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Actions of ATP and α,β-methylene ATP on neuromuscular transmission and smooth muscle membrane of the rabbit and guinea-pig mesenteric arteries

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1 In the rabbit mesenteric artery, adenosine triphosphate (ATP), showed two actions on the membrane potential of muscle cells: low concentrations (1–10 μM) hyperpolarized and high concentrations (> 50 μM) depolarized the membrane. Both changes in the potential were accompanied by increases in ionic conductance.

2 In the rabbit mesenteric artery, α,β-methylene ATP (MeATP), (> 30 nM) depolarized the muscle membrane at a lower concentration than ATP (> 50 μM), and increased the ionic conductance of the membrane. The depolarization induced by ATP was prevented by low concentrations of MeATP, but the hyperpolarization was retained. Furthermore, the hyperpolarization was not affected by theophylline (10 μM).

3 In the guinea-pig mesenteric artery, ATP and MeATP depolarized and increased the ionic conductance of muscle membrane, but to depolarize the membrane, higher concentrations of both agents were required, compared to those in the rabbit mesenteric artery.

4 In the mesenteric arteries from both species, perivascular nerve stimulation evoked excitatory junction potentials (e.j.ps). In both tissues, MeATP inhibited the amplitude of e.j.ps at lower concentrations than did ATP, and both agents had more potent inhibitory actions on rabbit than on guinea-pig. The inhibition of e.j.p. induced by low concentrations of these agents showed no relationship to depolarization, but the inhibition induced by high concentrations was paralleled by depolarization and increase in ionic conductance of the membrane.

5 In the rabbit mesenteric artery, overflow of noradrenaline (NA) and its metabolite (3,4-dihydroxyphenylglycol; DOPEG) produced by perivascular nerve stimulation was examined. ATP (0.1 mM) but not MeATP (0.1 μM) reduced the overflow of NA, whereas both agents had no effect on the overflow of DOPEG.

6 Exogenously applied high concentrations of NA (> 3 μM) depolarized the muscle membrane in both species. These NA-induced depolarizations were not affected by treatment with ATP or MeATP.

7 It is concluded that, in the rabbit mesenteric artery, ATP is more likely to be involved in generation of e.j.ps than is NA. A similar interpretation in the guinea-pig mesenteric artery is complicated by the depolarization produced by high concentrations of ATP or MeATP.

Introduction

In the rat tail artery, Burnstock *et al.* (1984) found that e.j.ps evoked by perivascular nerve stimulation ceased under treatment with α,β-methylene ATP (MeATP; a slowly degradable analogue of ATP) and the slow and maintained depolarization following e.j.ps ceased in the presence of phentolamine. They postulated that e.j.ps are generated by activation of the P_2-purinoceptor (Burnstock, 1971) due to release of ATP as a co-transmitter of noradrenaline (NA), from the nerve terminals. In the rabbit ear artery, Suzuki *et al.* (1984) noted that repetitive perivascular nerve stimulation evoked e.j.ps with a following slow depolarization. The e.j.ps were not blocked by reserpine pretreatment. However, the slow depolarization was inhibited by

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reserpine in proportion to a reduction in the amount of NA release from nerve terminals, as estimated from the bioassay of NA overflow. Furthermore, iontophoretic applications of ATP or NA to the tissue generated responses similar to e.j.ps and slow depolarization, respectively, and α-adrenoceptor blocking agents blocked only the slow depolarization. Therefore, they postulated the possible involvement of a neurotransmitter other than NA in generation of the e.j.p. (Suzuki, 1985).

If ATP generates the e.j.p. by activating the P_2-receptor, as a co-transmitter with NA in some vascular tissues, it may be that the nature of junctional receptors differs with the region and species. ATP potently inhibits the e.j.p. in the rabbit mesenteric artery, but the potency is weak in the guinea-pig mesenteric artery (Kuriyama & Makita, 1984). The adrenoceptor responsible for generation of e.j.p. in vascular tissues (guinea-pig mesenteric or rat basilar artery; Hirst & Neild, 1980; 1981; Hirst *et al.*, 1982) was termed a γ-adrenoceptor, or an intra-junctional adrenoceptor (Kuriyama & Suyama, 1983) because its responses to α-adrenoceptor blocking agents differed from that of extra-junctional adrenoceptors. These discrepancies related to the nature of neuromuscular transmission have not been elucidated.

ATP has multiple actions on vascular smooth muscle tissues, e.g. in the guinea-pig coronary artery and portal vein, it hyperpolarized or depolarized the membrane, respectively. In the portal vein, when the membrane was depolarized by ATP, the spontaneous spike generations were enhanced, while in the guinea-pig common jejunal vein, ATP hyperpolarized the membrane (Karashima & Takata, 1979; Takata & Kuriyama, 1980). In the rabbit and guinea-pig mesenteric arteries, ATP markedly or slightly inhibited the amplitude of e.j.ps respectively, with no effect on the facilitation of e.j.ps evoked by a train of stimuli with a frequency over 0.1 Hz (Kuriyama & Makita, 1984).

We attempted to determine whether or not ATP plays a main role in the generation of e.j.p. in mesenteric arteries of the rabbit and guinea-pig. For this purpose, the effects of ATP and its analogues, α,β-methylene ATP (MeATP) or adenyl-imidodiphosphate (AMP-PNP) on e.j.ps and muscle membrane potentials and their interactions with NA-mediated responses were investigated. In addition, the outflows of NA and a major metabolite in the presence or absence of ATP or MeATP were measured, to determine the sites of action of ATP or MeATP.

Methods

Guinea-pigs of either sex, weighing 250–300 g were stunned and bled. Albino rabbits of either sex, weighing 2–3 kg were anaesthetized by intravenous injection of sodium pentobarbitone (30 mg kg^{-1}) and bled. The arteries together with vein and lymph vessels running in parallel were excised from the mesenteric vascular bed of the jejunum. Arteries with an external diameter of between 120 and 200 μm and about 15 mm in length were mounted in 0.8 ml organ bath.

Recordings of electrical activities

The electrical activity generated from single smooth muscle cells was recorded by use of glass capillary microelectrodes filled with 3 M KCl of 60–80 MΩ resistance. A microelectrode was inserted into the muscle cell from the adventitial surface through the surrounding connective tissue. To record the electrotonic potential, the partition stimulating method was used (Abe & Tomita, 1968). Perivascular nerves were stimulated by drawing the proximal part of the artery into a suction electrode or by the point stimulation method (Suzuki & Fujiwara, 1982). Current pulses of 0.05–0.10 ms in duration and 50 V in intensity were supplied from an electric stimulator (Nihon Kohden SEN – 3013). Electrical activity was recorded on a recticorder (Nihon Kohden; RJB4024). The organ bath was superfused with Krebs solution at 33–35°C and the flow rate was 3 ml min^{-1}.

Normal Krebs bicarbonate solution contained (mM): Na^+ 137.4, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, HCO_3^- 15.5, $H_2PO_4^-$ 1.2, Cl^- 134 and glucose 11.5. This solution was bubbled with 95% O_2 and 5% CO_2 and the pH was 7.3–7.4.

Measurements of the overflow of noradrenaline (NA) and 3,4-dihydroxyphenylglycol (DOPEG)

To measure the NA and DOPEG overflow, a pair of Ag-AgCl wires (0.5 mm in diameter) was fixed vertically 1.0–1.2 mm apart, and the tissue was mounted between these wires by means of cotton thread. Krebs solution at 35°C was superfused over the tissue at a rate of 1 ml min^{-1} by means of a perfusion pump (Tokyo Rikakikai PO-1). The tissue was incubated under such conditions for at least 1 h and the perfusate was collected into a conical test tube placed at the bottom of the tissue for 5 min following the beginning of field stimulation of perivascular nerves (0.2 ms, 5 Hz, 50 V, and 300 pulses; Nihon Kohden SEN7103). The nerve stimulation was applied through the Ag-AgCl wires. ATP or MeATP were added to the superfusate 30 min before and during nerve stimulation. The solution containing ATP was newly prepared every 10 min to preserve the action of ATP. Perchloric acid solution (60%, 50 μl) was added to the perfusate, and the sample (each perfusate volume was 5 ml) was stored at − 20°C until assayed. At the end of experiments, the tissue was blotted and weighed (Suzuki *et al.*, 1984). NA and DOPEG were isolated by

the alumina adsorption method (Oishi *et al.*, 1983). An aliquot (50 μM) of the extract was subjected to high-performance liquid chromatography (Yanagimoto MGF, L-200L; Oishi *et al.*, 1983).

Drugs

The following drugs were used; noradrenaline (NA), ATP disodium salt, α,β-methylene ATP (MeATP) and theophylline (all from Sigma) and adenyl-imidodiphosphate tetralithium salt (AMP-PNP; Boehringer). Solutions containing the final concentrations of drugs were freshly prepared for each experiment.

Statistics

Values obtained from the electrophysiological experiments were expressed as mean ± s.d., number of measurements. The amount of overflow of NA or DOPEG was expressed as the mean ± s.e.mean, number of measurements. The statistical significance was assessed by Student's *t* test and a *P* value of less than 0.05 was considered significant.

Results

Effects of ATP and its analogues on the membrane properties of smooth muscle of the rabbit mesenteric artery

Figure 1 shows examples of typical changes in the membrane potential of smooth muscle cells of the rabbit mesenteric artery produced by ATP (0.1 mM) and MeATP (0.1 μM). Application of 0.1 mM ATP depolarized the membrane by over 15 mV within several minutes, then the cell gradually repolarized to the resting level within another 15 min. MeATP, 0.1 μM, also markedly depolarized the membrane (over 20 mV), then the membrane gradually repolarized to a new level sustained for over 15 min but did not repolarize to the resting level after 30 min. Consequently, the membrane potential in the presence of various concentrations of ATP or MeATP was measured 5–15 min after application.

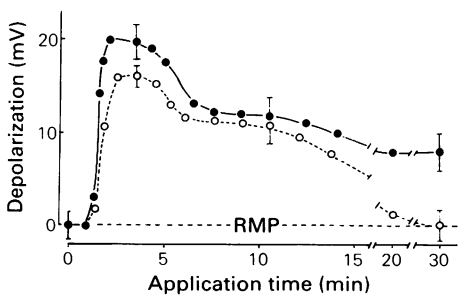

Figure 1 Effects of ATP and α,β-methylene ATP (MeATP) on the membrane potential of smooth muscle cells of the rabbit mesenteric artery. ATP (0.1 mM, ○) and MeATP (0.1 μM, ●) were applied for 30 min. The ATP-containing solution was replaced with fresh ATP-containing solution at 10, 20 and 30 min. Each point shows mean amplitude of depolarization (with s.d. shown for only selected points) measured from 5 tissues. (RMP: resting membrane potential; 69.5 ± 1.5 mV, $n = 25$)

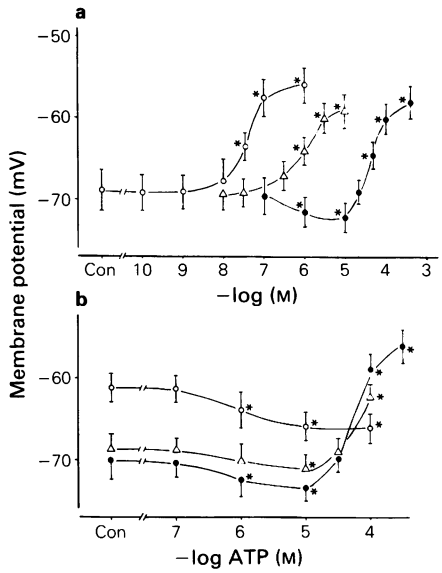

Figure 2 (a) Effects of various concentrations of ATP (●), α,β-methylene ATP (MeATP, ○) and adenylimidodiphosphate (AMP-PNP, △) on the membrane potential of smooth muscle cells of the rabbit mesenteric artery. The membrane potential was measured after 5–15 min superfusion with the drug-containing solution. Values are expressed as mean with s.d. shown by vertical lines, $n = 25–45$. (b) Effects of various concentrations of ATP on the membrane potential of smooth muscle cells of the rabbit mesenteric artery during treatment with 10 nM (△) or 0.1 μM (○) MeATP (mean with s.d., $n = 25–45$). Control (●). *Statistically significantly different from control ($P < 0.05$).

Figure 2a shows the effects of ATP, AMP-PNP and MeATP on the mean membrane potentials recorded from the rabbit mesenteric artery, the resting membrane potential being −69.3 ± 2.8 mV, $n = 109$. Applications of ATP produced two actions on the membrane potential, i.e. in low concentrations (1–10 μM), the membrane was marginally hyperpolarized and in high concentrations (> 50 μM), ATP depolarized the membrane. When MeATP (> 30 nM) or AMP-PNP (> 1 μM) was applied, the membrane was depolarized, concentration-dependently. Figure

2b shows the effects of ATP on the membrane potential of the rabbit mesenteric artery during treatment with MeATP. Application of 10 nM MeATP did not modify the membrane potential changes produced by applications of ATP. Application of 0.1 μM MeATP depolarized the membrane and blocked the depolarization induced by ATP without affecting the hyperpolarization. These results indicate that ATP is less potent than MeATP or AMP-PNP and its concentration-response curve is biphasic showing hyperpolarization at low concentrations, whereas the analogues only depolarize the membrane.

The effects of theophylline, a P_1-receptor blocker (Burnstock, 1981), on the hyperpolarization evoked by ATP during treatment with MeATP were studied (Table 1). Application of 10 μM theophylline did not modify the resting membrane potential, the depolarization induced by 0.1 μM MeATP or the hyperpolarization evoked by 0.1 mM ATP during treatment with 0.1 μM MeATP. This suggests that neither the hyperpolarization induced by ATP nor the depolarization produced by MeATP are due to activation of the P_1-receptor, but by default, may be via the P_2-receptor (Burnstock, 1981).

Table 2 shows the effects of NA (10 μM) on the membrane potential in the presence of ATP or MeATP. The membrane was depolarized by NA (control: -70.8 ± 2.8 mV, $n = 43$; in 10 μM NA: -62.3 ± 2.3, $n = 32$, $P < 0.001$). The size of the depolarization evoked by exogenous NA was not modified by the presence of ATP (0.1 mM, 30 min) or MeATP (0.1 μM, 30 min). Therefore, the NA-induced depolarization, in contrast to an ATP-induced depolarization of a similar magnitude, can occur in the presence of MeATP.

The current-voltage relationship was measured from single smooth muscle cells in an attempt to clarify both the basis of the biphasic concentration-response curve to ATP and of the depolarization induced by MeATP (in relation to the ionic conductance of the membrane) (Figure 3). The recording microelectrode was inserted into the cell at a distance of 0.1 mm from the stimulating electrode. With application of 10 μM ATP, the membrane was hyperpolarized by about 4.5 mV and amplitudes of the electrotonic potential evoked by various intensities of the inward and outward current pulses were reduced. Such reductions in the electrotonic potential were also observed at the same membrane potential level (the resting level) after displacement of the membrane potential by injections of the outward current in the presence of ATP (Figure 3a). The microelectrode was inserted into the same cell before and during application of ATP. The amplitude of the electrotonic potential evoked by the inward current after application of 10 μM ATP at the resting membrane potential level was 0.82 ± 0.05 times the control, ($n = 5$): therefore, the relative membrane resistance was reduced to 0.67 times the control (according to the estimation made by Hodgkin & Rushton, 1946; in the present experiments, the distance between the stimulating and recording electrodes was much less than the length constant of the tissue of 1.2 mm: Kuriyama & Makita, 1984). Furthermore, when the current-voltage relationships observed before and after application of ATP were extrapolated to the

Table 1 Effects of theophylline on the α,β-methylene ATP (MeATP)-induced depolarization and ATP-induced hyperpolarization during treatment with MeATP (rabbit mesenteric artery): theophylline does not affect membrane potentials produced by MeATP or by ATP with MeATP

Test drug	*Control* (mV)	*MeATP* (0.1 μM) (mV)	*ATP* (0.1 mM during treatment with MeATP 0.1 μM) (mV)
None	-70.3 ± 2.1 $n = 45$	-60.8 ± 1.3 $n = 35$	-66.0 ± 1.4 $n = 36$
Theophylline (10 μM)	-69.3 ± 1.2 $n = 29$	-61.6 ± 1.1 $n = 36$	-66.9 ± 1.8 $n = 35$

Mean ± s.d. is shown. n = number of observations.

Table 2 Effects of noradrenaline (NA)-induced depolarization in the presence of ATP or α,β-methylene ATP (MeATP)

A *Rabbit mesenteric artery*

Test drug	*Control* (mV)	*NA 10μM* (mV)
None	-70.8 ± 2.8 $n = 43$	-62.3 ± 2.3* $n = 32$
ATP 0.1 mM (30 min)	-70.6 ± 2.4 $n = 25$	-62.5 ± 1.5* $n = 18$
MeATP 0.1 μM (30 min)	-63.2 ± 1.6 $n = 29$	-54.5 ± 2.1* $n = 25$

B *Guinea-pig mesenteric artery*

Test drug	*Control* (mV)	*NA 30μM* (mV)
None	-69.7 ± 2.6 $n = 46$	-58.2 ± 3.5* $n = 28$
ATP 0.1 mM (30 min)	-67.9 ± 2.3 $n = 23$	-59.9 ± 3.6* $n = 34$
MeATP 0.1 μM (30 min)	-68.5 ± 3.3 $n = 17$	-55.3 ± 3.1* $n = 20$

Mean ± s.d. is shown. n = number of observations.
*Statistically significantly different from control ($P < 0.05$).

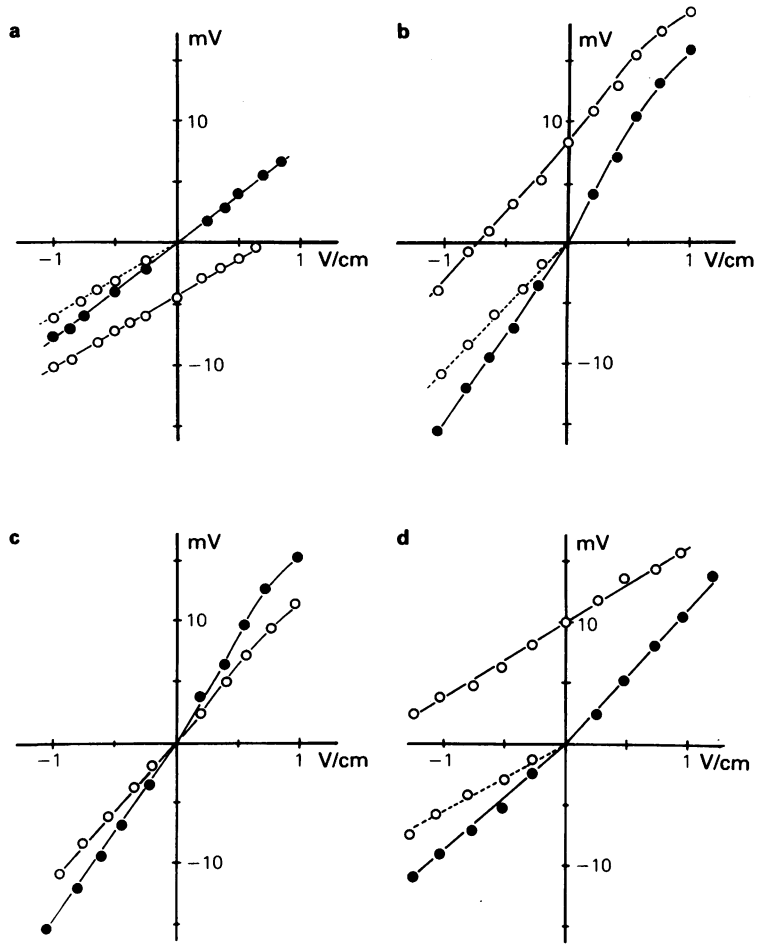

Figure 3 Effects of ATP and α,β-methylene ATP (MeATP) on the membrane resistance estimated from the change in amplitudes of the electrotonic potential on the rabbit mesenteric artery. Current-voltage relationships obtained before (control, ●) and after 10 min application of 10 μM ATP (O—O) (a), 10 min in 0.1 mM ATP (O—O) (b), 30 min in 0.1 mM ATP (O—O) (c) and 10 min in 0.1 μM MeATP (O—O) (d). The experimental procedures are described in the text. Dotted line indicates the current-voltage relationship observed after displacement of the membrane potential to the resting level, in the presence of ATP or MeATP. The microelectrode was inserted into the same cell before and during application of drug at 0.1 mm distant from the stimulating electrode. Each figure was obtained from a different cell.

hyperpolarized direction, both lines crossed at about − 85 mV, as expected from the increase in the K^+ conductance (the K^+ equilibrium potential is estimated to be − 80 to − 90 mV, Brading, 1981; Casteels, 1981).

When the current-voltage relationship was observered 10 min after application of 0.1 mM ATP (the membrane was depolarized as shown in Figure 3b), the relative change in the membrane resistance, calculated after the membrane potential had been displaced to the control level by the current injection, was reduced to 0.54 ± 0.09 times the control ($n = 5$). Furthermore, when this relationship was measured after the membrane repolarized to the control level (30 min after application of 0.1 mM ATP), the membrane resistance was still reduced over the control, as shown in Figure 3c (0.68 ± 0.11 times the control, $n = 5$). These results indicate that the hyperpolarization or depolarization of the membrane induced by ATP are due to increases in the ionic conductance of the membrane. When the membrane was repolarized to the resting level 30 min after the application of 0.1 mM ATP, the ionic conductance remained increased over the control.

Figure 3d shows the effects of 0.1 μM MeATP on the current-voltage relationship on single smooth muscle cells. The relative change in the membrane resistance was calculated to be 0.37 ± 0.04 times the control ($n = 3$) in the presence of 0.1 μM MeATP. This result indicates that the MeATP-induced depolarization is due to increases in the ionic conductance of the membrane and that MeATP is more potent than ATP in increasing ionic conductance.

Effects of ATP and MeATP on the membrane properties of smooth muscle of the guinea-pig mesenteric artery

The membrane potential of smooth muscle cells of the guinea-pig mesenteric artery was − 69.8 ± 1.1 mV, $n = 65$. ATP (> 1 μM) depolarized the membrane, concentration-dependently (Figure 4a). In the presence of 0.1 μM MeATP, the membrane potential was not modified and the depolarization induced by ATP (up to 0.1 mM) was inhibited. MeATP (> 0.3 μM) led to a dose-dependent depolarization (Figure 6d). NA (> 3 μM) depolarized the membrane; during treatment with 0.1 μM MeATP, this action of NA was not modified (Figure 4b).

MeATP (0.1 μM) did not modify the current-voltage relationship (Figure 5a) while MeATP (1 μM) depolarized the membrane and reduced the relative membrane resistance, as shown in Figure 5b (the mean membrane resistance 0.42 ± 0.08 times the control, $n = 4$; measured after displacement of the membrane potential to the control level). Much the same response was observed on the current-voltage relationship with 0.1 mM ATP, i.e. with depolarization of the membrane by ATP, the ionic conductance was increased (results not shown).

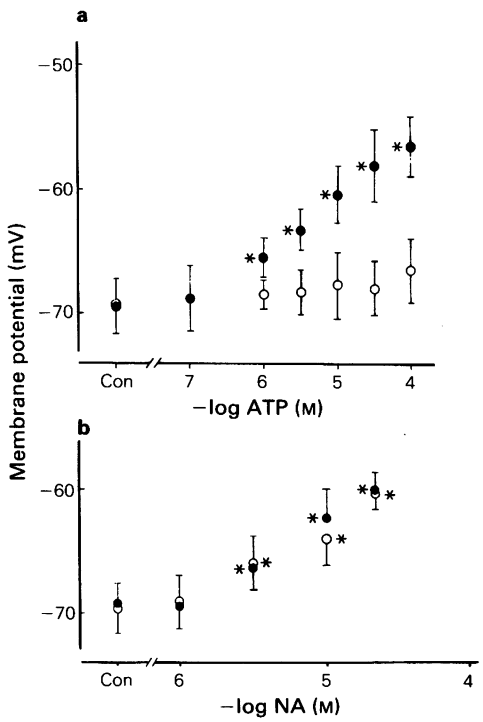

Figure 4 (a) Effects of various concentrations of ATP on the membrane potential recorded from smooth muscle cells of the guinea-pig mesenteric artery in the presence (○) or absence (●) of 0.1 μM α,β-methylene ATP (MeATP). (b) Effects of various concentrations of noradrenaline (NA) on the membrane potential in the presence (○) or absence (●) of 0.1 μM MeATP. The membrane potential was measured after 5–15 min applications of ATP or NA. MeATP was added 10 min before and during application of the above agents. Vertical bars indicate s.d., $n = 30–45$. *Statistically significantly different from the control ($P < 0.05$).

Effects of ATP and MeATP on e.j.ps from the rabbit mesenteric artery

Perivascular nerve stimulation produced e.j.ps in the rabbit mesenteric artery. E.j.p. was evoked by application of a train of field stimuli at frequencies below 0.5 Hz to avoid summation of e.j.ps due to the very long falling phase. Figure 6a and b shows the effects of ATP or MeATP on the e.j.ps evoked by stimulation at 0.5 Hz (6 pulses). With application of 0.1 mM ATP or 1 μM MeATP, e.j.ps were reduced by over 80%. When the mean amplitudes of e.j.p. and membrane potentials were measured in different tissues 5–15 min after application of ATP or MeATP, low concentrations of ATP or MeATP inhibited the amplitude of e.j.p. without changing the membrane potential. For these experiments, nerve stimulation was applied at intervals over 60 s. In concentrations over 1 μM, ATP continued to produce a concentration-dependent depression of the e.j.p. but this was accompanied by hyperpolarization then depolarization of the membrane as the concentration increased. After 3 min in 0.1 mM ATP, the amplitude of e.j.p. was reduced to 0.06 ± 0.03 times the control ($n = 25$) with depolarization of the membrane. When 0.1 mM ATP was applied for over 20 min, the membrane potential returned to the resting level, yet, the amplitude of e.j.p. remained reduced to 0.06 ± 0.04 that of the control ($n = 15$). MeATP (10 nM) reduced the amplitude of e.j.p. with no significant change in the membrane potential. At higher concentrations the e.j.p. was depressed but the membrane potential decreased; both actions were concentration-dependent. With 1 μM MeATP, the amplitude of e.j.p. was reduced to 0.18 ± 0.09 times the control ($n = 25$) with depolarization of the membrane − 57.8 ± 2.0 mV ($n = 30$).

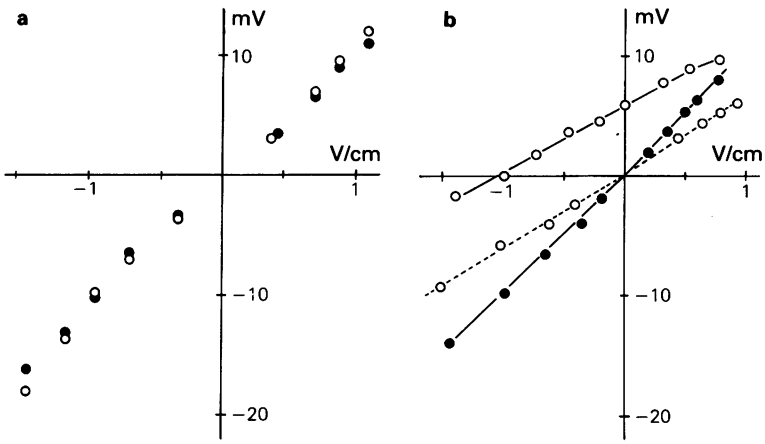

Figure 5 Effects of α,β-methylene ATP (MeATP) on the current-voltage relationship recorded from smooth muscle cells of the guinea-pig mesenteric artery (after 10–15 min): (a) 0.1 μM MeATP (○). (b) 1 μM MeATP (○). Dotted line indicates the relationship observed after displacement of the membrane potential to the control level; (●) control. Each figure was obtained from different cells. The experimental procedures were the same as those described in Figure 3.

To test for any effects of ATP and MeATP on the facilitation of e.j.ps, two pulses of field stimulation were applied at varying intervals (Figure 7). A plot was constructed of the facilitation (log scale) against interval. This gave a linear relationship which was completely unchanged by 20 μM ATP (Figure 7a) or 0.1 μM MeATP (Figure 7b). Thus, the facilitation of e.j.ps is not affected by ATP or MeATP.

Effects of ATP and MeATP on e.j.ps from the guinea-pig mesenteric artery

In this tissue, the falling phase of e.j.ps was much shorter than in the rabbit mesenteric artery. Therefore, the e.j.ps were evoked by 1.0 Hz stimulation (Figure 6 c and d). In the case of 1 μM ATP, the membrane was marginally depolarized (control; -69.8 ± 2.4 mV, $n = 30$ and in 1 μM ATP; -66.8 ± 1.4 mV, $n = 30$, $0.05 < P < 0.01$) with no change in the amplitude of e.j.p. With higher concentrations of ATP, the amplitude of the first e.j.p. in a train of stimuli was reduced and the membrane was depolarized, each in a concentration-related manner. However, at each concentration the reduction was less than that observed in the rabbit mesenteric artery (for guinea-pig in 0.1 mM ATP: e.j.p. was 0.50 ± 0.12 times the control, $n = 5$ at a membrane potential of -58.1 ± 1.4 mV, $n = 30$: cf. 0.06 ± 0.03 for rabbit, see above).

MeATP (0.1 μM) reduced the amplitude of e.j.p. (0.70 ± 0.10 times the control, $n = 24$, $P < 0.001$) with no depolarization of the membrane. With higher concentrations of MeATP (> 0.3 μM) the amplitudes of e.j.ps were reduced and depolarization of the membrane occurred; both effects were concentration-dependent. In the guinea-pig mesenteric artery, 0.1 mM ATP or 1 μM MeATP did not modify the facilitation (data not shown). AMP-PNP was less potent as an inhibitor of the amplitude of e.j.ps than were ATP or MeATP (data not shown).

Measurements of overflow of noradrenaline and 3,4-dihydroxyphenylglycol from rabbit mesenteric artery

Figure 8 shows the effects of ATP or MeATP on overflow of NA and DOPEG provoked by perivascular nerve stimulation (5 Hz, 0.2 ms and 300 pulse trains at 30 min intervals). With successive trains, overflow of NA and DOPEG was gradually reduced, but after the 4th stimulation (S4), the decline was small, i.e. expressed as a fraction of S1, mean ± s.e.mean, with S4, S5 and S6, NA was 0.87 ± 0.05, and 0.84 ± 0.07; DOPEG was 0.77 ± 0.05, 0.75 ± 0.04 and 0.72 ± 0.07 (Figure 8a). Thus when 0.1 mM ATP or 0.1 μM MeATP were added 30 min before and during S5, the effects were expressed as the ratio S5/S4 (Figure 8b). In control experiments, S5/S4 for NA and DOPEG respectively were 0.93 ± 0.04, $n = 8$ and 0.99 ± 0.07, $n = 6$. With 0.1 mM ATP the ratio for NA was smaller than in controls (0.66 ± 0.06, $n = 6$, $P < 0.005$), but the ratio for DOPEG was not (1.08 ± 0.12, $n = 6$, $P < 0.05$). MeATP (0.1 μM) had no effect on the ratio of S5/S4 for NA or DOPEG (0.98 ± 0.03, $n = 6$ and 0.92 ± 0.07, $n = 6$). This suggests that while ATP (0.1 mM) inhibits the NA release by field stimulation, MeATP (0.1 μM) does not.

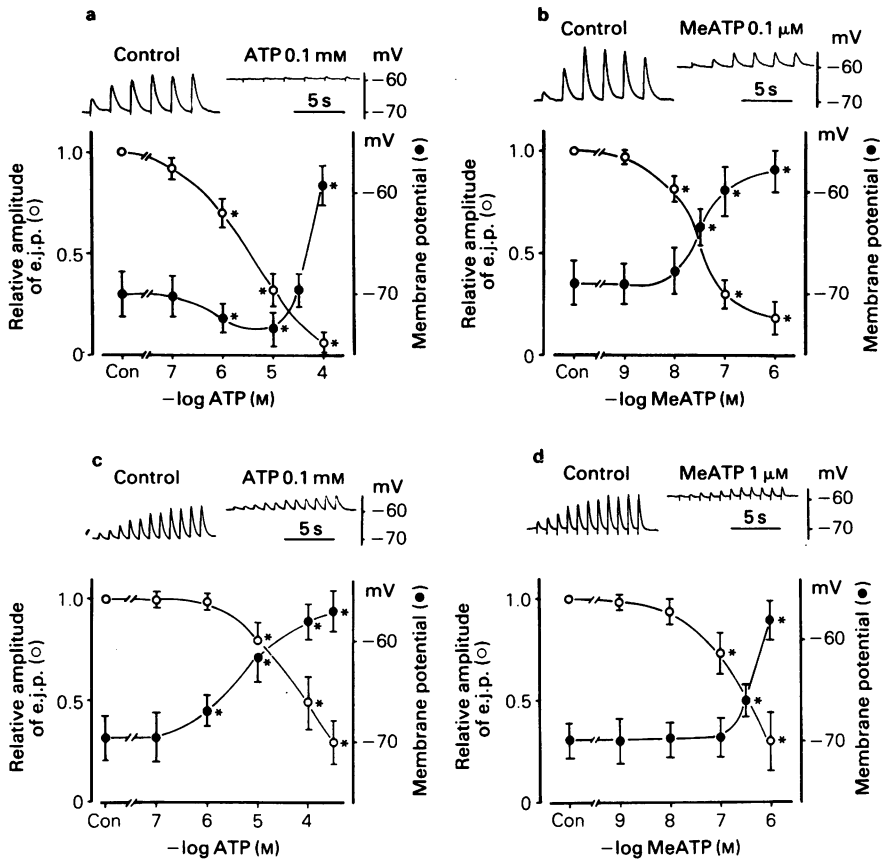

Figure 6 Effects of various concentrations of ATP or α,β-methylene ATP (MeATP) on the amplitude of e.j.p. and membrane potential recorded from smooth muscle cells of the rabbit (a,b) and the guinea-pig (c,d) mesenteric arteries. Actual records of e.j.ps before (control) and after application of ATP or MeATP are in the upper part of each figure (in the rabbit; 0.5 Hz, 6 pulses, in the guinea-pig; 1 Hz, 11 pulses). The amplitude of the e.j.p. in a train of stimuli was recorded and expressed as a fraction of drug-free control. The e.j.p. and membrane potential were recorded 10–15 min after application of these agents. Vertical bars indicate s.d., $n = 15-30$. *Statistically significantly different from the control ($P < 0.05$).

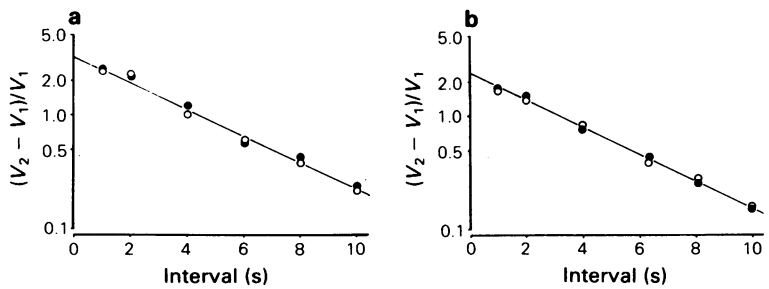

Figure 7 Effects of 20 μM ATP and 0.1 μM α,β-methylene ATP (MeATP) on the facilitation ratio of two e.j.ps to pulses of field stimulation at varying intervals in the rabbit mesenteric artery. Ordinates are log-scale of the value of $(V_2-V_1)/V_1$, where V_1 and V_2 are the amplitudes of the e.j.ps. recorded by the first and second stimulation. Abcissa scale indicates intervals between the pairs of pulses. Before (●) and 15 min after (○) application of (a) 20 μM ATP or (b) 0.1 μM MeATP.

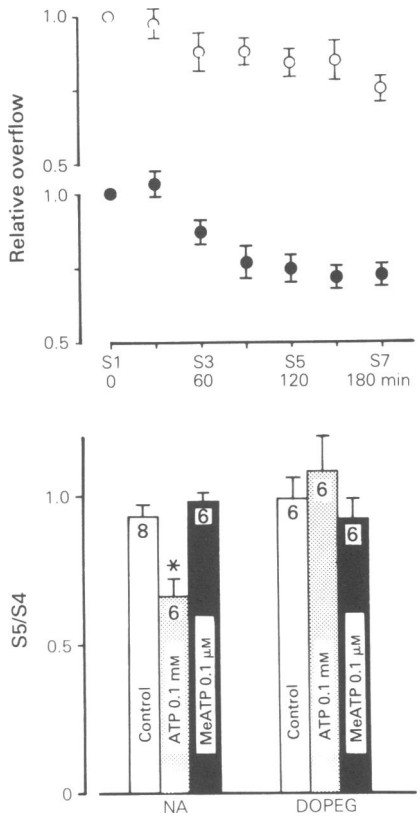

Figure 8 Effects of ATP or α,β-methylene ATP (MeATP) on the overflow of noradrenaline (NA) and 3,4-dihydroxyphenylglycol (DOPEG) provoked by field stimulation in the rabbit mesenteric artery. (a) Results are shown for control experiments without drugs. Relative amounts of NA or DOPEG released by successive periods of stimulation of the perivascular nerves (300 pulses at 5 Hz, S1 to S7 stimulation at 30 min intervals) were measured and expressed as a fraction of S1. Vertical bars indicate s.e.mean. $n = 6-10$. (b) The ratio of S5/S4 is shown. ATP (0.1 mM) or MeATP (0.1 μM) was present 30 min before and during S5. Vertical bars indicate s.e.mean. The number at the top of each column indicates n. *Statistically significantly different from the control ($P < 0.05$).

Discussion

Exogenously applied ATP has various actions on visceral smooth muscle. The responses to ATP are either hyperpolarization (guinea-pig taenia coli, Tomita & Watanabe, 1973; Shuba & Vladimirova, 1981; rabbit urinary bladder, Creed *et al.*, 1983; cat trachea, Ito & Takeda, 1982; guinea-pig coronary artery, Takata & Kuriyama, 1980), depolarization (guinea-pig ileum, Karashima & Takata, 1979; Bauer & Kuriyama, 1981), or hyperpolarization followed in time by depolarization (mouse uterus, Ninomiya & Suzuki, 1983). In all these tissues, ATP consistently increases the membrane conductance, as determined from the change in amplitude of the electrotonic potentials.

In the rabbit mesentric artery, ATP produces either hyperpolarization (1–10 μM) or depolarization (> 50 μM) with increase in ionic conductance. On the other hand, over the concentration ranges studied, MeATP and AMP-PNP only depolarized the membrane with an increase in ionic conductance. MeATP (0.1 μM) blocked the depolarization but not the hyperpolarization evoked by ATP. Theophylline (10 μM) had no effect on the hyperpolarization evoked by ATP (0.1 mM) in the continued presence of MeATP (0.1 μM) or on the depolarization to ATP (0.1 mM) or to MeATP (0.1 μM). These results suggest that in the rabbit mesenteric artery, both the hyperpolarization and the depolarization evoked by ATP may be due to activation of purinoceptors. This receptor population may not be homogeneous but may consist of at least two subclasses, i.e. one may be related to the increase in the K^+ conductance and the other to increase in the Na^+ conductance. Since MeATP does not cause hyperpolarization, it may activate only one (depolarizing) receptor subclass. On the other hand, in the guinea-pig mesenteric artery, the ATP-sensitive receptor may be composed of only one depolarization subclass or this may dominate over the agonist concentration-range tested.

In the rabbit mesenteric artery, 0.1 mM ATP evoked marked depolarization but repolarized to the resting membrane potential level in the presence of ATP after 30 min and with an ionic conductance exceeding that of the control. The nature of this phenomenon is obscure.

MeATP was approximately 100 times more potent than ATP in inhibiting the amplitude of e.j.ps in both species tested. Each was approximately 10 times more potent in the rabbit mesenteric artery than in the guinea-pig. AMP-PNP was a less potent inhibitor than these agents. However, neither agent affected the facilitation of e.j.ps. Su (1978) found that in rabbit blood vessels, the release of the labelled NA after incorporation into nerve terminals was reduced by treatment with ATP. The present results support these observations, although the size of the reduction was small even with 0.1 mM ATP which abolished e.j.ps. However, the release of DOPEG produced at nerve terminals due to the breakdown of NA by monoamine-oxidase (MAO), was not modified. MeATP (0.1 μM), which reduced the amplitude of e.j.ps, had no effect on the overflow of NA and DOPEG. Thus, the results show that in the rabbit mesenteric artery, the presynaptic purinoceptor is sensitive to ATP but not

to MeATP. However, the latter observation might be due to the low concentration of MeATP tested. The electrophysiological investigations indicate that effects of ATP or MeATP on the pre-junction site (nerve terminals) cannot be completely ruled out due to change in post-junctional membrane properties.

In the rat tail artery (Cheung, 1982; Itoh *et al.*, 1983) and in the rabbit ear artery (Suzuki *et al.*, 1984), responses of muscle membranes induced by perivascular nerve stimulation consist of an e.j.p. and a slow depolarization, only the latter being blocked by adrenoceptor blocking agents. On the other hand, in the mesenteric artery of the guinea-pig, there was an e.j.p. but no slow depolarization, even with strong repetitive stimulation. The e.j.p. generated by perivascular nerve stimulation was not suppressed, yet the contraction evoked by perivascular nerve stimulation was markedly inhibited by adrenoceptor blocking agents (Kuriyama *et al.*, 1982). Therefore, adrenoceptors which are activated by the overflow of NA from nerve terminals may produce the contraction with no change in the membrane potential, i.e. 'pharmaco-mechanical coupling mechanism' (Itoh *et al.*, 1981). In the guinea-pig vas deferens and rat tail artery (Sneddon & Burnstock, 1984; Burnstock *et al.*, 1984), MeATP was reported to inhibit the e.j.p. evoked by nerve stimulation. It was postulated that in these tissues, ATP as a co-transmitter is released from nerve terminals and generates the e.j.p. due to activation of P_2-receptors. In the rabbit and guinea-pig mesenteric arteries, low concentrations of MeATP inhibited e.j.ps with no change in the muscle membrane potential. The marked inibition of e.j.ps induced by high concentrations of ATP or MeATP might be explained by the depolarization and increase in the ionic conductance of the membrane. However, an equivalent depolarization to NA was not affected by ATP or MeATP, suggesting that NA released from nerve terminals may not be involved in generation of e.j.ps.

In conclusion, actions of ATP and MeATP differ on the muscle membrane of the rabbit and guinea-pig mesenteric arteries. In the rabbit mesenteric artery, ATP has a biphasic concentration-response relationship while MeATP only depolarizes the membrane. In the guinea-pig mesenteric artery, both ATP and MeATP only depolarize the muscle membrane. MeATP inhibits the ATP-induced depolarization but not the hyperpolarization. The receptor responsible for actions of ATP may consist of at least two subclasses in the rabbit mesenteric artery, each mediating a different effect, while in the guinea-pig mesenteric artery, where there is only one effect, ATP may activate only one receptor. In the rabbit and guinea-pig mesenteric arteries, both ATP and MeATP markedly reduce the amplitude but not the facilitation of e.j.ps. In the rabbit mesenteric artery, ATP but not MeATP inhibits the overflow of NA provoked by field stimulation in a concentration which inhibits the e.j.p. Thus part of the inhibitory effect of ATP, though not of MeATP, may be prejunctional. In low concentrations, ATP or MeATP inhibit the amplitude of e.j.ps with no change in the membrane properties, especially in the case of the rabbit mesenteric artery. Therefore, the present results may support the hypothesis that the chemical transmitter for the e.j.p. is a purinergic substance rather than NA in the case of the rabbit mesenteric artery. However, in the guinea-pig mesenteric artery, this interpretation is complicated by the depolarization at high concentrations of ATP and MeATP.

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