Published in final edited form as:

J Neurophysiol. 2007 February ; 97(2): 1236–1246. doi:10.1152/jn.00995.2006.

Serotonin Facilitates a Persistent Calcium Current in Motoneurons of Rats With and Without Chronic Spinal Cord Injury

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Abstract

In the months after spinal cord transection, motoneurons in the rat spinal cord develop large persistent inward currents (PICs) that are responsible for muscle spasticity. These PICs are mediated by low-threshold TTX-sensitive sodium currents (Na PIC) and L-type calcium currents (Ca PIC). Recently, the Na PIC was shown to become supersensitive to serotonin (5-HT) after chronic injury. In the present paper, a similar change in the sensitivity of the Ca PIC to 5-HT was investigated after injury. The whole sacrocaudal spinal cord from acute spinal rats and spastic chronic spinal rats (S2 level transection 2 mo previously) was studied in vitro. Intracellular recordings were made from motoneurons and slow voltages ramps were applied to measure PICs. TTX was used to block the Na PIC. For motoneurons of chronic spinal rats, a low dose of 5-HT (1 μ M) significantly lowered the threshold of the Ca PIC from -56.7 ± 6.0 to -63.1 ± 7.1 mV and increased the amplitude of the Ca PIC from 2.4 ± 1.0 to 3.0 ± 0.73 nA. Higher doses of 5-HT acted similarly. For motoneurons of acute spinal rats, low doses of 5-HT had no significant effects, whereas a high dose (about 30 μ M) significantly lowered the threshold of the L-Ca PIC from -58.5 ± 14.8 to -62.5 ± 3.6 mV and increased the amplitude of the Ca PIC from 0.69 ± 1.05 to 1.27 ± 1.1 nA. Thus Ca PICs in motoneurons are about 30-fold supersensitive to 5-HT in chronic spinal rats. The 5-HT-induced facilitation of the Ca PIC was blocked by nimodipine, not by the I_h current blocker Cs^+ (3 mM) or the SK current blocker apamin (0.15 μ M), and it lasted for hours after the removal of 5-HT from the nCSF, even increasing initially after removing 5-HT. The effects of 5-HT make motoneurons more excitable and ultimately lead to larger, more easily activated plateaus and self-sustained firing. The supersensitivity to 5-HT suggests the small amounts of endogenous 5-HT below the injury in a chronic spinal rat may act on supersensitive receptors to produce large Ca PICs and ultimately enable muscle spasms.

INTRODUCTION

A spasticity syndrome is often seen after CNS injury, featured as muscle hyperreflexia, hypertonus, clonus, and spasms (Ashby et al. 1987; Bennett et al. 1999; Heckman 1994; Taylor et al. 1997; Ward 2002). The motoneuron properties and ionic mechanisms underlying spasticity were recently investigated: the results indicate that the intrinsic excitability of motoneurons is profoundly increased over the months after spinal cord injury

and this plays a major role in the generation of spasticity, in both rats (Bennett et al. 2001a,b; Li et al. 2004a) and humans (Gorassini et al. 2004). That is, a brief stimulation can trigger long-lasting firing by activating voltage-dependent persistent inward currents (PICs) on motoneurons, which can cause a sustained depolarization (plateau potential) and firing that outlasts the stimulation by many seconds. These PICs can be triggered by dorsal root stimulation and produce self-sustained firing in the motoneurons and thus play a major role in the long-lasting spastic reflexes seen after chronic spinal cord injury. The PICs responsible for plateaus in motoneurons were previously shown to be mediated by L-type calcium channels (Li and Bennett 2003; Perrier et al. 2002; Simon et al. 2003) and tetrodotoxin (TTX)-sensitive persistent sodium currents (Li and Bennett 2003). The Na PICs are activated subthreshold, but partly inactivated over a few seconds. In contrast, the L-type calcium currents (Ca PICs) can persist for many seconds and contribute to roughly 60% of the sustained depolarization after PIC activation (plateau). After chronic spinal cord injury, there are dramatic changes in the Na and Ca PICs in motoneurons below the level of injury. These PICs induce large, rapidly activating plateaus that cause sustained muscle spasms (Bennett et al. 2001b; Li and Bennett 2003). The ability to activate plateaus in normal motoneurons relies on the facilitation of PICs by neuromodulators, including serotonin (5-HT) and norepinephrine (NE) acting on 5-HT₂ and α_1 -NE receptors on motoneurons, respectively (Lee and Heckman 1999; Perrier and Delgado-Lezama 2005; Perrier and Hounsgaard 2003). After acute spinal cord transection, the PICs in motoneurons are small and not sufficient to produce plateaus as a result of the loss of brain stem-derived neuromodulators such as 5-HT and NE (Harvey et al. 2006a,b,c). However, motoneurons somehow regain the ability to spontaneously exhibit large Na and Ca PICs and plateaus over the months after the injury, even though they are completely isolated from the brain stem (Bennett et al. 2001b). The origin of the large Na PICs in chronic spinal rats was recently suggested to arise from small amounts of residual 5-HT and NE in the spinal cord because 5-HT and NE receptor antagonists eliminate these Na PICs (Harvey et al. 2006a). Only 2–15% of normal 5-HT amounts remain below a chronic spinal transection (Hains et al. 2002; Newton and Hamill 1988), but motoneurons develop a supersensitivity to 5-HT that more than overcomes this reduction in normal 5-HT. That is, very small amounts of 5-HT (<1 μ M) facilitate Na PICs in chronic spinal rats, whereas much larger doses of 5-HT are needed in normal motoneurons (30 μ M; 30-fold supersensitivity) (Harvey et al. 2006b). The objective of the present study was to determine whether a similar supersensitivity to 5-HT develops for Ca PICs, like that described for Na PICs. If so, this may explain why the Ca PIC is also large in chronic spinal rats. That is, residual 5-HT may act on supersensitive receptors to enhance Ca PICs and ultimately contribute to spasticity. Our results confirmed the supersensitivity of the Ca PICs to 5-HT after chronic injury. Interestingly, 5-HT not only increased the amplitude of the Ca PICs at very low doses but dramatically lowered the onset threshold of the Ca PICs, making these persistent currents larger and easier to activate.

METHODS

Intracellular recordings were made from motoneurons in the sacrocaudal spinal cord of both normal adult rats (female Sprague–Dawley; 3–8 mo old) and spastic adult rats with chronic spinal cord injury (3–8 mo old). The chronic spinal rats were transected at 2 mo of age

(adult rat) and recordings were made after their affected muscles became spastic (1–6 mo after injury). [See Bennett et al. (1999) for details of the chronic spinal transection and spasticity assessment.] All recordings were made from the whole sacrocaudal spinal cord removed from the rat with an S2 sacral transection and maintained in vitro. This transection was made in chronic spinal rats just rostral to the chronic spinal injury, so as not to further damage the sacrocaudal cord. In contrast, in the normal rats, the spinal cord was necessarily acutely injured in the in vitro preparation (cut at S2); thus we refer to these motoneurons as from acute spinal rats. All experimental procedures were approved by the University of Alberta Health Sciences Animal Policy and Welfare Committee.

In vitro preparation

Details of the experimental procedures were described in previous publications (Li et al. 2004b,c). Briefly, all the rats were anesthetized with urethane (0.18 g/100 g; with a maximum dose of 0.45 g) and the cord between the T13 and L6 vertebrae was exposed and kept wet with modified artificial cerebrospinal fluid (mACSF). The rat was given pure oxygen for 5 min, after which the cord was removed and transferred to a dissection chamber containing mACSF. All spinal roots were carefully removed, except the S4 and Ca1 ventral roots. The cord was secured by gluing its dorsal surface onto a small piece of nappy paper. After an incubation of 1.5 h in the dissection chamber at room temperature (20–21°C), the cord was transferred to a recording chamber containing normal artificial CSF (nACSF) maintained near 24°C and with a flow rate >5 mL/min, then fixed to the bottom of the recording chamber with the ventral side up by pinning the nappy paper to the Sylgard base of the chamber. A 1-h period in nACSF was given to wash out the residual anesthetic and kynurenic acid, after which the nACSF was recycled as follows. The nACSF was oxygenated in a 200-ml source bottle and then run through the recording chamber. The nACSF running out of the recording chamber was collected, filtered, and returned to the source bottle with a pump. Because of the large volume (200 mL) of the source bottle and the small volume of the cord (<0.05 mL), any accumulation of possible metabolic by-products was negligible.

Intracellular recording

Intracellular recording methods were as described in Li and Bennett 2003 and are briefly summarized here. Sharp intracellular electrodes were made from thick-walled glass capillary tubes (1.5 mm OD; Warner GC 150F-10) using a Sutter P-87 micropipette puller and filled with 1 M K-acetate and 1 M KCl. Electrodes were beveled down from an initial resistance of 40–80 to 25–30 M Ω using a rotary beveler (Sutter BV-10). A stepper-motor micromanipulator (660, Kopf) was used to advance the electrodes through the tissue and break into cells. The S4 and Ca1 ventral roots were wrapped around Ag/AgCl electrodes above the recording chamber and sealed with high-vacuum grease (Dow Corning). Only motoneurons with a resting potential < –60 mV and antidromic spike overshoot >0 mV were considered healthy and used for recording. Recordings were also made from motoneurons penetrated after TTX was applied to block all the sodium channels, although they could not be directly identified as motoneurons by antidromic stimulation because TTX blocked the antidromic spike. However, these neurons could be indirectly identified as healthy motoneurons by their characteristic location (depth in motor nucleus), soma size (>25 μ m;

see *Histology*), Ca PIC, resting potential (V_m ; -75 to -60 mV), input resistance (R_m), and capacitance (C_m). The capacitance was measured from the response to a 0.4-nA hyperpolarizing current step; that is, from the time constant τ of an exponential fit to the response, which equals the product $R_m \times C_m$. Data were collected with an Axoclamp 2b intracellular amplifier (Axon Instruments, Burlingame, CA) running in discontinuous current clamp (DCC; switching rate: 5–8 kHz; output bandwidth: 3.0 kHz; sample rate: 6.7 kHz) or discontinuous single-electrode voltage clamp (SEVC; gain 0.8 to 2.5 nA/mV) modes.

Drugs and solutions

Two kinds of artificial cerebrospinal fluid (ACSF) were used in these experiments: a modified ACSF (mACSF) in the dissection chamber before recording and a normal ACSF (nACSF) in the recording chamber. The mACSF was composed of (in mM) 118 NaCl, 24 NaHCO₃, 1.5 CaCl₂, 3 KCl, 5 MgCl₂, 1.4 NaH₂PO₄, 1.3 MgSO₄, 25 D-glucose, and 1 kynurenic acid. Normal ACSF was composed of (in mM) 122 NaCl, 24 NaHCO₃, 2.5 CaCl₂, 3 KCl, 1 MgCl₂, and 12 D-glucose. Both types of ACSF were saturated with 95% O₂-5% CO₂ and maintained at pH 7.4. When the cord was transferred to the recording chamber, it was briefly exposed to nACSF solution containing 0.04% pronase E (Helixx Technologies) for 10 s to weaken the pia of the spinal cord and allow for easier penetration (Buschges 1994). This was done 1 h before recording and did not induce noticeable changes of the motoneuron properties. The main experiments involved testing the sensitivity of motoneurons to 1–50 μ M 5-HT (Sigma). This was done by applying 5-HT alone (in nACSF) or in the presence of channel blockers to isolate the Ca PIC, including 2 μ M TTX (Alamone Labs, Jerusalem, Israel) to block the Na PIC and action potentials (Li and Bennett 2003) and 0.15 μ M apamin (Alamone Labs) to block small conductance calcium-activated potassium currents (SK currents), which were shown to be activated by the L-type calcium current involved in the Ca PIC (Li and Bennett, unpublished observations). Also, in a separate group of cells, we blocked the hyperpolarization-activated cation current (I_h) with either 3 mM Cs⁺ or 50–100 μ M ZD7288.

Current- and voltage-clamp recordings

Slow triangular current ramps (0.4 nA/s) were applied to the motoneurons to measure basic cell properties and firing (the latter only when TTX was not present). The input resistance (R_m) was measured during the ramp over a 5-mV range near rest and subthreshold to PIC onset. The plateau potential produced by the PIC was seen as a subthreshold acceleration in membrane potential before firing and a long afterdepolarization. The degree to which the PIC helped sustain firing was quantified by subtracting the current at derecruitment (I_{end}) from the current at recruitment (I_{start}) ($I = I_{\text{start}} - I_{\text{end}}$). Positive values of I were termed self-sustained firing (firing that occurred after recruitment current was removed), as detailed in Li et al. (2004a). The spike voltage threshold (V_{th}), was averaged from five consecutive spikes starting with the second spike on the up ramp and was taken as the voltage just before when the rate of the membrane potential change (dV/dt) was >10 V/s (Li et al. 2004a). Slow triangular voltage ramps (3.5 mV/s) were applied to measure the PICs in voltage clamp to measure the current–voltage response (I – V ; Fig. 1). During the upward portion of the ramp, the current initially increased fairly linearly with voltage in response to

the passive leak conductance. A linear relation was fit in the region just below the PIC onset (5 mV below) and extrapolated to the whole range of the ramp (leak current). At depolarized potentials above the PIC onset threshold, there was a steep downward deviation from the extrapolated leak current and the PIC was estimated as the difference between the leak current and the total current (leak-subtracted current). The onset voltage for the PIC (V_{on} ; Fig. 1A) was defined as the voltage at which the slope of the current response first reached zero (Li and Bennett 2003). The amplitude of the initial peak PIC was measured as the peak amplitude of this downward deviation below the leak line (leak-subtracted current; downward arrow in most figures; e.g., Fig. 1A). The sustained PIC was likewise measured as the peak PIC, but on the downward ramp seconds later. Large PICs usually caused a negative-slope region (NSR) in the current response. Smaller PICs only caused a downward deflection in the current response (flattening of I - V slope) without the NSR. V_{on} is not possible to pick in cells without the NSR, although it could sometimes be estimated at the first minimum (rather than zero) slope in the current response. Similarly, the offset voltage for the PIC (V_{off}) was defined as the voltage at which the slope of the current response reached zero (or a minimum) on the downward ramp (Fig. 1A). The difference between V_{on} and V_{off} ($V_{on} - V_{off}$) was used to compute the hysteresis of the PIC. The currents corresponding to V_{on} and V_{off} are defined as I_{on} and I_{off} , respectively. A linear current response, not deviating downward from the leak current line (linear I - V relation), was used to indicate the absence of any PIC. The width of the valley formed by the NSR (V_w) was defined as the span between V_{on} and the voltage at which the current reached I_{on} for the second time on the upward section during the ramp (defined as V_{jump}), that is, $V_w = V_{jump} - V_{on}$ (Fig. 1B). Previously, it was shown that the width of the PIC corresponds to the amplitude of the plateau potential that can be produced by the PIC in current clamp (Li and Bennett 2003). The depth of the NSR caused by the PIC was defined as the difference between I_{on} and the second zero-slope point on the ramp (the peak of the PIC; downward arrow in Fig. 1B). The half-activation voltage of the PIC ($V_{1/2}$) was measured as the voltage where the PIC was half-activated (see Li et al. 2004a), approximately corresponding to where the steep NSR region occurred. For motoneurons lacking a NSR, $V_{1/2}$ was taken at the potential at which the derivative of the current reached a minimum as the PIC was activated.

Labeling of motoneurons

In a portion of the motoneurons, a fluorescent dye was included in the intracellular electrode solution (2% rhodamine dextran, 3,000 molecular weight; Molecular Probes, Eugene, OR) and a positive current of >1 nA was applied for 5 min (>5 nA min $^{-1}$) to fill the cell with dye. After recording PICs (>1 h later), the spinal cord was fixed overnight in 4% paraformaldehyde in phosphate-buffered saline and then cryoprotected in 20% sucrose (phosphate buffered). The cord was then frozen and sliced on a cryostat in 40- μ m transverse sections. The labeled neurons were visualized by fluorescent microscopy.

Data analysis

Data were analyzed in Clampfit 8.0 (Axon Instruments, Union City, CA), and figures were made in Sigmaplot (Jandel Scientific, San Rafael, CA). Data are shown as means $\pm$ SD. The number of motoneurons tested is indicated as n ; one cell was tested per rat per drug

condition, so n also equals the number of rats tested in a given condition. Unless otherwise specified, a paired Student's t -test was used to test for statistical differences before and after drug applications, with a significance level of $P < 0.05$. Unpaired t -tests were used to compare data in acute and chronic spinal rats. A Kolmogorov–Smirnov test for normality was applied to each data set, with a $P < 0.05$ level set for significance. All data sets were found to be normally distributed, as is required for a t -test.

RESULTS

Spontaneously occurring Ca PICs and plateaus in chronic spinal rats

Motoneurons of chronic spinal rats spontaneously exhibited a pronounced low-voltage-activated persistent calcium current (Ca PIC) that was seen in isolation by blocking the persistent sodium current, which is activated in a similar voltage range (Na PIC; with $2 \mu\text{M}$ TTX; $n = 11$; as in Li and Bennett 2003), and blocking SK currents with apamin ($0.15 \mu\text{M}$). This Ca PIC was previously shown to be nimodipine sensitive and is likely mediated by low-voltage-activated Cav1.3 L-type calcium channels (Li and Bennett 2003). In chronic spinal rats ($n = 11$), this Ca PIC was activated at $-56.7 \pm 6.0 \text{ mV}$ (V_{on}) and produced a downward deflection in the current response to an increasing voltage, which was quantified by subtracting the current from the extrapolated leak current just subthreshold to the PIC onset (thin line in Fig. 1A; see METHODS). On average, the initial peak of the Ca PIC (length of downward arrow in Fig. 1A) was $2.4 \pm 1.0 \text{ nA}$. Usually (10/11), the Ca PIC was large enough in chronic spinal rats to overcome the increasing leak current during a voltage ramp and produce an outright negative-slope region in the current response (NSR; labeled in Fig. 1), and the depth of this NSR was $1.51 \pm 0.8 \text{ nA}$ ($n = 10$; Table 1). After the NSR, the measured current increased again with increasing voltage and overcame the leak current (passing the onset current for the Ca PIC, I_{on} , $3.8 \pm 1.4 \text{ nA}$, $n = 11$) only when the potential was, on average, $10.2 \pm 6.2 \text{ mV}$ greater than V_{on} (width of valley formed by NSR, termed V_{w} ; Fig. 1). When the voltage was then decreased, there was a clearly sustained Ca PIC (sustained peak $1.7 \pm 1.1 \text{ nA}$, $n = 11$), which turned off only when the voltage was significantly below the onset voltage ($V_{\text{off}} = -65.3 \pm 6.6 \text{ mV}$, $n = 11$). This hysteresis ($V_{\text{on}} - V_{\text{off}}$) is likely attributable to the dendritic currents involved in the Ca PIC, which are only poorly clamped by the voltage clamp that we applied at the soma (Lee and Heckman 1996). Functionally, the large Ca PIC in chronic spinal rats produced a plateau potential when the membrane potential was free to change in current-clamp mode (Fig. 2A; $n = 10/11$). That is, during an increasing current injection, this plateau started with the same threshold (V_{on} , I_{on}) as the Ca PIC itself (Fig. 2A, arrowhead) and had a height that was equal to the width of the valley formed by the NSR (V_{w}). Also, the plateau was terminated only by reducing the current well below the onset current (I_{on} , Fig. 2B).

Indirect identification of motoneurons penetrated after TTX application

Because intracellular recordings from motoneurons can be mechanically unstable, it was not uncommon to lose a cell after applying TTX, but before the main 5-HT experiment could be performed. In this case, the TTX blocked the ventral roots and thus subsequent motoneurons could not be directly identified by antidromic ventral root stimulation. We salvaged these experiments by searching blindly for motoneurons and established an indirect criteria for

identifying motoneurons, with initial identification based on the characteristically slow time constant of motoneurons in response to a hyperpolarizing current pulse (recorded in DCC mode). In total, $n = 6$ putative motoneurons were penetrated and recorded while in TTX and indirectly identified with the following detailed criteria: 1) they were located in the motor nucleus; 2) they had a characteristically slow time constant ($\tau = 6.4 \pm 3.9$ ms, much slower than axons or glial cells encountered in the ventral cord); 3) they had moderately low input resistance ($R_m = 8.4 \pm 1.1$ M Ω , low compared with that of interneurons or axons); 4) their capacitance C_m was 850 ± 20 pF (determined from $\tau = R_m \times C_m$); 5) their resting potential (V_m) was < -60 mV; and 6) they had large Ca PICs (2.1 ± 0.7 nA). In these indirectly identified motoneurons ($n = 6$), these parameters (τ , R_m , C_m , V_m , Ca PIC) were not significantly different compared with those in motoneurons directly identified by antidromic ventral root activation. Some of the motoneurons indirectly identified in this manner were further confirmed to be motoneurons by histological analysis, all having extensive dendritic trees and cell bodies >25 μ m ($n = 5$), as previously reported for rat sacral motoneurons (Bennett et al. 2001b). Given their clear histological and electrophysiological identification, the indirectly identified motoneurons were grouped together with antidromically identified motoneurons ($n = 5$).

The Ca PIC is resistant to long-term block of spike-mediated transmission

In addition to blocking sodium channels on the motoneurons (spikes and Na PIC), TTX also blocks all spike-mediated synaptic inputs to the motoneurons. This synaptic blocking effect of TTX was previously shown to have very little effect on the Ca PIC, when studied for 20 min (Li and Bennett 2003). In the present motoneurons, we extended these findings, showing that large Ca PICs persisted for many hours in TTX ($n = 5$ cells held between 1 to 4 h; not all subsequently used for 5-HT application), and motoneurons penetrated >1 h after TTX application had large Ca PICs ($n = 5$) not significantly different from Ca PICs recorded <20 min after TTX application. Thus in <20 min a steady state was reached with TTX application, indicating that the receptors that endogenously facilitate the Ca PIC (e.g., 5-HT; Harvey et al. 2006c) are either constitutively active or activated by transmitters (5-HT) released in a spike-independent manner (TTX resistant). All experiments with 5-HT (see next section) were performed after the spikes and Na PIC were blocked by TTX and after a steady-state PIC was achieved (steady state usually occurred within 10 min of TTX application).

Low dose of 5-HT facilitates the Ca PIC in chronic spinal rats

When a low dose of 5-HT (1 μ M) was applied to motoneurons of chronic spinal rats (in the presence of TTX and apamin), the Ca PIC was dramatically altered in its onset threshold, width, and amplitude ($n = 11$; Fig. 1; Table 1), all of which led to dramatically enhanced plateaus (Fig. 2B) and thus greater overall excitability of motoneurons. This occurred despite the presence of TTX to block transmission, so the effects of 5-HT were most likely directly on the motoneurons themselves and not by indirect effects on interneurons. Furthermore, subsequent application of nimodipine blocked the Ca PIC ($n = 4/4$; not shown), verifying that the 5-HT-induced facilitation of the PIC was a persistent calcium current mediated by L-type calcium channels. On average, with 5-HT application (1 μ M), the onset threshold of the Ca PIC (V_{on}) was decreased significantly by 7.0 ± 4.7 mV (to

-63.7 ± 7.1 mV; $n = 11$; Table 1). Further, the amplitude of the PIC was also significantly increased, in both its initial (downward arrow in Fig. 2B; increased by on average 0.58 ± 0.68 nA) and sustained peaks (Fig. 2D and Table 1; increased by 0.51 ± 0.54 nA). The 5-HT-induced increase in PIC amplitude was variable, large in some cells (Fig. 1B) and smaller in other cells (Fig. 3B), whereas all cells showed a large reduction in the Ca PIC onset threshold (V_{on} ; Figs. 1B and 3B). The depth of the NSR produced by the PIC was also significantly larger with 5-HT (see Table 1) and the width of the valley formed by the NSR (V_w ; Fig. 1B) was significantly increased by 7.5 ± 3.7 mV ($n = 11$; V_w is a measure of the plateau size produced in current clamp; Li and Bennett 2003). Together, with these changes in the Ca PIC, the resting membrane potential and input conductance G_m (measured subthreshold to Ca PIC near rest) were both significantly reduced by the low dose of 5-HT ($n = 11$; see Table 1 and Figs. 1 and 2), consistent with previous reports of the action of 5-HT on basic membrane properties (Harvey et al. 2006b).

Interaction of 5-HT-induced changes in membrane properties and Ca PIC

Interestingly, with 5-HT application, the onset voltage (V_{on}) was lowered more than the offset voltage (V_{off}), so the amount of hysteresis ($V_{off} - V_{on}$) was significantly reduced, although not eliminated, by 5-HT (Table 1; Fig. 1). It is possible that this reduction in hysteresis might, in part, be related to the decreased input conductance (G_m), which could make dendritic Ca PICs more readily activated and clamped. However, we found no significant correlation between the change in conductance and change in hysteresis with 5-HT application ($r = 0.48$, $n = 11$, $P > 0.05$). Likewise, there was no significant correlation between the 5-HT-induced change in conductance and the change in PIC width (V_w , $r = 0.29$), onset voltage (V_{on} , $r = 0.18$), or amplitude ($r = 0.47$). Thus the 5-HT-induced change in input conductance cannot trivially account for the dramatic changes in PIC observed. A possible explanation for the reduced hysteresis with 5-HT might be an increased conductance of the channels underlying the Ca PIC, as opposed to a lowering in their threshold. On average, 5-HT depolarized the resting potential by about 7 mV (to -67.7 ± 7.3 mV) and hyperpolarized the onset of the Ca PIC by 7 mV (to -63.7 ± 7.1 mV; see above); thus cells rested very close to the Ca PIC threshold in 5-HT (Fig. 1B). This raised the concern that the Ca PIC may be partly inactivated if it were allowed to remain at rest between trials. To avoid this problem, between voltage ramp trials, we always held the potential at a fixed value of < -75 mV with a bias current.

The Ca PIC is initially increased after removal of 5-HT

After 5-HT application, the changes in the Ca PIC took about 10–15 min to reach a peak and, after that, remained stable while in 5-HT ($n = 11$). So, the Ca PIC is only slowly modulated by 5-HT and does not accommodate. Furthermore, after removal of 5-HT from the bath, the Ca PIC was not immediately reduced; remarkably, it instead *increased* for about 45 min (significant increase of 0.51 ± 0.1 nA, $n = 6$ followed). After this unexpected increase, the Ca PIC slowly decreased. At 2 h after washout of 5-HT, the Ca PIC was significantly reduced (portion of Ca PIC initially facilitated by 5-HT was reduced by $51.5 \pm 35.7\%$), but still greater than in control conditions ($n = 5$). We were not able to hold cells longer than this to evaluate full washout and reversal of the effects of 5-HT. It suffices to say that the effect of 5-HT on the Ca PIC must be long-lasting (similar slow action of 5-HT was

seen in acute spinal rats, described below). For comparison, the changes in membrane potential and input resistance induced by 5-HT were reversed within 15–20 min of removal of 5-HT (see Harvey et al. 2006b).

Higher 5-HT doses in chronic spinal rats had no further effect

Higher doses of 5-HT (10–30 μM) had similar effects to the standard low dose (1 μM) described above (six of six motoneurons tested), as shown in Fig. 3. In particular, the Ca PIC onset (V_{on}) and amplitude were significantly changed by high-dose 5-HT (Fig. 3C) compared with control (Fig. 3A), but not significantly more than that in low-dose 5-HT (Fig. 3B). Likewise, the hysteresis and width of the PIC were also not significantly different with high- and low-dose 5-HT, nor was the input conductance different. Thus the low 1 μM dose appears to provide a maximal effect on the Ca PIC in chronic spinal rats, similar to that described for 5-HT-induced changes in the Na PIC in motoneurons (Harvey et al. 2006b).

The Ca PIC is supersensitive to 5-HT in chronic compared with acute spinal rats

In contrast to chronic spinal rats, the low dose of 5-HT (1 μM) had no significant effect on the Ca PIC or other motoneuron properties of acute spinal rats ($n = 5$; Fig. 4B). A much higher dose of 5-HT (10–50 μM ; average dose, 30 μM ; $n = 9$) was needed to induce similar changes in the Ca PIC in acute spinal rats as seen in chronic spinal rats for low doses (Fig. 4C). The cell in Fig. 4 had a particularly large Ca PIC for an acute spinal rat before 5-HT, whereas, for most of the motoneurons from acute spinal rats, there was a smaller Ca PIC and no NSR before 5-HT ($n = 8/9$; Fig. 5A). In these typical cells, high-dose 5-HT (10–50 μM) usually increased the Ca PIC enough to produce the NSR ($n = 7/8$, as in Fig. 5) and always produced a plateau potential (in response to a current ramp) when there was not otherwise one before (resulting from a lack of the NSR; not shown). The onset threshold (V_{on}) was significantly reduced and the Ca PIC amplitude significantly increased by this high dose of 5-HT ($n = 9$; Table 2 and Fig. 4C). The 5-HT-induced facilitation of the Ca PIC was similar in absolute value to that of chronic spinal rats, but, because the Ca PICs in acute spinal rats were initially much smaller, 5-HT had a greater relative effect, nearly doubling the small PICs seen in acute spinal rats and bringing them closer to the PICs seen in chronic spinal rats (before 5-HT; Tables 1 and 2). The high dose of 5-HT (10–50 μM) also significantly reduced the input conductance and depolarized the motoneurons (Table 2), whereas, again, the low dose did not affect these parameters in acute spinal rats (Fig. 5). In summary, the Ca PIC, input conductance, and resting membrane potential are all supersensitive to 5-HT in motoneurons of chronic spinal rats compared with those in normal motoneurons in acute spinal rats (acutely isolated normal spinal cord). Between 10 to 50 (average 30) times more 5-HT is required in acute spinal rats to have the same effect as in chronic spinal rats, indicating an nearly 30-fold supersensitivity in chronic spinal rats.

Interaction of I_h current with Ca PIC measurements in chronic spinal rats

The I_h current is a persistent inward current that is peculiar because it is activated only at hyperpolarized potentials and is deactivated by depolarizations above about -65 mV (Mayer and Westbrook 1983). Previously, we ignored this current in our studies of the Ca PIC because the Ca PIC is normally activated well above -65 mV (Fig. 6A), where the I_h current should be mostly deactivated. However, our finding that 5-HT lowered the Ca PIC onset to

about -64 mV (see V_{on} above and Fig. 6B) forced us to directly evaluate the effect of I_h current on the Ca PIC estimation in chronic spinal rats. When the I_h channel blocker Cs^+ (3 mM) was applied under control conditions (without 5-HT), there was, as expected, no significant change in the PIC ($n = 5$; Fig. 7, A and B). In the presence of Cs^+ , subsequent application of 5-HT significantly reduced the Ca PIC onset voltage (from -59.9 ± 14.0 to -70.5 ± 8.8 mV) and increased the PIC amplitude by an average of 0.41 ± 0.11 nA (Fig. 6, C and D; $n = 5$). However, these 5-HT-induced changes in the Ca PIC were not significantly different from those measured without Cs^+ (described above; $n = 11$). Furthermore, the size of the 5-HT-induced facilitation of the Ca PIC in control conditions (without Cs^+) did not vary significantly with the onset voltage (V_{on}) of the Ca PIC in 5-HT ($r = 0.08$; $n = 11$), even though many cells had V_{on} values (in 5-HT) well below the potential at which the I_h current is deactivated (-66.1 mV; see details below; Fig. 6E). Together, these findings indicate that the presence of an I_h current does not affect the estimation of the Ca PIC, even when the onset of the Ca PIC is made very low by 5-HT, Cs^+ , or the more selective I_h blocker ZD7288, always reduced the conductance near rest (in region marked by line 1 in Fig. 7A) and appreciably hyperpolarized the resting potential ($n = 11/11$; six tested with Cs and five with ZD7288; average resting potential significantly reduced by a 9.6 ± 6.0 mV to near -80 mV), consistent with the block of a large depolarizing current active at rest (I_h current). In many cells (70%), the I_h current was so large that, as it turned off at more depolarized levels, it produced a clear reduction in the conductance (see fit lines 1 and 2 in Figs. 6A and 7A, at rest and > -65 , respectively), at the point which we termed the I_h bend in the current response. This I_h bend complicated the Ca PIC estimation because there was not a unique leak current line (see lines 1 and 2 in Fig. 6A) to extrapolate and subtract from the measured current to estimate the PIC (at vertical arrow in Fig. 6A). The best we could do was fit a line to the linear section of current just subthreshold to the PIC onset (5 mV below, line 2; standard leak line method, as described in METHODS) so that the extrapolated leak current best approximated the leak current present during the PIC. Cs^+ ($n = 6$) or ZD7288 ($n = 5$) always eliminated the downward I_h bend (Figs. 6B and 7B), and thus simplified the Ca PIC estimation by making the subthreshold current response more linear. This I_h bend was not produced by the Ca PIC itself because it persisted with a block of the Ca PIC with cadmium (Fig. 7C; $n = 8$) and was subsequently eliminated by Cs^+ or ZD7288 ($n = 8/8$; three tested with Cs^+ and five with ZD7288 after cadmium; Fig. 7D). In cadmium, large voltage steps from -80 to -60 mV revealed a slowly deactivating Cs^+ and ZD7288 sensitive current (I_h), which was again slowly activated on return to -80 mV (Fig. 7E). Current steps produced an associated slow voltage response (sag; not shown). To sum up, in sacral motoneurons, there is a large inward I_h current that is active at rest and contributes to about 10 mV of the resting potential, although this current slowly turns off at about -66 mV (at the I_h bend) and, although this nonlinearity complicates the leak-current estimation, it does not affect the Ca PIC estimation, even in 5-HT.

Effects on firing of 5-HT-induced increase in Ca PIC

Considering that the Ca PIC plays a major role in sustaining firing in motoneurons (Li et al. 2004a), we examined whether the increases in Ca PIC produced by 5-HT ultimately increased the ability of motoneurons to produce sustained firing. Thus in a separate group of neurons without TTX (in nACSF; $n = 9$ acute and $n = 14$ chronic spinal rats), we applied

slow triangular current ramps to evoke firing (Fig. 8) and quantified the difference I between the current needed to start firing on the upward ramp (I_{start}) and stop firing on the downward ramp (I_{end} , where $I = I_{\text{start}} - I_{\text{end}}$). Positive values of I indicate that the cell exhibits self-sustained firing (Fig. 8B), and this was previously shown to be attributed to the PICs that must be counteracted to stop firing (Li et al. 2004a). On average, in nACSF, there was significant self-sustained firing in chronic spinal rats ($I = 0.57 \pm 0.42$ nA) and no self-sustained firing in acute spinal rats ($I = 0.16 \pm 0.44$ nA). In the majority of cells, 5-HT increased I (Fig. 8A; $n = 6/9$ acute, $n = 9/14$ chronic; 5-HT 1–30 μM in chronic and 10–30 μM in acute spinal rats). However, in the remaining cells, I was unexpectedly not changed ($n = 2/9$ acute, $n = 2/14$ chronic) or even decreased ($n = 1/9$ acute, $n = 3/14$ chronic; Fig. 8B). Previously, we showed that, although the size of the PIC (especially Ca PIC) partly determines the degree of self-sustained firing, the half-activation voltage of the PIC ($V_{1/2}$; voltage at which the PIC is half activated; see METHODS and Li et al. 2004a) is equally, if not more, important. Specifically, self-sustained firing is most pronounced when the half-activation voltage ($V_{1/2}$) for the PIC is lower than (or near) the spike threshold (V_{th} ; as in control on *left* of Fig. 8B) because the PIC is able to be more fully activated [the membrane potential is kept below V_{th} by the afterhyperpolarizations (AHPs); Li et al. 2004a]. Thus the activation voltage of the PIC relative to spike threshold ($V_{1/2} - V_{\text{th}}$) is a critical factor in determining the degree of self-sustained firing. 5-HT hyperpolarizes both the spike threshold V_{th} (Harvey et al. 2006b) and the Ca PIC activation ($V_{1/2}$ in Fig. 8A and V_{on} described above), but often does not affect V_{th} and $V_{1/2}$ equally in the same motoneuron. In spite of this complexity, we found that 5-HT-induced changes in the critical $V_{1/2} - V_{\text{th}}$ parameter were significantly correlated with changes in self-sustained firing (I ; $r = 0.69$, $n = 16$, $P = 0.003$), as shown in Fig. 4C (only cells where both firing and PIC were measured are included). In cells where the half-activation voltage of the PIC shifted positively relative to the spike threshold ($V_{1/2} - V_{\text{th}}$ increased), the self-sustained firing decreased (*bottom right quadrant* in Fig. 8C; also see example in Fig. 4B), with an average decrease in I of -0.15 ± 0.13 nA ($n = 4$). This decreased self-sustained firing occurred despite an increase in the PIC by 0.96 ± 0.80 mV in these four cells, so the changes in $V_{1/2} - V_{\text{th}}$ dominated the response and led to the unexpected decrease in self-sustained firing. In contrast, in cells where $V_{1/2} - V_{\text{th}}$ was unchanged ($n = 4$) or decreased ($n = 8$) with 5-HT, the self-sustained firing significantly increased (*top left quadrant* of Fig. 8C; see example in Fig. 8A), with an average increase in I of 0.44 ± 0.28 nA ($n = 12$). For these cells, the PIC also increased with 5-HT by 1.05 ± 0.63 nA. Thus the increased PIC amplitude and hyperpolarization of $V_{1/2}$ relative to V_{th} together contributed to the increased self-sustained firing (greater I).

DISCUSSION

Our results demonstrate that the low-voltage-activated persistent calcium current (Ca PIC) in adult rat motoneurons is facilitated by 5-HT, with a marked reduction in threshold and increase in amplitude. This produces a larger and wider NSR in the I - V relation, which ultimately enhances plateau potentials (or enables them), making them larger in amplitude and lower in threshold. These results are consistent with previous reports of increases in the Ca PIC and Ca plateau with 5-HT in turtle motoneurons (Perrier and Hounsgaard 2003), although the marked reduction in the onset threshold were not previously described. The 5-

HT-induced facilitation of the Ca PIC is mediated by L-type calcium channels (nimodipine sensitive). This Ca PIC occurs in the presence of the SK channel blocker apamin (see RESULTS), and thus does not result from a 5-HT-induced change in the calcium-activated SK currents, even though there is recent evidence that the SK current is activated by the L-type calcium current underlying the Ca PIC (Li and Bennett, unpublished data). Likewise, the 5-HT-induced facilitation of the Ca PIC (or the spontaneous Ca PIC) is not affected by the I_h current blocker Cs^+ . We found that a large hyperpolarization-activated I_h current (ZD7288 or Cs^+ sensitive) does occur in rat sacral motoneurons and contributes to about a 10-mV depolarization of the resting potential. This I_h current can produce a substantial change in conductance subthreshold to the Ca PIC, which can interfere with the leak current estimation and subtraction used to measure the Ca PIC. However, in spite of this complication, on average, the 5-HT-induced facilitations of the Ca PICs estimated with and without the I_h current blocked are similar (Fig. 6).

We also found that the Ca PIC is much more sensitive to 5-HT applied to spinal cords of chronic spinal rats, compared with spinal cords taken from normal rats; likely, this has important implications for the understanding of spasticity, as examined at the end of this DISCUSSION.

Lower threshold of the Ca PIC helps to enable activation during firing

Functionally, a reduction in the Ca PIC threshold by 5-HT is as important as the increase in amplitude because only the portion of the Ca PIC that is activated below the firing threshold voltage (V_{th} ; about -50 mV) can be activated during firing. This is because, between spikes, the AHP always keeps the potential below the firing threshold and the Ca PIC is much too slow to respond to the millisecond depolarization during the spike (Li et al. 2004a). The Ca PIC that is activated during firing aids in sustaining firing and enables self-sustained firing [firing that outlasts a stimulation; the Na PIC plays a lesser role in self-sustained firing (Li et al. 2004a)]. When the Ca PIC activation is well below the firing threshold, self-sustained firing is pronounced and easily triggered (Li et al. 2004a). On the other extreme, when the Ca PIC activation is well above the firing threshold, it cannot be activated and does not produce self-sustained firing (Li et al. 2004a). Thus by lowering the activation voltage of the Ca PIC, 5-HT enables the Ca PIC to be more fully activated during firing, which usually increases sustained firing, as observed in Fig. 8. However, there are a few cells (25%) that unexpectedly become less excitable in 5-HT, with less sustained firing (Fig. 8 and Perrier and Hounsgaard 2003). This partly arises because 5-HT also lowers the firing threshold, V_{th} (Fedirchuk and Dai 2004; Harvey et al. 2006b). So when this reduction in firing threshold exceeds the reduction in the activation voltage of the Ca PIC (V_{on}) the Ca PIC is less able to activate during firing ($V_{th} > V_{1/2}$; Harvey et al. 2006b,c), even though it is bigger. Similar variable effects of 5-HT on self-sustained firing have also been seen in turtle motoneurons and seem to be associated with the balance of 5-HT receptor activation on the soma and dendrites: 5-HT receptor activation in distal dendrites is excitatory (by 5-HT₂ receptors), whereas somatic 5-HT application is inhibitory to sustained firing in motoneurons (Perrier and Hounsgaard 2003). Perhaps the latter is partly caused by the lowering of the firing threshold by 5-HT (Harvey et al. 2006b,c), as described above, considering that the spikes are generated in the soma.

The facilitation of the Ca PIC by 5-HT is long-lasting

Our results also demonstrate that not only does 5-HT facilitate the Ca PICs, but these effects are very long-lasting, continuing for as long as 5-HT is present and not reversing until about 2 h after removal of 5-HT. Unexpectedly, during the first 45 min after removal of 5-HT, the Ca PIC tends to increase further, rather than decrease, although this is consistent with the recent findings that 5-HT–receptor-induced facilitation of spinal reflexes is further augmented after removal of 5-HT agonists (Machacek et al. 2001). Thus reversal of the effect of exogenously applied 5-HT receptor agonists on the Ca PIC (see RESULTS) and spinal reflexes (Machacek et al. 2001) takes hours. Functionally, this leads to the surprising conclusion that normal 5-HT innervation of motoneurons, such as through transient brain stem activation, should increase motoneuron excitability for many hours. However, activation of the brain stem pathways that include 5-HT axons (raphae), instead lead to changes in motoneurons properties (including PIC-related hysteresis) that are *not* long-lasting (Hultborn et al. 2004). Possibly, this brain stem activation causes release of multiple transmitters, in addition to 5-HT, thus helping to turn off the PIC.

The long-lasting Ca PIC facilitation after removal of 5-HT could be attributed to: 1) constitutive activity of 5-HT receptors or 2) long-lasting downstream G-protein–coupled actions of 5-HT receptors. In favor of constitutive receptor action, we recently found that 5-HT–receptor antagonists that have inverse agonist properties can relatively rapidly reverse the 5-HT–induced facilitation of the Ca PIC (unpublished data).

Origins of spontaneously occurring Ca PICs

Because of the long-lasting effects of 5-HT, the spontaneously occurring Ca PICs that we measure in our in vitro spinal cord preparation could, in principle, be a result of 5-HT activation of motoneurons from the brain stem that occurs *at* or *before* the removal of the spinal cord from the animal. However, this is unlikely to be the case because we do not observe a rundown of Ca PICs many hours after removal of the spinal cord from normal rats, when the 5-HT effects should be slowly reversed. Furthermore, in chronic spinal rats, the brain stem innervation is completely gone months before the experiment and thus cannot be involved.

In chronic spinal rats, transmitters intrinsic to the spinal cord, including the residual amounts of 5-HT and NE in the chronic spinal animal (Newton and Hamill 1988), could in principle be responsible for enabling the large Ca PICs seen in these animals. These transmitters may be, in small part, released in a spike-mediated manner because there is a slightly larger Ca PIC (sensitive to Ca channel blockers) before compared with that after a block of spike-mediated transmission with TTX (Li et al. 2004a). However, the bulk of the Ca PIC persists in TTX and large Ca PICs can be observed even 3 to 5 h after application of TTX (see RESULTS), suggesting that whatever transmitter facilitates the Ca PIC (e.g., 5-HT) leaks from its terminal in a nonspike-mediated manner, if at all.

Ca PICs are supersensitive to 5-HT in chronic spinal rats

After chronic spinal cord injury, most of the 5-HT axons that innervate the spinal cord degenerate because they arise from the brain stem (Maxwell et al. 1996). Nevertheless,

roughly 2–15% of the normal levels of 5-HT remain below an injury site in a chronic spinal animal, in part resulting from intrinsic spinal 5-HT neurons, which can be fairly plastic after injury (Branchereau et al. 2002; Newton and Hamill 1988). The reduced 5-HT after injury is associated with a classic denervation supersensitivity because spinal reflexes are facilitated by unusually low doses of 5-HT (Bedard et al. 1979, 1987). Further, many motoneuron properties, including the input resistance, resting potential, Na PIC, and Ca PIC become supersensitive to 5-HT (RESULTS and Harvey et al. 2006b). Because of this supersensitivity, the small amounts of residual 5-HT may have large effects on the motoneurons and reflexes, ultimately leading to the hyperexcitable state of chronic spinal animals. Consistent with this idea, it was recently shown that pharmacologically blocking 5-HT₂ receptor activity reduces the spontaneously occurring Na PIC in chronic spinal rats (Harvey et al. 2006b), demonstrating that the 5-HT₂ receptors must be somehow tonically activated. This could be by residual 5-HT (as discussed above). Alternatively, the 5-HT₂ receptors may be constitutively active (active without 5-HT), as reported in other systems (Egan et al. 1998). Regardless of the mechanism, it is clear that there is some endogenous 5-HT₂ receptor activity in chronic spinal rats (Harvey et al. 2006c). This conclusion, taken with the findings that 5-HT₂ receptors facilitate the Ca PIC (Perrier and Hounsgaard 2003), suggests that endogenous 5-HT₂ receptor activation may play a role in producing the large Ca PICs in chronic spinal rats.

The mechanism underlying the supersensitivity of chronic spinal rats to 5-HT is unknown. It might include: changes in 5-HT receptors themselves, increased receptor numbers, changes in G-protein–coupled actions of the receptors, or even changes in the calcium channels underlying the Ca PIC. These possibilities are reviewed in Harvey et al. (2006b).

Summary and role of 5-HT in spasticity

The supersensitivity of the Ca PIC to 5-HT has important implications for the understanding of spasticity. It was previously shown that Ca PICs produce the many second-long spasms that are the hallmark of spasticity after spinal cord injury (Gorassini et al. 2004; Li et al. 2004a). Thus the small amounts of endogenous 5-HT below the injury in a chronic spinal rat may act on supersensitive receptors to produce large Ca PICs and ultimately enable muscle spasms. Furthermore, because the spinal sources of 5-HT are closely associated with the autonomic system (Newton and Hamill 1988), the Ca PICs and spasms might be modulated with autonomic input and help explain, for example, why bladder irritation can lead to increased spasms in spinal-cord–injured patients. Ultimately, understanding and treating spasticity will involve determining what 5-HT receptors are involved in facilitating the Ca PIC and what makes these receptors supersensitive.

Acknowledgments

Thanks to L. Sanelli for expert technical assistance.

GRANTS

This work was supported by National Institute of Neurological Disorders and Stroke Grant RO1 NS-47567-01, the Natural Sciences and Engineering Research Council, Canada Foundation for Innovation, the Canadian Institutes of Health Research, and the Alberta Heritage Foundation for Medical Research.

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FIG. 1.

Low-dose serotonin [5-hydroxytryptamine (5-HT)] facilitates an L-type calcium current (Ca PIC) in motoneuron from chronic spinal rat. *A*: current response to a triangular voltage ramp in voltage-clamp mode before 5-HT, with a Ca PIC activated at -62 mV [onset voltage (V_{on})] and the Ca PIC seen as the downward deviation below the leak line (thin line; initial peak Ca PIC downward vertical arrow). Persistent inward current (PIC) deactivation occurred at the offset voltage (V_{off}). *B*: low-dose 5-HT ($1 \mu\text{M}$) lowered the Ca PIC threshold, increased the Ca PIC amplitude (downward arrow) and width (V_w), depolarized the resting potential (at 0 nA, tick), and lowered the input conductance (slope of leak current). Negative-slope region (NSR) produced by the Ca PIC was also increased by 5-HT. However, V_{off} was not lowered as much as V_{on} . *A* and *B* recorded in the presence of $2 \mu\text{M}$ tetrodotoxin (TTX) and $0.15 \mu\text{M}$ apamin.

FIG. 2.

Low-dose 5-HT increases the Ca plateau in motoneuron from chronic spinal rat. *A*: Ca plateau potential induced by increasing current ramp, with onset at the steep rise in potential on the upward ramp. Same cell as in Fig. 1*A*, where Ca PIC measured. *B*: low-dose 5-HT increased the Ca plateau amplitude in the same cell, and lowered its onset voltage. Ca plateau is initiated by the Ca PIC, with the same onset voltage (Ca PIC onset V_{on} before and after 5-HT are indicated in *A* and *B* with an arrowhead). *A* and *B* recorded in the presence of 2 μ M TTX and 0.15 μ M apamin.

FIG. 3.

High- and low-dose 5-HT have similar effects on the Ca PIC in chronic spinal rat. *A*: Ca PIC activated by a voltage ramp in a motoneuron from a chronic spinal rat. *B*: low-dose 5-HT (1 μ M) facilitated the Ca PIC as in Fig. 1. *C*: a subsequent higher dose of 5-HT in the same cell had no further effects. *A–C* recorded in the presence of 2 μ M TTX and 0.15 μ M apamin.

FIG. 4.

In acute motoneurons, high-dose, but not low-dose 5-HT facilitates the Ca PIC. *A*: Ca PIC evoked by a voltage clamp mode in an acute spinal rat. *B*: low-dose 5-HT (1 μ M) had no effect on this cell. *C*: high-dose 5-HT (30 μ M) lowered the Ca PIC threshold, increased the Ca PIC amplitude, depolarized the resting potential, and lowered the conductance. *A–C* recorded in the presence of 2 μ M TTX and 0.15 μ M apamin.

FIG. 5. High-dose 5-HT facilitates a Ca PIC in acute spinal rats, even when the Ca PIC is initially very small. *A*: like in many motoneurons of acute spinal rats, the Ca PIC evoked by a voltage ramp was very small and did not produce an NSR. *B*: high-dose 5-HT induced a bigger Ca PIC and an NSR. *A* and *B* recorded in the presence of 2 μ M TTX and 0.15 μ M apamin.

FIG. 6.

I_h current does not affect the estimation of the 5-HT-induced facilitation of the Ca PIC, despite its effects on the subthreshold current at rest. **A:** I_h current was activated at rest during a voltage ramp, and then turned off when the potential was depolarized (at I_h bend), and reduced the leak current estimate (from thin line 1 to 2) used to measure the Ca PIC. **B:** 5-HT (1 μ M) lowered the Ca PIC onset so much that it overlapped with and obscured the I_h bend. **C:** a block of the I_h current with Cs^+ (3 mM) made the subthreshold current response more linear. **D:** subsequent application of 5-HT (1 μ M) in the same cell as in **C** increased the Ca PIC and lowered its threshold, just as occurred without Cs^+ (**B**). **E:** plot of increase of Ca

PIC with 5-HT application, as a function of the Ca PIC onset voltage in 5-HT. 5-HT induced facilitation of the Ca PIC amplitude was not correlated with the Ca PIC onset voltage in 5-HT, despite some cells having a lower threshold than that of the I_h bend (dashed line). Each cell is indicated by a point in the plot. *A–E* recorded in 2 μ M TTX.

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FIG. 7.

Block of the I_h current with Cs^+ does not affect the Ca PIC, but it hyperpolarizes the resting potential. *A*: Ca PIC evoked by a voltage ramp in a motoneuron of a chronic spinal rat. *B*: application of Cs^+ (3 mM) in the same cell as in *A* does not reduce the Ca PIC, although it does hyperpolarize the cell and eliminate the I_h bend. *C*: I_h bend is not eliminated by a block of the Ca PIC with cadmium (different cell than in *A* and *B*). *D*: I_h bend is eliminated by Cs^+ (or ZD7288, not shown), indicating that it does result from an I_h current. Resting potential is again hyperpolarized by Cs^+ . *E*: in cadmium, a voltage step evokes a slowly rising current, as the I_h current is deactivated, and then a slowing dropping current as the inward I_h current

is activated after the voltage step. This slow current was reduced by Cs^+ (overlay). *A–E* recorded in 2 μM TTX.

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FIG. 8.

Self-sustained firing is influenced by 5-HT-induced change in PIC activation voltage relative to firing threshold. Cells measured in normal artificial cerebrospinal fluid (ACSF), without Ca PIC blocked. *A*: motoneuron of a chronic spinal rat. *Top left*: slow current ramps, with instantaneous firing frequency shown above spikes. Recruitment and derecruitment thresholds indicated by dashed lines. Note that firing stopped at same current on down-ramp as the current at recruitment on up-ramp ($I = 0$, no self-sustained firing). *Bottom left*: standard slow voltage ramp, as in Fig. 7A. Large PIC is indicated by downward arrow from leak current (thin line). Note PIC half-activation voltage ($V_{1/2}$; solid vertical

line) was higher than spike voltage threshold (V_{th} ; dashed line). *Top right*: in 5-HT, current threshold for recruitment was greater than threshold at derecruitment ($I > 0$ nA, self-sustained firing). *Bottom right*: 5-HT increased PIC amplitude and shifted $V_{1/2}$ more hyperpolarized relative to V_{th} . *B*: a different chronic spinal rat motoneuron, in same format as *A*. *Top*: self-sustained firing was paradoxically *reduced* in 5-HT (smaller I). *Bottom*: 5-HT increased the total PIC amplitude in this cell, however 5-HT shifted $V_{1/2}$ more depolarized relative to V_{th} . *C*: each symbol represents an individual motoneuron. Change in self-sustained firing (I) with 5-HT is plotted against change in position of $V_{1/2}$ relative to V_{th} . In all cells shown, 5-HT increased total PIC amplitude. Self-sustained firing increased with 5-HT when $V_{1/2}$ was unchanged, or became more negative, relative to V_{th} [i.e., $(V_{1/2} - V_{th}) \leq 0$ mV, *top left quadrant*]. Self-sustained firing decreased when $(V_{1/2} - V_{th}) > 0$ mV (*bottom right quadrant*).

TABLE 1

Effects of 5-HT on motoneurons of chronic spinal rats

PIC Properties	Resting Potential, mV	Conductance Near Rest	V_{out} mV	Amplitude of Initial PIC, nA	Amplitude of Sustained PIC, nA	Width of Initial PIC, mV	Depth of NSR
nACSF	-76.7 ± 7.0	0.24 ± 0.09	-56.7 ± 6.0	2.4 ± 1.0	1.69 ± 1.1	10.2 ± 6.2	1.51 ± 0.8
5-HT	$-67.7 \pm 7.3^*$	$0.13 \pm 0.03^*$	$-63.7 \pm 7.1^*$	$3.0 \pm 0.73^*$	$2.2 \pm 0.75^*$	$17.7 \pm 4.9^*$	$1.9 \pm 0.63^*$

Values are means $\pm$ SD. 5-HT dose was 1 μ M (low dose).
A * indicates significant change, $P < 0.05$.

TABLE 2

Effects of 5-HT on motoneurons of acute spinal rats

PIC Properties	Resting Potential, mV	Conductance Near Rest	V_{out} mV	Amplitude of Initial PIC nA	Amplitude of Sustained PIC, nA	Width of Initial PIC, mV	Depth of NSR
nACSF	-77.9 ± 7.8	0.28 ± 0.09	-58.5 ± 14.8	0.41 ± 0.30	0.34 ± 0.29	N/A	N/A
5-HT	$-72.3 \pm 8.1^*$	$0.22 \pm 0.07^*$	-62.5 ± 3.6	$0.99 \pm 0.48^*$	$0.73 \pm 0.34^*$	$7.4 \pm 5.0^*$	$0.30 \pm 0.23^*$

Values are means $\pm$ SD. A 5-HT dose of 10–50 μ M is shown; 1 μ M had no effect on these motoneurons.
A * indicates significant change with 5-HT, $P < 0.05$.