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Neurotransmitter Modulation of Calcium Current in Rat Spinal Cord Neurons

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The modulation of Ca^{2+} currents by neurotransmitters was studied in freshly dissociated rat spinal cord neurons, using the whole-cell patch-clamp technique. GABA, baclofen, adenosine, ATP, serotonin, norepinephrine, somatostatin, and dynorphin A inhibited the current through Ca^{2+} channels in a substantial fraction of cells, while substance P, vasoactive intestinal polypeptide, [D-ala^2,d-leu^5]-enkephalin, cholecystokinin-8 (sulfated), calcitonin gene-related peptide, angiotensin II, neurotensin, vasopressin, and thyrotropin-releasing hormone had no effect. In the case of baclofen, the inhibition is mediated, at least in part, by a GTP-binding protein. Suppression of Ca^{2+} current by neurotransmitters may represent a mechanism of presynaptic inhibition in the spinal cord.

Calcium has a primary role in many cellular functions, including exocytosis and excitability. One of the main routes of Ca^{2+} entry into a cell is via voltage-dependent Ca^{2+} channels. In peripheral neurons, current through Ca^{2+} channels can be modulated by a number of neurotransmitters, including adenosine, GABA, norepinephrine, dynorphin A, and neuropeptide Y (for reviews, see Dunlap et al., 1987; Miller, 1987; Tsien et al., 1988; Bean, 1989a). However, much less is known about modulation of Ca^{2+} channels in central neurons, despite suggestions that presynaptic inhibition in the CNS involves depression of Ca^{2+} currents (Pierau and Zimmermann, 1973; Phillis et al., 1979; Murase et al., 1982). Recently, adenosine (Madison et al., 1987) and muscarine (Gahwiler and Brown, 1987) have been reported to inhibit Ca^{2+} currents in hippocampal pyramidal neurons, while norepinephrine has been reported to enhance Ca^{2+} currents in hippocampal granular neurons (Gray and Johnston, 1987). It is clearly of interest to determine whether neurotransmitter modulation of Ca^{2+} channels is a widespread phenomenon in the CNS and to identify the range of transmitters capable of such modulation.

I have recorded Ba^{2+} currents through Ca^{2+} channels from freshly dissociated rat spinal cord neurons, using the whole-cell patch-clamp technique, and examined the effects of a range of neurotransmitters, including GABA, baclofen, adenosine (ado), ATP, 5-HT, norepinephrine (NE), substance P (SP), somatostatin (SOM), dynorphin A (dyn A), [D-ala^2, d-leu^5]-enkephalin (DADLE), vasoactive intestinal polypeptide (VIP), chloecystokinin-8, sulfated (CCK-8_s), calcitonin gene-related peptide (CGRP), neurotensin (NT), angiotensin II (AT_{II}), thyrotropin-releasing hormone (TRH), and vasopressin (ADH). Here, I report that GABA, baclofen, adenosine, ATP, 5-HT, NE, SOM, and dyn A inhibit the current through Ca^{2+} channels in a substantial proportion of cells, while the remaining transmitters have no significant effect. These observations support the idea that suppression of Ca^{2+} current might represent a mechanism of presynaptic inhibition in central neurons. In peripheral neurons, transmitter inhibition of Ca^{2+} currents is mediated by GTP-binding proteins (Holz et al., 1986; Dolphin and Scott, 1987; Ewald et al., 1988). Similarly, inhibition of Ba^{2+} current by baclofen in spinal cord neurons involves G-proteins. When intracellular GTP is replaced by the hydrolysis-resistant analog, GTP-γ-S, inhibition by baclofen no longer reverses readily in most cells. Furthermore, after neurons are incubated with pertussis toxin, their Ba^{2+} currents are no longer responsive to baclofen. Thus, currents through Ca^{2+} channels in spinal cord neurons, like those in peripheral neurons, are modulated in an inhibitory manner by a number of neurotransmitters. In the case of baclofen, the inhibition probably involves G-proteins.

Materials and Methods

Experiments were performed on neurons freshly dissociated from the spinal cord of neonatal rats (P2–P11). Neurons were dispersed as previously described by Huettner and Baughman (1986), except that some cells were treated with trypsin Type XI (Sigma, 0.7 mg/ml). Two populations of cells were readily distinguishable by appearance: a predominant population with round or oval somata of relatively small diameter (10–20 μm) and a scarce population with irregular somata of relatively large diameter (>30 μm). Only cells from the former population were selected for study; these probably represent interneurons, while the others probably consist of motoneurons (Brown, 1981).

The whole-cell recording mode was used, and currents were filtered at 1 kHz. Patch pipettes had impedances of 5–15 MΩ and contained the following (in mM): 120 Cs-methanesulfonate, 5 $Mg(OH)_2$, 5 CsCl, 10 EGTA, 10 HEPES, and an ATP-regenerating solution (Forscher and Oxford, 1985), pH 7.40. The ATP-regeneration solution consisted of 14 mM creatine phosphate, 4 mM MgATP, 0.3 mM GTP, and 50 units/ml creatine phosphokinase (Type I, from rabbit muscle). Although series resistance compensation was not used, voltage errors were small; the product of peak current and series resistance (estimated from the time constant of the capacity transient divided by cell capacitance) was 4.2 $\pm$ 0.8 mV (SEM, n = 10). The cells were allowed to adhere to a glass coverslip and perfused slowly at room temperature with Tyrode's solution (pH 7.40) containing (in mM): 150 NaCl, 4 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 10 glucose, 10 HEPES, and, in most experiments, 4 $BaCl_2$. After the whole-cell recording mode was attained, control solution (160 mM TEA-Cl, 5 mM $BaCl_2$, 10 mM HEPES, and 1–3 μM tetrodotoxin) was applied to the cells, in order to isolate Ba^{2+} currents through Ca^{2+} channels. For application of agonists, a series of 6–8 microcapillary tubes (1 μl Drummond "Microcaps") was glued together, side-by-side. Solutions

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Table 1. Neurotransmitter modulation of Ba^{2+} current in spinal cord neurons

Agonist	Proportion of cells with >10% decrease in I_{Ba}	Mean decrease in I_{Ba} (range) %
GABA	30/30	51 (15–75)
Baclofen	30/30	41 (13–63)
Adenosine	15/16	32 (16–50)
ATP	4/11	26 (15–40)
5-HT	9/14	36 (18–60)
NE	7/10	28 (20–40)
Angiotensin II	0/10	
CCK-8_s	0/8	
CGRP	0/10	
DADLE	1/5	45
Dynorphin A	5/7	22 (15–35)
Neurotensin	1/8	17
Somatostatin	7/14	39 (26–50)
Substance P	0/12	
TRH	1/13	15
Vasopressin	0/4	
VIP	0/7	

Neurotransmitter modulation of Ba^{2+} current in spinal cord neurons. Conventional transmitters: 10 μM; peptides: 1 μM. Holding potentials: −100 to −70 mV; test potentials: −20 to 0 mV. Currents were measured 5–10 msec after stepping to the test potential. The mean decrease in current is the average inhibition in cells that responded (>10% decline).

were fed from separate reservoirs by gravity and, in some experiments, by additional pressure. The microcapillary tubes were mounted on a micromanipulator, which was used for moving the tubes relative to the cell in order to change solutions. All experiments were done at 20–25°C.

The following agonists were obtained from Sigma: GABA, adenosine, ATP, 5-HT-creatinine sulfate, NE-bitartrate, substance P, somatostatin, DADLE, CGRP, neurotensin, angiotensin II, and vasopressin. Dynorphin A, porcine VIP, CCK-8 (sulfated), and TRH were obtained from Peninsula, while (±) baclofen and muscimol were obtained from Research Biochemicals Inc. Frozen stocks of agonists were thawed and diluted on the day of the experiment into a solution (pH 7.40) containing 160 mM TEA-Cl, 5 mM $BaCl_2$, 10 mM HEPES, and 1–3 μM tetrodotoxin. Pertussis toxin was obtained from Sigma, while GTP-γ-S was obtained from Sigma or Boehringer-Mannheim.

Results

More than 90% of the cells obtained by the dissociation procedure were of small diameter (10–20 μm) and had round or oval somata, with no processes or very short processes (<40 μm in length); these probably represent interneurons, based on their size and predominance, and were selected for recording. Ba^{2+} currents through Ca^{2+} channels were evoked by stepping from holding potentials of −100 to −70 mV to test potentials of −20 to 0 mV. GABA (10 μM; Fig. 1*A*, Table 1) inhibited the inward voltage-dependent current by approximately half, on average, with more than 10% inhibition in every cell tested. However, effects of GABA on Ba^{2+} current were difficult to analyze in detail since GABA also activates a Cl^- conductance in these cells, via the $GABA_A$ receptor. The effects of GABA on Ba^{2+} current were mimicked by the selective $GABA_B$ agonist, baclofen (10 μM; Fig. 1*B*, Table 1), which inhibited Ba^{2+} currents by nearly half, on average, with more than 10% inhibition in every cell tested. Baclofen reduced Ba^{2+} currents without a shift in reversal potential, consistent with a selective effect on Ba^{2+} current. Furthermore, the inward current in the presence of

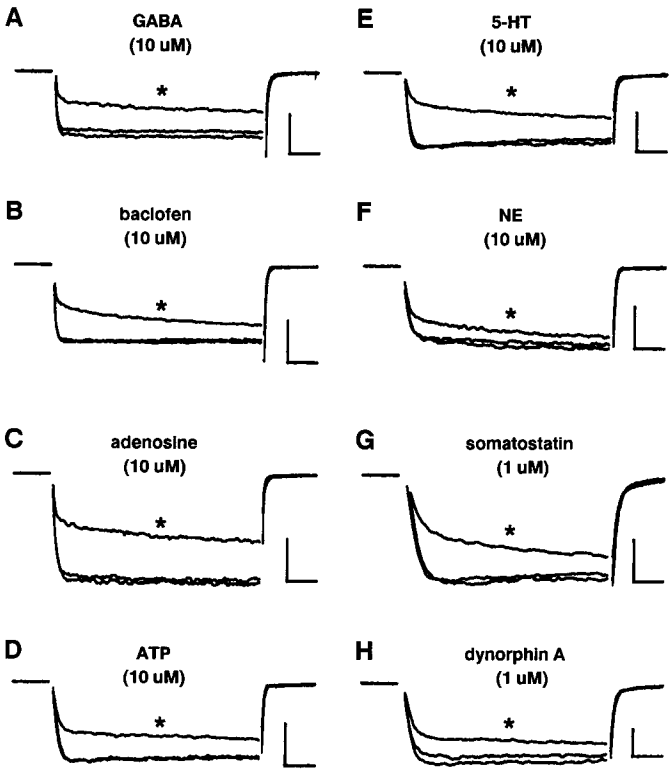

Figure 1. Effects of GABA (*A*), baclofen (*B*), adenosine (*C*), ATP (*D*), 5-HT (*E*), NE (*F*), somatostatin (*G*), and dynorphin A (*H*) on leak-subtracted Ba^{2+} currents in freshly dissociated spinal cord neurons. The agonists were applied in 160 mM TEA-Cl, 5 mM $BaCl_2$, 10 mM HEPES, and 1–3 μM TTX, pH 7.40. Holding potential: −70 mV (*A–F*) and −80 mV (*G–H*); test potential: −10 mV. Horizontal calibration bars: 10 msec; vertical calibration bars: 100 pA (*A, F–H*), and 200 pA (*B–E*). An *asterisk* indicates the current recorded in the presence of agonist; the other traces represent currents recorded in control solution, before and after application of agonist.

baclofen was completely blocked by 1–3 mM Cd^{2+} ($n = 3$). The effect of baclofen on Cd^{2+}-sensitive current is shown in Figure 2. Thus, baclofen selectively inhibits the voltage-dependent current through Ca^{2+} channels, while GABA probably inhibits inward current by 2 mechanisms—decreasing current through Ca^{2+} channels and increasing Cl^- current.

Adenosine (10 μM) inhibited the voltage-dependent current through Ca^{2+} channels in every cell tested, except one, with an average decrease of approximately one-third (Fig. 1*C*, Table 1). In contrast, ATP (10 μM) inhibited the current in fewer cells, with a somewhat smaller effect than adenosine (Fig. 1*D*, Table 1). The amines, 5-HT (10 μM; Fig. 1*E*) and NE (10 μM; Fig. 1*F*), each reduced the current in most cells, with an average inhibition of approximately one-third (Table 1). Of 11 neuropeptides tested (all at 1 μM), only 2, SOM and dyn A, affected the current through Ca^{2+} channels in a substantial proportion of cells (Table 1). SOM (Fig. 1*G*, Table 1) inhibited the current in half of the cells tested, by an average of 39%, while dyn A (Fig. 1*H*, Table 1) inhibited the current to a smaller extent. DADLE and NT were found to inhibit the current in only one case each, while SP, VIP, CCK-8_s, CGRP, AT_{II}, ADH, and TRH had no significant effect in any of the cells tested. There was no correlation between sensitivity to NE and sensitivity to SOM, 5-HT, or dyn A, nor was there a correlation between sensitivity to 5-HT and sensitivity to SOM.

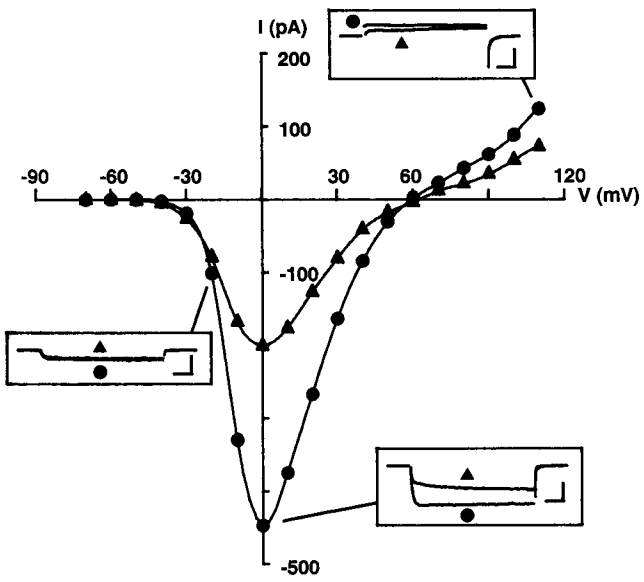

Figure 2. Effect of baclofen (10 μM) on current–voltage relation: ●, control; ▲, baclofen. Holding potential: −70 mV. The average current was measured between 4.9 and 6.6 msec after depolarization to the test potential. Current–voltage relations were measured in 160 mM TEA-Cl, 5 mM $BaCl_2$, 10 mM HEPES, and 1 μM TTX with and without 3 mM Cd^{2+} and with and without baclofen; the Cd^{2+}-sensitive component is plotted here. *Insets,* Currents are shown for test potentials of −20, 0, and +110 mV. Calibration bars: 10 msec and 200 pA.

In peripheral neurons, several different neurotransmitters inhibit Ca^{2+} current via G-proteins (Holz et al., 1986; Dolphin and Scott, 1987; Lipscombe and Tsien, 1987; Wanke et al., 1987; Ewald et al., 1988). To see if the inhibitory effect of baclofen in spinal cord neurons also involves G-proteins, modulation of Ba^{2+} currents was examined after treatment with PTx or after replacement of intracellular GTP with GTP-γ-S (Table 2). Overnight treatment with PTx (100–200 ng/ml) at 35°C blocked the effects of baclofen in 10/12 cells (Fig. 3*B*, Table 2). Furthermore, the 2 responsive cells exhibited only small reductions in Ba^{2+} current (13 and 17%). Cells incubated in parallel with vehicle had normal responses to baclofen, with an inhibition of Ba^{2+} current in every cell tested (38% on average).

Table 2. Effect of pertussis toxin and intracellular GTP-γ-S on inhibition of Ba^{2+} current by baclofen

Addition	Proportion of cells with >10% decrease in I_{Ba}	Mean decrease in I_{Ba} (%)	Proportion of cells with >50% recovery from inhibition
GTP, 0.3 mM			
GTP-γ-S, 100 μM	30/30	41	30/30
Vehicle	15/15	40	5/15
Pertussis toxin,	6/6	38	–
100–200 ng/ml[a]	2/12	15	–

Effect of pertussis toxin and intracellular GTP-γ-S on inhibition of Ba^{2+} current by baclofen, 10 μM. Holding potentials: −100 to −70 mV; test potentials: −20 to 0 mV. Currents were measured 5–14 msec after stepping to the test potential. The mean decrease in current is the average inhibition in cells that responded (>10% decline).

[a] Overnight at 35°C.

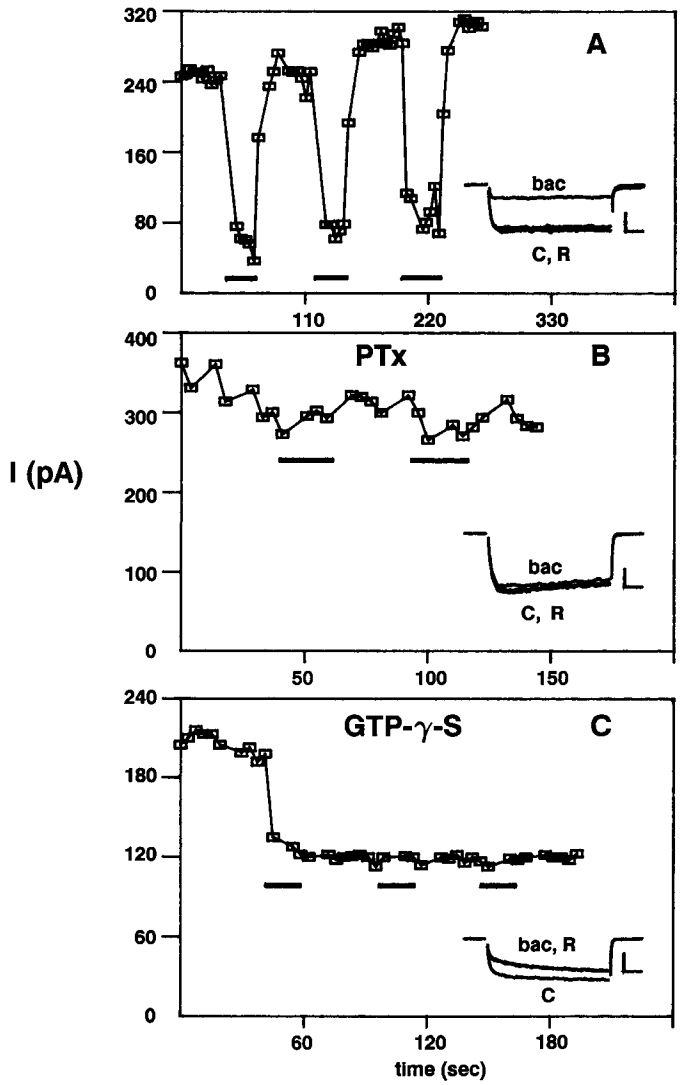

Figure 3. Effect of pertussis toxin and GTP-γ-S on baclofen (10 μM) inhibition of Ba^{2+} currents. *A,* control, with intracellular GTP (0.3 mM). Baclofen was applied 3 times; the inhibition reversed rapidly and completely each time. Holding potential: −90 mV; test potential: −10 mV. *B,* Pertussis toxin treatment (200 ng/ml, 23 hr at 35°C). Baclofen was applied twice; there was no significant change in Ba^{2+} current either time. *C,* Intracellular GTP-γ-S (100 μM). The first application of baclofen produced a 35% inhibition of the Ba^{2+} current. However, the effect was irreversible, and there was no effect of 2 subsequent applications. Currents were measured between 6.4 and 14 msec. The *solid bars* represent the periods of baclofen application. *Insets*: *bac,* baclofen; *c,* control; *r,* recovery; calibration bars: 10 msec and 100 pA.

Normally, the inhibition of Ba^{2+} current by baclofen reverses completely and rapidly (within 8 sec, Fig. 3*A*). When intracellular GTP was replaced with GTP-γ-S (100 μM), the frequency and magnitude of the baclofen effect was the same as that with intracellular GTP. However, in most cells, more than half of the inhibition was irreversible (Fig. 3*C*, Table 2), suggesting that at least that part of the baclofen effect is mediated by a G-protein.

Discussion

Previous studies of Ca^{2+} current modulation in central neurons demonstrated inhibitory effects of adenosine (Madison et al., 1987) and muscarine (Gahwiler and Brown, 1987) in rat hippocampal pyramidal neurons, and stimulatory effects of NE via

β-receptors in rat hippocampal granular neurons (Gray and Johnston, 1987). Here, it has been shown that Ca^{2+} channels in spinal cord neurons are modulated in an inhibitory manner by the conventional neurotransmitters, GABA (via B-receptors), adenosine, ATP, 5-HT, and NE. Although a number of peptidergic transmitters are present in the spinal cord (Hökfelt et al., 1976, 1977; Khachaturian et al., 1982), only SOM and dyn A altered Ba^{2+} currents through Ca^{2+} channels in a subpopulation of cells; AT_{II}, SP, DADLE, VIP, CCK-8_S, CGRP, NT, ADH, and TRH had little effect. None of the transmitters tested increased Ba^{2+} currents; this parallels the predominance of inhibition in peripheral neurons (Tsien et al., 1988; Bean, 1989a).

GABA is a major inhibitory transmitter in the CNS. In the spinal cord, GABA is present in interneurons (Hunt et al., 1981). GABA was originally described to activate a Cl^- conductance, via $GABA_A$ receptors. More recently, an additional effect was ascribed to GABA in the peripheral nervous system, a decrease in Ca^{2+} conductance (Dunlap and Fischbach, 1981) via $GABA_B$ receptors. The specific $GABA_B$ receptor agonist, baclofen, has inhibitory actions on central neurons which have been attributed to 2 mechanisms: an increase in potassium conductance (Newberry and Nicoll, 1984) and presynaptic inhibition, resulting in suppression of postsynaptic potentials (Pierau and Zimmermann, 1973). The mechanism of presynaptic inhibition was postulated to be a reduction in Ca^{2+} current; however, baclofen had no detectable effect on Ca^{2+} action potentials in slices from adult rat cortex (Howe et al., 1987) or on Ca^{2+} currents in hippocampal neurons cultured from neonatal rats (Gahwiler and Brown, 1985). In contrast, in spinal cord neurons freshly dissociated from neonatal rats, baclofen substantially decreases currents through Ca^{2+} channels. The discrepancy between the results reported here and those in cortical slices may be explained by regional tissue differences (spinal cord versus cortex and hippocampus), animal age (neonatal versus adult), culture age (freshly dissociated versus 3–5 weeks *in vitro*), or sensitivity of method (direct measurement of current through Ca^{2+} channels with voltage-clamp versus length of Ca^{2+} action potential). There is actually no discrepancy between the observations described here and the experiments with cultured hippocampal neurons (Gahwiler and Brown, 1985), since in the latter study, the effect of baclofen was only examined on currents elicited with 20 mV depolarizing steps. For small depolarizations, there is similarly no effect of baclofen on Ba^{2+} currents in spinal cord neurons (see Fig. 2). (The reason for the lack of effect with small depolarizations remains to be determined; it could arise from differential effects on different types of Ca^{2+} channels—see Lipscombe and Tsien, 1987). The inhibitory effects of baclofen on current through Ca^{2+} channels in spinal cord neurons are qualitatively similar to those reported in sensory neurons (Dunlap and Fischbach, 1981; Dolphin and Scott, 1987) and may play a role in presynaptic inhibition of transmission in central neurons.

G-proteins have been shown to mediate inhibition of Ca^{2+} current by NE and baclofen in chick DRG neurons (Holz et al., 1986); adenosine and baclofen (Dolphin and Scott, 1987), and NPY (Ewald et al., 1988) in rat DRG neurons; enkephalin in neuroblastoma–glioma cells (Hescheler et al., 1987); and SOM in a pituitary cell line (Lewis et al., 1986). Similarly, baclofen modulation of currents through Ca^{2+} channels in spinal cord neurons appears to be mediated, at least in part, by G-proteins, since the inhibition was blocked by preincubation with pertussis toxin, and the recovery from inhibition (in most cells) hampered with intracellular GTP-γ-S. However, in 5 of 15 cells perfused internally with GTP-γ-S, more than 50% of the baclofen effect reversed readily (within 30 sec). This may have been due to the presence of residual intracellular GTP. Unlike rat DRG neurons (Dolphin and Scott, 1987), the magnitude of the inhibitory effects of baclofen in spinal cord neurons is not altered with GTP-γ-S. Nevertheless, the action of baclofen on currents through Ca^{2+} channels in spinal cord neurons is consistent with the current model of G-protein signal transduction (Dunlap et al., 1987; Neer and Clapham, 1988).

Adenosine is a potent inhibitor of firing rate in central neurons, while ATP has strong excitatory effects (Phillis et al., 1979). A number of experiments on transmitter release (Fredholm and Hedqvist, 1980; Dolphin and Archer, 1983) and synaptic transmission (Phillis et al., 1979; Dunwiddie and Hoffer, 1980) indicate that the inhibitory effects of adenosine are probably due to inhibition of transmitter release from presynaptic terminals (Stone, 1981). Since adenosine-receptor agonists inhibit Ca^{2+} influx into synaptosomes (Wu et al., 1982), presynaptic inhibition may result from a decrease in presynaptic Ca^{2+} current. The results reported here, as well as those reported for sensory neurons (Dolphin et al., 1986; Macdonald et al., 1986) and hippocampal pyramidal neurons (Madison et al., 1987), support such a mechanism.

ATP excites a population of neurons from the dorsal horn of the spinal cord (Fyffe and Perl, 1984) by increasing a TTX-insensitive sodium conductance (Jahr and Jessell, 1983). The inhibitory effects on current through Ca^{2+} channels seen here may be due to an additional effect of ATP or to adenosine after enzymatic degradation of ATP (Stone, 1981).

The spinal cord receives descending serotonergic and adrenergic inputs from the raphé nucleus (Fuxe, 1965; Hökfelt et al., 1978; Steinbusch et al., 1978) and locus coeruleus (Carlsson et al., 1964), respectively; these projections arborize in the dorsal and ventral horns. Electrophysiological studies suggest that 5-HT and NE may contribute to analgesia by inhibiting spinal transmission of nociceptive impulses (Akil and Mayer, 1972; Basbaum et al., 1976; Headley et al., 1978; Reddy and Yaksh, 1980). The inhibitory effects of 5-HT and NE on current through Ca^{2+} channels reported here are qualitatively similar to their effects in sensory neurons (Forscher and Oxford, 1985; Holz et al., 1986; Deisz and Lux, 1988) and may mediate suppression of synaptic transmission.

In the rat spinal cord, immunocytochemical studies have provided evidence for the presence of neuropeptides. Johansson et al. (1984) have demonstrated that there are numerous SOM-positive cells in lamina II, with a high density of immunoreactive fibers in the dorsal horn, particularly in lamina II; some of these fibers represent terminals of a population of sensory neurons that contain SOM (Hökfelt et al., 1976). Similarly, dynorphin is localized within fibers and a few cells of the marginal zone of the dorsal horn (Khachaturian et al., 1982), while enkephalin is localized within interneurons and descending terminals (Hökfelt et al., 1977). The physiological effects of these peptides in the spinal cord are generally inhibitory (Basbaum et al., 1976; Kelly, 1982). In the cat, SOM depresses the firing rate of nociceptive dorsal horn neurons (Randic and Miletic, 1978). Intracellular recordings from rat dorsal horn neurons in the slice preparation (Murase et al., 1982) showed that SOM and enkephalin hyperpolarized most cells, with a decrease in membrane resistance and firing rate; Murase et al. suggest that at least part of this effect may be presynaptic since it was reduced

by a low Ca^{2+}/high Mg^{2+} solution. The results reported here are consistent with presynaptic inhibitory effects of SOM and dyn A via reductions in Ca^{2+} current. The effect of SOM in the spinal cord is qualitatively similar to those reported in rat sympathetic neurons (Ikeda et al., 1987) and pituitary (Lewis et al., 1986) and neuroblastoma–glioma (Tsunoo et al., 1986) cell lines, while that of dyn A resembles its action on mouse sensory neurons (Gross and Macdonald, 1987).

In the present study, there was no detectable effect of SP on currents through Ca^{2+} channels in spinal cord neurons. In contrast, Murase et al. (1986) report that SP's excitatory effects in the spinal cord slice preparation are mediated by an augmentation of Ca^{2+} current, in addition to suppression of K^+ current (Nowak and Macdonald, 1982). A possible explanation for the discrepancy is enzymatic degradation of the SP receptor during the dissociation procedure used for the experiments described here. However, voltage-clamp data in slice preparations may be difficult to interpret due to poor voltage control and contaminating K^+ currents. Furthermore, the effect of SP in the slice preparation may be at least partially transsynaptic, consistent with its mimicry by agents that enhance Ca^{2+} current and blockade by agents that suppress Ca^{2+} current (Murase et al., 1986).

Whole-cell and single-channel recordings in peripheral neurons have recently demonstrated the existence of 3 channel types—L, N, and T (Nowycky et al., 1985); multiple types of Ca^{2+} current have been described in central neurons as well (see Tsien et al., 1988; Bean, 1989a). Selective modulation of N-current has been reported for ACh (Wanke et al., 1987), adenosine (Madison et al., 1987), and dyn A (Gross and Macdonald, 1987). In the present study, suppression of current through Ca^{2+} channels was generally greater at the beginning of the voltage step than at the end (see Fig. 1), with no consistent differences among the effective transmitters (excluding GABA). The implications of this slowing in kinetics, with respect to channel type (Tsien et al., 1988; Bean, 1989a) and mechanism (Bean, 1989b) remain to be determined.

The great majority of neurons in the spinal cord are interneurons, outnumbering motoneurons 30:1 (Henneman, 1980). They serve as points of convergence; most primary sensory fibers and descending fibers terminate on interneurons, and most of the synaptic boutons on motoneurons represent terminations from interneurons. Thus, interneurons play a dominant role in information processing within the spinal cord. The neurons studied in the experiments described here are likely to be interneurons, based on their predominance and size. Inhibition of their Ca^{2+} currents by GABA (via B-receptors), adenosine, ATP, 5-HT, NE, SOM, and dyn A may represent a mechanism of modulating their output *in vivo*.

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