Modulation of N-Type Ca\(^{2+}\) Currents by A\(_1\)-Adenosine Receptor Activation in Male Rat Pelvic Ganglion Neurons

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ABSTRACT

Modulation of voltage-activated Ca\(^{2+}\) channels by adenosine was investigated in male rat major pelvic ganglion (MPG) neurons by using the whole-cell variant of the patch-clamp technique. Adenosine inhibited high voltage-activated (HVA) Ca\(^{2+}\) currents in a concentration-dependent manner with an EC\(_{50}\) of 313 nM and a maximal inhibition of 36%, respectively. Inhibition of HVA Ca\(^{2+}\) currents in adrenergic and cholinergic MPG neurons was similar. Adenosine did not modulate T-type Ca\(^{2+}\) channels present in adrenergic MPG neurons. Reverse transcription-polymerase chain reaction analysis indicated that MPG neurons express mRNAs encoding A\(_1\) and A\(_2a\) receptors. Ca\(^{2+}\) current inhibition by adenosine was mimicked by \(N^\circ\)-cyclopentyladenosine, an A\(_1\)-selective agonist, and prevented by 100 nM \(8\)-cyclopentyl-1,3-dipropylxanthine, an A\(_2a\)-selective antagonist. Conversely, CGS 21680, an A\(_2a\)-selective agonist, displayed a relatively low potency (EC\(_{50}\) = 2200 nM) for inhibiting Ca\(^{2+}\) currents. The action of adenosine was significantly attenuated by 2 mM guanosine-5'-thiodiphosphate or 500 ng/ml pertussis toxin. The voltage dependence of adenosine-induced current inhibition was evident by 1) a bell-shaped profile between the current inhibition and test potentials, 2) kinetic slowing in the presence of agonist, and 3) relief of the current inhibition by a conditioning prepulse to +80 mV. Finally, 1 \(\mu\)M \(\omega\)-conotoxin GVIA occluded adenosine-induced current inhibition. Taken together, we concluded that adenosine inhibits N-type Ca\(^{2+}\) currents by activation of A\(_1\) receptors via a voltage-dependent and PTX-sensitive pathway in rat MPG neurons. Our data may explain how adenosine acts as an inhibitory modulator of ganglionic and neuromuscular transmission in the pelvic plexus.

The pelvic ganglia provide autonomic innervation to the lower bowel and various urogenital organs, including the urinary bladder, prostate, and penis (for review, see Keast, 1999). Physiologically, the ganglia play important roles in various autonomic reflexes, including micturition and penile erection (de Groat and Booth, 1993a; de Groat et al., 1993). A distinctive feature of the pelvic ganglia that differentiate them from other autonomic ganglia is the colocalization of both sympathetic and parasympathetic postganglionic neurons within the same ganglion capsule (Keast, 1999). Anatomical structures of the pelvic ganglia show variability among different species and genders. Because of their relatively simple anatomy and thus, ease of isolation, manipulation and quantification (Keast, 1999), male rat pelvic ganglia, termed the major pelvic ganglia (MPG), have been used as a model system for studying physiological and pathophysiological aspects of the neural control of pelvic viscera.

MPG neurons are known to express various putative neurotransmitters, including neuropeptide Y (NPY), vasoactive inhibitory peptide, and nitric oxide in addition to classical neurotransmitters such as norepinephrine and acetylcholine (Keast and de Groat, 1989; Keast, 1995; Zhu et al., 1995). Electrophysiological studies have shown that these neurotransmitters act as modulators of voltage-activated Ca\(^{2+}\) channels, which play important roles in synaptic transmission and neuronal excitability (Zhu et al., 1995; Zhu and Yakel, 1997). Indeed, MPG seem to function as a potential integration site where both adrenergic and cholinergic synaptic transmission toward effector organs would be edited (Theobald and de Groat, 1989; Zoubek et al., 1993; Warren and Lavvidis, 1996; Félix et al., 1998; Keast, 1999).

Considerable evidence has accumulated suggesting that purines such as ATP and adenosine act as neurotransmitters/modulators in the pelvic plexus (De Groat and Booth, 1993b). Generally, ATP exerts excitatory effects via \(P_2\) receptors on the urogenital smooth muscles, including the vas deferens. However, adenosine is known to inhibit ganglionic transmission in the pelvic plexus as well as in other autonomic ganglia (Keast and de Groat, 1989; Keast, 1995; Zhu et al., 1995). HVA Ca\(^{2+}\) currents play a key role in synaptic transmission in the pelvic plexus.
deferens, urinary bladder, and urethra (Fujii, 1988). A recent study has shown that ionotropic P_{2x} receptors are also present in rat MPG neurons although their roles in excitatory ganglionic transmission are unclear (Zhong et al., 1998).

In comparison with ATP, adenosine is known to produce inhibitory effects on ganglionic and neuromuscular transmission via adenosine (P_{1}) receptors presumably located on post-ganglionic pelvic neurons and nerve terminals (Akasu et al., 1984; Theobald and de Groat, 1989). To date, however, the nature of the adenosine receptor subtype and the cellular mechanisms underlying the inhibitory actions of adenosine remain unclear. In preliminary experiments with rat MPG neurons, we observed that adenosine, but not ATP, was capable of inhibiting voltage-activated Ca^{2+} currents. In the present study, thus, we identified the subtype of adenosine receptor and the signaling pathway responsible for the adenosine-induced Ca^{2+} current inhibition in rat MPG neurons by using patch-clamp and RT-PCR techniques. Our data suggest that adenosine inhibits N-type Ca^{2+} currents by activation of A_{1} receptors via a voltage-dependent and pertussis toxin (PTX)-sensitive pathway in rat MPG neurons.

**Materials and Methods**

**Preparation of Pelvic Ganglion Neurons.** MPG neurons were enzymatically dissociated with some modifications of the method described previously (Zhu et al., 1995). Briefly, adult (200–300 g) male Sprague-Dawley rats were anesthetized with pentobarbital sodium (50 mg/kg i.p.). MPG clusters were dissected out from the lateral surface of the prostate gland and placed in cold Hank’s balanced salt solution. The ganglia were then deassembled, cut into small pieces, and incubated in Earle’s balanced salt solution containing 0.7 mg/ml collagenase type D (Roche Molecular Biochemicals, Indianapolis, IN), 0.1 mg/ml trypsin type I (Roche Molecular Biochemicals), and 0.1 mg/ml DNase type I (Sigma Chemical, St. Louis, MO) at 35°C for 1 h in a shaking water bath. After incubation, ganglia were dispersed into single neurons by vigorous shaking of the culture flask containing the ganglia. After centrifugation at 50g, the neurons were resuspended in RPMI 1640 containing 10% fetal calf serum and 1% penicillin-streptomycin (all from Invitrogen, Carlsbad, CA). Neurons were then plated onto culture dishes (35-mm) coated with poly-L-ornithine and maintained in a humidified atmosphere (95% air, 5% CO2 incubator at 37°C) for 2 days. As appropriate, neurons were incubated overnight 37°C after plating. As appropriate, neurons were used within 24 h.

**RT-PCR Analysis.** Total RNA from dissociated MPG neurons was prepared using a modified guanidinium thiocyanate-phenol-chloroform extraction method (Chomczynski and Sacchi, 1987). Synthesis of the first strand of cDNA was performed in an RT-PCR buffer containing 2 μg of total RNA, 25 of nmol of dNTP, 0.5 μg of random hexamer, 20 U of RNase inhibitor, and 200 U of murine leukemia virus reverse transcriptase (all from Promega, Madison, WI) in a final volume of 25 μl at 37°C for 60 min. Specific sense and antisense primer pairs were designed based on the known cloned rat adenosine receptor sequences deposited in GenBank (Table 1). Single-stranded cDNA products were denatured at 94°C for 5 min and then subjected to PCR amplification (32 cycles). Each PCR cycle consisted of denaturing at 94°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 1 min in a GeneAmp thermocycler (PerkinElmer Instruments, Norwalk, CT). The PCR buffer (50 μl) contained the transcribed cDNA, 10 pmol of primers, 10 nmol of dNTP, and 1.25 U of Taq polymerase (PerkinElmer Instruments). As a PCR control, amplification of rat 28S RNA was performed for 24 cycles under the same temperature conditions. The resultant PCR products were separated and visualized on a 1.1% agarose gel containing ethidium bromide.

**Electrophysiology.** Ca^{2+} channel currents were recorded using the whole-cell variant of the patch-clip technique. Patch electrodes were fabricated from a borosilicate glass capillary (Corning 7052; Garner Glass Co., Claremont, CA) by using a P-97 Flaming Brown micropipette puller (Sutter Instrument Co., San Rafael, CA). The patch electrodes were fire polished on a microforge (Narishige, Tokyo, Japan) and had resistances of 1 to 3 MΩ when filled with the internal solution described below. An Ag/AgCl wire was used to ground the bath. The cell membrane capacitance and series resistance were compensated (>80%) electronically using the patch-clamp amplifier (Axopatch 1D; Axon Instruments, Foster City, CA). Voltage protocol generation and data acquisition were performed using pClamp 6.03 software on an IBM computer equipped with an analog-to-digital converter (Digidata 1200; Axon Instruments). Current traces were filtered at 2 to 5 kHz by using the four-pole Bessel filter in the clamp amplifier and stored on the computer hard drive for later analysis.

**Solution and Drugs.** Ca^{2+} currents were isolated using patch electrodes filled with an internal solution containing 120 mM N-methyl-D-glucamine-heptanesulfonate (MS), 20 mM tetraethylammonium-MS, 20 mM HCl, 11 mM EGTA, 1 mM CaCl_2, 10 mM HEPES, 4 mM Mg-ATP, 0.3 mM Na_2-GTP, 14 mM creatine phosphate, pH 7.2. External recording solution contained 145 mM tetraethylammonium-MS, 10 mM HEPES, 10 mM CaCl_2, 15 mM glucose, 0.0003 mM tetrodotoxin, pH 7.4. Drugs were applied to single neurons via a gravity-fed fused silica capillary tube connected to an array of seven polyethylene tubes. The outlet of the perfusion system was located within 100 μm of the cell. The bath superfusion rate was approximately 1 to 2 ml/min. All experiments were performed at room temperature (20–24°C). Drugs used in experiments were obtained as follows: α-conotoxin GVIA (α-CgTx GVIA) from Peninsula Laboratories (Belmont, CA); guanosine-5’-thiodiphosphate (GDPβS), N^6-cyclopentyladenosine (CPA), CGS 21680, and 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) from Sigma/RBI (Natick, MA); and adenosine, tetrodotoxin, PTX, and nimodipine from Sigma Chemical. For stock solutions (1–100 mM), all drugs were dissolved in distilled water, filter sterilized, and stored at -20°C.

**TABLE 1**

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<th>Position</th>
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<td>CCT TGG CTG TGG TTT GCC T</td>
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S, sense; AS, antisense.
water except CPA and CGS 21680, which were dissolved in dimethyl sulfoxide.

Data Analysis. Amplitudes of step currents were usually determined isochronally 10 ms after the onset of a test pulse, normalized to membrane capacitance, and expressed as pA/pF. Membrane capacitance ($C_m$) was measured by applying a 20-ms, 10-mV hyperpolarizing step from a holding potential of $-80$ mV and calculated according to the following equation (Benitah et al., 1993): $C_m = \tau \cdot I / \Delta V_m$, where $\tau$ is the time constant of the capacitive current, $I_o$ is the maximum capacitative current value, $\Delta V_m$ is the amplitude of a voltage step, and $I_s$ is the amplitude of the steady-state current. Concentration-response curves and IC$_{50}$ values for half-maximal Ca$^{2+}$ current inhibition were obtained from fitting to a single-site binding isotherm with least-squares nonlinear regression. Data were presented as means $\pm$ S.E.M. Statistical significance was determined using Student's $t$ test, and $p < 0.05$ was considered significant.

Results

Characteristics of Ca$^{2+}$ Currents in MPG Neurons. Figure 1A illustrates inward Ca$^{2+}$ currents in a MPG neuron elicited by a voltage ramp to $+80$ mV from a holding potential of $-80$ mV. Based on the absence or presence of low-voltage-activated (LVA) T-type Ca$^{2+}$ currents, two different subpopulations of MPG neurons could be distinguished. According to a previous report (Zhu et al., 1995), adrenergic MPG neurons express T-type Ca$^{2+}$ channels, whereas cholinergic MPG neurons do not. In a subpopulation of the tested neurons, there were prominent voltage humps between $-50$ and $-20$ mV on the current-voltage (I-V) curves, indicating the presence of LVA Ca$^{2+}$ currents (Fig. 1A). The average $C_m$ of neurons expressing T-type Ca$^{2+}$ channels was $82 \pm 3$ pF ($n = 50$), compared with $47 \pm 3$ pF ($n = 54$) for neurons lacking T-type Ca$^{2+}$ channels ($p < 0.01$) (Fig. 1B). The correlation between $C_m$ (an indirect measurement of membrane surface area) and expression of T-type channels was consistent with previous findings in rat MPG neurons (Zhu et al., 1995). HVA Ca$^{2+}$ current density was not significantly different for neurons with or without T-type Ca$^{2+}$ channels ($44 \pm 4$ versus $49 \pm 5$ pA/pF, respectively; $p > 0.05$) (Fig. 1C).

Inhibition of Ca$^{2+}$ Currents by Adenosine. We tested whether adenosine modulates Ca$^{2+}$ currents elicited by the ramp protocol in MPG neurons. As shown in Fig. 2A, application of 30 $\mu$M adenosine significantly inhibited HVA, but not LVA Ca$^{2+}$ currents recorded from adrenergic neurons. The I-V relationships for the Ca$^{2+}$ currents in the absence or presence of adenosine are shown in Fig. 2B. On average, adenosine inhibited the peak Ca$^{2+}$ currents by $36 \pm 3\%$ ($n = 9$). We also determined whether Ca$^{2+}$ currents were differentially modulated by adenosine in adrenergic and cholinergic neurons. The degree of Ca$^{2+}$ current inhibition by adenosine at concentrations ranging between 0.01 and 30 $\mu$M was not different for the two subpopulations (Fig. 2C). This is in contrast to the previous findings for $\alpha_2$-adrenergic receptor-mediated Ca$^{2+}$ current inhibition in rat MPG neurons (Zhu and Yakel, 1997). Accordingly, we did not discriminate between the two subpopulations in the following experiments.

Identification of Adenosine Receptor Subtype Involved in Ca$^{2+}$ Current Inhibition. To identify subtypes of adenosine receptors expressed in MPG neurons, we performed RT-PCR with four pairs of primers specific to adenosine receptor isoforms (A$_1$, A$_{2a}$, A$_{2b}$, and A$_3$) (Table 1). We confirmed that all the primers did properly work in control PCR experiments by using the brain (A$_1$, A$_{2a}$, and A$_{2b}$) and the testis (A$_1$, A$_{2b}$, and A$_3$) (data not shown). In addition, the possibility of genomic DNA contamination was eliminated by PCR experiments without prior reverse transcription (data not shown). As shown in Fig. 3, A$_1$ and A$_{2b}$ receptor mRNAs, predicted as 205- and 371-bp products, respectively, were detected after the RT-PCR reaction in MPG neurons. In contrast, A$_{2b}$ and A$_3$ receptor mRNAs were not detected in MPG neurons.

To pharmacologically identify the receptor subtypes underlying adenosine-induced Ca$^{2+}$ current inhibition, normalized concentration-response curves for Ca$^{2+}$ current inhibition in the presence of different agonists were acquired (Fig. 4A). The EC$_{50}$, as determined with least-squares nonlinear regression, was $313$ and $63$ nM for adenosine and CPA (an A$_1$-selective agonist), respectively. In contrast, CGS 21680, an A$_2$-selective agonist, was much less potent (EC$_{50}$ = 2200 nM) compared with CPA. In other experiments, MPG neurons were pretreated with DPCPX (100 nM), an A$_1$-selective antagonist, for 10 min. DPCPX alone did not affect the amplitude of Ca$^{2+}$ currents but significantly blocked the inhibitory effects of adenosine and CPA on Ca$^{2+}$ currents (Fig. 4B). Furthermore, the inhibitory action of CGS 21680 was also prevented by DPCPX ($n = 3$; data not shown). In contrast, the effect of norepinephrine (NE) was not affected by DPCPX pretreatment, indicating specific actions on adenosine receptors. In addition, a P$_2$-purinergic agonist, ATP, also produced Ca$^{2+}$ current inhibition that was prevented by DPCPX, indicating that the inhibition was attributed to adenosine produced by a partial degradation of ATP. Other P$_2$-selective agonists, 2-methylthio ATP (100 $\mu$M; $n = 5$) and UTP (100 $\mu$M; $n = 6$), did not modulate the Ca$^{2+}$ currents

*Fig. 1. Whole-cell Ca$^{2+}$ currents in male rat MPG neurons. A, representative traces of inward Ca$^{2+}$ currents in adrenergic and cholinergic neurons. The Ca$^{2+}$ currents were elicited by voltage ramps from $-80$ to $+80$ mV in neurons held at $-80$ mV. Adrenergic neurons were distinguished from cholinergic neurons by the presence of low voltage-activated T-type Ca$^{2+}$ currents. B and C, summary of average $C_m$ and current densities (pA/pF) in adrenergic ($n = 50$) and cholinergic ($n = 54$) neurons, respectively. The peak Ca$^{2+}$ currents were elicited by test pulses to $+10$ mV from a holding potential of $-80$ mV. Current amplitudes were determined isochronally 10 ms after onset of test pulses and normalized to membrane $C_m$. Data represent the mean $\pm$ S.E.M. $^{***}p < 0.001$.}
that T-type Ca\textsuperscript{2+} elicited by the protocol described in Fig. 1 in an adrenergic neuron. Note A, representative traces of Ca\textsuperscript{2+} currents in absence (solid line) and presence (dotted line) of 30 \textmu M adenosine. The ramp currents were elicited by the protocol described in Fig. 1 in an adrenergic neuron. Note that T-type Ca\textsuperscript{2+} currents were not modulated by adenosine. B, averaged I-V relationship in the absence (○) and presence (●) of adenosine for adrenergic neurons (n = 9). C, concentration-response curves for adenosine-induced Ca\textsuperscript{2+} current inhibition in adrenergic (n = 9; ○) and cholinergic (n = 8; ○) neurons. Inhibition (%) was determined as (1 – I\textsubscript{adreno}/I\textsubscript{control}) × 100%. The smooth curves were obtained by fitting data to a single-site binding isotherm with a nonlinear least-squares regression program. In B and C, data represent the mean ± S.E.M.

Effects of GDP\beta S and PTX on Adenosine-Induced Ca\textsuperscript{2+} Current Inhibition. Dialysis of neurons with GDP\beta S, a hydrolysis-resistant GDP analog, has been shown to abolish the G protein-mediated effects of agonists by acting as a competitive inhibitor of GTP binding to the Gs subunits (Holz et al., 1986; Jeong and Wurster, 1997). As summarized in Fig. 5A, GDP\beta S significantly decreased the Ca\textsuperscript{2+} current inhibition produced by adenosine (from 33 ± 4%; n = 5 to 8 ± 1%; n = 12) and NE (from 47 ± 4%; n = 9 to 6 ± 1%; n = 11). In general, \textalpha\textsubscript{1} adenosine receptors are coupled to PTX-sensitive G\alpha\textsubscript{s} proteins (Fredholm et al., 1994; Ralevic and Burnstock, 1998). To identify the nature of G protein coupling between the adenosine receptors and Ca\textsuperscript{2+} channels, MPG neurons were incubated overnight in a medium containing PTX (500 ng/ml). The PTX treatment significantly attenuated the adenosine-induced Ca\textsuperscript{2+} current inhibition from 29 ± 2% (n = 9) to 9 ± 1% (n = 7). In control experiments, NE-induced inhibition was also reduced by PTX treatment from 40 ± 4% (n = 7) to 10 ± 1% (n = 11) (Fig. 5B). Taken together, these data suggest that adenosine-induced Ca\textsuperscript{2+} current inhibition is primarily mediated by PTX-sensitive G\alpha\textsubscript{s} proteins.

Voltage-Dependence of Adenosine-Induced Ca\textsuperscript{2+} Current Inhibition. As determined from the I-V curves in Fig. 2B, the relationship between the adenosine-induced Ca\textsuperscript{2+} current inhibition and test potentials displayed a "bell-shaped" profile (Fig. 6A). The voltage-dependent inhibition of Ca\textsuperscript{2+} currents by adenosine was also demonstrated using a double pulse protocol consisting of two identical test pulses to +10 mV separated by a large depolarizing conditioning pulse to +80 mV (Fig. 6B, bottom). The time course of the adenosine-induced Ca\textsuperscript{2+} current inhibition is shown in Fig. 6B (top). The adenosine-induced Ca\textsuperscript{2+} current inhibition displayed the hallmarks of voltage-dependent inhibition, i.e., kinetic slowing and relief of current inhibition by the conditioning pulses (Elmslie et al. 1990) (Fig. 6B). Facilitation, defined as the ratio of the postpulse to prepulse current amplitude, increased from 1.09 to 1.55 after adenosine application.

Modulation of ω-Conotoxin GVIA-Sensitive N-Type Ca\textsuperscript{2+} Channels by Adenosine. As established previously (Zhu et al., 1995; Zhu and Yakel, 1997), ω-CgTx GVIA-sensitive N-type Ca\textsuperscript{2+} channels contribute to the majority (60 ± 4%; n = 9) of HVA Ca\textsuperscript{2+} channel currents in MPG neurons (Fig. 7B). Thus, we tested whether N-type Ca\textsuperscript{2+} channels...
Discussion

Identical Modulation by Adenosine in Adrenergic and Cholinergic MPG Neurons. The present study describes identification of an adenosine receptor subtype and signal transduction pathway responsible for the adenosine-induced HVA Ca\(^{2+}\) current inhibition in rat MPG neurons. As described previously, the MPG contains distinct populations of adrenergic and cholinergic neurons providing motor inputs to pelvic effectors (Keast, 1999). Accordingly, it is possible that the expression level of a certain receptor and the resultant modulation of Ca\(^{2+}\) currents are phenotype-specific. For example, NE and NPY have been found to produce much larger Ca\(^{2+}\) current inhibition in adrenergic MPG neurons compared with cholinergic MPG neurons (Zhu et al., 1995; S. K. Cha, K. S. Park, and S. W. Jeong, unpublished observations). This phenotype-dependent differential modulation might be explained by the fact that both NE and NPY are colocalized in adrenergic nerve terminals in MPG neurons (Keast and de Groat, 1989; Keast, 1991; Keast, 1999). However, this is not the case for adenosine-induced Ca\(^{2+}\) current inhibition because the potency and efficacy of adenosine were almost identical in the two types of MPG neurons (Fig. 2C). This suggests that adenosine can act as an inhibitory modulator for both adrenergic and cholinergic MPG neurons (see below).

Identification of Adenosine Receptor Subtype Involved in Ca\(^{2+}\) Current Inhibition. Based on amino acid sequence and pharmacology, adenosine/P1 receptors are subdivided into four subtypes, A\(_1\), A\(_2\)a, A\(_2\)b, and A\(_3\) (Fredholm et al., 1994; Ralevic and Burnstock, 1998). A comprehensive study has revealed that all subtypes of adenosine receptor are expressed in rat brain tissues (Dixon et al., 1996). However, RT-PCR analysis delineated only A\(_1\) and A\(_2\)a in rat MPG neurons, suggesting tissue-specific distribution of adenosine receptors. Several lines of evidence indicate that adenosine-induced Ca\(^{2+}\) current inhibition is mainly mediated via A\(_1\) receptors in MPG neurons. First, CPA, an A\(_1\)-selective agonist, potently mimicked adenosine-induced Ca\(^{2+}\) current inhibition. In contrast, CGS 21680, an A\(_2\)-selective agonist, was an order of magnitude less potent than CPA. The rank order of potency for adenosine and adenosine analogs (CPA > adenosine > CGS 21680) in MPG neurons is reminiscent of that in superior cervical ganglion (SCG) neurons where activation of A\(_1\) receptors inhibits Ca\(^{2+}\) currents (Zhu and Ikeda, 1993). Second, pretreatment of DPCPX, the most widely used A\(_1\)-selective antagonist, completely blocked the
adenosine-induced Ca\(^{2+}\) current inhibition. Finally, the pharmacological data described above are consistent with the experiments with PTX. It is well established that A\(_1\) receptors are coupled to PTX-sensitive Go/i, whereas A\(_2\) receptors are coupled to cholera toxin-sensitive Gs (Ralevic and Burnstock, 1998). Recently, Jeong and Ikeda (2000) have demonstrated that A\(_1\) receptors can couple to Gi2, GoA, and GoB to inhibit Ca\(^{2+}\) currents in rat SCG neurons. Indeed, blockade of adenosine-induced Ca\(^{2+}\) current inhibition by PTX supports the involvement of A\(_1\) receptors. It should be noted, however, that A\(_1\) receptors in supraoptic neurons are coupled to a PTX-insensitive G protein, probably Gz (a member of Gi family) (Noguchi and Yamashita, 2000; but see Jeong and Ikeda, 1998). Although cholera toxin-sensitive Gs has been shown to couple vasoactive inhibitory peptide receptors to Ca\(^{2+}\) current inhibition (Zhu and Ikeda, 1994), most studies have reported that activation of Gs-coupled A\(_2\) receptors potentiates Ca\(^{2+}\) currents (mostly P-type) to facilitate synaptic transmission via a cyclic AMP/protein kinase A-dependent pathway (Mogul et al., 1993; Umemiya and Berger, 1994; Gubitz et al., 1996). Because there appear to be few, if any, P/Q-type Ca\(^{2+}\) channels in MPG neurons (Zhu et al., 1995; Zhu and Yakel, 1997), it is unlikely that stimulation of A\(_2\) receptor modulates Ca\(^{2+}\) currents in MPG neurons.

**Voltage-Dependent Inhibition of N-Type Ca\(^{2+}\) Currents by Adenosine.** In most neurons, PTX-sensitive G

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**Fig. 6.** Voltage-dependent inhibition of Ca\(^{2+}\) currents by adenosine. A, bell-shaped relationship between inhibition (%) and test potential. The profile was derived from the data shown in Fig. 2B (n = 16). B, time course of current inhibition (top) and superimposed current traces (bottom) in the absence and presence of 30 μM adenosine. The currents were elicited by a double pulse protocol consisting of two identical test pulses to +10 mV separated by a large depolarizing conditioning pulse to +80 mV. The facilitation was defined as the ratio of the postpulse (□) to prepulse (○) current amplitude (post/pre). Data represent the means ± S.E.M.

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**Fig. 7.** Effects of adenosine on ω-CgTx GVIA-sensitive Ca\(^{2+}\) currents. A, time course of the effects of the consecutive application of 30 μM adenosine (ADO), 1 μM ω-CgTx GVIA, and 10 μM nimodipine on Ca\(^{2+}\) currents. The currents were elicited and amplitudes were determined as in Fig. 1. B, contribution of N-type (ω-CgTx sensitive), L-type (nimodipine sensitive), and non-N/L-type (ω-CgTx and nimodipine-resistant, respectively) currents to the total Ca\(^{2+}\) current. C, summary of inhibition (%) of Ca\(^{2+}\) current by adenosine in the absence and presence of ω-CgTx GVIA. In B and C, data (acquired from 5 to 9 neurons) represent the mean ± S.E.M. ***p < 0.01.
proteins transduce the voltage-dependent and membrane-delimited inhibition of Ca\(^{2+}\) currents (Hille, 1994). Likewise, the voltage-dependence of adenosine-induced Ca\(^{2+}\) current inhibition was also evident by 1) the bell-shaped relationship between the current inhibition and test potentials, 2) slowing of the activation kinetics, and 3) greatly increased prepulse facilitation (Elmslie et al., 1990). The latter two characteristics can be explained by interconversion between “willing” and “reluctant” channels (Bean, 1989; Zhu and Ikeda, 1993) that requires direct binding of G\(\beta\)\(\gamma\) subunits released from Go/i to Ca\(^{2+}\) channels (for review, see Ikeda and Dunlap, 1999). Ca\(^{2+}\) current inhibition mediated by A\(_1\) receptors has also been described in other neurons, including those of dorsal root ganglia (Dolphin et al., 1986), hippocampus (Scholz and Miller, 1991; Mogul et al., 1993; Wu and Saggau, 1994), brain stem (Unemiyi and Berger, 1994), spinal cord (Mynieff and Beam, 1994), SCG (Zhu and Ikeda, 1993), and supraoptic nucleus (Noguchi and Yamashita, 2000). In all cases, N-type Ca\(^{2+}\) currents were inhibited by adenosine. Previous studies have shown that MPG neurons express at least three different HVA Ca\(^{2+}\) channels, i.e., N-, L-, and non-N/L-type (Zhu et al., 1995; Zhu and Yakel, 1997). Consistent with previous studies, the major target of A\(_1\) receptor activation in this study was the \(\omega\)-CgTx GVI-A-sensitive N-type Ca\(^{2+}\) channels, which underlie the majority (60%) of whole-cell Ca\(^{2+}\) currents in MPG neurons (Fig. 7).

**Functional Relevance of Adenosine-Induced Ca\(^{2+}\) Current Inhibition.** It is well known that ATP can be released from both adrenergic and cholinergic nerve terminals in pelvic viscera (Fuji, 1988; Hoyle, 1992). Thus, ATP mediates neurally elicited contraction of effectors via P2 receptors, whereas adenosine, catabolized from ATP by ectonucleotidases at an extracellular side, exerts inhibitory effects on neuromuscular transmission via adenosine receptors located in presynaptic terminals (Theobald and de Groat, 1989). On the other hand, a study has proposed that adenosine is released by stimulation of preganglionic nerves as a potential source of endogenous adenosine. Regardless of the source, on-going neuronal activity seems to influence the local concentration of adenosine, which should in turn provide an activity-dependent autoregulatory mechanism to control excessive excitation in the pelvic plexus. In this scenario, a strong stimulation of pre- and postganglionic nerves facilitates corelease of ATP with NE or acetylcholine. The subsequent breakdown of ATP by ecto-nucleotidases at an extracellular side, exerts inhibitory effects on neuromuscular transmission via adenosine receptors located in presynaptic terminals (Theobald and de Groat, 1989).

In conclusion, we have demonstrated that adenosine inhibits its N-type Ca\(^{2+}\) currents by activation of A\(_1\) receptors via voltage-dependent and PTX-sensitive pathways in rat MPG neurons.

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**References**


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