5-HT$_2$ receptors promote plateau potentials in turtle spinal motoneurons by facilitating an L-type calcium current

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Abstract

The effects of serotonin (5-HT) on intrinsic properties of spinal motoneurons were investigated with intracellular recordings in a slice preparation from adult turtles. In 55% of the cells recorded from, addition of 5-HT to the extracellular medium promoted plateau potentials as revealed by the response to depolarizing current pulses applied through the intracellular electrode. In the remaining 45% of cells, 5-HT had an inhibitory effect. However, when tested with an applied electric field that preferentially polarizes distal dendrites, 5-HT facilitated plateau potentials in 100% of the cells. Plateau potentials were also promoted by 5-HT focally applied on a dendrite by iontophoresis. Applied near the soma, 5-HT either promoted plateau potentials or inhibited spike generation. The latter effect was accompanied by a decrease in input resistance. Voltage clamp recordings showed that the facilitation of plateau potentials mediated by L-type Ca\(^2+\) channels was due to activation of 5-HT\(_2\) receptors. These findings show that 5-HT regulates intrinsic properties of motoneurons in opposite ways: activation of 5-HT receptors in the soma region inhibits spike generation and plateau potentials, while activation of 5-HT\(_2\) receptors in the dendrites and the soma region promotes spiking by facilitation of plateau potentials mediated by L-type Ca\(^2+\) channels.
**Introduction:**

Raphé spinal neurons are the main source of serotonin (5-HT) in the spinal cord (Schmidt and Jordan 2000). They project extensively and 5-HT affects most cell types in the spinal cord including motoneurons (Jacob and Azmitia 1992; Kiehn et al. 1992). Several studies have shown that the activity of raphé spinal neurons is related to the level of motor output (Heym et al. 1982; Veasey et al. 1995), suggesting a functional role of 5-HT in motor control. Accordingly, 5-HT has a variety of effects on spinal motoneurons, ranging from depolarization to hyperpolarization and from decrease to increase in input resistance (Rekling et al. 2000). Some of these effects are known to be mediated by modulation of specific postsynaptic ion channels including Ih and SK potassium channels (Rekling et al. 2000). Recently it was shown that the excitatory effect of 5-HT on spinal motoneurons is in part mediated by 5-HT\textsubscript{1A} receptors, which inhibit a TASK-1 like resting conductance (Perrier et al. 2002, submitted). In the adult spinal cord, 5-HT also facilitates plateau potentials (Hounsgaard et al. 1988; Conway et al. 1988; Hounsgaard and Kiehn 1989; Bennett et al. 2001). Plateau potentials in spinal motoneurons are mediated by slowly inactivating, low-voltage activated L-type calcium channels of the Ca\textsubscript{V}1.3 subtype (Hounsgaard and Mintz 1988; Perrier and Hounsgaard 1999; Carlin et al. 2000; Perrier et al. 2002). However, the effect of 5-HT on plateau potentials is not fully understood. Several questions remain to be answered. 1) Since 5-HT modulates several conductances, what is the net effect on motoneurons? 2) Which 5-HT receptor facilitates plateau potentials? 3) Does 5-HT promote plateaus by facilitating a persistent inward current or by inhibiting an outward current? 4) What is the identity of the modulated current? 5) Are the effects of 5-HT homogeneously spatially distributed in the motoneuron?
The facilitation of plateau potentials by 5-HT was originally suggested to be an indirect consequence of inhibition of the slow afterhyperpolarization mediated by SK K⁺ channels (Hounsgaard & Kiehn 1989). Other neurotransmitters, however, modulate plateau potentials in spinal motoneurons by activating pathways positively coupled to L-type calcium channels. These include group I metabotropic receptors for glutamate (mGluRI) and muscarinic receptors (Svirskis and Hounsgaard 1998). Their action may be exerted via the phospholipase C (PLC) - diacylglycerol - inositol trisphosphate cascade, since their effects are mimicked by a transient increase in intracellular Ca²⁺ concentration and prevented by chelation of intracellular Ca²⁺ (Perrier et al. 2000). Among the 5-HT receptors, only the 5-HT₂ group couples positively to PLC (Barnes and Sharp 1999). For this reason it is tempting to hypothesize that these receptors are responsible for the promotion of plateaus by 5-HT.

The present study was undertaken to analyze the modulation of plateau potentials in spinal motoneurons by 5-HT. Our findings suggest that activation of 5-HT₂ receptors facilitates plateau potentials by modulating the properties of L-type Ca²⁺ channels. This facilitation can be evoked in the cell body region and in proximal and distal dendrites. Activation of other 5-HT receptors, on the other hand, inhibits spiking and masks plateau properties. These receptors seem to be mainly located in the soma region.
Materials and Methods:

Transverse slices (1-2 mm thick) were obtained from the lumbar enlargement of adult turtles (*Chrysemys scripta elegans*) anaesthetized by intraperitoneal injection of 100 mg sodium pentobarbitone and killed by decapitation. The surgical procedures comply with the Danish legislation and are approved by the controlling body under The Ministry of Justice. Experiments were performed at room temperature (20-22 °C) in a solution containing (in mM): 120 NaCl; 5 KCl; 15 NaHCO₃; 2 MgCl₂; 3 CaCl₂ and 20 glucose saturated with 98% O₂ and 2% CO₂ to obtain pH 7.6.

Recordings. Intracellular recordings in current-clamp and voltage clamp mode were performed with an Axoclamp 2B amplifier (Axon Instruments). Pipettes were filled with 1 M K-Acetate. Voltage-clamp recordings were performed in discontinuous service mode at a sample rate of 7-8.9 kHz, gain of 0.7-1.5 nA.mV⁻¹ and low-pass filter of 0.1 kHz. A triangular voltage waveform command (7-7.6 s duration) was used to depolarize the motoneurons from the resting potential (for details see Svirskis and Hounsgaard, 1997). Motoneurons were selected for study if they had a stable membrane potential of more than -60 mV. Data were sampled at 10 kHz with a 12-bit analogue-to-digital converter (DIGIDATA 1200 from Axon Instruments) and displayed by means of Axoscope software. Input resistance of the cells recorded from was calculated as the steady state voltage to current ratio, and estimated with current pulses applied from the resting membrane potential (amplitude between –0.2 and –1 nA; duration 200 ms). Plateau potential amplitude was measured as the maximal amplitude of the afterdepolarization following a 2s depolarizing current pulse at an intensity subthreshold for spike generation during the afterpotential.
Field stimulation. The slice was placed in the recording chamber between two silver electrodes positioned laterally to the slice (Hounsgaard and Kiehn 1993; Svirskis and Hounsgaard 1997; Delgado-Lezama et al., 1999). The electric field was applied in the lateral direction of motoneuronal dendrites. With this orientation of the electric field, maximal polarization is obtained in the distal compartments of lateral and medial dendrites while an indifferent point with no change in membrane potential is located proximally in lateral dendrites (Baginskas et al. 1993; Hounsgaard and Kiehn 1993; Svirskis et al. 1997; Svirskis and Hounsgaard 1998). With the cathode lateral to the ventral horn recorded from, the lateral dendrites are depolarized while the medial dendrites and the soma are hyperpolarized. This configuration, that allows a selective depolarization of part of the dendritic compartment, is termed a soma-hyperpolarizing field (S.). When the recording electrode was withdrawn from a motoneuron the extracellular potentials induced by the fields applied during intracellular recording were measured. Offline, the transmembrane potential was obtained as the difference between intra and extracellular potentials for each intensity of the electric field applied.

Iontophoresis. Micropipettes filled with 150 mM serotonin hydrochloride, pH 4-4.5 were used for microiontophoresis. The pH value was chosen so that 5-HT was ejected by a positive current. During experiments, diffusion of 5-HT from the pipette was minimized by applying a constant holding current of –40nA. The microiontophoresis electrode was either positioned close to the recording electrode and therefore presumably close to the cell body (fig. 3E) or in the white matter (fig. 3A). In the latter case, any effect induced by 5-HT was considered to occur on a dendrite.
**Electrical stimulation.** Extracellular stimulation in the dorsolateral funiculus (DLF) was performed by applying thirty constant current pulses (0.2 ms duration) at 25 Hz by means of a bipolar wire electrode.

**List of drugs:** Serotonin (5-HT; 10µM; Sigma), (+/-)-1-[2,5]-dimethoxy-4-iodophenyl-2-aminopropane (DOI; 10µM; Sigma), tetrodotoxin (TTX; 1-2 µM; Alomone), nifedipine (10 µM; Sigma), SB 206553 hydrochloride (Sigma).

For all the experiments, the synaptic potentials were inhibited by blocking fast synaptic receptors with a mixture of CNQX (25µM; Tocris), Strychnine (10µM; Sigma), DL-AP5 (50µM; Tocris) or DL-AP7 (25µM; Tocris).

Data were analyzed statistically by using two populations (paired or independent) t-test (Microcal Origin software, Northampton, MA). Significance was accepted when \( P<0.05 \). Data are presented as a means value ± standard error.
Results:

**General effects of serotonin on motoneurons**

The ability of 5-HT to promote plateau potentials in motoneurons was monitored by the response to depolarizing current pulses of different amplitudes. Generation of plateau potentials was indicated by an accelerated discharge of action potentials during the current pulse and/or an afterdepolarization or afterdischarge following the current pulse (Hounsgaard and Kiehn 1989). By this measure, 5-HT facilitated plateau potentials in 55% (12 out of 22) of the motoneurons recorded from (fig. 1A; increase in plateau calculated in 9 of the 12 cells: 309 ± 159% (see methods); in the 3 remaining cells, the increase in plateau properties could not be quantified because the plateau was only present after addition of 5-HT). However, in 45% of motoneurons recorded from (10 out of 22), addition of 5-HT did not facilitate plateau potentials (n=6/10) and could even suppress them when present in control conditions (fig. 1B1-2; mean inhibition 63% ± 10, n=4/10). These experiments show that serotonin has at least two separate effects on motoneurons: an excitatory effect involving facilitation of plateau potentials and an inhibitory effect that may be strong enough to prevent facilitation of plateau potentials.

**Compartmentalization of the effects of serotonin**

In order to investigate whether the different effects induced by 5-HT were spatially homogenous, lateral dendrites of the motoneurons recorded from were selectively depolarized by means of an electric field (S-configuration) applied through the slice. When such a field is applied, the soma and medial dendrites are hyperpolarized, while the lateral dendrites are depolarized (fig. 2A, see methods; Hounsgaard and Kiehn 1993; Delgado-Lezama et al. 1999). Therefore, if a plateau potential is induced by the
field, one can conclude that it originates from the lateral dendrites. For these experiments, the intensity of the field in control condition was adjusted to a level low enough not to induce a plateau. When 5-HT was added to the extracellular medium the same S- field was always able to generate a plateau potential (fig. 2BC; n=14/14). This result, which contrasts the results obtained with depolarizing current pulses applied to the soma (fig. 2C), suggests that the 5-HT receptors responsible for plateau facilitation are present in dendrites and that there is a differential distribution between these receptors and the receptors responsible for the inhibition.

To examine the spatial segregation of the receptor types further, 5-HT was focally applied by means of iontophoresis (a microiontophoresis pipette; see methods). When iontophoresed close to a lateral dendrite (fig. 3A), 5-HT only induced excitatory effects which consisted of a sudden depolarization followed by a sustained discharge similar to the one recorded during a plateau potential (fig. 3B; n=5). The latency of the effect decreased with increasing current passed through the 5-HT pipette, i.e. with the amount of 5-HT released. When released close to the soma (fig. 3E), 5-HT had more complex effects: it could either depolarize the motoneuron and promote plateau potentials (fig. 3C; n=10) or reduce excitability as revealed by decreased spiking during test depolarizations (fig. 3D; n=15). The latter effect was accompanied by lower input resistance (-8.2 ±3%; n=6) and a small hyperpolarization (-0.7 ±0.3mV; n=15). The hyperpolarization was not the only reason for the reduction of depolarization induced spiking. Even when the change in membrane potential was cancelled by means of a positive bias current, the generation of action potentials remained inhibited (insert in fig. 3; n=2).
This result confirms that at least two populations of receptors were activated by 5-HT: receptors mediating inhibition located on or close to the cell body and receptors promoting plateau potentials located both on the cell body and on the dendrites.

5-HT$_2$ receptor activation promotes plateau potentials

To test if plateau potentials could be promoted by activating 5-HT receptors coupled to a PLC dependent pathway, we tested the effect of DOI, a broad agonist for 5-HT$_2$ receptors. For all the motoneurons tested, DOI promoted plateau potentials (fig. 4A; mean increase 343% ± 163; n=5/5). The currents modulated by 5-HT$_2$ receptor activation were investigated in voltage clamp using depolarizing ramp commands. Addition of DOI always facilitated a slowly developing voltage sensitive inward current (fig. 4B2; n=7/7), responsible for the clockwise hysteretic configuration in IV plots (fig. 4B3). In all cells tested, DOI had no significant effect on the slope of the rising phase of the IV representation of the recordings (Slope in DOI / Slope in control = 97 ± 5%; n=7; P>0.05), illustrating the absence of change in input resistance. As already shown in previous reports (Svirskis and Hounsgaard 1997, 1998; Delgado-Lezama et al. 1997), addition of nifedipine blocked the facilitated plateau potential recorded in current clamp and the facilitated inward current recorded in voltage clamp (n=5/5; not illustrated) suggesting a facilitatory coupling between 5-HT$_2$ receptors and L-type Ca$^{2+}$ channels.
Discussion

The main finding of this study is that 5-HT$_2$ receptor activation promotes plateau potentials in spinal motoneurons. 5-HT$_2$ receptors exert their effect by facilitating the dihydropyridine sensitive, slowly activating voltage dependent inward current responsible for the hysteresis in IV representation. Added to the fact that 5-HT$_2$ receptor activation does not affect input resistance, this result strongly suggests that 5-HT$_2$ receptors facilitate the L-type Ca$^{2+}$ channels responsible for plateau potentials in spinal motoneurons.

Other effects induced by serotonin

Beside facilitation of plateau potentials, 5-HT has other obvious effects on motoneurons. Iontophoresis of serotonin induced either a depolarization that could trigger the plateau potential or a hyperpolarization associated with a shunt (fig. 3). The depolarization could be caused by activation of 5-HT$_{1A}$ receptors, which inhibit a resting K$^+$ conductance mediated by a TASK-1 like channel in spinal motoneurons (Perrier et al. 2002). The hyperpolarisation has not been systematically studied in this article and has not yet been ascribed to a particular 5-HT receptor. The differential voltage sensitivity of the two effects reported in the present study and their differential time courses are fully in line with the excitatory effect of 5-HT in the dendrites and the inhibitory action confined to the soma region. The dual inhibitory and excitatory action of 5-HT on motoneurons has precedent in cortical neurons (Davies et al. 1987; Araneda and Andrade 1991). The functional significance of apparently opposing effects of a neuromodulator on a neuron is not clear. The finding that the two effects are associated with different cellular compartments in
motoneurons raises the possibility that different serotonin receptor subtypes are activated by different presynaptic serotonergic neurons. The somatically located inhibitory action of serotonin could be related to an arrangement similar to cervical motoneurons in primates in which $5\text{-HT}_{1\text{A}}$ receptors are densely expressed in the axon hillock and diffusely expressed in the soma (Kheck et al. 1995; Azmitia et al. 1996).

5-HT receptors responsible for the facilitation of plateau potentials

We have previously implicated $5\text{-HT}_{1\text{A}}$ in synaptically induced facilitation of L-channels and plateau potentials based on the effects of the receptor antagonists NAN-190 and pindobind-$5\text{-HT}_{1\text{A}}$ (Delgado-Lezama et al. 1997). However, these antagonists have considerable affinity for a range of 5-HT receptors, including $5\text{-HT}_{1\text{A}}$, $5\text{-HT}_{1\text{B}}$, $5\text{-HT}_{1\text{C}}$, $5\text{-HT}_{2}$, $5\text{-HT}_{3}$ and $5\text{-HT}_{7}$ (Millan et al. 1994; To et al. 1995). Another potential problem with our previous study arises from using microelectrodes containing lidocaine for the voltage clamp experiments. In addition to sodium channels, lidocaine also blocks calcium channels (Talbot and Sayer 1996). It is conceivable that the persistent inward current was gradually blocked by lidocaine rather than the receptor antagonists applied. The lack of specificity of the $5\text{-HT}_{1\text{A}}$ receptor antagonists used in our previous study necessitated a clarification of the serotonin facilitation of plateau potentials. In the present study we show that DOI, in the presence of TTX, activated an inward current with the same electrical and pharmacological properties as the L-type $\text{Ca}^{2+}$ current. As DOI is considered a very selective agonist for $5\text{-HT}_{2}$ receptors (Barnes and Sharp 1999), we conclude that serotonin binding to $5\text{-HT}_{2}$ receptors, facilitates L-type $\text{Ca}^{2+}$ channels. This could be the mechanism for up-regulation of the excitability of extensor motoneurons by DOI in acute spinal cats (Miller et al 1996).
**Presumed intracellular pathway**

Like mGluRI receptors and M1 muscarine receptors, 5-HT2 receptors are positively coupled to PLC and, through IP3, to secondary release of Ca^{2+} from intracellular stores. Facilitation of plateau potentials by mGluRI and muscarinic receptors has been shown to be mediated by a Ca^{2+} dependent pathway (Perrier et al. 2000). By analogy, it seems reasonable to hypothesize that 5-HT2 receptors also facilitate the L-type Ca^{2+} channels by increasing the intracellular Ca^{2+} concentration. This suggests that all the metabotropic receptor pathways known to facilitate plateau potentials in spinal motoneurons converge on PLC to facilitate L-type Ca^{2+} channels (Perrier et al. 2000).

**Functional considerations**

At rest, motoneurons have a highly negative membrane potential, well below the threshold for generation of action potentials. The activity of raphé neurons, and presumably the release of 5-HT, are highly correlated with motor activity (Heym et al. 1982; Veasey et al. 1995; Jacobs and Fornal 1997). One obvious function of serotonin could be to increase excitability of motoneurons. This could be mediated by block of the spike afterhyperpolarization mediated by SK channels (Hounsgaard and Kiehn 1989), by the activation of 5-HT_{1A} receptors which inhibit a leak conductance and depolarize the cell (Perrier et al. 2002), and by facilitation of plateau potentials by 5-HT2 receptors. Metabotropic synaptic facilitation of plateau potentials induced by 5-HT2 receptors could also provide a mechanism for selectively adapting the excitability of motoneurons and could be used by the central nervous system to change the recruitment order among pools of motoneurons.
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References:


Figure legends:

**Figure 1.** Effect of serotonin on two different motoneurons. A1, control response to a depolarizing current pulse applied from a positive bias current (+0.5nA). A2, after addition of 5-HT (10 µM), the same current protocol induced a train of action potentials followed by an afterdepolarization with a superimposed afterdischarge, indicative of an underlying plateau potential. The sustained activity was turned off by a negative current pulse. A3, A stronger pulse of current applied from the resting membrane potential induced a discharge of action potentials and a small afterdepolarization (black trace). Addition of 5-HT increased the discharge frequency and the amplitude of the afterdepolarization (gray trace). Action potentials truncated to fit in the frame of the figure. B1, response of another motoneuron to a depolarizing current pulse. Note the activation of a plateau potential as indicated by the afterdepolarization and the afterdischarge in control medium. B2, addition of 5-HT (10 µM) eliminated the plateau properties. For each recording, upper trace: membrane potential, lower trace: current.

**Figure 2.** 5-HT selectively facilitates plateau potentials in the dendritic compartment. A, scheme showing a spinal cord slice between the two silver electrodes used to apply the electrical field. With the cathode positioned laterally to the motoneuron recorded from, the medial dendrites and the soma were hyperpolarized (S- configuration), while the lateral dendrites were depolarised. B, lower trace: control response induced by the S- field (black bar). Note the transient hyperpolarisation. Upper trace: response
to the same field stimulation after addition of 5-HT (10µM). Note the supplementary depolarisation, the amplitude of which was strong enough to generate an afterdischarge when the field was stopped. C, summary diagram: 5-HT facilitates plateau potential in 55% of the motoneurons when tested with depolarising current pulses, and in 100% of the motoneurons when tested with the S-field.

**Figure 3.** Spatial segregation of the effects induced by serotonin on motoneurons. All recordings from the same cell. A, scheme of preparation and electrode arrangement with a 5-HT microiontophoresis pipette near a distal dendrite and the recording electrode at the soma. B, three superimposed recordings from a motoneuron to release of 5-HT at 3 different intensities close to a dendrite (sweeps and stimulus bars labelled with iontophoresis current). Sweeps separated for clarity (the resting potential was the same throughout the experiment). With more 5-HT released the plateau potential was triggered faster. C, response of the same motoneuron when the iontophoresis pipette was moved to the cell body (as in E). The excitability of the motoneuron was monitored by regular depolarising current pulses. Within a few seconds, iontophoresis of 5-HT (bar) induced a plateau potential. D, response of the same motoneuron to the same intensity of 5-HT iontophoresis as in C. Plateau potentials were prevented by a negative intracellular bias current. The excitability of the motoneuron was monitored by regular depolarising current pulses. Under control conditions the cell responded to the intracellular pulse with 6 action potentials (D1). Iontophoresis of 5-HT (bar) gradually hyperpolarized the cell and inhibited the spiking until complete suppression (D2). The hyperpolarization was not sufficient to explain the inhibition of the spiking since a partial release of the bias current, adjusted
so that the membrane potential was the same as in control (i.e. –78mV), did not prevent the spike suppression (insert; recording obtained during a separate 5-HT application). The effect was fully reversible (D3). E, scheme showing the position of the iontophoresis pipette close to the cell body.

**Figure 4.** 5-HT₂ receptor activation promotes a plateau potential. A1, control response to a depolarising current pulse. A2, after addition of DOI (10 µM), the same current pulse induced a train of action potentials followed by an afterdepolarization on which was superimposed an afterdischarge. The plateau potential was turned off by a negative current pulse. B, response of another motoneuron to triangular voltage command in the presence of TTX (1µM). B1, control response. B2, addition of DOI (10µM), facilitated an inward current during the repolarisation phase. The dot line represents the position of the control trace. B3, I-V plot of the current generated during the triangular voltage command before and after addition of DOI. DOI induced the clockwise hysteresis.
Figure 1
A - SOMA HYPERPOLARIZED CONFIGURATION

B

5-HT (10μM)

5-HT (10μM)

20 mV

2 s

C - FACILITATION OF PLATEAU POTENTIALS BY 5-HT

(n = 12 / 22) (n = 14/14)

Current pulse S- field

Figure 2
Figure 3