Stimulation of adenosine A1 and A2A receptors by AMP in the submucosal plexus of guinea pig small intestine

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Gao N, Hu H-Z, Liu S, Gao C, Xia Y, Wood JD. Stimulation of adenosine A1 and A2A receptors by AMP in the submucosal plexus of guinea pig small intestine. Am J Physiol Gastrointest Liver Physiol 292: G492–G500, 2007. First published October 5, 2006; doi:10.1152/ajpgi.00257.2006.—Actions of adenosine 5′-monophosphate (AMP) on electrical and synaptic behavior of submucosal neurons in guinea pig small intestine were studied with�sharp’’ intracellular microelectrodes. Application of AMP (0.3–100 μM) evoked slowly activating depolarizing responses associated with increased excitability in 80.5% of the neurons. The responses were abolished by the adenosine A2A receptor antagonist ZM-241385 but not by pyridoxal-phosphate-6-azophenyl-2,4-disulfonic acid, trinitrophenyl-ATP, 8-cyclopentyl-1,3-dimethylxanthine, suramin, or MRS-12201220. The AMP-evoked responses were insensitive to AA-COCF3 or ryanodine. They were reduced significantly by 1) U-73122, which is a phospholipase C inhibitor; 2) cyclopiazonic acid, which blocks the Ca2+ pump in intraneuronal membranes; and 3) 2-aminoethoxy-diphenylborane, which is an isoinositol (1,4,5)-trisphosphate receptor antagonist. Inhibitors of PKC or calmodulin-dependent protein kinase also suppressed the AMP-evoked excitatory responses. Exposure to AMP suppressed fast nicotinic ionotropic postsynaptic potentials, slow metabotropic excitatory postsynaptic potentials, and slow noradrenergic inhibitory postsynaptic potentials in the submucosal plexus. Inhibition of each form of synaptic transmission reflected action at presynaptic inhibitory adenosine A1 receptors. Slow excitatory postsynaptic potentials, which were mediated by the release of ATP and stimulation of P2Y1 purinergic receptors in the submucosal plexus, were not suppressed by AMP. The results suggest an excitatory action of AMP at adenosine A2A receptors on neuronal cell bodies and presynaptic inhibitory actions mediated by adenosine A1 receptors for most forms of neurotransmission in the submucosal plexus, with the exception of slow excitatory purinergic transmission mediated by the P2Y1 receptor subtype.

Gastrointestinal tract; neurogastroenterology; enteric nervous system; synaptic transmission; purinergic receptors

The cellular neurophysiology of purinergic receptors in the enteric nervous system (ENS), which encompasses both P1- and P2-type receptors, continues to be a focus of attention for neurogastroenterology. Adenosine is generally accepted as the ligand for the P1 receptor; ATP is the ligand for P2 receptors (1).

P1 receptors are further classified as A1, A2A, A2B, and A3 receptors according to their pharmacology, molecular sequences, and signal transduction pathways. Both A2A and A2B adenosine receptors are coupled through a Gs protein to adenylate cyclase, elevation of cAMP, and stimulation of neuronal excitability. On the other hand, A1 and A3 adenosine receptors are negatively coupled to adenylate cyclase through the same Gs protein (35, 43) and inhibit cAMP formation and neuronal excitability.

In the ENS, adenosine acts as a presynaptic neuromodulator to influence the release of the inhibitory neurotransmitters norepinephrine and somatostatin (2, 53) and the excitatory neurotransmitters acetylcholine (ACh) and tachykinins (6, 7, 9, 12, 13, 38, 40). Presynaptic inhibition by adenosine is mediated by adenosine A1 receptors, which act via pertussis toxin (PTX)-sensitive G proteins to suppress voltage-activated calcium currents (7).

Tonic stimulation of postsynaptic A1 adenosine receptors suppresses cAMP formation in dissociated myenteric ganglia (52). Suppression of histamine-activated chloride and calcium conductance (2, 47) by adenosine in myenteric neurons is also mediated by A1 adenosine receptors. Exposure to adenosine increases K+ conductance and clamps the membrane potential at hyperpolarized levels in myenteric neurons in guinea pig small intestine (15, 42). Stimulation of A2A adenosine receptors and activation of protein kinase A in the coupled signal transduction cascade are suggested to account for the excitatory actions of adenosine in the guinea pig small intestinal submucosal plexus (3, 4). Presynaptic inhibitory A1 receptors in the submucosal plexus suppress neurotransmitter release at excitatory synapses and thereby inhibit mucosal secretory reflexes (3, 17).

P2 receptors are subdivided into P2X and P2Y receptor subtypes. P2X receptors are ligand-gated ion channels, which mediate ATP-evoked responses in neurons of the myenteric and submucosal divisions of the ENS (5, 6, 54). P2Y receptors are G protein-coupled metabotropic receptors. The P2Y1 purinergic receptor subtype expressed by enteric neurons in the submucosal division of the ENS is activated by presynaptic release of ATP from other enteric neurons and sympathetic postganglionic fibers (32). It is a metabotropic receptor that is linked to activation of phospholipase C (PLC), synthesis of inositol (1,4,5)-trisphosphate and mobilization of Ca2+ from intracellular stores (28, 32).

Following synaptic release, ATP is hydrolyzed by several families of ectonucleotidases to ADP, AMP, and adenosine in this sequence (55). Actions of both ADP and adenosine on neuronal excitability and neurotransmission in the ENS are known; less is known about the actions of AMP (5, 54). The aim of the present study was to gain better understanding of the actions of AMP, the receptors that mediate its action, and

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submucosal plexus of guinea pig small intestine. A preliminary report has appeared in abstract form (26).

MATERIALS AND METHODS

Tissue preparation. Tissues were obtained from 30 male Hartley-Dawley guinea pigs (300–350 g), which were euthanized by stunning followed by exsanguination from the cervical blood vessels. This procedure was approved by the Ohio State University Institutional Laboratory Animal Care and Use Committee and Veterinary Inspectors from the United States Department of Agriculture. Segments of intestine were removed and placed in ice-cold Krebs solution, which contained 1 μM nicardipine. Composition of the Krebs solution was (in mM) 120.9 NaCl, 5.9 KCl, 1.2 MgCl₂, 1.2 NaH₂PO₄, 14.4 NaHCO₃, 2.5 CaCl₂, and 11.5 glucose. The segments of ileum were opened along the mesenteric border and the contents were flushed away with Krebs solution. Submucosal plexus preparations were obtained from segments of small intestine removed 10–20 cm proximal to the ileocecal junction. The segments were microdissected for selective cation channels accounts for the membrane depolarization in AH-type neurons (Figs. 1–5; see Ref. 50 for a review of S-type neurons). The membrane hyperpolarization occurred in a concentration-dependent manner (Fig. 1A). The mean for the hyperpolarizing responses to 30 μM AMP in AH-type neurons was 7.4 ± 3.5 mV for five animals. Presence in the bathing solution of the selective adenosine A₁ receptor antagonist 8-CPT (1 μM) suppressed the hyperpolarizing action of AMP in each of 7 AH-type neurons (Fig. 1A). The presence of the selective adenosine A₂A receptor antagonist ZM-241385 (1 μM) did not alter the AMP-evoked hyperpolarizing responses in any of the five neurons that were tested (data not shown).

Application of AMP (0.3–100 μM) to submucosal neurons with S-type electrophysiological behavior and uniaxonal morphology evoked slow activating depolarizing responses associated with elevated excitability in 80.5% (66/82) of the neurons (Figs. 1, 2, 4). The depolarizing responses for 15 neurons (Figs. 1–5; see Ref. 50 for a review of S-type neurons). Elevated excitability was reflected by an increase in the number of action potentials evoked by intracellular injection of constant-current depolarizing current pulses and by an increase in the spontaneous discharge of action potentials in some of the neurons (Figs. 1, 2, 4). The depolarizing responses for 15 neurons were associated by either a decrease or no change in the duration of the AMP-evoked responses without any significant change in the peak amplitude of the depolarization in AH-type neurons (Figs. 1B and 4).

Because it was known that increased conductance in nonselective cation channels accounts for the membrane depolarization during metabotropic slow EPSPs and slow EPSP-like responses to excitatory agonists in S-type submucosal neurons, we examined the effects of Na⁺ depletion on the depolarizing responses to AMP (33, 49). Depletion of Na⁺ in the bathing medium suppressed the depolarizing responses to AMP (Figs. 1B and 4).
Fig. 1. Exposure to AMP evoked hyperpolarizing responses in neurons with AH-type electrophysiological behavior and Dogiel II multipolar morphology and depolarizing-excitatory responses in neurons with S-type electrophysiological behavior and uniaxonal morphology in the guinea pig submucosal plexus. A: hyperpolarizing responses to AMP in an AH-type neuron were concentration dependent over a range of 3 to 30 μM and were suppressed by the selective adenosine A1 receptor antagonist 8-CPT. Morphology of the neuron appears in the inset. Downward deflections on the voltage traces are electrotonic potentials produced by repetitive injection of 100-ms rectangular hyperpolarizing constant-current pulses. B: depolarizing responses evoked by AMP in an S-type neuron were suppressed by reduction of the concentration of Na+ in the bathing medium and were enhanced by reduction of Ca2+ in the bathing medium. Morphology of the neuron appears in the inset.

Responses evoked by 10 μM AMP were suppressed to 6.5 ± 2.5% of control (n = 5 neurons) when the Na+ concentration was reduced from 145 mM to 26.2 mM by substitution of equimolar N-methyl-d-glucamine or choline chloride for NaCl (Fig. 4). The resting membrane potential was unchanged by reduction of the extracellular Na+ concentration from 145 to 26.2 mM.

Pharmacology of excitatory responses. We used selective antagonists for P1 and P2 receptors as pharmacological tools in an attempt to identify the purinergic receptor that might be responsible for the AMP-evoked excitatory responses in the S-type submucosal neurons. The presence of a supramaximal concentration (1 μM) of the specific A1 receptor antagonist 8-CPT in the bathing medium did not suppress the AMP-evoked depolarizing responses (Figs. 2B and 3). Depolarizing responses to 10 μM AMP were 11.0 ± 1.9 mV for seven neurons in the presence of 8-CPT and were not different from responses of 11.6 ± 2.0 mV in eight neurons evoked by 10 μM AMP in the absence of 8-CPT (P > 0.05). Exposure to the selective adenosine A2 receptor antagonist MRS-1220 (100 nM) likewise had no effect on the depolarizing responses evoked by 10 μM AMP (Figs. 2C and 3). Depolarizing responses to 10 μM AMP were 13.1 ± 2.9 mV for seven neurons in the presence of MRS-1220 and were not different from responses of 12.9 ± 3.0 mV in seven neurons evoked by 10 μM AMP in the absence of MRS-1220 (P > 0.05). On the other hand, coapplication of AMP with the selective A2A receptor antagonist ZM-241385 (300 nM) suppressed responses to 10 μM AMP to 24.5 ± 3.7% of the responses in the absence of ZM-241385 (Figs. 2D and 3). The depolarizing responses to 10 μM AMP alone amounted to 10.5 ± 0.6 mV in 16 neurons and were reduced to 2.4 ± 0.4 mV for 15 neurons when coapplied with ZM-241385 (P < 0.01). A selective A2A receptor agonist CGS-21680, evoked depolarizing responses with amplitudes of 7.6 ± 1.2 mV for five neurons when applied at 300 nM. The AMP-like responses to CGS-21680 were reduced to 1.8 ± 0.8 mV when it was coapplied with 300-nM ZM-241385 in five S-type neurons.

We also tested a number of selective and nonselective selective purinergic receptor antagonists on the depolarizing responses to AMP and found that none altered the AMP-evoked responses (Fig. 3). These included 1) the nonselective P2 receptor antagonist suramin (300 μM), 2) the P2X receptor antagonist pyridoxal-phosphate-6-azophenyl-2,4-disulfonic acid (30 μM), 3) trinitrophenyl-ATP (1 μM), and 4) the selective P2Y1 receptor antagonist MRS-2179 (3 μM).

Signal transduction for excitatory responses. We used pharmacological agents known to interfere with specific steps in
We tested a hypothesis that the postreceptor signal transduction cascade for AMP involves activation of PLC, synthesis 1,4,5-inositol trisphosphate (IP3) and elevation of intraneuronal Ca2+ by incubating the preparations with the PLC inhibitor U-73122 before application of AMP. Preincubation with 10 μM U-73122 reduced the depolarizing responses to 10 μM to 6.0 ± 2.2% of the responses to 10 μM AMP in the absence of U-73122 for five neurons (P < 0.01, Fig. 5). U-73343, which is the inactive analog of U-73122, did not affect AMP-evoked depolarizing responses (Fig. 5). Depolarizing responses to 10 μM AMP were 9.8 ± 1.5 mV for eight neurons in the presence of 10 μM U-73343 and were not different from responses of 9.9 ± 1.7 mV evoked by 10 μM AMP in the absence of U-73343 (P > 0.05). Preincubation of the preparations with the phospholipase A2 inhibitor AAOCOF3 (30 μM) had no effect on the AMP-evoked depolarizing response in six neurons (Fig. 5).

Preincubation with the IP3 receptor antagonist 2-APB (100 μM) for 3 min reduced the amplitude of the depolarizing responses to 10 μM AMP to 24.6 ± 5.5% of control responses obtained without 2-APB pretreatment (Fig. 5). The mean depolarizing response to 10 μM AMP was 9.4 ± 1.4 mV in the absence of 2-APB in seven neurons, and this was reduced to 2.6 ± 0.9 mV by 2-APB (P < 0.01).

In view of reports by others that stimulation of ryanodine receptors might be a significant factor for excitation of neurons in the myenteric plexus (30, 34), we investigated the effects of ryanodine on AMP-evoked depolarizing responses in neurons of the submucosal plexus. Ryanodine was used as a tool for studying involvement of Ca2+ mobilization from intraneuronal membranes in the AMP signal transduction cascade because it is a high-affinity ligand for the Ca2+-induced Ca2+ release channel (i.e., the ryanodine receptor). Ryanodine promotes activation of the channel at low concentrations and blocks the channels when present in concentrations greater than 10 μM (24). Depolarizing responses to 10 μM AMP were 10.0 ± 2.5 mV for six neurons in the presence of 10 μM ryanodine and were not different from responses of 9.5 ± 2.3 mV evoked by 10 μM AMP in the absence of ryanodine (P > 0.05; Figs. 4B and 5).

Preincubation with the selective protein kinase A inhibitor H89 for 30 min had no effect on AMP-evoked responses. The mean depolarization to 10 μM AMP was 9.7 ± 0.9 mV for seven neurons and was not significantly different from the

Fig. 3. Data showing suppression of AMP-evoked depolarizing responses in submucosal neurons with S-type electrophysiological behavior and uniaxonal morphology by the selective adenosine A2A receptor antagonist ZM-241385 and lack of effect of other purinergic receptor antagonists. PPADS, pyridoxal-phosphate-6-azophenyl-2,4-disulfonic acid; TNP-ATP, trinitrophenyl-ATP. Numbers of neurons appear in parentheses. *P < 0.001.

Fig. 4. Signal transduction for AMP-evoked depolarizing responses in submucosal neurons with S-type electrophysiological behavior and uniaxonal morphology. A: response to AMP alone. B: a high concentration of ryanodine did not suppress AMP-evoked excitatory response. C: the Ca2+-ATPase inhibitor CPA suppressed excitatory responses to AMP. D: the 1,4,5-inositol trisphosphate (IP3) receptor antagonist 2-APB suppressed excitatory responses to AMP. E: response to AMP alone following washout of the last pharmacological agent. F: the neuron from which each of the electrophysiological records was obtained. Downward deflections on the voltage traces are electrotonic potentials produced by repetitive injection of 100-ms rectangular hyperpolarizing constant-current pulses.
mean of 9.6 ± 0.7 mV in the presence of 1 μM H89 (P > 0.05). On the contrary, preincubation for 30 min with the selective PKC inhibitor GF109203X (1 μM) reduced the amplitude of responses evoked by 10 μM AMP to 29.7 ± 7.2% of the control responses in the absence of GF109203X for five neurons (P < 0.05, Fig. 5). Furthermore, 30-min pretreatment with 3 μM KN-62, which inhibits calmodulin kinases, reduced responses to 10 μM AMP to 31.5 ± 5.7% (P < 0.01) of the amplitude of the depolarizing responses in the absence of 3 μM KN-62 in five neurons (Fig. 5).

Presynaptic inhibition (fast EPSPs). Nicotinic fast EPSPs were evoked in the submucosal plexus by focal electrical stimulation of interganglionic fiber tracts. Application of AMP reversibly suppressed the fast EPSPs in all of 15 uniaxonal neurons that had S-type electrophysiological behavior (Fig. 6). Suppression of the fast EPSPs by AMP was concentration dependent with an IC50 of 3.9 ± 1.2 μM for five neurons (Fig. 6, A–C). Maximal inhibition was a reduction in amplitude to 34.3 ± 7.9% of control by 100 μM AMP. The inhibitory action of AMP was abolished by the selective adenosine A1 receptor antagonist 8-CPT (1 μM), which was pre- and coapplied with 30 μM AMP for six neurons (Fig. 6D). Micropressure pulses of ACh evoked characteristic EPSP-like depolarizing responses that were suppressed or abolished by 100 μM hexamethonium. The EPSP-like responses to ACh in six neurons were unaltered by the presence of 100 μM AMP in the tissue bath (data not shown).

Presynaptic inhibition (slow EPSPs). Slow EPSPs were also evoked in the submucosal plexus by focal electrical stimulation of interganglionic fiber tracts. The submucosal slow EPSPs were subdivided into three categories according to their sensitivity to the P2Y1 receptor antagonist MRS-2179 as follows: 1) “pure” purinergic slow EPSPs were abolished by 10 μM MRS-2179; 2) partially purinergic slow EPSPs were suppressed, but not abolished, by 10 μM MRS-2179; and 3) nonpurinergic slow EPSPs were unaltered by 10 μM MRS-2179. The pure and partially purinergic slow EPSPs were recorded in neurons with S-type electrophysiological behavior and uniaxonal morphology, and the nonpurinergic slow EPSPs occurred in neurons with AH-type electrophysiological behavior and Dogiel type II multipolar morphology as we reported earlier (32).

The effects of AMP on the slow EPSPs in neurons with S-type electrophysiological behavior and uniaxonal morphology were different from the effects on slow EPSPs in neurons with AH-type electrophysiological behavior and Dogiel type II multipolar morphology. In 5 AH/Dogiel II neurons, AMP suppressed the stimulus-evoked nonpurinergic slow EPSPs in a concentration-dependent manner with an EC50 of 2.3 ± 0.2 μM (Fig. 7, A and D). Exposure to 100 μM AMP abolished the slow EPSPs in each of the 7 AH/Dogiel II neurons tested (Fig. 7A). Bath application of either substance P or serotonin evoked slow EPSP-like depolarizing responses in the AH/Dogiel II neurons (Fig. 7, B and C). The presence of 100 μM AMP, in the bathing medium, did not change the amplitude of the depolarizing responses to either substance P or serotonin (Fig. 7, B and C). Presence in the bathing solution of the selective

**Fig. 5.** Data showing suppression of AMP-evoked depolarizing responses in submucosal neurons with S-type electrophysiological behavior and uniaxonal morphology by agents that interfere with the PLC → IP3 → Ca2+ → PKC postreceptor signal transduction cascade. Numbers of neurons appear in parentheses. *P < 0.001.

**Fig. 6.** AMP suppressed fast excitatory postsynaptic potentials (EPSPs) in a concentration-dependent manner in a submucosal neuron with S-type electrophysiological behavior and uniaxonal morphology. A: suppression of fast EPSPs by 10 μM AMP. B: suppression of fast EPSPs by 30 μM AMP. C: suppression of fast EPSPs by 100 μM AMP. D: presence of the selective adenosine A1 receptor antagonist 8-CPT in the bathing medium suppressed the inhibitory action of AMP on the fast EPSPs. E: the neuron from which each of the electrophysiological records was obtained. Selected EPSPs marked by arrows are shown on an expanded time scale above in the uppermost records.
adenosine A₁ receptor antagonist 8-CPT reduced suppression of the slow EPSPs by AMP. On the other hand, the selective adenosine A₂A receptor antagonist ZM-241385 affected neither suppression on stimulus-evoked slow EPSPs by AMP nor AMP-evoked hyperpolarizing responses (n = 5 neurons; data not shown).

In contrast to the AH/Dogiel II neurons, application of AMP did not change the amplitude of the purinergic slow EPSPs, which were sensitive to MRS-2179 in the neurons with S-type electrophysiological behavior and uniaxonal morphology (Fig. 8A). We applied 100 μM AMP to five S-type submucosal neurons that had stimulus-evoked pure purinergic slow EPSPs and found no effect on the amplitude of the EPSPs. We also applied 100 μM AMP to five S-type neurons that had partially purinergic slow EPSPs (i.e., MRS-2179 suppressed the EPSP amplitude by only 53.3 ± 3.3%). These particular neurons received both a purinergic slow excitatory synaptic input mediated by ATP and a slow excitatory synaptic input mediated by a nonpurinergic transmitter as we reported earlier (32). Application of 100 μM AMP reduced the amplitude of these partially purinergic slow EPSPs by 50.0 ± 4.1% in five neurons. Coapplication of AMP with the selective P2Y₁ receptor antagonist MRS-2179 abolished the partially purinergic slow EPSPs (Fig. 8, B and C). The effect of AMP on partially purinergic slow EPSPs was abolished by 1 μM 8-CPT (n = 5, data not shown).

Presynaptic inhibition (slow IPSPs). Focal electrical stimulation of interganglionic fiber tracts in the submucosal plexus evoked characteristic noradrenergic slow inhibitory postsynaptic potentials (IPSPs), in addition to fast and slow EPSPs. Application of AMP suppressed the amplitude and duration of the slow IPSPs in a concentration-dependent manner with an EC₅₀ of 36.3 ± 7.5 μM for seven neurons (Fig. 9, A and C). Maximal inhibition occurred with 300 μM AMP, which suppressed the slow IPSP amplitude by 87.5 ± 1.5% for seven neurons. The presence of 1 μM 8-CPT in the tissue bath abolished the inhibitory effect of 300 μM AMP in each of seven neurons (Fig. 8A). The presence of the selective adenosine A₂A...
receptor antagonist ZM-241385 (300 nM) did not alter the inhibitory action of 30 μM AMP on the slow IPSPs (n = 5, data not shown).

Micropressure pulses of norepinephrine evoked characteristic IPSP-like hyperpolarizing responses that were suppressed or abolished by 1 μM phentolamine. The slow IPSP-like responses evoked by microejection application of norepinephrine to the impaled neurons were unchanged by the presence of 10 μM AMP in the bathing medium (Fig. 9B).

DISCUSSION

Exposure to AMP suppressed excitability in neurons with AH-type electrophysiological behavior and Dogiel type II morphology. This action for AH-type neurons was essentially the same as reported for adenosine in AH-type neurons (42). Both adenosine and AMP act to increase K+ conductance and thereby clamp the membrane potential of AH neurons to a hyperpolarized level near the K+ equilibrium potential (i.e., −90 mV) and hold the cell soma in a state of low excitability or inexcitability. The hyperpolarizing action of AMP for AH-type neurons most likely reflected stimulation of the adenosine A1 receptor subtype because, like the action of adenosine (14, 15), it was suppressed by the selective A1 receptor antagonist 8-CPT.

In contrast to AH-type neurons, exposure to AMP evoked depolarization of the membrane potential and enhanced excitability in neurons with S-type electrophysiological behavior and uniaxonal morphology. Adenosine A1 receptors could not be implicated in this action because pretreatment with the selective adenosine A1 receptor antagonist 8-CPT did not alter the excitatory responses to AMP. Likewise, neither the adenosine A3 receptor subtype nor P2Y receptor subtypes were implicated in the excitatory action of AMP because the excitatory responses were unaffected by pretreatment with selective antagonists for adenosine A3 and P2Y receptors. On the other hand, application of the selective adenosine A2A receptor antagonist ZM-241385 suppressed or abolished the AMP-evoked excitatory action. This was consistent with the adenosine A2A subtype as the receptor responsible for the AMP-evoked excitatory responses in the S-type neurons.

The postreceptor signal transduction mechanism for the adenosine A2A receptor has been reported to be Gs protein coupling to adenylate cyclase and elevation of intracellular cAMP (20, 21, 23). Nevertheless, we found that the cAMP-dependent protein kinase inhibitor H89 had no effect on the AMP-evoked excitatory responses in the S-type neurons. This suggests that postreceptor signal transduction for the adenosine A2A receptor in guinea pig submucosal S-type neurons differs from other tissues. PTX-sensitive G proteins were not involved in the AMP-evoked responses because preincubation with PTX had no effect on the actions of AMP. Most of our pharmacological evidence supports the hypothesis that a PLC → IP3 receptor activation → intracellular Ca2+ mobilization → calmodulin-dependent protein kinase and/or PKC cascade was involved in the AMP-evoked excitatory responses. Essentially the same signal transduction pathway for the adenosine A2A receptor was reported for rat neostriatal neurons, in which the adenosine A2A receptor mediates inhibition of NMDA receptor-activated inward ionic current (48).

The A2A receptor can now be added to a growing list of G protein-coupled metabotropic receptors expressed by enteric neurons with S-type electrophysiological behavior and uniaxonal morphology, which share the same postreceptor signal transduction cascade. The list includes the P2Y1 receptor (32) protease activated receptors (27) and the B2 bradykinin receptor (33).

Our findings for the adenosine A2A receptor underscore an important aspect of metabotropic slow excitatory neurotransmission, which distinguishes enteric neurons with AH-type electrophysiological behavior and Dogiel type II multipolar morphology from neurons with S-type electrophysiological behavior and uniaxonal morphology (reviewed in Ref. 49). Slow excitatory responses in neurons with AH-type electrophysiological behavior and multipolar Dogiel type II morphology are characterized by membrane depolarization associated with closure of Ca2+-gated K+ channels, which is reflected by...
increased neuronal input resistance. Slow excitatory responses in neurons with S-type electrophysiological behavior and uniaxonal morphology are characterized by membrane depolarization associated with opening of cationic channels and either decreased neuronal input resistance or negligible change in the input resistance. Postreceptor signaling, which involves activation of adenylate cyclase, stimulation of cAMP formation, and activation of protein kinase A, generates excitatory responses characterized by increased neuronal input resistance in AH-type neurons. Postreceptor signaling, which involves activation of PLC, release of IP3 and diacylglycerol, and activation of PKC and calmodulin kinases, generates excitatory responses characterized by decreased neuronal input resistance in S-type neurons.

**Presynaptic inhibition (fast EPSPs).** All actions of AMP on synaptic transmission were at presynaptic inhibitory receptors, which suppressed both excitatory and inhibitory neurotransmission. Exposure to AMP suppressed fast nicotinic EPSPs without any reduction of nicotinic depolarizing responses to exogenously applied ACh. This was evidence for presynaptic suppression of ACh release. Blockade by the selective adenosine A1 receptor antagonist 8-CPT is consistent with identification of the presynaptic inhibitory receptor as the adenosine A1 subtype.

**Presynaptic inhibition (slow EPSPs).** Metabotropic slow EPSPs in AH- and S-type neurons are mimicked by a variety of signal substances including biogenic amines, neuropeptides, and mast cell proteases (reviewed in Refs. 29 and 50). AMP suppressed the slow EPSPs in AH-type neurons without affecting slow EPSP-like responses evoked by application of substance P or serotonin, which like the results for fast EPSPs suggest presynaptic inhibition of release of excitatory neurotransmitters. A1 receptors also mediated this presynaptic inhibitory action.

An exception to the suppression of slow metabotropic synaptic excitation by AMP was the finding that slow EPSPs, which were mediated by the release of ATP and its action at purinergic P2Y1 receptors, were not suppressed by AMP. Secretomotor neurons with S-type electrophysiological behavior, uniaxonal morphology, noradrenergic IPSPs, and expression of the neurotransmitter vasoactive intestinal peptide comprise the primary population of neurons in the guinea pig submucosal plexus that receive purinergic P2Y1 excitatory synaptic input. Absence of presynaptic inhibitory receptors at these purinergic synapses is likely to be of significance in the normal and/or pathophysiological minute-to-minute functioning of the ENS; nevertheless, the specific significance is unclear.

**Presynaptic inhibition (slow IPSPs).** One kind of slow IPSP in S-type neurons of the guinea pig submucosal plexus reflects the intramural release of norepinephrine from sympathetic postganglionic axons (41, 53). Our results suggest that accumulation of AMP would act to suppress norepinephrine release from the sympathetic innervation. Suppression of norepinephrine release appeared to be a direct action at presynaptic inhibitory receptors on the sympathetic nerve terminals because AMP did not suppress the hyperpolarizing action of exogenously applied norepinephrine. Blockade of the presynaptic inhibitory action of AMP on the noradrenergic IPSPs by the selective A1 receptor antagonist 8-CPT suggests that suppression of noradrenergic synaptic transmission by AMP was mediated by the adenosine A1 receptor.

Others have reported that a subset of IPSPs in submucosal neurons is mediated by transmitter(s) other than norepinephrine (31, 37). The nonadrenergic transmitter has not been identified unequivocally; nevertheless, somatostatin emerges as the most likely candidate (36, 45). Unlike the adrenergic IPSP, the nonadrenergic IPSP was seldom observed in our study and actions of AMP were not investigated.

Most of the neurons in the guinea pig submucosal plexus, which receive inhibitory noradrenergic synaptic input, are secretomotor neurons with S-type electrophysiological behavior and uniaxonal morphology. Suppression of norepinephrine release by an accumulation of AMP in the functioning intestine would be expected to remove sympathetic braking action from the neurons. This, together with AMP-evoked elevation of excitability in the neuronal cell bodies, is expected to enhance secretion of H2O, electrolytes and mucus and thereby increase the liquidity of the luminal contents, such as might occur in inflammatory states where ATP release is enhanced (8, 10, 11, 16, 18, 22, 25, 39, 44, 51).

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