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ADENOSINE 5′-TRIPHOSPHATE MODULATES MEMBRANE POTASSIUM CONDUCTANCE IN GUINEA-PIG MYENTERIC NEURONES

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SUMMARY

1. Intracellular recordings were made from myenteric neurones isolated from the guinea-pig small intestine to study actions of adenosine 5′-triphosphate (ATP). ATP was applied by superfusion (10 nM-100 μM) or pressure ejection from ATP-containing glass pipettes.

2. Myenteric neurones have been classified into two groups: type I/S neurones and type II/AH neurones. ATP produced a membrane hyperpolarization in 80% of AH neurones and a membrane depolarization in 90% of S neurones in a dose-dependent manner. Adenosine caused responses similar to those induced by ATP in both AH and S neurones, but was less effective than ATP.

3. The ATP-induced hyperpolarization was associated with a fall in input resistance, but the ATP-induced depolarization was accompanied by an increase in input resistance. Both responses reversed in polarity near the potassium equilibrium potential (−84 to −87 mV) and the reversal potential varied with extracellular potassium concentration, as predicted by the Nernst equation. These results indicate that the hyperpolarization is due to an increase, while the depolarization is due to a decrease in potassium conductance.

4. Both the hyperpolarization and the depolarization induced by ATP persisted in calcium-free solution containing 1·2 mM-magnesium, but were markedly reduced or abolished in calcium-free solutions containing 3·7–10 mM-magnesium and by 1 mM-nickel or cobalt. Both responses to ATP persisted in tetraethylammonium (1–10 mM) or tetrodotoxin (1–3 μM)-containing solutions.

5. Quinine and quinidine (1–100 μM) reversibly depressed both the ATP-induced responses. Caffeine (100 μM), theophylline (100 μM) and 3-isobutyl-1-methylxanthine (1–10 μM) did not significantly affect the ATP-induced depolarization but did reversibly depress the ATP-induced hyperpolarization.

6. These results suggest that the ATP-induced hyperpolarization may be due to activation, and the ATP-induced depolarization to inactivation, of a calcium-sensitive potassium conductance.

INTRODUCTION

It has been reported that adenosine 5′-triphosphate (ATP) is released onto gut smooth muscles from nerve terminals of colonic myenteric neurones (Burnstock, Campbell & Rand, 1966; Campbell, 1966). An ATP-mediated (purinergic) inhibitory neurotransmission system has been proposed for the gut (see reviews by Burnstock, 1972, 1981). Consistent with these ideas, intracellular recordings from gut smooth muscles demonstrated that ATP and its derivatives cause a membrane hyperpolarization, due presumably to an increase in potassium conductance (Burnstock, Campbell, Satchell & Smythe, 1970; Tomita & Watanabe, 1973; Shuba & Vladimirova, 1980). However, in autonomic ganglion neurones ATP and its derivatives produce a membrane depolarization accompanied by a decrease in membrane conductance and an increase in cellular excitability (Siggins, Gruol, Padjen & Forman, 1977; Akasu, Hirai & Koketsu, 1983).

White & Leslie (1982) have reported a depolarization-induced release of ATP from isolated varicosities derived from the myenteric plexus. Since there are abundant varicosities within the plexus, the released ATP may act on myenteric neurones. The present electrophysiological experiments were performed to study actions of ATP on single myenteric neurones. We demonstrate that ATP causes a membrane depolarization in type I/S neurones and a membrane hyperpolarization in type II/AH neurones.

METHODS

The small intestine was removed from adult guinea-pigs (250–300 g) which had been stunned and bled from the neck. The myenteric ganglia were dissected out and pinned in a tissue bath (volume: 0·8 ml), as described previously (Nishi & North, 1973). Individual neurones were visualized with Nomarski optics (×400). Membrane potentials were recorded with intracellular microelectrodes containing 3 M-KCl, having DC resistances of 30–60 MΩ (Nishi & North, 1973; Morita, North & Tokimasa, 1982*a*). Classification of individual neurones was made according to published criteria: type I/S neurones have nicotinic fast excitatory postsynaptic potentials (EPSPs) and type II/AH neurones show a long-lasting after-hyperpolarization (AHP) following an action potential (Nishi & North, 1973; Hirst, Holman & Spence, 1974). The ganglia were superfused at constant flow (5 ml/min) with heated (35–37 °C) modified Krebs solution of the following composition (mM): NaCl, 117; KCl, 4·7; $MgCl_2$, 1·2; $CaCl_2$, 2·5; NaH_2PO_4, 1·2, $NaHCO_3$, 25; glucose, 11; and gassed with 95% O_2 and 5% CO_2. Calcium-free/high-magnesium Krebs solutions were made by substituting $CaCl_2$ with 3·7 or 10 mM-$MgCl_2$. Solutions containing different potassium, calcium or magnesium concentrations were maintained isosmotic by adjusting the NaCl concentration. Adenosine 5′-triphosphate (ATP, Wako), adenosine (Wako), substance P (Peptide Institute, Osaka), atropine sulphate (Wako), quinine chloride (Wako), quinidine (Sigma), 1,3,7-trimethylxanthine chloride (caffeine, Wako), theophylline chloride (Wako), 3-isobutyl-1-methylxanthine (IBMX, Aldrich), ethyleneglycol-bis-(β-aminoethylether)-*N,N,N′,N′*-tetraacetic acid (EGTA, Sigma), tetraethylammonium chloride (TEA, Wako), and tetrodotoxin (TTX, Sankyo) were applied by adding the drug to the superfusing solution. ATP and substance P were also applied by pressure ejection (760–1000 mmHg for 20–100 ms) from drug-containing micropipettes (100 μM–5 mM-ATP or 100 nM–1 μM-substance P, freshly dissolved in the superfusing solution before each experiment). The pressure pipettes were usually located 100–500 μm distant from the impaled ganglion cell to reduce the effect of drug leakage. Glass microelectrodes filled with modified Krebs solution were used for extracellular focal stimulation in order to evoke synaptic responses; stimuli comprised single or repeated pulses (0·5 ms duration at 1–10 Hz for 1–5 s). Atropine (1–3 μM) was added to the superfusing solution from time to time, to prevent the muscarinic excitation of myenteric neurones and that of longitudinal muscles (see Morita, North & Tokimasa, 1982*b*). All quantitative results are expressed as mean ± S.E. of the mean.

RESULTS

The present results were based on intracellular recordings from more than eighty AH and thirty S neurones with membrane potentials of −50 mV or better.

ATP hyperpolarizes type II/AH neurones

Superfusion of ATP (1–10 μM) produced a membrane hyperpolarization in the majority of AH neurones tested (forty-seven out of sixty); the response developed slowly after its onset (Fig. 1*A*). The ATP-induced hyperpolarization continued as

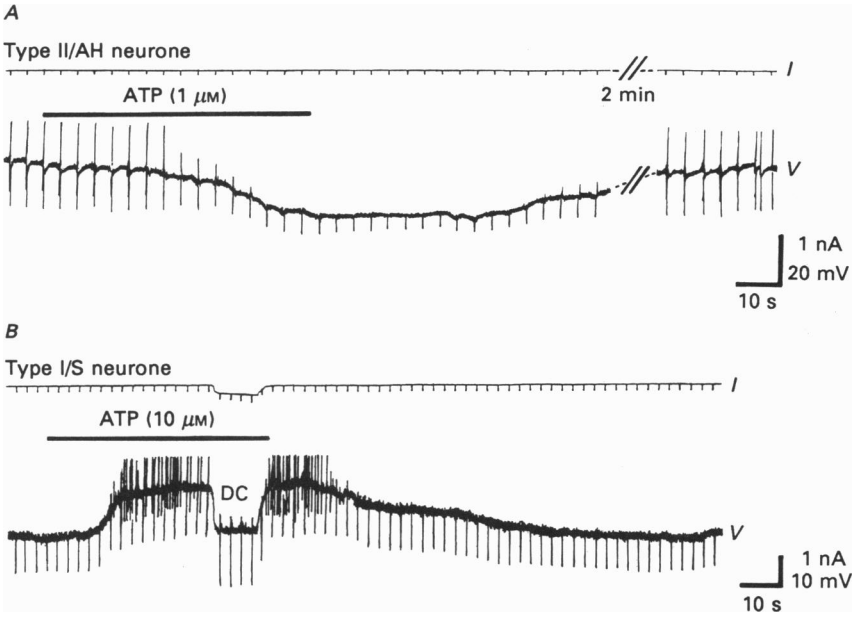

Fig. 1. ATP-induced hyperpolarization in an AH neurone (*A*) and ATP-induced depolarization in an S neurone (*B*). ATP was applied by superfusion during the periods indicated by continuous bars. *I*, applied transmembrane current; *V*, voltage recordings. In this and all subsequent figures, downward voltage deflections were hyperpolarizing potentials evoked by constant-current pulses applied through the recording microelectrode (0·1–0·4 nA for 100–200 ms). *A*, ATP caused a slowly developing membrane hyperpolarization. Note that the anodal brake response disappeared during the ATP-induced hyperpolarization (action potential amplitude was truncated by the frequency-response characteristics of the pen recorder). The resting membrane potential was −54 mV. *B*, ATP produced a depolarization accompanied by action potential discharges. An increase input resistance was apparent when the depolarization was nullified by passing hyperpolarizing current, indicated by DC. The resting membrane potential was −56 mV.

long as ATP was present (up to 10 min) and disappeared within 3–6 min after washing with ATP-free solution. The mean amplitude of the ATP-induced hyperpolarization at 1 μM was 11·2 ± 0·3 mV (forty-seven AH neurones whose resting potential was −63 ± 0·3 mV). This hyperpolarization could be evoked consistently when ATP was applied for short periods (1–3 min) at intervals longer than 10 min. The peak amplitude of the ATP-induced hyperpolarization was concentration-

dependent within the range tested (10 nM-100 μM; Fig. 2*A*). The rise time of the response usually became longer with increasing concentrations of ATP. In seven neurones ATP caused no detectable change in the resting membrane potential or input resistance. In six neurones ATP produced a small, slow depolarization (2–3 mV) accompanied by an increase in input resistance like that seen in S neurones. In about half the neurones tested, a small depolarization (2–3 mV) was observed after withdrawal of ATP (not shown).

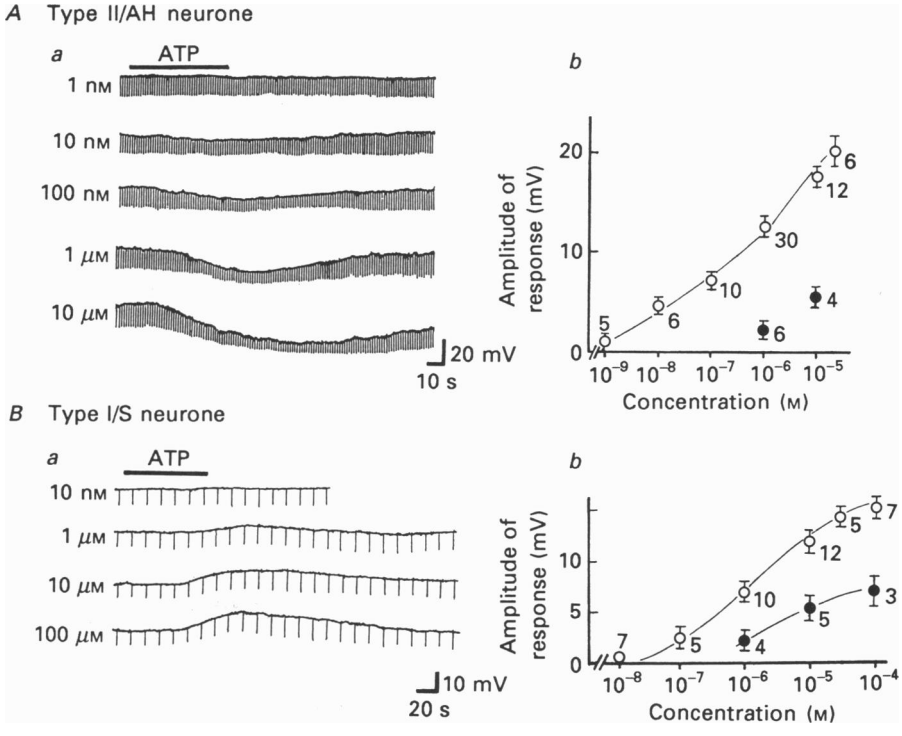

Fig. 2. Concentration dependence of the ATP-induced hyperpolarization in an AH neurone (*A a*) and the ATP-induced depolarization in an S neurone (*B a*). ATP was applied by superfusion during the periods indicated by continuous bars. The resting membrane potential was −62 mV in *A a* and −56 mV in *B a*. Open circles in *A b* and *B b* plot the peak amplitude of ATP-induced hyperpolarizations (*A b*) and depolarizations (*B b*) (ordinate) as a function of the concentration of ATP (abscissa, logarithmic scale). The peak amplitude of adenosine-induced responses is indicated by filled circles. Values are mean ± S.E. of the mean for the number of observations indicated; lines were drawn by eye. The amplitude of the responses was measured at the resting membrane potential (range −50 to −68 mV).

Application of ATP (100 μM–1 mM) by pressure ejection also induced a hyperpolarization in thirty-seven out of forty neurones. The ATP-induced hyperpolarization could be evoked consistently at 1 min intervals. The time course of the ATP-induced hyperpolarization varied from cell to cell and/or according to the position of the ejection pipette; the time to peak and the half-decay time of the response were 1–20 and 3–30 s, respectively.

The ATP-induced hyperpolarization persisted in the presence of TTX (1–3 μM),

suggesting that this response might be due to a direct action of ATP on AH neurones.

The ATP-induced hyperpolarization caused either by superfusion or by pressure ejection was always associated with a fall in input resistance (see Figs 2*A a* and 4*A*). For example, in 1 μM-ATP the input resistance was reduced to 60±3% ($n = 34$) of control. This reduction in input resistance persisted when the ATP-induced hyperpolarization was nullified by passing depolarizing current into the neurone (53±3% of control, $n = 10$). The reduction in input resistance was greatest at high ATP concentrations, as shown in Fig. 2*A a*.

Adenosine (1–10 μM) also produced a slowly developing hyperpolarization associated with a fall in input resistance in ATP-responsive AH neurones. This effect of adenosine was concentration-dependent, but a given concentration of adenosine was always much less effective than a similar concentration of ATP (compare open and filled circles in Fig. 2*A b*).

ATP depolarizes type I/S neurones

In the majority of S neurones (seventeen out of twenty), ATP (1–10 μM) caused a membrane depolarization, usually associated with an increase in input resistance (Figs 1*B* and 2*B*). This depolarization often reached the threshold for generating action potentials or off-responses (see Fig. 1*B*). The ATP-induced depolarization developed slowly after its onset, persisted during ATP application for up to 10 min, and gradually disappeared within 3–8 min following wash-out of ATP. The ATP response could be evoked consistently when ATP was added for short periods (1–3 min) at intervals longer than 10 min. The peak amplitude of the depolarization produced by 10 μM-ATP was 13·8±1·6 mV (twelve S neurones whose resting potential was −55±0·7 mV), and increased with increasing ATP concentration (Fig. 2*B*). The time to peak was not greatly altered by changing the ATP concentration. The associated increase in input resistance became marked when the ATP-induced depolarization was nullified (Fig. 1*B*); in 10 μM-ATP the input resistance increased to 160±3% ($n = 8$) of control.

Application of ATP (100 μM–5 mM) by pressure ejection also induced a consistent depolarization at intervals of 2–3 min. The time to peak and the half-decay time of the depolarization were relatively constant, 3±0·8 s ($n = 16$) and 18±1·8 s ($n = 16$), respectively. The amplitude of the ATP-induced depolarization increased when the amount of ejected ATP was increased by changing the duration or the number of pressure pulses.

In three S neurones, pressure ejection and/or bath application of high concentrations of ATP (10–100 μM) produced a membrane depolarization, but low concentrations (10 nM–1 μM) produced a membrane hyperpolarization accompanied by a fall in input resistance like that seen in AH neurones.

The ATP-induced depolarization was observed in solutions containing TTX (1–3 μM) (see above).

Adenosine (1–10 μM) also produced a membrane depolarization associated with an increase in input resistance in ATP-sensitive S neurones. The effect of adenosine was concentration-dependent, but was always weaker than that of ATP (compare open and filled circles in Fig. 2*B b*).

Effects of ATP on action potentials

We examined whether or not ATP could affect active membrane properties as other biogenic substances could, e.g. substance P (see North, Morita & Tokimasa, 1982).

Type II/AH neurones

The large hyperpolarization and conductance increase produced by high concentrations of ATP made it very difficult to evoke an action potential by current

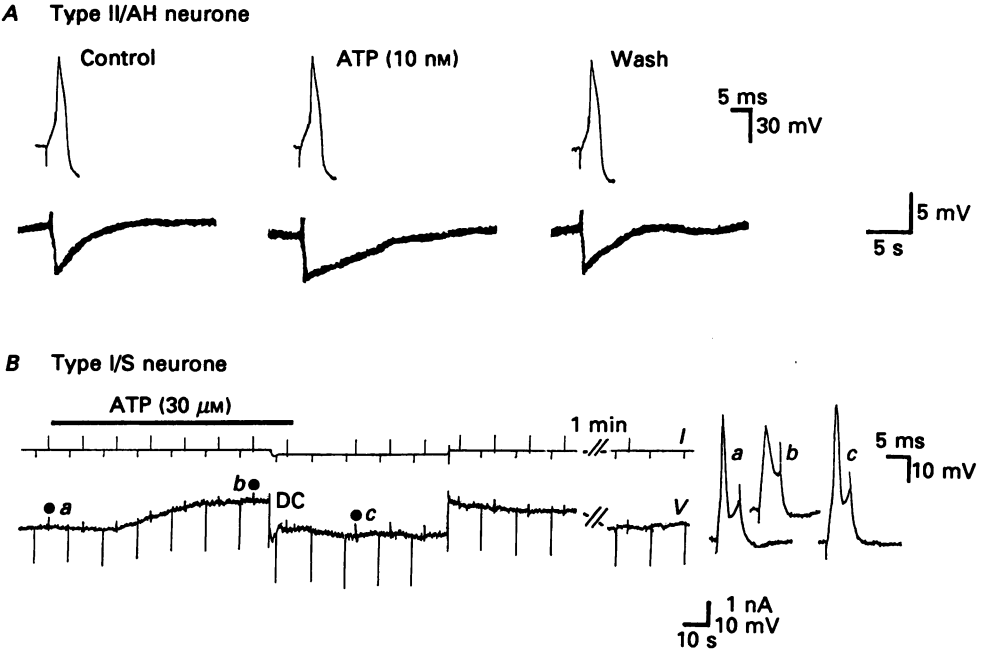

Fig. 3. Effect of ATP on action potentials in an AH neurone (*A*) and an S neurone (*B*). *A*, action potentials were elicited by depolarizing pulses (duration: 5 ms). Upper trace, fast-sweep recordings of action potential; lower trace, slow-sweep recordings of the slow after-hyperpolarization following the action potential. Control (left), after 2 min superfusion with ATP (centre) and after a 5 min wash (right). In the AH neurone 10 nM-ATP prolonged the slow after-hyperpolarization without changing the shape of action potential (resting membrane potential −62 mV). *B*, ATP was applied by superfusion during the period indicated by the continuous bar. *I*, current recordings; *V*, voltage recordings. Depolarizing pulses (●; duration, 5 ms) evoked action potentials, some of which are shown in the fast-sweep recordings on the right. In the S neurone 30 μM-ATP prolonged the action potential, even after the ATP-induced steady depolarization was nullified by passing hyperpolarizing current (indicated by DC). The resting membrane potential was −56 mV.

injection. At lower concentrations ($\leqslant$ 10 nM) ATP reversibly prolonged the slow after-hyperpolarization (AHP) without significantly affecting the action potential (Fig. 3*A*). The half-decay time of the slow AHP gradually increased to $160 \pm 12\%$ ($n = 4$) of control, without significant change in the peak amplitude ($102 \pm 2\%$) after about 2 min application of 10 nM-ATP. The prolongation of the slow AHP by ATP persisted when the ATP-induced hyperpolarization was nullified (not shown) ($n = 2$).

During the large membrane hyperpolarization induced by higher concentrations of ATP ($\geqslant$ 100 nM), the amplitude of the action potential increased and its duration was shortened slightly, and the slow AHP was prolonged without consistent change in amplitude (not shown).

Type I/S neurones

During the ATP-induced depolarization the action potential was prolonged but reduced in amplitude (30 μM, $n = 4$ and 100 μM, $n = 3$). However, both the amplitude and the duration of the action potential were increased when the ATP-induced depolarization was nullified (Fig. 3*B*).

Voltage dependence of ATP-induced responses

Hyperpolarization in type II/AH neurones

The amplitude of the ATP-induced hyperpolarization decreased when the membrane was hyperpolarized. The response reversed polarity when the membrane was hyperpolarized to potentials more negative than -90 mV (Fig. 4*Aa*). The peak amplitude of the ATP-induced hyperpolarization was almost linearly related to membrane potential between -60 and -100 mV, as seen in Fig. 4*B* (○). The changes in time course and amplitude of the ATP-induced response were similar to those changes in the slow AHP which occur when AH neurones are depolarized above -60 mV (Morita *et al.* 1982*a*). The reversal potential of the ATP-induced hyperpolarization was -87 ± 2 mV ($n = 8$), similar to that of the slow AHP of the AH neurones (-91 mV, see Morita *et al.* 1982*a*).

Depolarization in type I/S neurones.

The peak amplitude of the ATP-induced depolarization decreased with membrane hyperpolarization and increased with membrane depolarization (Fig. 4*Ab*). The response reversed polarity at -84 ± 2 mV ($n = 6$). The peak amplitude of the ATP-induced depolarization was almost linearly related to membrane potential in the range between -50 and -100 mV, as seen in Fig. 4*B* (●). The decay time course of the response became faster with membrane hyperpolarization and slower with membrane depolarization (Fig. 4*Ab*).

Effect of changing extracellular potassium concentration on the ATP-induced responses

Elevation of the external potassium concentration to 10 mM caused a membrane depolarization (4–7 mV) associated with a fall in input resistance and reduced the amplitude of the ATP-induced hyperpolarization and depolarization by 30–50%. The reversal potential of the hyperpolarization was -66 ± 3 mV ($n = 3$) in 10 mM-potassium and -44 ± 2 mV ($n = 3$) in 20 mM-potassium (○, Fig. 4*C*). The reversal potential of the depolarization was -62 ± 3 mV ($n = 3$) in 10 mM-potassium solution (●, Fig. 4*C*). The reversal potentials of both ATP responses were dependent mainly on the external potassium concentration as predicted by the Nernst equation (Fig. 4*C*). All of the present findings indicate that ATP hyperpolarization is due mainly to activation, while the ATP depolarization is due mainly to inactivation of potassium conductance.

Effects of TEA on the ATP response

At low concentrations ($\leqslant$ 1 mM) TEA did not induce any detectable change in the resting membrane potential or input resistance in either cell type, but at high concentrations (10–20 mM) TEA always caused a slowly developing depolarization accompanied by an increase in input resistance and sometimes by action potentials (see Fig. 5). Both of the ATP-induced responses persisted in 1–10 mM-TEA (1 mM: five

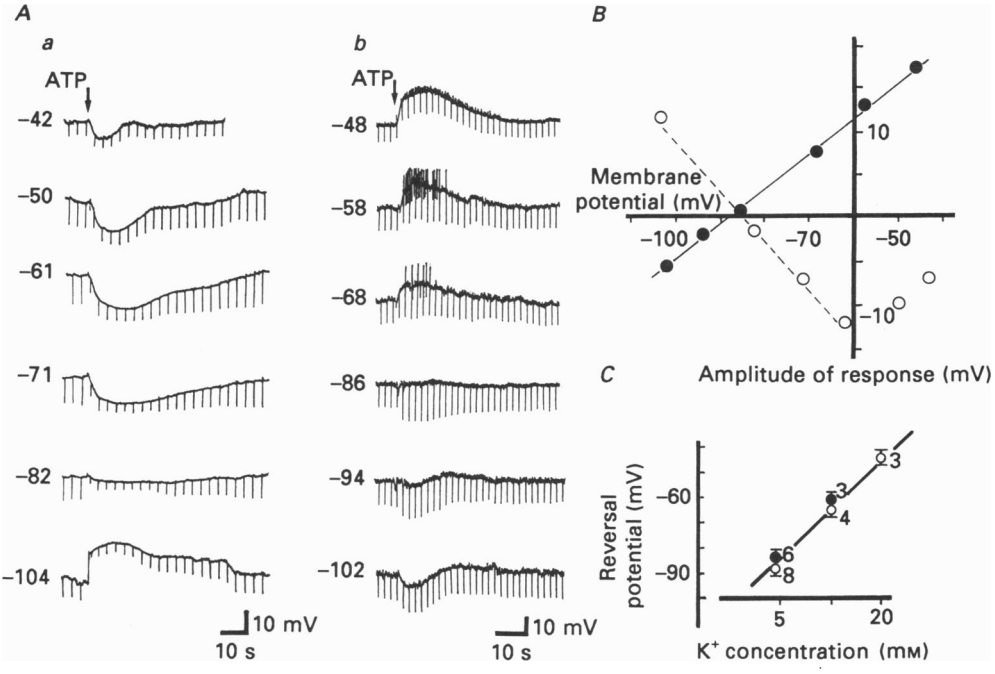

Fig. 4. Relationship between the amplitude of the ATP-induced responses and the membrane potential. *A*, the ATP-induced hyperpolarization (*a*) and depolarization (*b*) were evoked at various membrane potentials, indicated on the left (mV), in the presence of atropine (1 μM in *a* and 3 μM in *b*). ATP was applied by pressure ejection (indicated by arrow, 100 μM for 50 ms in *a* and 1 mM for 100 ms in *b*). The resting membrane potential was −61 mV (*a*) and −58 mV (*b*). Note a fast component in the rising phase of the ATP response (bottom trace of *a*). The fast component was often observed when cells were strongly hyperpolarized. *B*, the peak amplitudes (ordinate) of the ATP-induced hyperpolarization (○) and depolarizations (●) are plotted as a function of membrane potential (abscissa). Values were taken from *A*. Continuous and dotted lines were drawn by eye. *C*, the mean reversal potential (ordinate) of the ATP-induced hyperpolarization (○) and depolarization (●) are plotted as a function of the external potassium concentration (abscissa in logarithmic scale). Each vertical bar represents the S.E. of the mean for the number of observations indicated. The slope of the line is 61 mV per tenfold change in potassium concentration.

AH and four S neurones, 10 mM: eleven AH and six S neurones; Fig. 5*Aa* and *Ba*), but 20 mM-TEA reversibly depressed the ATP-induced hyperpolarization ($n = 3$) and depolarization ($n = 2$). In S neurones both the ATP-induced depolarization and the slow EPSP increased in amplitude in 10 mM-TEA (Fig. 5*B*). However, these effects vanished when the TEA-induced depolarization was nullified by passing

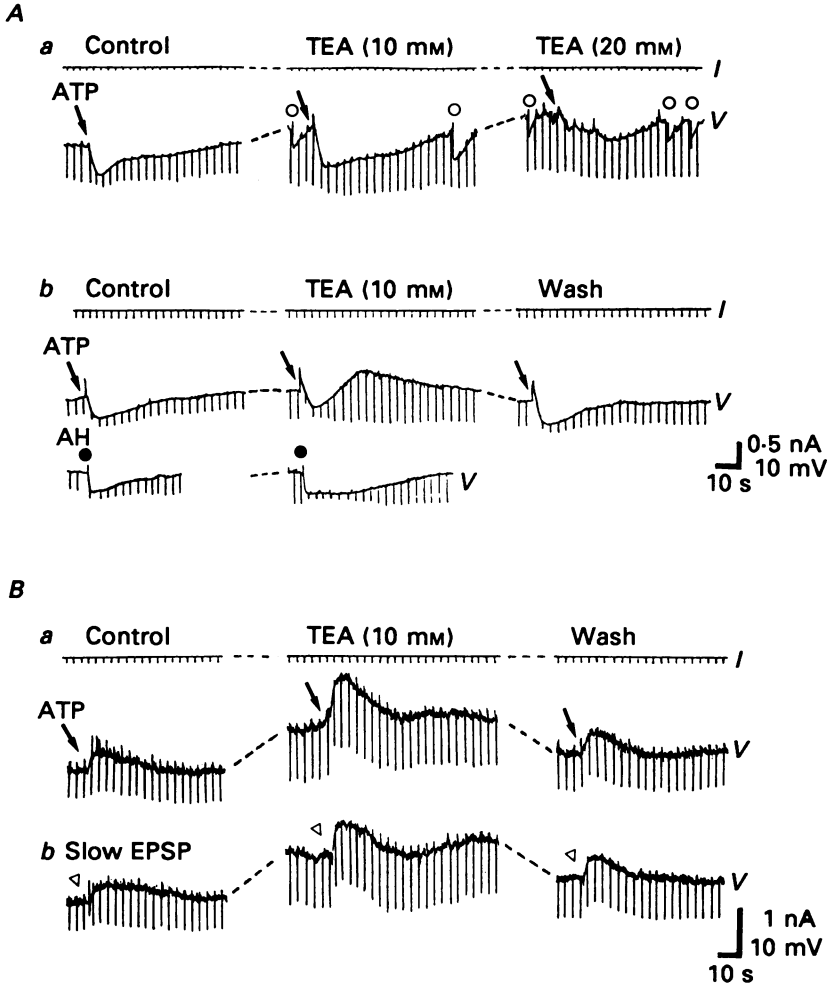

Fig. 5. Effects of TEA in two AH neurones (*A*) and an S neurone (*B*). ATP was applied by pressure ejection (indicated by arrow, 1 mM for 20 ms in *Aa*, 100 μM for 40 ms in *Ab* and 1 mM for 80 ms in *Ba*). *I*, current recordings; *V*, voltage recordings. *Aa*, the ATP-induced hyperpolarization in TEA-containing solution. Control (left), and 10 min after exposure to TEA (centre, 10 mM; right, 20 mM). The slow after-hyperpolarization following an off-response (○) persisted after treatment with TEA. *Ab*, the ATP-induced response (upper voltage traces) and the slow after-hyperpolarization following an action potential (●, lower traces). Control solution (left), after 10 min superfusion with 10 mM-TEA (centre), and after a 20 min wash (right). In this AH neurone the response to ATP became biphasic in TEA, and TEA prolonged the slow after-hyperpolarization. Resting membrane potential: −63 mV (*Aa*), −68 mV (*Ab*). *B*, the ATP-induced depolarization (*a*) and slow EPSP (*b*) evoked by repetitive focal stimulation (△), in the presence of 3 μM-atropine. Control (left), after a 10 min exposure to 10 mM-TEA (centre), and after a 10 min wash (right). Resting membrane potential, −56 mV.

hyperpolarizing current, suggesting that they resulted from the change in membrane potential.

In two AH neurones exposed to 10 mM-TEA, the ATP-induced response became biphasic; the hyperpolarization accompanied by a fall in input resistance was followed by a slow depolarization associated with an increased input resistance (Fig. 5*Ab*). As shown previously (see Morita *et al.* 1982*a*), the slow AHP was markedly prolonged in TEA (lower trace, Fig. 5*Ab*).

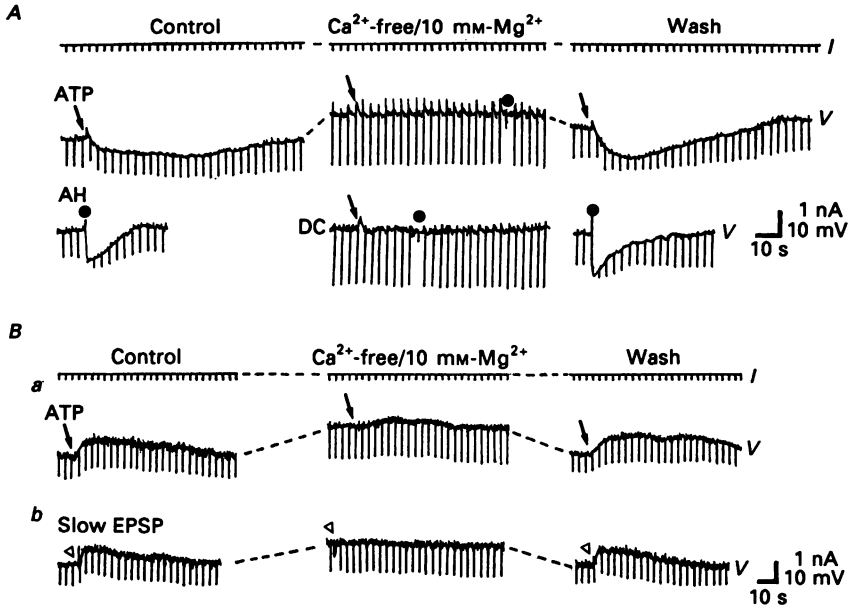

Fig. 6. Effect of calcium-free/high (10 mM)-magnesium solution on the ATP-induced responses, the slow after-hyperpolarization in an AH neurone (*A*) and the slow EPSP in an S neurone (*B*). ATP was applied by pressure ejection (indicated by arrow, 1 mM for 40 ms in *A* and 80 ms in *B*). *I*, current recordings; *V*, voltage recordings. *A*, the upper traces show the ATP-induced hyperpolarization and the lower traces show the slow after-hyperpolarization following an action potential evoked by an applied depolarizing pulse (●). Left, control; centre, 20 min after changing to the calcium-free/high-magnesium solution; and right, 20 min after washing. In the lower centre trace the depolarization caused by calcium removal was nullified by passing hyperpolarizing current, indicated by DC. *B*, the upper traces show the ATP-induced depolarization (*a*) and the lower traces show the slow EPSP (*b*) recorded from an S neurone in the presence of 1 μM-atropine. The slow EPSP was evoked by focal stimulation (5 pulses at 5 Hz), indicated by open triangles. Left, control; centre, 20 min (*a*) and 5 min (*b*) after changing to the test solution; and right, 20 min after washing. Resting membrane potential: −66 mV in *A*, −56 mV in *B*.

Effect of calcium and calcium blockers on the ATP-induced responses

Since the ATP-induced hyperpolarization was similar in nature to the slow AHP which is calcium-dependent (Morita *et al.* 1982*a*), it was examined whether or not the ATP responses could be sensitive to calcium. In both AH and S neurones superfusion with calcium-free solutions containing 1·2–10 mM-magnesium usually pro-

duced a membrane depolarization (5–10 mV in 1·2 mM-magnesium) associated with an increase in input resistance (Fig. 6). Both the ATP-induced hyperpolarization in AH neurones and depolarization in S neurones persisted when calcium was simply omitted from the normal modified Krebs solution (1·2 mM-magnesium) (not shown); this solution blocked evoked synaptic responses and the slow AHP. However, superfusion with calcium-free solutions containing higher magnesium concentrations progressively reduced the amplitude of the ATP-induced hyperpolarization and depolarization, even when the steady depolarization associated with calcium-free solutions was cancelled by passing hyperpolarizing current. For example, in AH neurones 20–30 min superfusion with a calcium-free/3·7 mM-magnesium solution reduced the ATP-induced hyperpolarization to 10–20% of control amplitude ($n = 2$), or abolished it altogether ($n = 4$). Calcium-free/10 mM-magnesium solution abolished the hyperpolarization within 10–20 min (Fig. 6*A*, $n = 6$). Similarly, the ATP-induced depolarization in S neurones was reduced to 20–30% of control amplitude ($n = 3$) or abolished ($n = 4$) after 20–30 min superfusion with calcium-free/10 mM-magnesium solutions (Fig. 6*B*). The inhibitory effects of these calcium-free/high-magnesium solutions were reversible. ATP-induced responses were also blocked during superfusion with calcium-free solutions containing 1–2 mM-EGTA, but this effect was not reversible ($n = 2$).

Solutions containing nickel (0·1–1 mM) or cobalt (1–3 mM) caused a membrane depolarization (up to 10 mV) associated with an increased input resistance in both AH and S neurones similar to the effect produced by calcium-free/1·2 mM-magnesium solution (see above and Fig. 6). The ATP-induced hyperpolarization ($n = 4$) and depolarization ($n = 3$) were markedly depressed or abolished in the presence of these calcium blockers (data not shown).

Pharmacological properties of ATP-induced responses

ATP receptors have been classified into several subtypes, based on their sensitivity to adenosine, quinine and methylxanthines (Burnstock, 1972; Stone, 1981). The following experiments were undertaken to identify the receptor subtypes mediating the ATP-induced responses in myenteric neurones.

ATP-induced hyperpolarization

This response was reversibly inhibited by quinine and its stereoisomer, quinidine. Low concentrations of these antagonists (1–10 μM) depressed the ATP-induced hyperpolarization without significant change in the membrane potential. A high concentration (100 μM) of these drugs always caused a membrane depolarization and almost completely blocked the response to ATP (Fig. 7*A a* and *b*). It took 10 and 30 min to wash out the effects of 100 μM-quinine and quinidine, respectively. Caffeine (100 μM, $n = 6$), theophylline (100 μM, $n = 2$) and IBMX (1 μM, $n = 3$ and 10 μM, $n = 4$) decreased the peak amplitude by 30–60%, and markedly shortened the duration of the ATP-induced hyperpolarization without significant change in the membrane potential (Fig. 7*A c*). However, caffeine at low concentration (1 μM) prolonged the duration of the ATP-induced hyperpolarization. The effects of low concentrations of caffeine on the ATP-induced hyperpolarization resembled those on the slow AHP (Morita *et al.* 1982*a*). As described above, adenosine produced a

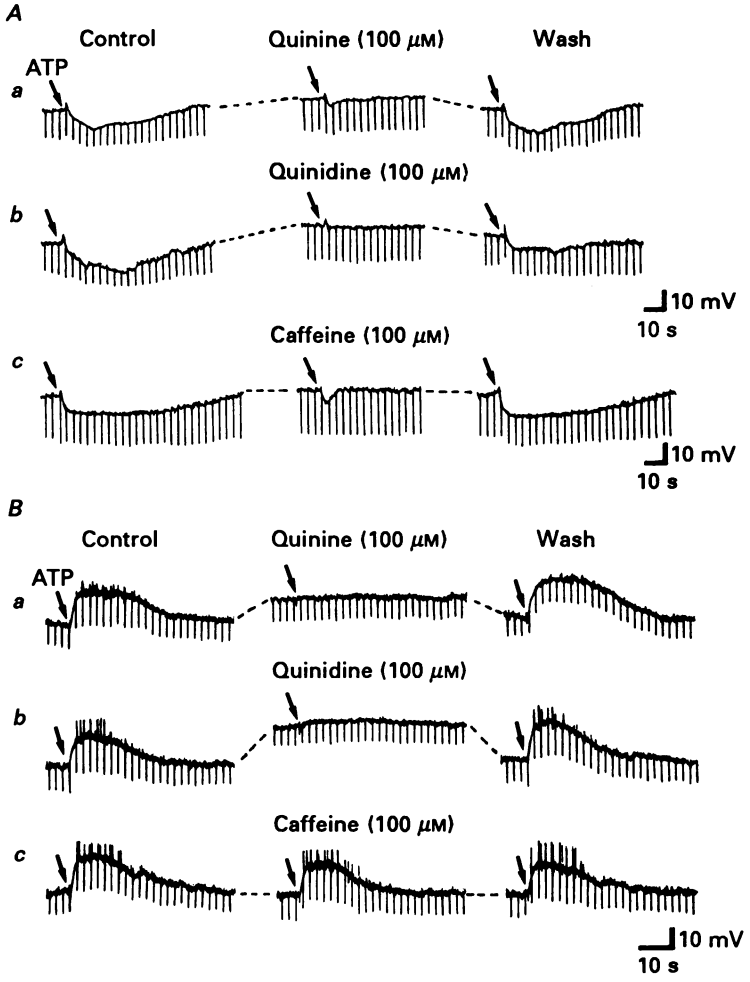

Fig. 7. Actions of quinine, quinidine and caffeine on the ATP-induced hyperpolarization (*A*) and depolarization (*B*). ATP was applied by pressure ejection (indicated by arrow, 1 mM for 40 ms in *A* and 100 ms in *B*). Control (left), after 5–10 min exposure to the drug-containing solutions (centre) and after a 10–30 min wash (right). Resting membrane potential: −65 mV in *A a* and *b*, −66 mV in *A c*, and −54 mV in *B*. Records in *a* and *b* of *A* were taken from the same AH neurone. All records in *B* were obtained from the same S neurone.

smaller hyperpolarization than ATP (see Fig. 2 and Palmer, Wood & Zafirov, 1987). These findings indicate that the receptor subtype for the ATP hyperpolarization might be different from classical P_1 or P_2 (see Burnstock, 1972; Stone, 1981).

ATP-induced depolarization

This response was reversibly blocked by quinine and quinidine. A low concentration of these drugs (10 μM) reduced the peak amplitude to 30–40% (quinine, $n = 4$) and 40–60% (quinidine, $n = 4$) of control. A higher concentration (100 μM)

depolarized the neurones by 5–20 mV and abolished the ATP-induced depolarization (quinine, $n = 4$; quinidine, $n = 3$; Fig. 7*Ba* and *b*). Complete recovery from the depressant effect of 100 μM-quinidine was achieved only after a 30 min wash. The blocking action of quinine and quinidine was not due to their depolarizing effect, because the block persisted both when the depolarization was cancelled by injected current and in those cases where the drugs produced no appreciable change

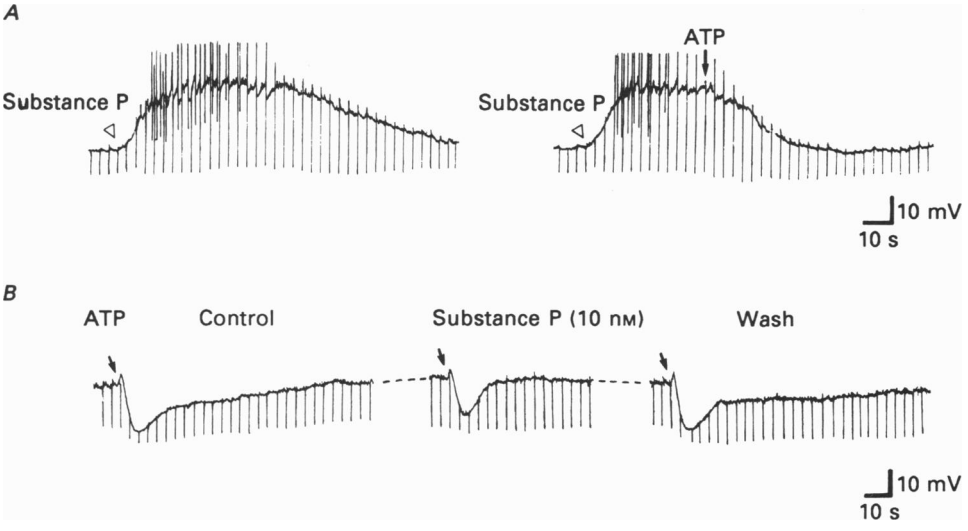

Fig. 8. Interaction between the ATP- and substance P-induced responses in AH neurones. ATP (*A*, 100 μM; *B*, 1 mM) was applied by pressure ejection (indicated by arrows; duration: 40 ms in *A*, 20 ms in *B*). Substance P (100 nM) was applied by pressure ejection in *A* (△; duration: 40 ms) and by superfusion in *B*. *A*, responses to substance P alone (left trace), and to substance P followed by ATP (right trace). The decay time course of the substance P-induced depolarization became more rapid following ATP application. *B*, responses to ATP recorded before exposure to substance P (left trace), following a 10 min exposure to substance P (centre), and 10 min after wash-out of substance P (right). Substance P inhibited the ATP-induced hyperpolarization. Resting membrane potential: −66 mV in *A*, −62 mV in *B*.

in membrane potential. Sometimes the response to ATP was transiently augmented after the withdrawal of these blocking drugs (see Fig. 7*Ba* and *b*). Caffeine (100 μM, $n = 5$), theophylline (100 μM, $n = 2$) and IBMX (10 μM, $n = 3$) did not significantly affect the peak amplitude of the ATP-induced depolarization, the membrane potential or the input resistance (Fig. 7*Bc*). However, caffeine decreased the half-decay time of the ATP-induced response to $62 \pm 3\%$ ($n = 5$) of control (Fig. 7*Bc*). Theophylline and IBMX also shortened the decay time, though their effect was weaker than that of caffeine. These results suggest that the receptor subtype for the ATP-induced depolarization is most likely a P_2 type.

Interaction between substance P and ATP

The present findings indicate that the ATP responses are due either to an increase or to a decrease in potassium conductance. Since substance P decreased potassium

conductance in both AH and S neurones (see Katayama, North & Williams, 1979), the interaction between the substance P and ATP responses was examined.

In AH neurones superfusion with 10 μM-ATP reduced the amplitude of the substance P-induced depolarization. This inhibitory effect persisted even when the ATP-induced hyperpolarization was nullified. The substance P-induced depolarization decayed more quickly when ATP was applied immediately after the substance P-induced response reached its peak, as shown in Fig. 8*A*. Likewise, the ATP-induced hyperpolarization decayed more quickly in the presence of 10 nM-substance P, as shown in Fig. 8*B*. These interactions between ATP and substance P were confirmed in four additional AH neurones.

In S neurones 10 μM-ATP also reduced the amplitude of the substance P-induced depolarization (not shown). This effect of ATP was reversible, and was observed even when the ATP-induced depolarization was nullified. Reciprocally, the ATP-induced depolarization was also inhibited by substance P (1–10 nM); this inhibitory action of substance P became marked when the substance P-induced depolarization was nullified.

DISCUSSION

ATP applied either by superfusion or by pressure ejection caused a dose-dependent membrane hyperpolarization in most AH neurones, but a depolarization in most S neurones (Figs 1 and 2). The hyperpolarization produced by ATP was associated with a decrease in input resistance, whereas the ATP-induced depolarization was associated with an increase in input resistance. The reversal potentials of both responses were close to the potassium equilibrium potential (−91 mV, see Morita *et al.* 1982*a*) and varied with the concentration of extracellular potassium ions as predicted by the Nernst equation (Fig. 4). All of these results indicate that the ATP-induced hyperpolarization is due to an increase, while the depolarization is due to a decrease in potassium conductance.

Calcium sensitivity of the ATP responses in myenteric neurones

Both responses to ATP persisted in TTX-containing or calcium-free/normal-magnesium solution, and were reduced or abolished in calcium-free/high-magnesium solution (Fig. 6*A*) and in solutions containing cobalt or nickel. These results suggest that the effects of ATP are not presynaptic, and that the potassium conductance modulated by ATP is calcium-dependent, or at least calcium-sensitive.

Because calcium-free solutions depolarized AH neurones and decreased their input conductance (Fig. 6; see also Grafe, Mayer & Wood, 1980; Hirst, Johnson & Helden, 1985; North & Tokimasa, 1987), it appears that some of the resting potassium conductance is also calcium-sensitive. The calcium-dependent potassium conductance underlying the slow after-hyperpolarization (AHP) is thought to be the same as that which contributes to the resting potential (North & Tokimasa, 1987). Another similarity between the calcium-sensitive potassium conductance activated by ATP and that which contributes to the resting potential is that both are persistent, i.e. do not appear to inactivate (see North & Tokimasa, 1987). From the present data, the calcium-sensitive potassium conductance modified by ATP appears

to be identical to the calcium-sensitive potassium conductance(s) open in resting neurones or following action potentials in AH neurones. There are several mechanisms by which ATP may activate a calcium-sensitive potassium conductance in AH neurones. It is suggested that the action of ATP on smooth muscles is due partially to release of calcium from internal stores (Den Hertog, 1982). This mechanism seems plausible in AH neurones as well, because the ATP-induced hyperpolarization persisted in calcium-free solution with normal (1·2 mM) magnesium concentration, and low ATP concentrations prolonged the slow AHP without detectable change in the action potential (Fig. 3*A*). Indeed, caffeine, which releases calcium from intracellular store sites, prolonged both the ATP-induced hyperpolarization and the slow AHP (Morita *et al.* 1982*a*; Cherubini, Morita & North, 1984).

ATP inactivates M-type potassium channels in bull-frog sympathetic ganglion cells (Akasu *et al.* 1983). However, it is unlikely that the ATP-induced depolarization in myenteric S neurones is due to blocking M channels, because the amplitude of the ATP response was linearly related to the membrane potential in the range −60 to −100 mV (see Brown & Adams, 1980). This suggests that the ATP-induced depolarization may instead be due to blockage of 'calcium-sensitive resting' or 'leakage' potassium conductance, as also occurs during the slow EPSP (Morita & North, 1985) and the muscarinic depolarization (Morita *et al.* 1982*b*) in myenteric neurones.

Actions of quinine or quinidine and methylxanthines on the ATP responses

Most of the drugs used to examine the receptor subtypes for the ATP responses are known to act on intermediate processes. Indeed, both ATP responses developed slowly; this is consistent with involvement of a second messenger system. Methylxanthines inhibit cyclic AMP phosphodiesterase and hence result in an accumulation of cytoplasmic cyclic AMP (see Butcher & Sutherland, 1962). Since in the present study methylaxanthines inhibited the ATP-induced hyperpolarization in AH neurones, involvement of cyclic AMP might be considered in the action of ATP on AH neurones (see Palmer, Wood & Zafirov, 1986).

In myenteric AH neurones, quinine depressed both the slow AHP and the hyperpolarization produced by intracellular injection of calcium (Cherubini, North & Surprenant, 1984). In liver and red blood cells quinine and quinidine inhibited activation of a calcium-dependent potassium conductance by biogenic substances and a calcium ionophore (Burgess, Claret & Jenkinson, 1981; Cook & Haylett, 1985). These results suggest that the inhibitory action of quinine and quinidine on the ATP-induced responses in myenteric neurones may be due to an effect on calcium-dependent processes contributing to the ATP-induced potential changes, rather than to direct blockade of ATP receptor sites. It seems likely that these drugs may distinguish between intracellular mechanisms rather than different receptor types.

Physiological significance

The ATP-induced hyperpolarization reduces excitability of most AH neurones, while the ATP-induced depolarization increases excitability of most S neurones. Thus ATP can differentially modulate activity in the myenteric plexus by activating

or inhibiting calcium-sensitive potassium channels as acetylcholine can in the central nervous system (see Egan & North, 1986). Furthermore, the time to peak and decay time of responses to pressure ejection of ATP in AH neurones varies greatly but not in S neurones. It is possible that this might reflect a difference in the site of action of ATP in the two cell types, e.g. processes in AH neurones and soma in S neurones. Indeed, AH and S neurones are morphologically different (Hodgkiss & Lees, 1983).

There was an interaction between ATP- and substance P-induced responses (Fig. 8). This suggests that ATP might modulate slow EPSPs, because substance P is a putative neurotransmitter for the slow EPSPs (Johnson, Katayama, Morita & North, 1981; Bornstein, North, Costa & Furness, 1984). From the present results, however, we cannot determine whether ATP and substance P might share some intermediate processes or sites of actions, e.g. calcium-sensitive potassium channels. Mechanisms underlying this interaction remain to be further analysed.

It is concluded that ATP may act as a neuromodulator in the myenteric plexus as in other preparations (Akasu *et al.* 1983; Morita, Katayama, Ketetsu & Akasu, 1984; Silinsky, 1984). It is also possible that ATP mediates slow EPSPs in S neurones and slow IPSPs in AH neurones, because ATP mimics these synaptic responses (see Johnson, Katayama & North, 1980).

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