicited an inward shift of the holding current under voltage clamp (F2). Recordings were performed using whole cell recording techniques in the presence of TTX (1 μM), GABA(zine) (10 μM), DNQX (20 μM), and APV (50 μM).
of the membrane potential and fitted with a Boltzmann equation,
\[ I/I_{\text{Max}} = \frac{1}{1 + \exp \left( \frac{V - V_{1/2}}{k} \right) \left( V_{1/2} \right) / H_{11001} \exp \left( \frac{V - V_{1/2}}{k} \right) \]]
where \( I_{\text{Max}} \) is maximal tail current, \( V_{1/2} \) is the potential at which the current is half-maximally activated, and \( k \) is a slope factor. This analysis indicated that the membrane potential at half-maximal activation and slope factor were not altered by 5-HT (\( V_{1/2} \) was 97.3 ± 4.8 mV in control and 99.8 ± 4.5 mV in 5-HT, \( P = 0.2 \); \( k \) was 9.4 ± 0.5 in control and 9.3 ± 0.4 in 5-HT, \( P = 0.7 \), \( n = 7 \)). The characterization of \( I_{H} \) was sometimes hampered by an overlap of \( I_{H} \) with other voltage-activated ionic currents, such as inward rectifier K⁺ currents that activate over a similar range of potentials. To rule out the possibility that 5-HT-induced reduction of K⁺ conductance masked the change of \( I_{H} \) following 5-HT application, we repeated the \( I_{H} \) experiment in

FIG. 5. Effect of 5-HT on \( I_{H} \) in STN neurons examined with whole cell recording technique. A: top: hyperpolarizing voltage steps used to elicit current responses (bottom) during control (A1) and application of ZD 7288 (50 μM; A2). A time-dependent increase in the inward current activated by hyperpolarization resembled the characteristics of \( I_{H} \) and was blocked by ZD 7288. B: top: hyperpolarizing voltage steps used to elicit current responses (bottom) during control (B1) and application of 5-HT (10 μM; B2). Instantaneous current response (\( I_{\text{inst}} \)), steady-state current response (\( I_{ss} \)), and tail current (\( I_{\text{tail}} \)) were measured at latencies as indicated by open bars. C: current-voltage (I-V) relationship for \( I_{\text{inst}} \) and \( I_{ss} \) in control and 5-HT (C1), for current difference of \( I_{\text{inst}} \) in control and 5-HT and current difference of \( I_{ss} \) in control and 5-HT (C2), and for \( I_{H} \) (determined by difference between \( I_{ss} \) and \( I_{\text{inst}} \)) in control and 5-HT (C3). Note that 5-HT decreased membrane conductance (C1 and C2), but did not enhance the \( I_{H} \) amplitude (C3). D: normalized \( I_{H} \) was plotted as a function of voltage and fitted with a Boltzmann equation, \[ I/I_{\text{Max}} = \frac{1}{1 + \exp \left( \frac{V - V_{1/2}}{k} \right) \left( V_{1/2} \right) / H_{11001} \exp \left( \frac{V - V_{1/2}}{k} \right) \]} \]. Normalized \( I_{H} \) was determined from the tail current relative to that obtained at −140 mV as indicated in A. Note that 5-HT did not change the half-maximal activation voltage (\( V_{1/2} \)) or the slope factor (\( k \)). \( V_{1/2} \) was −107.0 mV in control and −105.2 mV in 5-HT, and \( k \) was 10.1 in control and 11.0 in 5-HT.
the presence of potassium channel blocker barium. A similar lack of the effect on $I_{H}$ amplitude and activation curve by 5-HT was observed ($n = 4$). These results indicate that the 5-HT–induced excitation in STN neurons could not be attributed to the enhancement of $I_{H}$ or change in the voltage dependence of $I_{H}$ activation.

**Effect of 5-HT on the relationship between firing frequency and level of depolarization by current injection**

The effects of 5-HT on repetitive firing frequency in response to intracellular injection of depolarizing current pulses were examined in seven STN neurons. As shown in Fig. 6A, a given intensity of depolarization current injection elicited a higher frequency spike train in the presence of 5-HT (10 μM) than the control. Figure 6B shows the relationships between the firing frequencies ($f$; 1st ISI and average frequency) versus injected current intensities ($I$). The linear portion of the $f$-$I$ relationship was fit with a linear regression line. As shown in Fig. 7B, the $f$-$I$ relationships for both first ISI and average frequency were shifted to the left following the application of 5-HT. A slight increase in the slope of linear regression line for first ISI was observed with 5-HT application ($0.529 \pm 0.068$ Hz/pA in 5-HT compared with $0.483 \pm 0.062$ Hz/pA in control, $P = 0.01, n = 8$), whereas the slope for the average frequency was not significantly altered ($0.397 \pm 0.048$ Hz/pA in control and $0.424 \pm 0.059$ Hz/pA in 5-HT, $P = 0.2, n = 8$). In contrast, applying cadmium caused a very robust increase in the slope of $f$-$I$ relationships for both first ISI and average frequency ($0.372 \pm 0.049$ Hz/pA in control vs. $0.723 \pm 0.040$ Hz/pA in cadmium for the 1st ISI, $P = 0.03, n = 6; 0.298 \pm 0.035$ Hz/pA in control vs. $0.664 \pm 0.034$ Hz/pA in cadmium for the average frequency, $P = 0.001, n = 6$).

**FIG. 6.** Effect of 5-HT on the relationship between the firing frequency and depolarizing current intensity in STN neurons examined with perforated-patch recording technique. A: firing of a STN neuron in response to different intensity of depolarizing current injection in control (left) and after application of 10 μM 5-HT (right). B: frequency-current ($f$-$I$) relationship for the 1st interspike interval (1st ISI; left) and mean firing frequency (right) before and after 5-HT application in the same neuron as in A. Linear regions of the $f$-$I$ relationship were fitted by linear regression lines. Note an increase in $f$-$I$ slope for the 1st ISI (left) but not for the mean firing frequency (right) after application of 5-HT. C: $f$-$I$ relationship for the 1st ISI (left) and mean firing frequency (right) before and after application of cadmium (600 μM) in a different STN neuron. Linear regions of the $f$-$I$ relationship were fitted by linear regression lines. Note an increase in $f$-$I$ slope for both the 1st ISI (left) and the mean firing frequency (right) after cadmium application.
0.029 Hz/pA in control vs. 0.606 ± 0.028 Hz/pA in cadmium for the average frequency, \( P = 0.03, n = 6; \) Fig. 6C). These results indicate that 5-HT plays a limited role in modulating input-output function of STN neurons. It also supports the conclusions that 1) different mechanisms underlie 5-HT–induced and cadmium-induced increase in firing frequency, and 2) HVA calcium channel and AHP current are not involved in the serotonergic modulation of STN neurons.

5-HT\(_4\) and 5-HT\(_{2C}\) receptor subtypes involved in serotonin action in STN neurons

To determine 5-HT receptor subtypes involved in modulating firing frequency in STN neurons, we examined the effects of various 5-HT receptor agonists and antagonists in STN neurons. Local application of 5-HT\(_4\) receptor agonist mCPBG (3 \( \mu M \)) had negligible effect on the ISI in STN neurons (198.7 ± 58.2 ms in control and 177.1 ± 36.5 ms in mCPBG, \( P = 0.8, n = 10 \)). In contrast, \( \alpha \)-m-5-HT (20 \( \mu M \)), an agent commonly used as a 5-HT\(_2\) receptor agonist, mimicked the action of 5-HT (Fig. 7). It reduced the ISI by 34% on average (165.6 ± 19.8 vs. 109.1 ± 12.1 ms for ISI in control and \( \alpha \)-m-5-HT, respectively, \( P = 0.02, n = 7 \)) with no effect on CV of ISI (0.082 ± 0.030 vs. 0.062 ± 0.011 for CV of ISI in control and \( \alpha \)-m-5-HT, \( P = 0.5, n = 7 \); Fig. 7E). The magnitude of increase in firing frequency by \( \alpha \)-m-5-HT was comparable to 5-HT–induced action (cf. Fig. 1). On the other hand, another 5-HT\(_2\) receptor agonist, DOI (10 \( \mu M \)), was less effective in modulating firing frequency in STN neurons (Fig. 8A). DOI reduced the ISI by 18% (129.7 ± 21.3 ms in control and 105.4 ± 16.8 ms in DOI, \( P = 0.02, n = 7 \); Fig. 8A). Furthermore, preperfusion with 5-HT\(_2\) receptor antagonist, ketanserin (10 \( \mu M \)), failed to prevent the action of 5-HT (Fig. 8B). In the presence of 10 \( \mu M \) ketanserin, 5-HT decreased the ISI by 31% (151.8 ± 20.8 ms in ketanserin-containing ACSF as control and 101.3 ± 16.5 ms during co-application of 5-HT and ketanserin, \( P = 0.04, n = 5 \); Fig. 8B2). These data suggest that receptors other than 5-HT\(_2\) subtypes are also engaged in modulating firing in STN neurons.

Because \( \alpha \)-m-5-HT has been shown to have good affinity for 5-HT\(_4\) receptor (Bockaert et al. 1992; Gerald et al. 1995), we examined the effect of a selective 5-HT\(_4\) agonist cisapride in STN neurons. As shown in Fig. 8C, application of cisapride (1 \( \mu M \)) decreased the ISI by 25% (178.0 ± 30.7 ms in control and 134.1 ± 27.7 ms in cisapride, \( P = 0.01, n = 8 \)).

To further confirm the roles of 5-HT\(_4\) and 5-HT\(_2\) receptors in serotonergic modulation of STN neurons, we examined the effects of 5-HT\(_4\) receptor antagonist RS 23597-190 together with 5-HT\(_2\) receptor antagonist ketanserin on 5-HT action. As shown in Fig. 8D1, application of RS 23597-190 (30 \( \mu M \)) reversed a large fraction of the 5-HT–induced decrease in ISI, and the addition of ketanserin (10 \( \mu M \)) completely reversed the action of 5-HT. Figure 8D2 summarizes the effects of RS 23597-190 and ketanserin on the 5-HT–induced decrease of firing frequency in STN neurons.
ISI, expressed as a percentage of the control value. RS 23597-190 reversed the 5-HT action by 62% (from 51.3 ± 4.8% in 5-HT to 81.6 ± 5.5% in 5-HT and RS 23597-190, P = 0.03, n = 6), and the addition of ketanserin fully reversed the 5-HT action (from 81.6 ± 5.5% in 5-HT and RS 23597-190 to 107.2 ± 5.8% after the addition of ketanserin, P = 0.03, n = 6).

Because it has been reported that STN neurons express 5-HT2C receptor mRNA at a high level and 5-HT2A receptor mRNA at a low level (Pompeiano et al. 1994), we examined the effect of a selective 5-HT2C receptor antagonist RS 122201 (Bonhaus et al. 1993) on 5-HT-induced increase in firing frequency. As shown in Fig. 9, RS 122201 (1 μM) partially reversed the 5-HT action by 45% (from 66.2 ± 2.7% in 5-HT to 81.5 ± 1.6% in 5-HT and RS 122201, P = 0.03, n = 6). It is interesting to note that the magnitude of the antagonizing effect of RS 122201 on 5-HT action was comparable with that of ketanserin (45 vs. 40%, respectively, P = 0.8), which suggested that the 5-HT2 receptor–mediated component was due to activation of 5-HT2C receptors. Taken together, the results indicated that 5-HT–induced increase in firing frequency in STN neurons was mediated by 5-HT4 and 5-HT2C receptor subtypes, with a larger fraction of action attributed to activation of 5-HT4 receptors.

**DISCUSSION**

Our results clearly indicated a role of 5-HT in modulation of firing of subthalamic neurons, exerting an excitatory effect on...
5-HT has been shown to be involved in serotonergic modulation of neuronal excitability across different brain regions. The importance of 5-HT in modulating neuronal excitability (McCormick and Wang 1991; Zhang 2003). This observation indicates that 5-HT–induced depolarization leading to an increase in neuronal excitation has been observed in various neurons in the CNS (Andrade and Nicoll 1987; Bockaert et al. 1992; Davies et al. 1987; Eriksson et al. 2001; Foehring et al. 2002; McCormick and Wang 1991; Zhang 2003). This observation indicates the importance of 5-HT in modulating neuronal excitability across different brain regions.

Several membrane conductances have been reported to be involved in serotonergic modulation of neuronal excitability. 5-HT has been shown to 1) reduce resting potassium conductance and voltage-dependent potassium conductance (Bockaert et al. 1992; Davies et al. 1987; Zhang 2003), 2) decrease amplitude of calcium-dependent potassium current-mediated AHP (Andrade and Chaput 1991; Bockaert et al. 1992; Lorenzo and Foehring 1992; Torres et al. 1994), 3) facilitate $I_{\text{H}}$ (McCormick and Pape 1990; Zhang 2003), and 4) inhibit HVA calcium channel currents (Bayliss et al. 1995, 1997; Foehring 1996). Data from this study provided evidence that 5-HT–induced increase in firing frequency in STN neurons was due to depolarization of membrane potential caused by a reduced potassium conductance. HVA calcium channel currents and calcium-dependent potassium currents were not involved in this action, because cadmium failed to occlude the effect of 5-HT, and 5-HT had no effect on AHP current. Although a blockade of $I_{\text{H}}$ did not significantly alter the basal firing in STN neurons (Bevan and Wilson 1999), in principle, an increase in $I_{\text{H}}$ or positive shift of the $I_{\text{H}}$ activation curve could increase the excitability of STN neurons. However, our voltage-clamp analysis on $I_{\text{H}}$ showed that 5-HT had no significant effect on $I_{\text{H}}$ amplitude and voltage dependence of $I_{\text{H}}$ activation. We therefore concluded that $I_{\text{H}}$ was not involved in 5-HT modulation on firing of STN neurons.

### Ionic mechanism underlying 5-HT–induced excitation

5-HT–induced depolarization leading to an increase in neuronal excitation has been observed in various neurons in the CNS (Andrade and Nicoll 1987; Bockaert et al. 1992; Davies et al. 1987; Eriksson et al. 2001; Foehring et al. 2002; McCormick and Wang 1991; Zhang 2003). This observation indicates the importance of 5-HT in modulating neuronal excitability across different brain regions.

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### 5-HT modulation of f-I relationship

5-HT application shifted the f-I relationships for both first ISI and average frequency to the left. At the same time, it increased the slopes of the linear portion of f-I relationship for the first ISI but not the average frequency. Such constancy of the f-I slopes for average frequency following 5-HT application was probably due to the insensitivity of AHP current to 5-HT. This notion was supported by the observation that cadmium, which inhibits AHP current, not only caused a shift of the f-I relationships for both first ISI and average frequency to the left but also caused a robust increase in the f-I slope for both first ISI and average frequency (Fig. 6C). The increase in the slope of f-I relationships for both first ISI and steady-state frequency following 5-HT application has been reported in neonatal hypoglossal and trigeminal motoneurons and attributed to 5-HT–induced attenuation of mAHP (Berger et al. 1992; Hsiao et al. 1997; Inoue et al. 1999). In contrast, the left-shifted f-I relationships without change in the f-I slope for the average frequency indicated that serotonergic activation only increased overall firing frequency without changing average input-output gain of STN neurons. The increase in the f-I slope for the first ISI suggested that the small changes in incoming synaptic inputs would produce large alterations in initial firing frequency of STN neurons.

### Receptor subtypes involved in 5-HT–induced excitation

Among the 5-HT receptor families, 5-HT$_2$, 5-HT$_3$, and 5-HT$_4$ receptors have been shown to mediate excitatory responses in the brain (Bockaert et al. 1992; Chapin et al. 2002; Davies et al. 1987; McMahon and Kauer 1997; Xiang and Prince 2003; Zhang 2003). The 5-HT$_2$ receptor family consists of three receptor subtypes (5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$), which have similar pharmacological profiles, molecular structures, and signal transduction pathways (Barnes and Sharp 1999). The similarity of the pharmacological profiles of 5-HT$_2$ receptor subtypes makes it difficult to characterize each subtype because of a lack of selective agonists or antagonists (Baxter et al. 1995). The 5-HT$_4$ receptor has at least four splice variants: 5-HT$_{4A}$, 5-HT$_{4B}$, 5-HT$_{4C}$, and 5-HT$_{4D}$ (Barnes and Sharp 1999; Hoyer and Martin 1997). These splice variants display an identical pharmacological profile and show a similar ability to stimulate adenylyl cyclase activity in the presence of 5-HT (Barnes and Sharp 1999; Blondel et al. 1998). 5-HT$_{4A}$...
and 5-HT₄(b) were previously designated as 5-HT₄₅ and 5-HT₄₄ for the short and long form of the receptor, respectively (Hoyer and Martin 1997). 5-HT₁ receptors are the only ligand-gated ion channel receptors in the 5-HT receptor family, which are nonselectively permeable to cations such as Na⁺, Ca²⁺, and K⁺ (Barnes and Sharp 1999; Hoyer et al. 2002). It has been shown that mRNA that code 5-HT₂ (particularly 5-HT₂C) and 5-HT₄ (particularly 5-HT₄(a)) receptors is highly expressed in the STN (Pompeiano et al. 1994; Vilaro et al. 1996; Wright et al. 1995). However, no studies have reported the presence of 5-HT₃ receptors in the STN.

In this study, we used several agonists and antagonists to determine 5-HT receptor subtypes involved in 5-HT action in STN neurons. First, we examined the effect of 5-HT₃ receptor agonist mCPBG. Local application of mCPBG had no effect on firing frequency of STN neurons. It is not likely that the failure to obtain the 5-HT₃ receptor–mediated action was due to a rapid desensitization of 5-HT₁ receptors (Yakel and Jackson 1988; Yang et al. 1992), because the local application technique we used allowed relatively fast solution exchange. In addition, other studies have shown that even with bath application of mCPBG, 5-HT₃ responses can still be induced (Koyama et al. 2000; Zhou and Hablitz 1999). Thus we concluded that the 5-HT₃ receptor was not involved in 5-HT–mediated excitation in the STN.

We then examined the effect of α-m-5-HT on firing of STN neurons. α-m-5-HT is usually used as a 5-HT₂ receptor agonist, which has higher affinity for 5-HT₂B/2C receptors than for 5-HT₂A receptors (Barnes and Sharp 1999; Baxter et al., 1995). In addition, α-m-5-HT also shows a good affinity for 5-HT₄ receptors (Gerald et al. 1995). At the concentration (20 μM) used in this study, α-m-5-HT would likely have acted on all three 5-HT₂ receptor subtypes, as well as 5-HT₄ receptors. We found that α-m-5-HT mimicked the action of 5-HT. Quantitative analysis revealed that the magnitude of increase in firing frequency by α-m-5-HT was comparable with that induced by 5-HT, suggesting that 5-HT action was mediated by 5-HT₂ and/or 5-HT₄ receptors. To further dissect the subtypes involved in this action, we examined the effect of DOI, a 5-HT₂ receptor agonist with similar affinity for all three 5-HT₂ receptor subtypes (Barnes and Sharp 1999; Baxter et al., 1995). We found that DOI was less effective than 5-HT or α-m-5-HT. This observation suggested that the 5-HT action was partially mediated by 5-HT₂ receptors. To substantiate this notion, we applied ketanserin, a commonly used 5-HT₂ receptor antagonist with about two orders of magnitude more selective for the 5-HT₂A receptors than 5-HT₂B/2C receptors (Barnes and Sharp 1999; Baxter et al. 1995). At the concentration of 10 μM used in this study, it would block all three subtypes of 5-HT₂ receptors. We found that preperfusion with ketanserin failed to prevent the 5-HT action. This result confirmed the idea that activation of 5-HT₂ receptors could only account for a part of the 5-HT action.

To determine whether 5-HT₄ receptors have also participated in the 5-HT–induced excitation, we examined the effect of a 5-HT₄ receptor agonist cispapride (Briejer et al. 1993) and the effect of a 5-HT₄ receptor antagonist RS 23597-190 (Eglen et al. 1993, 1995) on the 5-HT action. We found that an application of cispapride caused an increase in firing frequency. However, the action of cispapride was less effective than 5-HT or α-m-5-HT. On the other hand, an application of RS 23597-190 partially antagonized the action of 5-HT, and an addition of ketanserin to the RS 23597-190–containing perfusate completely reversed the 5-HT action. These results indicated that both 5-HT₂ and 5-HT₄ receptors were involved in 5-HT–induced excitation in STN neurons.

Last, we also used a 5-HT₂C receptor antagonist RS 102221 to determine the contribution of 5-HT₂C receptor subtype in 5-HT action. We found that an application of RS 102221 partially reversed the action of 5-HT and that the magnitude of its antagonizing effect of RS 122201 was comparable with that of ketanserin. These results suggested that 5-HT₂ receptor–mediated component was due to the action of 5-HT₂C receptors.

Based on these pharmacological experiments, we concluded that 5-HT₄ and 5-HT₂C receptors were involved in the excitatory action of 5-HT in STN neurons and that 5-HT₄ and 5-HT₂C receptor subtypes may be co-localized in a single STN neuron. Since RS 23597-190 was more effective than ketanserin or RS 122201, we further concluded that most of the 5-HT effect was mediated by 5-HT₄ receptors.

5-HT₂ receptor–mediated excitation has been reported in a variety of brain regions. Evidence suggests that in some of these cases the excitation is attributed to 5-HT₂A receptors in the neurons in the pyriform cortex and neocortex (Araneda and Andrade 1991; Marek and Aghajanian 1994; Sheldon and Aghajanian 1991), whereas others involve 5-HT₂C receptors in the neurons in the pyriform cortex, substantia nigra pars reticulata, hypothalamus, and nucleus tractus solitarius (Eriksson et al. 2001; Rick et al. 1995; Sevoz-Couche et al. 2000; Sheldon and Aghajanian 1991). As for the mechanism of the action of these receptors, the 5-HT₂A receptor–mediated excitation is mainly associated with a decrease in potassium conductances (Aghajanian 1995; Zhang 2003). There are also reports suggesting that the excitatory responses to 5-HT₂C receptor activation are mediated by the closing of potassium channels (Panicker et al. 1991) or by activation of Na⁺/Ca²⁺ exchange (Eriksson et al. 2001). Activation of 5-HT₄ receptors has also been shown to reduce potassium conductances and to enhance membrane depolarization in CNS neurons (Bockaert et al. 1992; Fagni et al. 1992). Our results agree with the above studies concerning the reduction of potassium conductances underlying 5-HT₂C and 5-HT₄ receptor–mediated excitation. On the other hand, we failed to detect a 5-HT₄ receptor–mediated broadening of action potentials by inhibiting a voltage-gated potassium current reported in mouse colliculi neurons (Ansanay et al. 1995). In contrast, our data indicated that 5-HT₁ receptor–mediated action had no effect on repolarization of action potentials in STN neurons. It is interesting to note that an activation of 5-HT₄ receptor results in an increase in I₄ in hippocampal neurons (Bickmeyer et al. 2002), whereas in a similar preparation, 5-HT₄ receptor–mediated depolarization was shown to be independent of I₄ (Chapin et al. 2002). Our results showed that 5-HT did not enhance I₄ or change the activation curve (Fig. 5). Furthermore, in the presence of the I₄ blocker 2D 7288, 5-HT₁ receptor agonist cispapride was still able to induce an inward current in STN neurons (data not shown). These data suggested that I₄ was not engaged in 5-HT₁ receptor–mediated excitation in STN neurons.

In addition to 5-HT₂, 5-HT₄, and 5-HT₃ receptors, 5-HT₇ receptors were recently shown to mediate membrane potential depolarization in thalamic neurons by acting on I₄ (Chapin and...
Andrade 2001a,b). However, our preliminary study revealed that 5-HT$_3$ receptor antagonist SB 269970 (10 µM) had no effect on 5-HT–induced increase in firing in STN neurons (data not shown), suggesting that the 5-HT$_3$ receptor was not involved in 5-HT action in STN neurons.

In conclusion, our study showed that 5-HT, acting on 5-HT$_{2C}$ and 5-HT$_3$ receptors, 1) depolarizes membrane potential due to a reduction of potassium conductance and 2) leads to an increase in firing frequency without changing firing pattern of STN neurons. STN is the only nucleus in the basal ganglia whose output neurons are excitatory and plays an important role in motor functions (DeLong 1990; DeLong et al. 1985; Matsumura et al. 1992; Wichmann et al. 1994). It is interesting to note that serotonergic neurons in the dorsal raphe nucleus have also been implicated in various aspects of motor control (Jacobs and Fornal 1993; Steinfields et al. 1983; Trulson et al. 1981). Anatomical studies have shown that the STN receives serotonergic innervations from the dorsal raphe nucleus (Bobillier et al. 1976; Lavoie and Parent 1990) and that both the STN and the dorsal raphe nucleus receive inputs from the prefrontal cortex (Kitai and Deniau 1981). We may therefore speculate that, on the cortical activation for a specific movement, a group of raphe serotonergic neurons that project to the STN might be activated in concert with STN neurons, which would amplify subthalamic activity, and in turn, facilitate the excitation in the output structures in the basal ganglia, which in turn inhibits the thalamic motor area.

REFERENCES


Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, Saxena PA, and Humphrey PP. International Union of Pharmacology


