The Journal of Neuroscience, June 1990, 10(6): 1922–1928

Serotonin Enhances a Low-Voltage-Activated Calcium Current in Rat Spinal Motoneurons

Albert J. Berger and Tomoyuki Takahashi

Department of Physiology, Kyoto University Faculty of Medicine, Kyoto, Japan

Calcium currents and the effects of 5-HT on these currents were investigated in visually identified motoneurons using whole-cell recording in the neonatal rat spinal cord slice preparation. In current-clamp recording, step depolarizations from a holding potential of about −90 mV produced a low-threshold transient depolarizing response and a high-threshold long-lasting spike. In voltage-clamp recording, low (LVA) and high (HVA) voltage-activated Ca^{2+} currents were recorded in response to depolarizing voltage steps. Low concentration of Cd^{2+} (50 μM) did not reduce the amplitude of the LVA current but markedly diminished the HVA current.

Bath application of 5-HT (10–50 μM) markedly increased the amplitude of the LVA current without causing a shift in the current (*I*)–voltage (*V*) relation. In contrast, 5-HT did not appreciably affect the amplitude of the HVA current. We conclude that 5-HT specifically enhances the LVA Ca^{2+} current and that this effect together with the previously reported 5-HT-induced inward current (Takahashi and Berger, 1990), would facilitate the excitation of motoneurons.

Peripheral and central neurons contain both low (LVA) and high (HVA) voltage-activated Ca^{2+} currents (see reviews by Miller, 1987; Tsien et al., 1988). Previous studies, using intracellular microelectrodes, have shown that Ca^{2+} currents are present in mammalian spinal motoneurons (Harada and Takahashi, 1983; Walton and Fulton, 1986). Recently, Ca^{2+} currents have been observed in dissociated embryonic chick spinal motoneurons (McCobb et al., 1989). Modulation of Ca^{2+} currents by 5-HT has been demonstrated in invertebrates (Pellmar and Carpenter, 1980; Paupardin-Tritsch et al., 1986), as well as in mammalian neurons (Nedergaard et al., 1988). It has been suggested also that 5-HT-induced plateau potentials in turtle spinal motoneurons may arise from modulation of Ca^{2+} currents (Hounsgaard and Kiehn, 1989).

In our recent study, using tight-seal whole-cell recording from neonatal rat spinal motoneurons in slice, we demonstrated that 5-HT exerts a direct excitatory effect on spinal motoneurons (Takahashi and Berger, 1990). During the course of those experiments, we also observed an inward current that was evoked by depolarization in the presence of TTX and the current was enhanced by 5-HT. Since that inward current was blocked by 1 mM Cd^{2+} or by replacing Ca^{2+} with Mg^{2+}, the current appeared to be carried by Ca^{2+}

The present study is aimed at extending these findings. We demonstrate here that spinal motoneurons possess both the LVA and HVA Ca^{2+} currents and that 5-HT increases the magnitude of the LVA current.

Materials and Methods

The thin-slice preparation of the neonatal rat spinal cord and whole-cell recording methods from motoneurons have been previously described (Takahashi, 1990a, b). The present experiments were conducted on 3- and 4-d-old Wistar rats. Thin slices were prepared from the mid-cervical spinal region. The identification of motoneurons was made visually by location and cell size, as previously described (Takahashi, 1990a).

Slices were incubated at 37°C for about an hour and were initially perfused with a normal Krebs solution that contained the following (concentrations expressed in mM): NaCl, 113; KCl, 3; $NaHCO_3$, 25; NaH_2PO_4, 1; $CaCl_2$, 2; $MgCl_2$, 1; D-glucose, 11. In order to isolate Ca^{2+} currents and to minimize both Na^+ and K^+ currents, the perfusion fluid was switched to one containing the following (concentrations expressed in mM): tetraethylammonium chloride (TEA), 116; 4-aminopyridine (4-AP), 4; KCl, 3; NaH_2PO_4, 1; $NaHCO_3$, 10; $CaCl_2$, 10; $MgCl_2$, 1; D-glucose, 11; and TTX, 0.4 μM. In some experiments, 2 μM strychnine was added to this perfusing fluid to minimize spontaneous miniature inhibitory postsynaptic currents (Takahashi, 1984). When Cd^{2+} and Ni^{2+} were added to the perfusing fluid, NaH_2PO_4 was omitted from the solution to prevent precipitation. All perfusing fluids were gassed with 95% O_2 and 5% CO_2. The patch pipette was filled with the following solution (composition expressed in mM): CsCl, 140; NaCl, 11; HEPES (as free acid), 10; $MgCl_2$, 1; ATP, 2; BAPTA, 5; and the pH was adjusted to 7.3 using CsOH. The DC resistance of the pipette was about 5 MΩ.

Following establishment of whole-cell recording conditions, series resistance of the pipette was usually 15–50 MΩ and was compensated by 40–50%. Ca^{2+} currents were activated by applying 200 msec depolarizing command voltages (V_c) by steps from a holding voltage (V_h) of approximately −90 to −100 mV. (In a few cells, HVA currents were generated from a V_h of about −75 mV.) In the current traces shown in the figures, leak currents were subtracted with hyperpolarizing steps of one-quarter the V_c amplitude, and liquid junction potential between the perfusion fluid and the pipette solution (3 mV) was corrected. All experiments were carried out at room temperature (23–25°C).

The data were recorded on a PCM video tape recorder (10 kHZ) and analyzed using a digital computer by sampling current and voltage records at 2–5 kHz. All records were drawn by a digital plotter.

Results

Voltage-clamp recordings in normal Krebs solution

We first made whole-cell voltage-clamp recordings from motoneurons in normal Krebs solution containing TTX. Ramp depolarization from a V_h of about −90 mV produced a transient inward current (Fig. 1, top trace, asterisk) much slower in time course than the Na^+ current observed in the absence of TTX (Takahashi, 1990a). The current was activated at the potential

Received Oct. 30, 1989; revised Jan. 12, 1990; accepted Jan. 16, 1990.

We are grateful to Dr. Motoy Kuno for his critical reading of the manuscript. This study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (No. 62480106) to T.T. and by a Fogarty Senior International Fellowship from the USPHS to A.J.B.

Correspondence should be addressed to Dr. Albert J. Berger, Department of Physiology and Biophysics, SJ-40, University of Washington School of Medicine, Seattle, WA 98195.

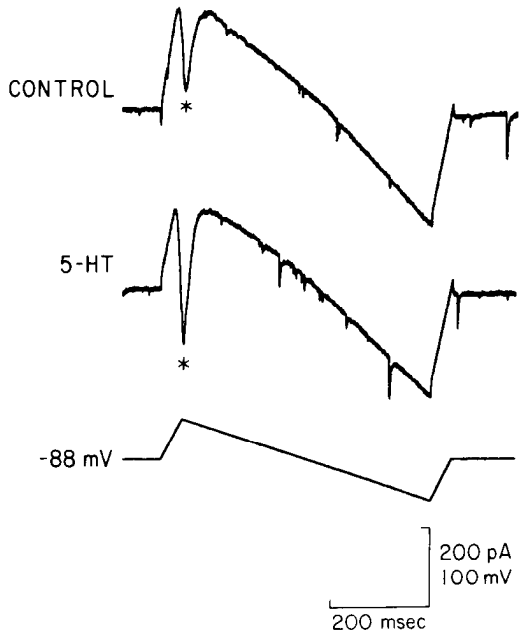

Figure 1. Effects of 10 μM 5-HT application on the transient inward current observed in a motoneuron recorded in voltage-clamp mode. Voltage command ramps of ±50 mV from a V_h of −88 mV (*bottom trace*) were applied before (*Control*) and during 5-HT application (*5-HT*). *Asterisks* indicate the transient inward current that was enhanced by 5-HT. Slice bathed in normal Krebs solution containing TTX. Patch pipette contained following solution (in mM): KCl, 18; KGluconate, 123; NaCl, 9; HEPES, 10; $MgCl_2$, 1; EGTA, 0.2.

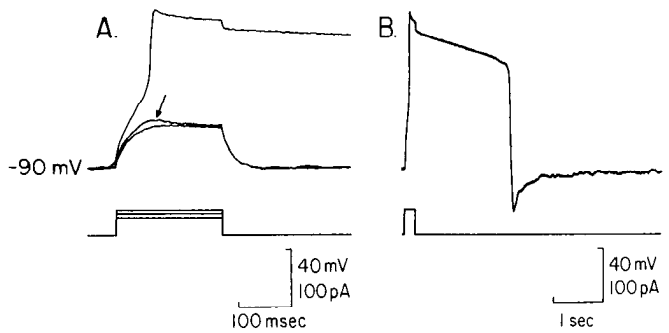

Figure 2. Current-clamp recording from a spinal motoneuron showing local response (*arrow*) and all-or-none spike. *Bottom traces in A,* Three current steps of 200 msec duration. *B,* Slow time-base record of the spike. Initial membrane potential is −90 mV.

of about −55 mV and was abolished either by replacing external Ca^{2+} with Mg^{2+} or by addition of Cd^{2+} (1 mM) to the perfusate (not shown). Thus, the current appears to be carried by Ca^{2+} through LVA Ca^{2+} channels. When 5-HT was applied to the solution, the transient inward current was markedly enhanced in amplitude (Fig. 1, middle trace). These results seem to imply that 5-HT may enhance the LVA Ca^{2+} current. In the following section, we have studied this possibility under optimal conditions to evoke Ca^{2+} currents (see Materials and Methods).

Current-clamp recordings

In current-clamp recording from a motoneuron, a small depolarizing current step produced a subthreshold local response (Fig. 2*A*, arrow). By increasing the current amplitude, a large all-or-none action potential was evoked (Fig. 2*A*, top trace). The spike lasted for several seconds after cessation of the depolarizing current pulse (Fig. 2*B*). Such a long-lasting spike was observed only with the K^+ channel blockers (see Materials and Methods), suggesting that in normal conditions, K^+ conductances have a repolarizing function. Because most Na^+ in the external solution had been replaced with TEA and the solution contained TTX in concentration to almost abolish the Na^+ current (Takahashi, 1990a), the long-lasting spike is most likely to be produced by Ca^{2+} currents.

Voltage-clamp recordings

In voltage-clamp recording from a motoneuron, depolarizing command voltage steps of increasing amplitude from V_h of −94 mV revealed an inward current with apparent transient and steady components. The current was detectable already at about −67 mV (Fig. 3*A*) with the range of −40 to −67 mV (10 motoneurons). The amplitude of the current in the motoneuron progressively increased as the depolarization V_c increased until a much larger and slower inward current was abruptly generated at about −20 mV (Fig. 3*A*). Figure 3*B* shows the current (*I*)–voltage (*V*) relation plotted at the peak and at the end of depolarizing pulses. Both the LVA and HVA currents were abolished after reducing external Ca^{2+} concentration to 0.1 mM (Fig. 3*C*), indicating that the currents are carried by Ca^{2+}. The characteristics of the currents thus conform to those designated as LVA and HVA Ca^{2+} currents in other systems (Carbone and Lux, 1984, 1987; Fox et al., 1987; Akaike et al., 1989; Blaxter et al., 1989; Crunelli et al., 1989; Huang, 1989).

Separation of the Ca^{2+} currents with divalent cations

We have tested whether Cd^{2+} and Ni^{2+} can pharmacologically separate the LVA and HVA currents. As shown in Figure 4*Aa*, 50 μM Cd^{2+} consistently reduced the HVA currents but not the LVA currents (6 motoneurons). The HVA current was partially recovered after washing out Cd^{2+}. This result further supports that the LVA and the HVA Ca^{2+} currents in motoneurons are mediated by a distinct class of Ca^{2+} channels. At a higher concentration of Cd^{2+} (600 μM), both the LVA and HVA currents were largely suppressed (Fig. 4*Ab*). Similarly, 500 μM Ni^{2+} substantially blocked both the LVA and HVA currents (Fig. 4*B*).

Effect of 5-HT on Ca^{2+} currents

Voltage-clamp recordings. LVA and HVA Ca^{2+} currents were evoked in a motoneuron with the paradigm similar to those illustrated in Figure 3 (Fig. 5). Bath application of a single dose of 10 μM 5-HT consistently enhanced the amplitude of the LVA Ca^{2+} current. The peak LVA current was increased by 66 ± 41% (mean ± SD; range, 24–141%, $n = 7$ motoneurons). Similar enhancement was observed for the amplitude of the inward current remaining at the end of the command pulse. In contrast, 5-HT did not potentiate the HVA Ca^{2+} current in all 7 motoneurons tested, whereas the amplitude of HVA current gradually declined, presumably owing to the run-down of the current (Tsien et al., 1988).

Because of the apparent run-down of the Ca^{2+} currents, it seemed necessary to follow the time course of the 5-HT effect on Ca^{2+} currents. Figure 6 shows the peak amplitude and the amplitude of the inward current remaining at the end of the command pulse for the LVA (Fig. 6*A*) and HVA (Fig. 6*B*) currents before, during, and after application of 5-HT. Enhance-

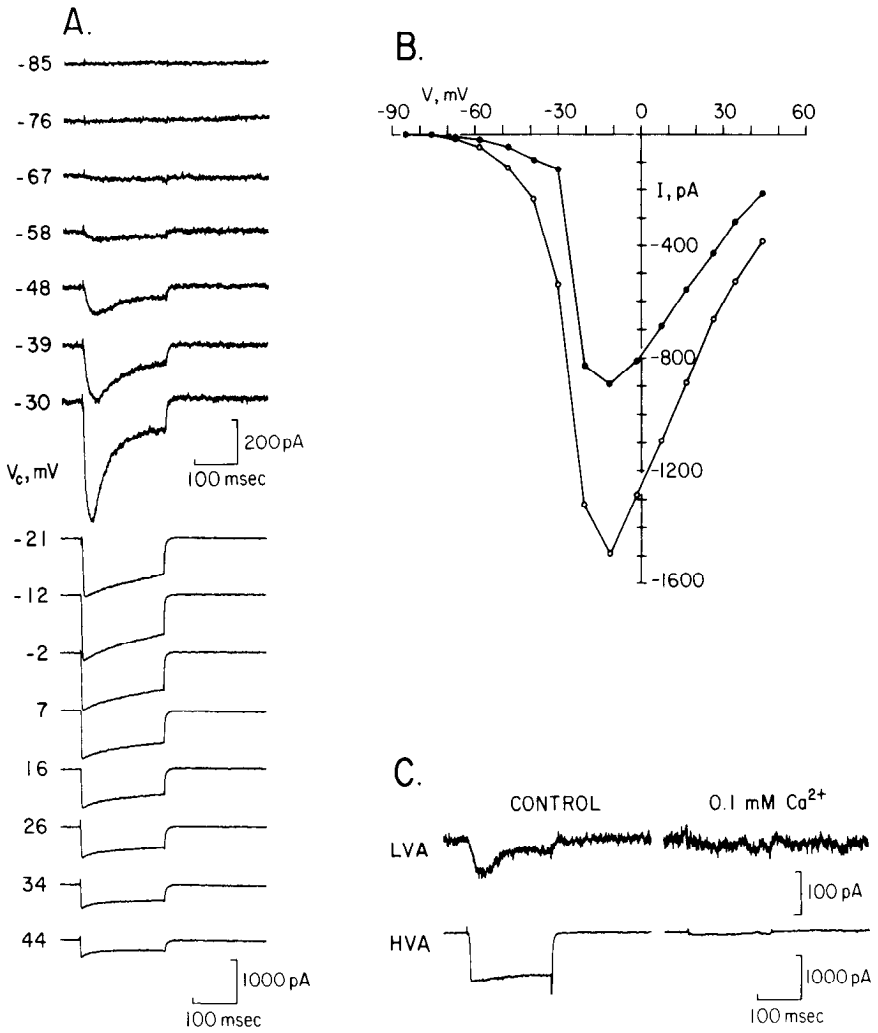

Figure 3. LVA and HVA Ca^{2+} currents observed in a voltage-clamped motoneuron. *A*, Recordings show the response to 200 msec depolarizing V_c steps from a V_h of −94 mV. Note that V_c steps to −30 mV are at a higher gain than the remainder of the V_c steps. *B*, The resultant *I–V* relationships for the peak inward current (*open circles*) and the residual current (inward current remaining at the end of the 200 msec test pulse—*filled circles*). Data in *A* and *B* from the same motoneuron. *C*, Effect of reducing Ca^{2+} concentration to 0.1 mM and raising Mg^{2+} concentration to 10 mM. Data shown for reduced Ca^{2+} taken at 6 min after switching to low Ca^{2+} mixture. For LVA generation: V_h = −95 mV, V_c = −42 mV; for HVA generation: V_c = −16 mV.

ment of the LVA current was detected about 20 sec after 5-HT application and reached a maximum in 2 min (Fig. 6*A*). After washing out the drug, the current amplitude returned to about the same level as control within 2 min. In contrast, peak HVA current declined time dependently (Fig. 6*B*), but showed no appreciable change in the declining slope during 5-HT application. The current amplitudes at the end of the 200 msec command pulses were marginally enhanced by 5-HT for LVA but not for HVA currents (Fig. 6).

Present results are indicative of differential enhancement of the LVA current by 5-HT. However, it cannot be excluded that the apparent enhancement of the LVA current may arise from a negative shift of the *I–V* relation by the drug. This possibility was tested by studying the 5-HT effect on the *I–V* relationship. As shown in Figure 7, 5-HT enhanced the amplitude of Ca^{2+} current only in the low-voltage range (see also Fig. 7, left, for the expanded *I–V* in the low-voltage range); the current in the high-voltage range apparently was suppressed after 5-HT application. In fact, the 2 *I–V* curves, before and during 5-HT application, crossed at around −10 mV. This presumably is due to run-down of the HVA current (see Fig. 6*B*). However, before, during, and after 5-HT application, the whole *I–V* relation showed no appreciable shift. Thus, it may be concluded that 5-HT specifically potentiates the LVA Ca^{2+} current.

Dose–response of the 5-HT-enhanced LVA current

We studied the dose dependency of the 5-HT effect on LVA Ca^{2+} current (Fig. 8). Since prolonged (up to 5 min) or repeated (twice) application of 5-HT did not appreciably reduce the sensitivity of LVA current to 5-HT (not shown), we applied increasing doses of 5-HT consecutively without intervening washout. At the submicromolar 5-HT concentrations, LVA current was not affected. A clear effect was observed only above 10 μM, being more pronounced at 50 μM. The average increase in amplitude of the LVA current at a dose of 10 μM 5-HT was less than that observed in a different population of cells to a single application of 10 μM 5-HT (66% increase in the latter vs. 16% in the former case). This difference may indicate time-dependent run-down of intracellular messengers or receptor desensitization due to the consecutive application of lower doses of 5-HT. Nevertheless, the results presented in Figure 8 indicate that micromolar concentrations of 5-HT are required to activate the 5-HT receptor mediating the potentiating effect on the LVA current. This conclusion is supported by the observations shown in Figure 8 that first applications of low doses (100 or 400 nM 5-HT) did not significantly change the amplitude of the LVA current from control values (Student's *t*-test, $p > 0.5$).

Current-clamp recordings. As shown in Figure 2*A*, injection

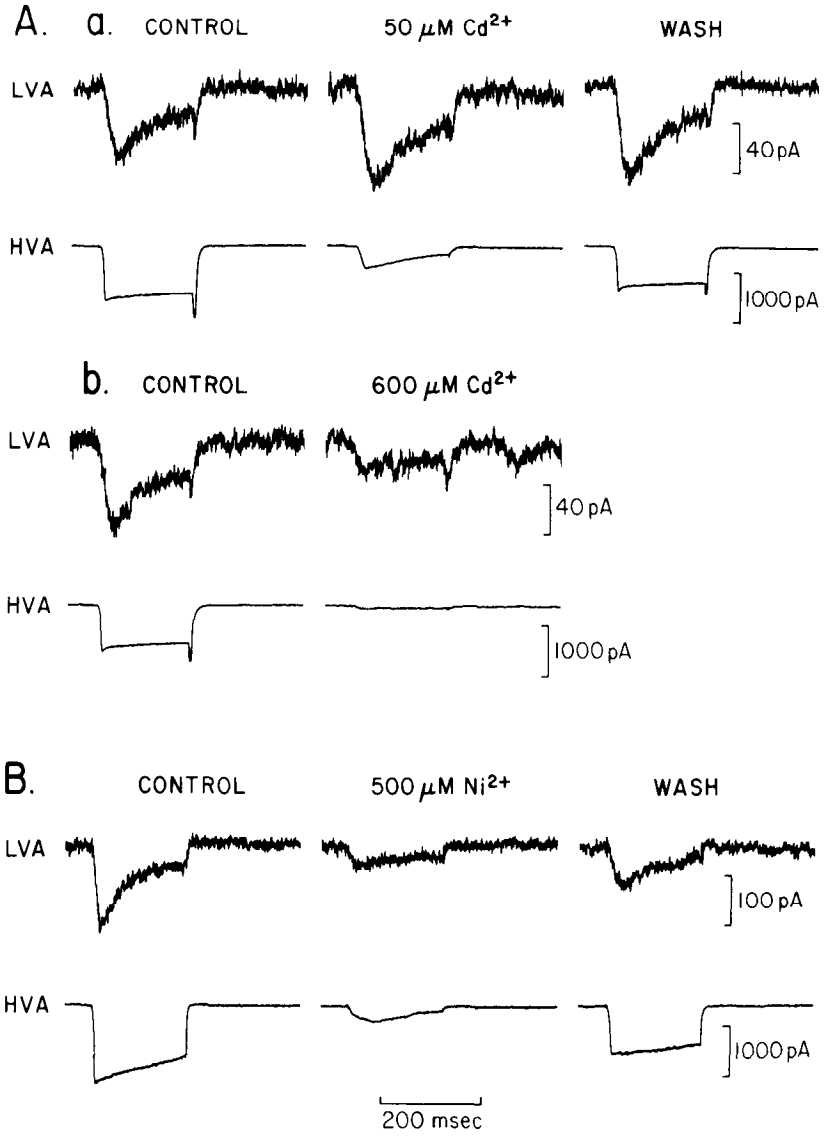

Figure 4. Effect of divalent cations on the LVA and HVA Ca^{2+} currents recorded in voltage-clamped motoneurons. *A*, Effect of 50 and 600 μM Cd^{2+}, respectively, in same motoneuron. Low dose of Cd^{2+} results in differential block of LVA and HVA currents. Cd^{2+} applied for 5 min at 50 and 600 μM concentrations. Wash data taken at 6 min after 50 μM Cd^{2+} removal. *B*, Effect of 500 μM Ni^{2+}. Ni^{2+} applied for 3 min and wash data obtained at 10 min after Ni^{2+} removal. In *A* and *B*, $V_h = -94$ and -93 mV, respectively; for LVA generation, $V_c = -46$ and -45 mV, respectively, and for HVA generation, $V_c = -22$ and -21 mV, respectively. Time base same for *A* and *B*.

of depolarizing current caused a transient depolarizing response in motoneurons. Bath application of 10 μM 5-HT markedly enhanced the transient depolarization (Fig. 9) in a reversible manner. Therefore, these results, in conjunction with the voltage-clamp data, provide evidence that the transient depolarization seen in current clamp is due to activation of a LVA Ca^{2+} current and that this transient depolarization is specifically enhanced by 5-HT.

Discussion

In the present study, we have demonstrated that LVA and HVA Ca^{2+} currents in mammalian spinal motoneurons are similar in characteristics to those reported in other mammalian neurons. These include the activation threshold, inactivation time course, and the differential sensitivities to low concentration of Cd^{2+} (Carbone and Lux, 1984, 1987; Nowycky et al., 1985; Fox et al., 1987; Akaike et al., 1989; Blaxter et al., 1989; Crunelli et al., 1989; Huang, 1989). HVA currents, particularly those of noninactivating type, are known to run down during whole-cell recording. Similar run-down was observed for HVA currents, but not for LVA currents in spinal motoneurons.

It may be argued that motoneurons with dendritic arborization cannot be adequately voltage-clamped. In fact, signs of inadequate space-clamp were observed in normal Krebs solution for Na^{2+} currents, which occurred in all-or-none fashion above threshold, and their latencies became shorter with more intense depolarization (Takahashi, 1990a). However, Ca^{2+} currents recorded in the present condition behaved differently in most cells. For the incremental depolarizing command pulses, the peak amplitude of the Ca^{2+} increased gradually with more or less constant latency, suggesting a rather reasonable space-clamp condition (Fig. 3). It is possible that blockade of K^+ conductances resulted in an increase in the length constant of the neurites.

The main result of the present study is the enhancement of LVA-Ca^{2+} current amplitude by 5-HT. Differential modulation of certain types of Ca^{2+} channels by endogenous substances has been reported. For example, angiotensin II enhances HVA but not LVA Ca^{2+} channel currents in adrenal cortical cell lines (Hescheler et al., 1988). Selective suppression of LVA current by catecholamines has been reported in chick autonomic ganglion cells (Marchetti et al., 1986) and selective enhancement

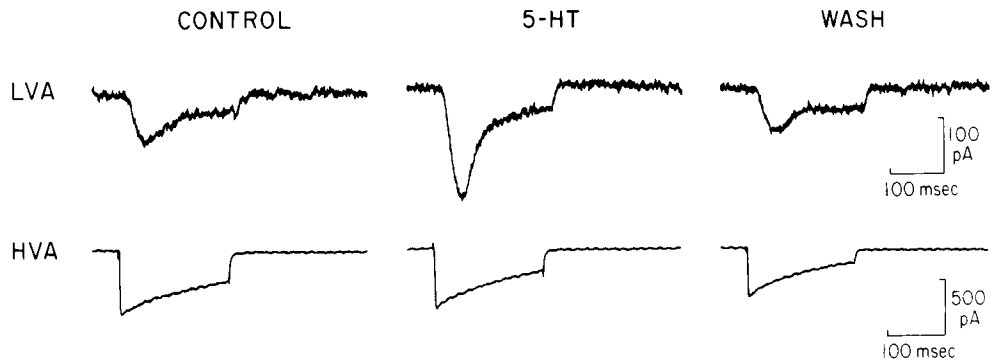

Figure 5. Effect of 10-μM 5-HT application on LVA and HVA Ca^{2+} currents recorded in 2 voltage-clamped motoneurons shows that 5-HT enhanced the LVA and not the HVA current. $V_h = -94$ and -74 mV, $V_c = -46$ and -4 mV for LVA and HVA data, respectively. 5-HT data for both cells obtained 2 min after start of 5-HT application. Wash data obtained at 3.5 and 3 min after cessation of 5-HT for LVA and HVA data, respectively.

of LVA current has been reported in rat sensory ganglion cells (Wanke et al., 1989). It has been suggested that 5-HT has no direct effect on the low-threshold Ca^{2+} conductance in dorsal raphe neurons (Burlhis and Aghajanian, 1987). More recently, 5-HT has been shown to enhance a dendritic high-threshold Ca^{2+} spike in nigrostriatal neurons (Nedergaard et al., 1988). Enhancement of an HVA Ca^{2+} current has been reported also in invertebrate neurons (Pellmar and Carpenter, 1980; Paupardin-Tritsch et al., 1986). Recently, we have demonstrated that 5-HT causes inward current or depolarization of lumbar motoneurons (Takahashi and Berger, 1990). However, the present 5-HT effect on LVA current is distinct from these for the following reasons. First, the 5-HT-induced inward current that we reported previously did not require external Ca^{2+}. Second, the 5-HT induced inward current was produced at holding potentials well below the threshold of LVA Ca^{2+} current. Third, the inward current was evoked by 5-HT at concentrations that were submicromolar, whereas the enhancement of the LVA current was observed for 5-HT above 10 μM. It is suggested that different types of 5-HT receptors mediate these different 5-HT effects (see also Peroutka et al., 1981).

In reptile and cat spinal motoneurons, 5-HT has been reported to induce sustained plateau potentials (Hounsgaard et al., 1988; Hounsgaard and Kiehn, 1989). However, we could not see a similar effect of 5-HT in neonatal rat motoneurons under the present condition.

The functional significance of the 5-HT enhancement of the LVA Ca^{2+} current in central neurons is not clear. Llinás and his colleagues (Llinás and Yarom, 1981a, b; Jahnsen and Llinás, 1984) have shown that low-threshold Ca^{2+} spikes in central neurons can be activated only after membrane hyperpolarization and therefore have attributed to them a role in membrane oscillation. The LVA Ca^{2+} current underlies the low-threshold Ca^{2+} spike (Suzuki and Rogawski, 1989). Since oscillatory be-

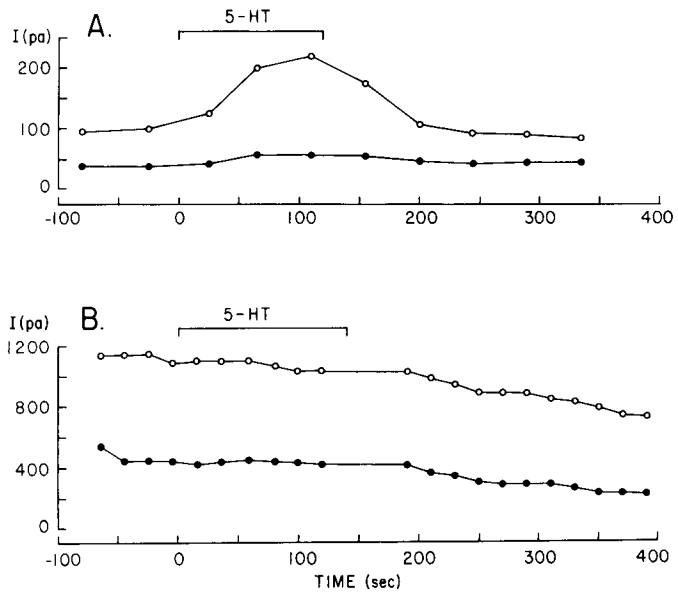

Figure 6. Temporal pattern of response of LVA (*A*) and HVA (*B*) Ca^{2+} currents to 10 μM 5-HT application in 2 voltage-clamped motoneurons. Same cells and same conditions for LVA and HVA generation as in Figure 5. *Open circles,* peak inward current; *filled circles,* remaining inward current at the end of the 200 msec depolarizing voltage command pulse.

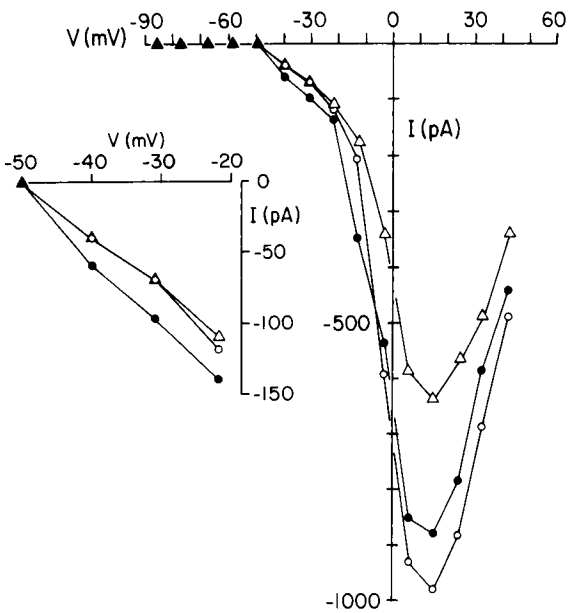

Figure 7. Effect of 10 μM 5-HT on the *I–V* relationship of the peak calcium current amplitude. *Open circles,* prior to; *filled circles,* 90 sec after the start of; and *triangles,* 3 min after cessation of 5-HT application. *Insert,* expanded plot of *I–V* relation in range of LVA activation. $V_h = -95$ mV.

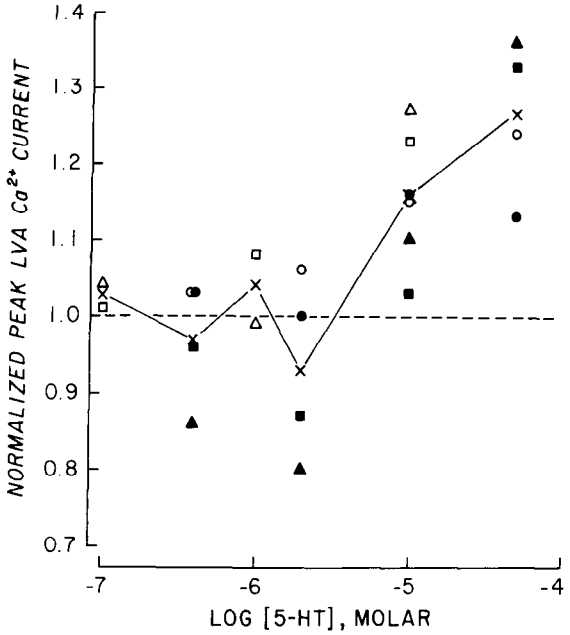

Figure 8. Normalized LVA Ca^{2+} peak current amplitude as a function of logarithmic 5-HT concentrations. Values normalized with respect to the peak inward current in control solution prior to 5-HT application. Data obtained from consecutive, 2 min applications of increasing doses of 5-HT without intervening washout. *Crosses* (connected by *solid line*) represent mean values at each concentration. The other symbols represent data points for 6 different motoneurons, respectively. Range of values: V_h, −94 to −96 mV; V_c, −36 to −44 mV.

havior has been reported in neonatal rat spinal motoneurons (Walton and Llinás, 1986), it is possible that 5-HT may physiologically facilitate such oscillatory behavior in these cells.

It is also possible that 5-HT may shorten the interspike interval of motoneurons by enhancing the LVA Ca^{2+} current (White et al., 1989). This, together with the direct depolarizing effect of 5-HT (Takahashi and Berger, 1990), may synergetically raise the excitability of motoneurons.

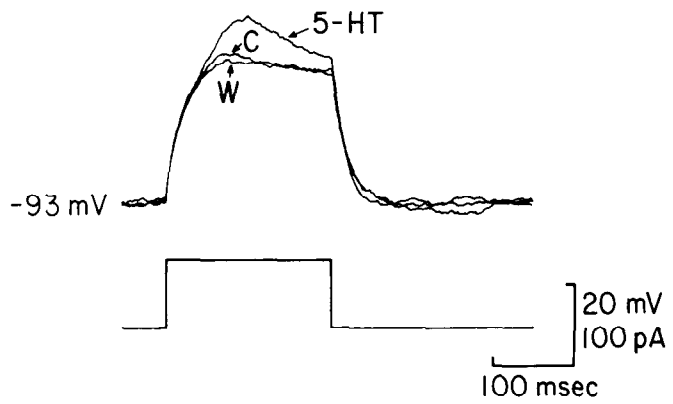

Figure 9. Effect of 10 μM 5-HT application on the low-threshold depolarizing response in a motoneuron recorded in current-clamp mode. Same amplitude, 200 msec current steps applied before (*C*), during (*5-HT*), and after (*W*) 5-HT application. Holding membrane potential was −93 mV.

References

Akaike N, Kostyuk PG, Osipchuk YV (1989) Dihydropyridine-sensitive low-threshold calcium channels in isolated rat hypothalamic neurones. J Physiol (Lond) 412:181–195.

Blaxter TJ, Carlen PL, Niesen C (1989) Pharmacological and anatomical separation of calcium currents in rat dentate granule neurones *in vitro*. J Physiol (Lond) 412:93–112.

Burlhis TM, Aghajanian GK (1987) Pacemaker potentials of serotonergic dorsal raphe neurons: contribution of a low-threshold Ca^{2+} conductance. Synapse 1:582–588.

Carbone E, Lux HD (1984) A low voltage-activated, fully inactivating Ca channel in vertebrate sensory neurones. Nature 310:501–502.

Carbone E, Lux HD (1987) Kinetics and selectivity of a low-voltage-activated calcium current in chick and rat sensory neurones. J Physiol (Lond) 386:547–570.

Crunelli, V, Lightowler S, Pollard CE (1989) A T-type Ca^{2+} potential in cells of the cat and rat lateral geniculate nucleus. J Physiol (Lond) 413:543–561.

Fox, AP, Nowycky MC, Tsien RW (1987) Kinetic and pharmacological properties distinguishing three types of calcium currents in chick sensory neurones. J Physiol (Lond) 394:149–172.

Harada Y, Takahashi T (1983) The calcium component of the action potential in spinal motoneurones of the rat. J Physiol (Lond) 335:89–100.

Hescheler J, Rosenthal W, Hinsch K-D, Wulfern M, Trautwein W, Schultz G (1988) Angiostensin II-induced stimulation of voltage-dependent Ca^{2+} currents in an adrenal cortical cell line. EMBO J 7: 619–624.

Hounsgaard J, Kiehn O (1989) Serotonin-induced bistability of turtle spinal motoneurones caused by nifedipine-sensitive calcium plateau potential. J Physiol (Lond) 414:265–282.

Hounsgaard J, Hultborn H, Jespersen B, Kiehn O (1988) Bistability of α-motoneurones in the decerebrate cat and in the acute spinal cat after intravenous 5-hydroxytryptophane. J Physiol (Lond) 405:354–367.

Huang L-Y M (1989) Calcium channels in isolated rat dorsal horn neurones, including labelled spinothalamic and trigeminothalamic cells. J Physiol (Lond) 411:161–177.

Jahnsen H, Llinás R (1984) Ionic basis for the electroresponsiveness and oscillatory properties of guinea-pig thalamic neurones *in vitro*. J Physiol (Lond) 349:227–247.

Llinás R, Yarom Y (1981a) Electrophysiology of mammalian inferior olivary neurones *in vitro*. Different types of voltage-dependent ionic conductances. J Physiol (Lond) 315:549–567.

Llinás R, Yarom Y (1981b) Properties and distribution of ionic conductances generating electroresponsiveness of mammalian inferior olivary neurones *in vitro*. J Physiol (Lond) 315:569–584.

Marchetti C, Carbone E, Lux HD (1986) Effects of dopamine and noradrenaline on Ca channels of cultured sensory and sympathetic neurons of chick. Pfluegers Arch 406:104–111.

McCobb DP, Best PM, Beam KG (1989) Development alters the expression of calcium currents in chick limb motoneurons. Neuron 2:1633–1643.

Miller RJ (1987) Multiple calcium channels and neuronal function. Science 235:46–52.

Nedergaard S, Bolam JP, Greenfield SA (1988) Facilitation of a dendritic calcium conductance by 5-hydroxytryptamine in the substantia nigra. Nature 333:174–177.

Nowycky M, Fox AD, Tsien RW (1985) Three types of neuronal calcium channels with diffuse calcium agonist sensitivity. Nature 316: 440–443.

Paupardin-Tritsch D, Hammond C, Gershenfeld HM (1986) Serotonin and cyclic GMP both induce an increase of the calcium current in the same identified molluscan neurons. J Neurosci 6:2715–2723.

Pellmar TC, Carpenter DO (1980) Serotonin induces a voltage-sensitive calcium current in neurons of *Aplysia californica*. J Neurophysiol 44:423–439.

Peroutka SJ, Lebovitz RM, Snyder SH (1981) Two distinct central serotonin receptors with different physiological functions. Science 212: 827–829.

Suzuki S, Rogawski MA (1989) T-type calcium channels mediate the transition between tonic and phasic firing in thalamic neurons. Proc Natl Acad Sci USA 86:7228–7232.

Takahashi T (1984) Inhibitory miniature synaptic potentials in rat motoneurons. Proc R Soc London [Biol] 221:103–109.

Takahashi T (1990a) Tight seal whole-cell recording of membrane currents from motoneurones visually identified in thin slices of new born rat spinal cord. J Physiol (Lond) (in press).

Takahashi T (1990b) Inward rectification in rat spinal motoneurones. J Physiol (Lond) (in press).

Takahashi T, Berger AJ (1990) Direct excitation of rat spinal motoneurones by serotonin. J Physiol (Lond) (in press).

Tsien RW, Lipscombe D, Madison DV, Bley KR, Fox AP (1988) Multiple types of neuronal calcium channels and their selective modulation. Trends Neurosci 11:431–438.

Walton K, Fulton BP (1986) Ionic mechanisms underlying the firing properties of rat neonatal motoneurons studied *in vitro*. Neuroscience 19:669–683.

Walton K, Llinás R (1986) Calcium-dependent low threshold rebound potentials and oscillatory potentials in neonatal rat spinal motoneurons *in vitro*. Soc Neurosci Abstr 12:386.

Wanke E, Becchetti A, Bertollini L, Ferroni A (1989) Cross-talk between receptors on a calcium current in adult but not in neonatal rat sensory neurones. Abstracts, Bruno Ceccarelli Memorial Symposium, Milan, Italy, p 16.

White G, Lovinger DM, Weight FF (1989) Transient low-threshold Ca^{2+} current triggers burst firing through an afterdepolarizing potential in an adult mammalian neuron. Proc Natl Acad Sci USA 86: 6802–6806.