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ATP Modulation of Synaptic Transmission in the Spinal Substantia Gelatinosa

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Actions of adenosine triphosphate (ATP) on neurons of the substantia gelatinosa (SG) were evaluated in spinal cord slices using tight-seal, whole-cell recordings. Bath-applied ATP activated a fast inward current and potentiated both glutamate-induced and synaptically evoked currents by acting through a purinergic receptor with the pharmacology of the P_2 type. ATP also induced a delayed slow outward current and depressed synaptic currents that appeared to result from hydrolysis of ATP to adenosine. The inhibitory actions had features suggesting mediation by a P_1-like purinergic receptor. Suramin, a putative P_2 antagonist, inhibited ATP-induced fast inward currents but did not suppress synaptic currents evoked by dorsal root stimulation. It was concluded that in the SG, ATP released in synaptic regions acts as a synaptic modulator by augmenting excitatory amino acid actions and possibly by also producing a secondary adenosine inhibition.

[Key words: adenosine triphosphate (ATP), substantia gelatinosa, spinal cord, glutamate, synaptic transmission, suramin, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), synaptic modulation, EPSC]

Proposals that adenosine triphosphate (ATP) may be an excitatory chemical transmitter for primary afferent fibers have surfaced periodically subsequent to the initial suggestion by Holton and Holton (1954; Holton, 1959). Support for this idea has come from histochemical evidence for the presence of a fluoride-resistant acid phosphatase (FRAP) in the substantia gelatinosa (SG) of certain small rodents and in a subset of primary afferent neurons (Coimbra et al., 1970, 1974; Knyihár, 1971; Knyihár et al., 1974). The demonstration that a 5′-nucleotide is a proper substrate for this phosphatase also has been argued to be consistent with the concept (Dodd et al., 1983, 1988). Furthermore, ATP has been shown to cause fast depolarization of cultured dorsal horn neurons (Jahr and Jessell, 1983) and to excite a subset of superficial dorsal horn neurons *in vivo* (Hill and Salt, 1982; Fyffe and Perl, 1984; Salter and Henry, 1985). The SG, the focus of attention in the FRAP studies, receives its principal dorsal root input from thin afferent fibers (Ranson, 1913; Light and Perl, 1979a,b). This implicates the SG in afferent processing related to pain and other somatic sensations and gives the question of synaptic action by ATP there a recognizable significance. Nonetheless, the evidence favoring ATP as an excitatory synaptic agent for primary afferent fibers is largely circumstantial.

The present experiments analyze the physiological characteristics and the pharmacological profile of ATP effects on neurons of the rodent SG using tight-seal whole-cell recordings from transverse slices of the spinal cord *in vitro*. The observations suggest that ATP, rather than being a principal mediator of fast synaptic excitation from primary afferent fibers in the region, may serve as a modulator of such excitation.

Some of the results have been preliminarily reported (Li and Perl, 1991).

Materials and Methods

The methods have been described in detail elsewhere (Li and Perl, 1994) and are briefly outlined here.

Slice preparation. Young (21–28 d) Syrian golden hamsters were deeply anesthetized with urethane (1.6 g/kg intraperitoneally) and then cooled for several minutes in an ice bath. A segment of spinal cord including three to five lower thoracic and lumbar dorsal roots was quickly removed and immersed in ice-cold, sucrose-substituted physiological saline (sucrose saline; in mM: sucrose, 234; KCl, 3.6; $CaCl_2$, 2.5; $MgCl_2$, 1.2; NaH_2PO_5, 1.2; $NaHCO_3$, 25; glucose, 11). In ice-cold sucrose artificial cerebral spinal fluid (ACSF) the spinal cord was cut transversely into 500 μm slices with a Vibratome. Three to five slices with attached dorsal rootlets were collected and incubated at room temperature in a beaker containing ACSF equilibrated with 95% O_2 and 5% CO_2 (ACSF; in mM: NaCl, 125; KCl, 2.5; $CaCl_2$, 2; $MgCl_2$, 1; NaH_2PO_5, 1.25; $NaHCO_3$, 26; glucose, 12). In the recording chamber, the slice was superfused with oxygenated ACSF at 6 ml/min.

Electrophysiology. Viewed with a dissecting microscope, the SG appeared as a dark belt in the dorsolateral part of the dorsal horn. Tight-seal, whole-cell recordings were obtained from cells in the middle of this zone with pipette electrodes of 5–8 MΩ using a blind-patch method modified from Blanton et al. (1989). A Dagan 8900 amplifier was used for current-clamp or voltage-clamp recordings. All voltage-clamp recordings used a holding potential of −70 mV unless otherwise noted. Values are given as mean ± the standard deviation (n = number of samples).

Two pipette solutions were employed, composed of the following (mM)—(1) K solution: K-gluconate, 130; NaCl, 5; $CaCl_2$, 1; $MgCl_2$, 1; EGTA, 11; HEPES, 10; Na-ATP, 4. (2) Cs-TEA solution: Cs-gluconate, 130; NaCl, 5; $CaCl_2$, 1; $MgCl_2$, 1; EGTA, 11; HEPES, 10; Na-ATP, 4; tetraethylammonium (TEA), 20. Fast excitatory currents were studied using pipette electrodes filled with Cs^+ and TEA to suppress contributions from potassium currents (Li and Perl, 1994). For neurons recorded with the Cs-TEA pipette solution in current clamp, a 'holding current' of −20 to −10 pA was used to bring the resting membrane potential from near −40 mV to −60 mV (Li and Perl, 1994).

The dorsal rootlets were stimulated through a suction electrode using constant-voltage pulses. Analog data were digitized with an Axolab 1100 analog-to-digital and digital-to-analog interface in an MS-DOS microcomputer. Data acquisition was controlled through the computer by the pCLAMP program (version 5.5) and stored as computer files. Although the data was acquired at 0.5–0.8 msec sample intervals, to save storage space and shorten plotting time most drug-induced current traces were stored and plotted as the average of 50 samples. Averages

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Table 1. Classification of ATP effects on SG neurons

	Cs-TEA	K	Total
Excitatory	60/106	1/28	61/132
Inhibitory	1/106	18/28	19/132
Biphasic	2/106	10/28	12/132

"Excitatory" indicates that ATP evoked inward current and/or membrane depolarization. "Inhibitory" indicates that ATP evoked outward current and/or membrane hyperpolarization. "Biphasic" designates a biphasic response in which excitation was followed by an inhibition. "Cs-TEA" and "K" denote the pipette solutions used to reach responses. Data are presented as number of neurons exhibiting the indicated ATP effect/number of neurons tested.

of three trials were generally used for synaptic currents elicited by dorsal root stimulation. Data were also plotted on a paper chart recorder and stored in parallel on magnetic tape using a modified video recorder.

Pharmacological procedures and agents. Nucleotides including ATP, ADP, AMP, UTP, GTP, ATPγS, and adenosine were purchased from Boehringer–Mannheim Biochemicals. Sigma (St. Louis, MO) was the source of Tetrodotoxin (TTX), Miles Inc. (West Haven, CT) of sodium suramin, and Research Biochemicals (Boston, MA) of CNQX.

Pharmacological agents were applied by switching the superfusion to preprepared solutions. The superfusion inlet to the recording chamber was adjustable and positioned close to the slice to ensure rapid delivery of chemical agents; however, the timing of reactions and concentrations cannot be compared to those observed in experiments in which similar agents are applied to isolated cells or in cultures. The organized tissue of slices from the fully formed spinal cord has diffusional barriers. Very few of our recordings were made from neurons at the cut surface. Both ATP and GLU currents desensitize quickly (Trussell and Fischbach, 1989; Bean, 1990). Regardless of how rapidly an agent reaches a tissue slice, appreciable time is necessary for it to diffuse into the vicinity of the neurons being recorded in the depth of a section of tissue. Moreover, both ATP and GLU are quickly removed from the extracellular fluid via hydrolysis or uptake, further slowing the buildup of drug concentration at a neuron. Both of these processes would enhance the opportunity for desensitization. To speed the concentration buildup at neurons and thereby reduce chances for desensitization, relatively high concentrations of ATP and GLU were used in these experiments. In turn, higher concentrations of antagonists (e.g., suramin) were needed to block effects. Nonetheless, properties of the observed currents and the selective and reversible effects of antagonists suggest that the actions reported were specifically related to the agents and their antagonists.

Results

ATP induces a fast inward current in SG neurons

In confirmation of Jahr and Jessell's (1983) observation on cultured spinal cord neurons, in current-clamp conditions ATP (1–5 mM) in the ACSF superfusion induced a fast membrane depolarization of SG neurons and the discharge of action potentials. This ATP depolarization was analyzed in voltage-clamp recordings using pipettes filled with Cs^+-TEA solution to minimize K^+ currents and 1 μM TTX to block Na^+-dependent action potentials. Under these conditions, ATP evoked an inward current in approximately half of neurons examined (Table 1) at the usual holding potential of −70 mV (e.g., Fig. 1). Glutamate (GLU) also induced an inward current in about 70% of the SG neurons tested under similar conditions (Table 2). The amplitudes of the ATP-induced currents, while varying from one neuron to another, were concentration dependent over the range tested (0.5 to 5 mM). For ATP concentrations of 3 mM, the inward current in different neurons ranged from a few to more than 200 pA, averaging 86 ± 49 pA (n = 46). The slope of the rising phase of this current also varied from one neuron to another, with a mean of 4.5 ± 5.2 second (n = 46) for 10–90% rise times. Figure 2 shows examples of the limits of differences we

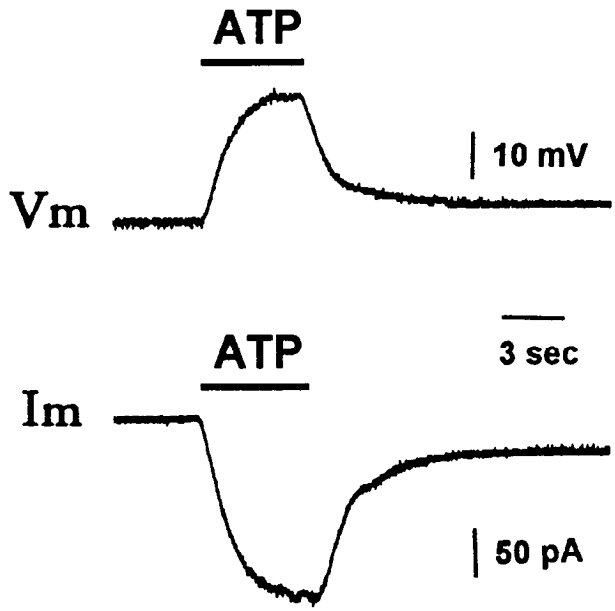

Figure 1. Activation of a SG neuron by ATP. *Top,* Current clamp recording of a SG neuron showing membrane depolarization by ATP (3 mM). Holding current of −10 pA was used (see Materials and Methods). *Bottom,* Voltage-clamp recording of ATP (3 mM)-induced current in the same neuron. Both recordings obtained with Cs-TEA pipette solution and with TTX (1 μM) in the superfusion.

observed in the rising phase of the currents. In many cases, ATP currents with a rapid-rising phase also declined rapidly to a steady state (Fig. 2*A*). ATP currents with a slower rising slope typically increased gradually in amplitude to a plateau and persisted (Fig. 2*B*).

In a given neuron, the GLU- and ATP-induced currents had similar rising phases and like adaptations to persisting application of the agents (e.g., Fig. 2), although they differed in other aspects. The GLU-evoked currents, regardless of rate of rise, were consistently blocked by 5 μM CNQX (6-cyano-7-nitroquinoxaline-2,3-dione), a non-NMDA GLU receptor antagonist. Therefore, GLU-induced currents, in spite of differences in rate of rise, appeared to be the non-NMDA type; we presumed the variation in the rising phase resulted from differential diffusion of the agent to neurons variously located in the slice. The sim-

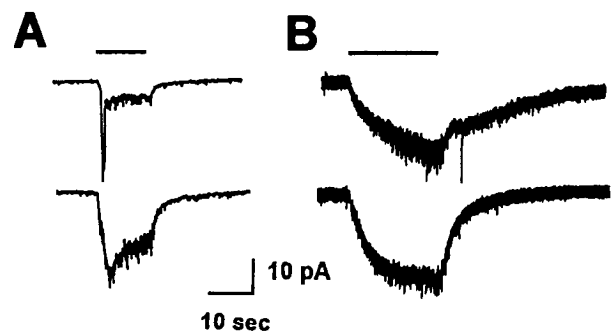

Figure 2. Comparison of ATP and GLU-activated inward currents in the same neurons. Panels *A* and *B* are records from two different neurons. The *upper trace* in each panel is the ATP (3 mM) current, and the *lower trace*, the GLU (1 mM) current. Note the similarity of the slope of initial phases for each neuron. All recordings were performed with Cs-TEA pipette solution with TTX (1 μM) in the superfusion. The *horizontal bars* indicate the times of drug application to the superfusion.

Table 2. Classification of the SG neurons tested by both GLU and ATP application

	Excitatory	Inhibitory	Biphasic	Unresponsive	Total
ATP only	6	—	1	—	7
ATP & GLU	20	—	11	—	31
GLU only	—	8	—	10	18
Neither	—	5	—	17	22

"ATP only," "ATP & GLU," "GLU only," and "Neither" designate neurons that are *excited* by the indicated agent. "Excitatory," "Inhibitory," "Biphasic," and "Unresponsive" denote the type of ATP effect observed (see Table 1).

ilarity in the rising phase and adaptation of ATP- and GLU-induced currents in a neuron made it likely that the various time courses observed for the ATP currents also were a product of differential access in the slice preparation. For this reason, we did not classify ATP-induced inward currents on the basis of the dynamics of the evoked response. Most of the observations presented here were made on relatively rapidly rising currents because this variety often was larger.

The current–voltage (*I/V*) relationship of the conductance activated by ATP was measured in nine neurons. In five neurons the current induced by ATP gradually decreased to zero as the holding potential was changed systematically from −90 to 0 mV (e.g., Fig. 3*A*). This was taken to indicate that in these neurons the equilibrium potential for the ATP current was at, or close to, zero transmembrane potential.

The ATP current showed strong inward rectification; intracellular potentials as large as +50 mV did not generate significant amounts of current (Fig. 3*A*). In contrast, in the same neurons GLU initiated significant outward currents at positive holding potentials (Fig. 3*B*). Inward rectification of the ATP-induced current was partially related to intracellular Mg^{2+} because when a Mg^{2+}-free solution was used for the recording pipette (two neurons), a clear reversal occurred at positive potentials (e.g., Fig. 4). The reversal potentials for the ATP-evoked currents were 0 and +20 mV in two neurons recorded with Mg^{2+}-free pipette solution. The reversal potential of GLU-evoked currents also ranged from 0 to +30 mV (e.g., Fig. 3*B*). Thus, the current-voltage properties of the ATP-activated inward conductance generally matched those of the nonselective cation channels activated by GLU.

ATP produces inward current through P_2 purinergic receptors

The relative effectiveness of various purine derivatives of ATP were used to gain insight on the receptor type mediating the fast inward current. Exogenous ATP was consistently more potent than ADP (adenosine diphosphate) and neither AMP (adenosine monophosphate) nor adenosine evoked appreciable inward current (Fig. 5*A*). GTP (guanosine triphosphate; tested in two cells) and UTP (uridine triphosphate; tested in one cell) were found to be much less effective than ATP in inducing an inward current, suggesting that the ATP action was selective. Therefore, mediation of the ATP inward current in SG neurons appeared to be

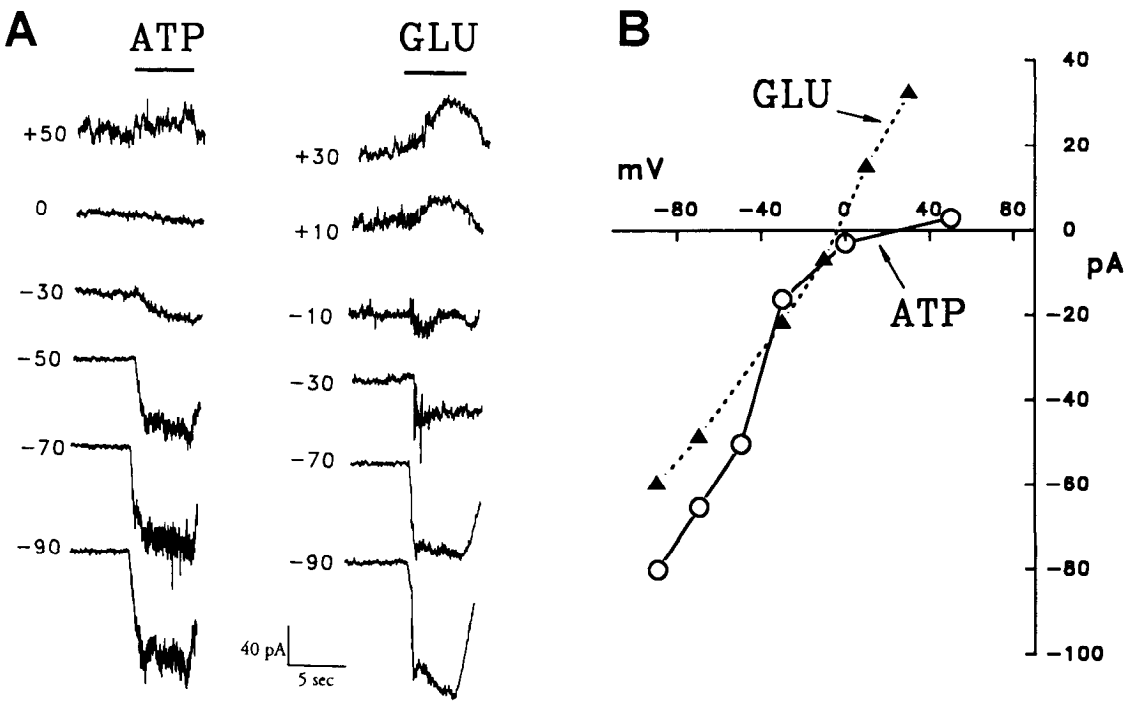

Figure 3. Comparison of current–voltage (*I/V*) properties evoked in a SG neuron by superfusion with ATP (5 mM) and GLU (1 mM). Recordings obtained using Cs-TEA pipette solution under voltage-clamp conditions in the presence of TTX (1 μM) in the superfusion. *A*, ATP- and GLU-currents at various holding potentials indicated in mV on the *left*. *B*, Plot of the peak current as function of holding potential from the data of *A*. The amplitudes of a current trace was determined from the average of 500 points (0.85 msec/point) bracketing the peak value.

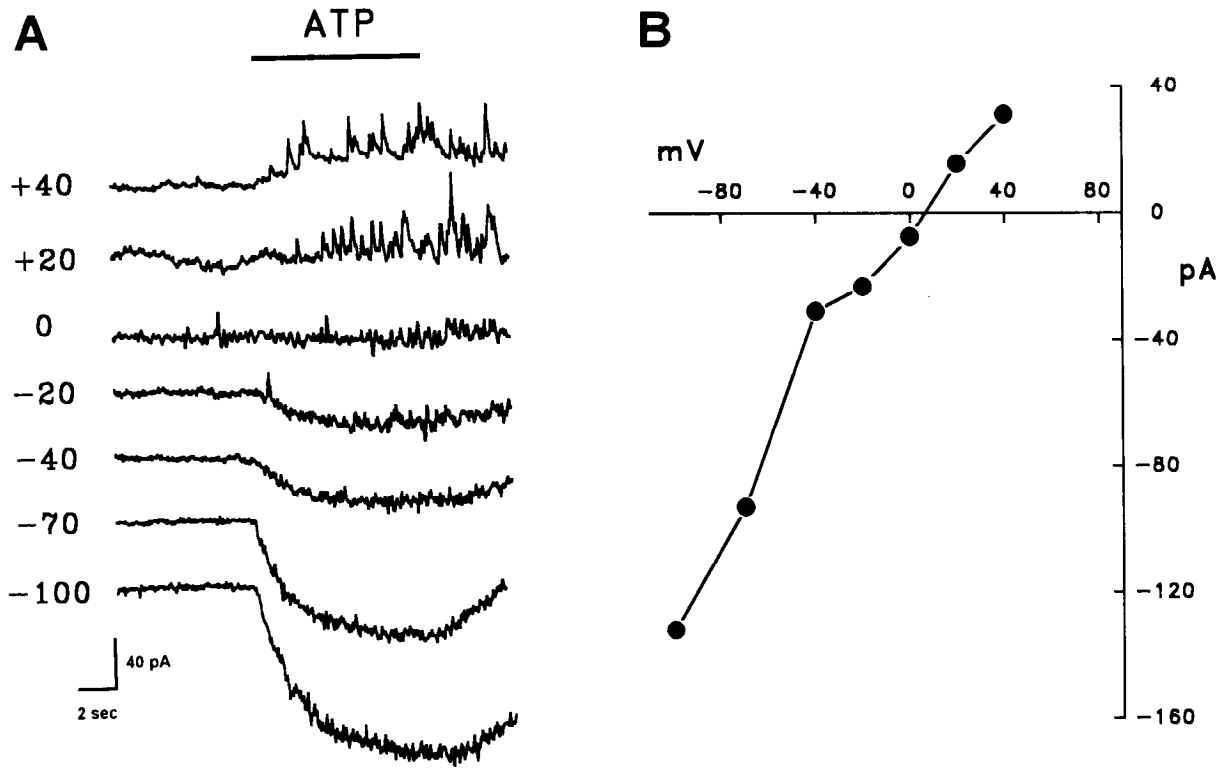

Figure 4. Current–voltage relationships of ATP-induced inward currents in a SG neuron. Records obtained with Mg^{2+}-free pipette solutions. *A*, ATP-induced current at various holding potentials (indicated in mV to the *left*). Note the outward current at the +40 mV holding potential. *B*, Plot of peak ATP current amplitude as function of holding potential. See Figure 3*B* legend for additional details.

through the P_2 type of purinergic receptor (see Burnstock and Buckley, 1985), a conclusion supported by the lack of effect on the ATP inward current by both 5 μM of CNQX and the nonspecific P_1 purinergic receptor antagonist, theophylline (1 mM). The nonhydrolyzable analog of ATP, ATPγS [adenosine-5′-*o*-(3-thiotriphosphate)], proved much more potent than ATP in evoking inward current in all five neurons tested. As shown in Figure 5*B*, ATPγS produced a current that had the same amplitude and lasted substantially longer than that induced by a magnitude greater concentration of ATP. Suramin, a P_2 antagonist in several other cellular systems (Dunn and Blakeley, 1988; Nakazawa et al., 1990) substantially reduced the inward current generated by ATP (Fig. 5*C*); this provides additional support for the concept that the ATP depolarization was mediated by a P_2 type of purinergic receptor. Suramin, in concentrations sufficient to suppress the ATP inward current, had no demonstrable effect upon membrane potential and input resistance. Even prolonged washings in normal ACSF did not always reverse the suramin suppression of ATP excitation.

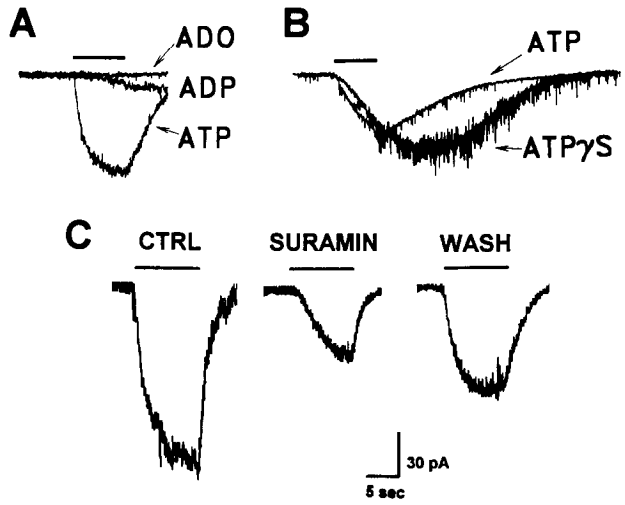

Figure 5. Pharmacological properties of ATP-activated inward currents in SG neurons. *Horizontal bars* indicate the application of agents in the superfusion. Recordings made with Cs-TEA pipette solution in the presence of TTX (1 μM) in the superfusion. *A*, Comparison of ATP, ADP, and adenosine (*ADO*) effects (all at 3 mM). *B*, Comparison of ATP (3 mM) and ATPγS (0.3 mM) currents in another SG neuron. *C*, Effects of suramin (0.5 mM) on ATP (5 mM) inward current for a third neuron.

Suramin, in concentrations of 0.5 or 1 mM, while consistently suppressing the amplitude of the inward ATP current, had little effect on the amplitude of dorsal root evoked EPSCs. In trials on six neurons a modest increase (20%) in EPSC latency in one neuron was the only demonstrable suramin effect on the dorsal root evoked response. In contrast, CNQX (5 μM) consistently and substantially suppressed dorsal root-evoked EPSCs unaffected by suramin (Fig. 6).

ATP potentiation of GLU currents

The dorsal root-evoked EPSCs, resistant to suramin, had the stability of latency expected for a monosynaptic connection (Li and Perl, 1994). Therefore, while ATP induces a fast inward current in many SG neurons, it does not appear to be the principal mediator of fast EPSCs produced by primary afferent activity. To examine alternative possibilities, ATP's action upon GLU-induced currents was evaluated. Simultaneous application of ATP and GLU did not significantly enhance the fast inward

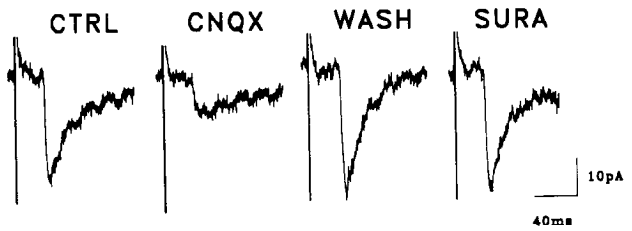

Figure 6. Effects of antagonist on dorsal root-evoked EPSCs in a SG neuron. CNQX (5 mM) or suramin (SURA, 0.5 mM) were applied in the superfusion prior to the indicated test. Each trace is an average of three trials. Recordings obtained containing Cs-TEA pipette solution.

current produced by GLU. On the other hand, if ATP was applied 5–10 sec prior to GLU, the GLU current was considerably increased in 6 of 11 neurons. In the example shown in Figure 7*A*, ATP by itself caused a fast inward current of short duration. GLU applied after the ATP conditioning resulted in a marked increase compared to the current induced by GLU alone. The enhanced GLU current was much larger than the summation of the fast currents independently induced by the two agents. ATP potentiation of GLU action appeared to be specific. GLU did not enhance ATP-induced currents. Sequential applications of either GLU or ATP alone did not result in measurable potentiation.

There was close correlation between the magnitude of the ATP-produced inward current and the enhancement of GLU-evoked current. Neurons that exhibited small ATP currents had only small ATP-induced increases of the size of GLU currents (Fig. 7*B*). If ATP did not evoke a fast inward current, it did not potentiate the GLU current. Interestingly, in one neuron suramin, which inhibited the ATP current, did not notably affect the ATP-induced potentiation, suggesting that the potentiation was not directly linked to the inward current. Neither adenosine nor AMP altered GLU current in a neuron that showed a sizable enhancement by ATP.

The potentiation of GLU-activated inward currents by ATP opens the possibility that this agent acts as an excitatory synaptic modulator. However, ATP is rapidly hydrolyzed to adenosine, which generates an outward current in many SG neurons (Li and Perl, 1994). This ATP outward current would mask excitatory actions. Therefore, to test ATP effects on evoked synaptic action it was necessary to study neurons unaffected by adenosine. As shown in Figure 8, ATP enhanced the dorsal root evoked EPSC by approximately 25% in a neuron that was not inhibited by adenosine. The ATP potentiation of GLU currents and this action on evoked EPSCs suggests that ATP can act postsynaptically to enhance the glutamatergic synaptic transmission in the SG.

Indirect effects of ATP mediated by P_1 purinergic receptors

In experiments using potassium pipette solutions, ATP was noted to produce a membrane hyperpolarization or, under voltage clamp, a delayed slow outward current. In many neurons recorded from by potassium containing pipettes, ATP evoked a biphasic response, i.e., an initial inward current followed by an outward current (Fig. 9*B*). Table 1 classifies observations obtained on the ATP responses with potassium internal electrode solutions according to whether they were excitatory, inhibitory, or biphasic.

In a given neuron, adenosine in the superfusion mimicked the delayed ATP outward current (Fig. 9*A*). In contrast to its suppression of the fast inward current, the ATP slow outward current was not affected by suramin (Fig. 9*A*). Adenosine, ADP, and AMP evoked only slow outward currents in neurons exhibiting a biphasic ATP response (e.g., for adenosine; Fig. 9*B*). The delayed slow outward current elicited by ATP appeared to result from adenosine produced by hydrolysis of ATP. This supposition was supported by the parallel suppression of ATP and adenosine hyperpolarizations by theophylline (200–500 μM), an adenosine receptor blocker (Fig. 9*C*). Furthermore, ATPγS (0.5 μM), a nonhydrolyzable ATP analog, mimicked the ATP depolarization but not its hyperpolarization (Fig. 9*D*).

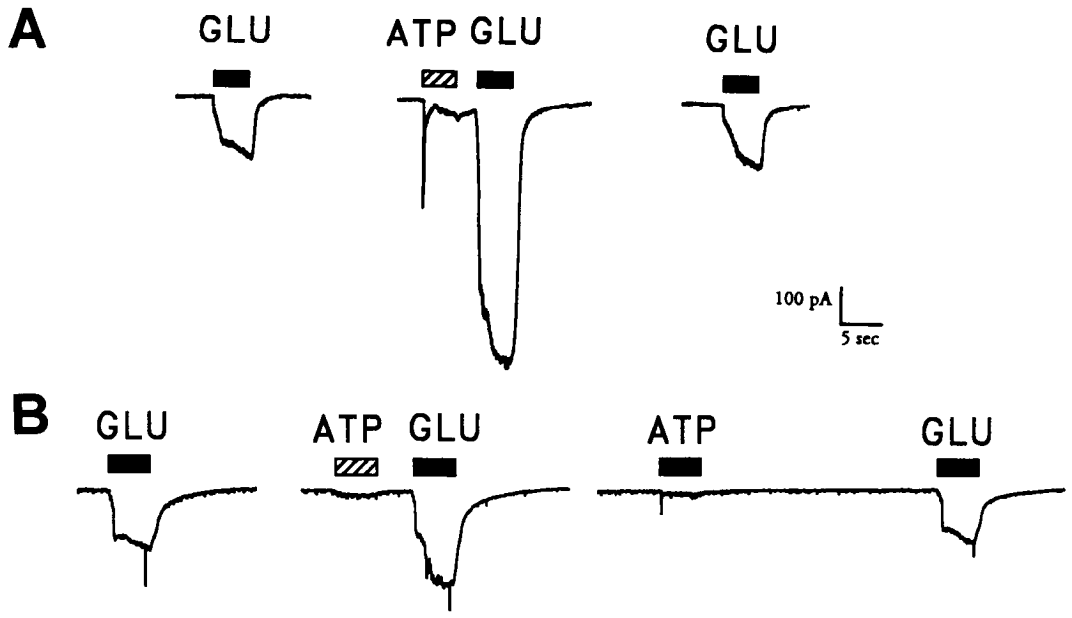

Figure 7. ATP action on GLU-evoked currents in SG neurons. *A* and *B* are from different cells. *Solid* and *hatched bars* represent the time of application of GLU (1 mM) and ATP (3 mM) in the superfusion fluid, respectively. Recordings obtained using Cs-TEA pipette solution with TTX (1 μM) in the superfusion.

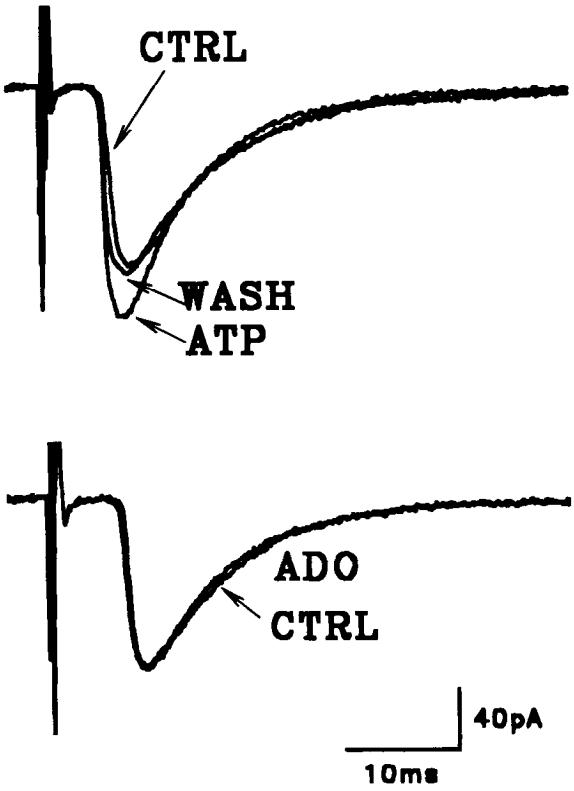

Figure 8. ATP and adenosine (*ADO*) effects on dorsal root EPSCs in a SG neuron. Each trace is the average of three trials. Voltage-clamp recording with potassium pipette solution. *Top*, ATP (1 mM) in superfusion. *Bottom*, Adenosine (0.5 mM) in superfusion. Control and wash (initial) after change to superfusion solution in each instance.

ATP did not initiate an outward current when recording was done with Cs^+-TEA containing electrodes. In other work we showed that adenosine activates a potassium conductance in SG neurons (Li and Perl, 1994). *I/V* relationships of the ATP-evoked outward currents in three cells obtained under voltage clamp gave reversal potentials at about −90 mV (not shown), consistent with the ATP outward current to result from increased potassium conductance.

Discussion

The ATP induction of a fast inward current in a substantial number of *in situ* neurons of the mammalian spinal substantia gelatinosa suggests an excitatory synaptic action. The spinal cord slices used for the observations were from young, free-ranging animals and, therefore, presumably represent features of mature spinal cord. In the center of the SG, where we usually sampled, dorsal root evoked EPSCs were consistently inhibited by non-NMDA GLU receptor antagonists (Schneider and Perl, 1988; Yoshimura and Jessell, 1990; Li and Perl, 1992, 1994). Suramin, which suppressed ATP-evoked currents in the same neurons, did not measurably depress dorsal root initiated EPSCs in these neurons. The primary afferent input to the SG stems mainly from unmyelinated or thinly myelinated fibers (Ranson, 1913; Light and Perl, 1979a,b; Sugiura et al., 1986). Thus, it is unlikely that ATP is a principal mediator of fast synaptic currents at central terminals of most thin primary afferent fibers. A variety of observations suggest L-glutamate or a similar amino acid to serve that function in the mammalian SG (Johnson and Aprison, 1970; Zieglgansberger and Puil, 1973; Jahr and Jessell, 1985; Jessell et al., 1986; Schneider and Perl, 1988; Yoshimura and Jessell, 1990; Li and Perl, 1992, 1994).

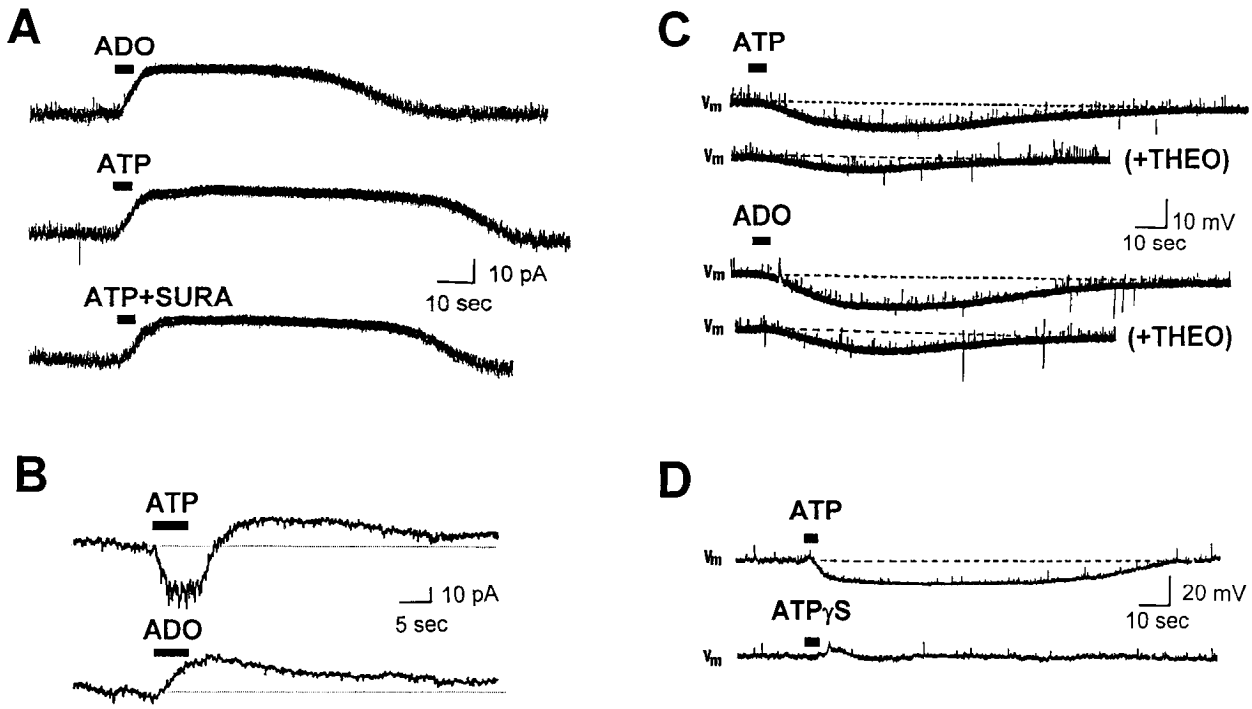

Figure 9. Adenosine-like actions of ATP applied in superfusion to SG neurons. *A*, Adenosine (*ADO*; 0.5 mM) and ATP (3 mM) initiated slow outward currents. ATP + SURA illustrates lack of suramin effect on the ATP current. *B*, ATP (5 mM) and ADO (1 mM)-evoked currents in the same neuron. *A* and *B* are voltage-clamp recordings. *C*, Theophylline (0.3 mM) effects on ATP (5 mM) and adenosine (1 mM) produced membrane hyperpolarization. *D*, Comparison of superfusion of ATP (5 mM) and ATPγS (0.5 mM) on membrane potentials. *C* and *D* are current-clamp recordings. All the records (*A–D*) were obtained with TTX (1 μM) in the superfusion and potassium solutions in the recording pipettes.

Our observations do provide an alternative function for ATP in synaptic transmission to SG neurons. There is sound evidence favoring the packaging of ATP in synaptic vesicles (Dowdall et al., 1974; Nagy et al., 1976; Carlson et al., 1978; Tashiro and Stadler, 1978) and the release of ATP by synaptic activity (Fredholm et al., 1982; Levitt et al., 1984) or manipulations causing release of neurotransmitters (White et al., 1985; Unsworth and Johnson, 1990). Therefore, the enhancement or facilitation of GLU suggests that ATP acts to potentiate GLU excitation at some synaptic junctions in the spinal SG. The long latency of the ATP potentiation of GLU current raises the possibility that ATP is involved in certain kinds of activity-dependent, short-term synaptic plasticity. For example, the synaptically released ATP may have little effect on the concurrent glutamatergic synaptic transmission, but would enhance the synaptic efficacy immediately following nerve impulses.

In contrast to the ATP-induced inward currents, the ATP potentiation of GLU currents was not blocked by suramin. The substantial delay in the ATP potentiating effect suggests that it may operate through a second messenger system, whereas the fast ATP current appears to be the product of ligand-gated channels. This difference is consistent with mediation of the two effects by different purinergic receptor subtypes. Receptor phosphorylation by both Ca/calmodulin-dependent protein kinase II (CaM Kinase II; McGlade-McCulloh et al., 1993) and protein kinase A (Raymond et al., 1993; Wang et al., 1993) have been reported to potentiate non-NMDA GLU receptor currents. ATP can activate phospholipase C (PLC) to elevate the intracellular level of Ca^{2+} through P_{2Y} or P_{2U} purinergic receptors (Pirotton et al. 1987; Cooper et al., 1989; Hirano et al. 1991; O'Connor et al., 1991; Murrin and Boarder, 1992).

In vivo, electrophoresed ATP preferentially excites a subgroup of dorsal horn neurons that are activated by innocuous mechanical stimuli (Fyffe and Perl, 1984; Salter and Henry, 1985). Much of the latter afferent input to the spinal cord involves large-diameter myelinated fibers that principally terminate deep to the SG. Our studies focused on neurons of the SG and, therefore, did not address the role of ATP for such deeper projections. Given that ATP causes a fast excitation in SG neurons and that it does act as the principal fast excitatory transmitter at other synapses (Burnstock, 1990; Edwards, et al., 1992; Evans et al., 1992), it also remains possible that ATP acts as a synaptic mediator for descending fibers or interneurons in the SG or other areas of the spinal dorsal horn.

Mimicry of the ATP fast inward current in SG neurons by ATPγS supports the idea that the ATP action is mediated by P_2 purinergic receptors and is not a product of energy release by ATP hydrolysis. The ATP-induced inward current appears too rapidly to be consistent with a kinase-mediated thiophosphorylation (Gratecos and Fischer, 1974). The short latency and brief duration of the ATPγS inward current also does not fit an irreversible protein thiophosphorylation triggered by ATPγS activation of protein kinases (Gratecos and Fischer, 1974; Sherry et al., 1978; Cassidy et al., 1979) and ectoprotein kinases (Ehrlich et al., 1990). Jahr and Jessell (1983) showed that the ATP-induced fast excitation of dorsal horn neurons in culture is not the result of chelation of divalent ions; disodium, magnesium, calcium, and Tris salts of ATP proved equally effective in depolarizing the neurons, but EDTA and pyrophosphate were ineffective. For these reasons, we conclude the ATP-induced inward current is a direct effect mediated by P_2 receptors, possibly of the P_{2X} type, since this subtype appears to be a ligand-gated channel (Burnstock, 1990).

One conceivable mechanism for an ATP potentiation of glutamatergic synaptic current is disinhibition of the NMDA component through chelation of Mg^{2+} (Mayer et al., 1984; Nowak et al., 1984). An ATP-induced potentiation based on NMDA action implies a long time course (Wieraszko and Seyfried, 1989) since NMDA-mediated glutamatergic synaptic excitation has a slower rise time and slower decay than non-NMDA events (Dale and Roberts, 1985; Collingeridge et al., 1988; Forsyth and Westbrook, 1988; Bekkers and Stevens, 1989; Hestrin et al., 1990). In our experiments, ATP increased the amplitude of evoked EPSCs, but changed neither their rise time nor duration (e.g., Fig. 8, top). This argues against involvement of NMDA receptors in the ATP potentiation.

The rise time and reversal potentials of the fast inward current evoked by ATP and by GLU were similar. On the other hand, the current–voltage characteristics of the two currents were distinctly different in several ways. Only the ATP currents showed strong inward rectification (Fig. 3). The ATP currents lacked the negative slope attributed to NMDA receptor activation (Mayer and Westbrook, 1984). Even in the same neurons, the ATP and GLU currents showed substantial differences in kinetics (compare Fig. 2). Finally, the ATP initiated currents were sensitive to suramin but not to CNQX, while the sensitivity of GLU currents to the two antagonists was the reverse.

Voltage-clamp experiments on *in situ* neurons with the complex dendritic tree typical of the SG are problematic, since clamping spatially distant arborizations would not be possible. Nevertheless, the *I/V* characteristics in our observations were similar to those found for ATP currents in other tissues: (1) from acutely dissociated dorsal root ganglion neurons with a much simpler geometry (Krishtal et al., 1983, 1988; Bean, 1990; Bean et al., 1990), (2) from smooth muscle (Benham and Tsien, 1987), (3) from sympathetic neurons (Nakazawa, 1994). The characteristics of GLU induced currents recorded in the experiments were also similar to those reported in cultured spinal neurons (Mayer and Westbrook, 1984). These similarities to other situations suggest a reasonably good voltage control of the recorded neurons for the currents analyzed in the present observations. Therefore, reversal potentials near zero for the ATP current would appear to represent a reasonable estimate. Since this reversal is essentially identical to that of GLU current, it implies that ATP and GLU act through similar nonselective cation channels.

The significance of the effects deduced to result from ATP hydrolysis to adenosine are intriguing but difficult to assess. Ectonucleotidases rapidly hydrolyze ATP. From this it can be expected that ATP released by presynaptic terminals in the SG would lead to the presence of adenosine in synaptic regions (Slakey et al., 1986; Welford et al., 1987; Culic et al., 1990; Torres et al., 1990; James and Richardson, 1993). A high density of adenosine receptors has been shown in the SG. An enzyme catalyzing the conversion of AMP to adenosine 5′-nucleotidase with both intracellular and extracellular isoforms is also concentrated in the region (Zimmerman, 1992). Inasmuch as adenosine has potent pre- and postsynaptic inhibitory actions in SG neurons (Li and Perl, 1994), it is conceivable that synaptically released ATP after enzymatic conversion to adenosine could also have inhibitory effects through action on P_1 receptors. The complex synaptic geometry in the SG, in which some thin primary afferent fibers form arrangements with a central terminal sur-

rounded by synaptic junctions with excitatory and inhibitory features (Maxwell and Réthelyi, 1987) could support a form of biphasic action. At the same time, the high concentrations of adenosine itself in this part of the spinal cord makes such an indirect effect a less compelling concept.

The SG has been linked to pain mechanisms for many years on the basis of its relationship to the smaller diameter primary afferent fibers and more recently from demonstration of nociceptive neurons within it; however, it serves other sensory functions as well (Pearson, 1952; Perl, 1984). The synaptic modulatory actions we attribute to ATP only could be demonstrated for some SG neurons. Therefore, ATP modulation cannot be automatically assigned to nociceptive functions. In fact, earlier work from this laboratory suggests that ATP excitatory action on SG neuron recorded *in vivo* occurs for neurons most effectively excited by innocuous mechanical stimuli (Fyffe and Perl, 1984). Regardless, ATP's facilitation of glutamate excitation adds another facet to the complex synaptic arrangements in this part of the spinal cord.

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