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PRE- AND POST-JUNCTIONAL EFFECTS OF ADENOSINE TRIPHOSPHATE ON NORADRENERGIC TRANSMISSION IN THE RABBIT EAR ARTERY

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SUMMARY

1. The effects of adenosine triphosphate (ATP), 5′-adenylylimidodiphosphate (AMP-PNP) or α,β-methylene ATP (mATP) on the excitatory junction potential (e.j.p.) and slow depolarization evoked by perivascular nerve stimulation were studied in smooth muscle cells of the rabbit ear artery.

2. ATP (above 10^{-6} M), AMP-PNP (above 10^{-6} M) and mATP (above 10^{-8} M) transiently (10–15 min) depolarized the membrane. The membrane remained depolarized after prolonged exposure (over 20 min) to ATP (above 3×10^{-5} M), AMP-PNP (above 10^{-5} M) or mATP (above 3×10^{-8} M).

3. ATP (above 10^{-5} M), AMP-PNP (above 5×10^{-6} M) or mATP (above 3×10^{-8} M) decreased the membrane resistance. Increasing the external K^+ concentration (K_o^+) to 10·1 mM also decreased the membrane resistance, with an associated depolarization.

4. ATP (10^{-6}–5×10^{-5} M) or AMP-PNP (over 10^{-6} M) transiently decreased and then increased amplitudes of the e.j.p. and of the slow depolarization, the latter component increasing more than the former.

5. Depolarization of the membrane by 10·1 mM-K_o^+ solution or mATP (10^{-7} M) decreased the amplitude of e.j.p.s, with no change in the facilitation, and the slope of the relationship between amplitude of e.j.p. and that of slow depolarization decreased with mATP but not with 10·1 mM-K_o^+ solution.

6. The outflows of noradrenaline and 3,4-dihydroxyphenylglycol (DOPEG) induced by perivascular nerve stimulation increased with ATP (above 10^{-6} M) or AMP-PNP (above 10^{-5} M), while there was no change with mATP (10^{-8}–10^{-5} M) or 10·1 mM-K_o^+ solution.

7. Pre-treatment with mATP inhibited the ATP-induced increase in the outflow of noradrenaline and DOPEG, and also the ATP-induced enhancement of the amplitude of the e.j.p.

8. Therefore ATP and AMP-PNP have predominantly excitatory actions on both pre- and post-junctional membranes, while mATP has an excitatory action on the post-junctional membrane but antagonizes the facilitatory action of ATP on release of noradrenaline from the nerve terminal.

INTRODUCTION

Stimulation of perivascular nerves releases adenosine triphosphate (ATP), dopamine β-hydroxylase, chromogranin A and metabolites of noradrenaline (NA) concomitantly with NA (Smith, De Potter, Moerman & De Schaepdryver, 1970; Su, Bevan & Burnstock, 1971; Cubeddu, Barnes, Langer & Weiner, 1974; Su, 1975; Langer & Pinto, 1976; Henseling, Graefe & Trendelenburg, 1978; Janssens & Verhaege, 1983; Mishima, Miyahara & Suzuki, 1984). Of these substances, NA and ATP depolarize and contract smooth muscle cells of muscular arteries (Bolton & Large, 1986), while most of the metabolites of NA such as 3,4-dihydroxyphenylglycol (DOPEG), 3,4-dihydroxymandelic acid (DOMA) or normetanephrine (NM) produce no detectable change in electrical responses of smooth muscle membrane (Mishima *et al.* 1984).

ATP released from perivascular nerves is degraded to many purine compounds which stimulate prejunctional purine receptor mechanisms to modulate the subsequent release of transmitter, or are taken up into the nerve terminals (Vanhoutte, Verbeuren & Webb, 1981). Exogenously applied ATP reduces the release of NA from adrenergic nerves distributed in tissues such as blood vessels, vas deferens or seminal tract (Enero & Saidman, 1977; Su, 1978; Su, Tsuru & Su, 1978; Bevan, Bevan & Duckles, 1980; Vanhoutte *et al.* 1981; Ishikawa, 1985). Release of NA is also inhibited by adenosine, one of the metabolites of the released ATP (Hedqvist & Fredholm, 1976; Su, 1978; Katsuragi & Su, 1980).

Electrical responses of smooth muscle cell membrane of various blood vessels to perivascular nerve stimulation consist of an excitatory junction potential (e.j.p.) or slow depolarization, or both, and α-adrenoceptor antagonists can block only the slow depolarization, while guanethidine blocks both electrical components (Kuriyama, Ito, Suzuki, Kitamura & Itoh, 1982; Bolton & Large, 1986). The resistance of the e.j.p. to α-adrenoceptor antagonists was explained by assuming a specific NA receptor (γ-receptor) located only at the junctional region (Hirst & Neild, 1981). However, the e.j.p. can be evoked in tissues depleted of NA stored in the nerves (Cheung, 1982; Sneddon & Westfall, 1984; Suzuki, Mishima & Miyahara, 1984), and ionophoretic application of NA produces depolarization which decays with a longer time course than that of the e.j.p. (Suzuki *et al.* 1984).

As in the guinea-pig vas deferens (Sneddon, Westfall & Fedan, 1982; Sneddon & Westfall, 1984), ionophoretic application of ATP to the rabbit ear artery evokes electrical responses similar in form to the e.j.p. (Suzuki *et al.* 1984; Suzuki, 1985). In the rat tail artery, the e.j.p., but not the slow depolarization, is blocked selectively by long application of a stable ATP analogue, α,β-methylene ATP (mATP) to desensitize the receptor mechanism for ATP (Sneddon & Burnstock, 1985). However, in the rat basilar artery, mATP reduces the depolarization with both NA and ATP, thereby indicating that mATP is a specific agonist not only for ATP but also for NA (Byrne & Large, 1986). Thus, it is controversial whether the e.j.p. is generated by ATP released concomitantly with NA.

We investigated the effects of ATP on noradrenergic transmission in the rabbit ear artery by recording electrical responses of the smooth muscle cell membrane and by measuring outflow of NA into the superfusate. Effects of mATP and adenyl-

ylimidodiphosphate (AMP-PNP) on the noradrenergic transmission were also studied, to determine whether the apparent effects of ATP were in fact due to metabolites of ATP. Some of the results were reported at the fifty-eighth annual meeting of the Japanese Pharmacological Society (Suzuki & Miyahara, 1985).

METHODS

Albino rabbits of either sex, weighing 1·9–2·2 kg, were anaesthetized by sodium pentobarbitone (40 mg kg^{-1}, I.V.) and bled. The central ear artery was dissected and cleaned by removing connective tissues surrounding the vessel in the Krebs solution at room temperature.

To record electrical responses of smooth muscle cells, a segment of the artery (1·5–2 cm long) was taken from the proximal part of the ear and was mounted in a 2 ml volume recording chamber made of Lucite plate. The mounted tissue was superfused with warmed (35 °C) Krebs solution, at a flow rate of 2–3 ml min^{-1}. The electrical responses were recorded using a glass capillary microelectrode filled with 3 M-KCl. The tip resistance of the electrode ranged between 40 and 70 MΩ. The electrode was impaled from outside of the vessel. The tissues were stimulated by the partition stimulating method (Abe & Tomita, 1968) to produce electrotonic potentials or by the point stimulation method (Suzuki, 1983) to evoke neurogenic electrical responses (i.e. e.j.p. and slow depolarization; Suzuki & Kou, 1983). The electrical responses were displayed on a pen recorder (Recticorder RJG 4024, Nihonkohden).

To measure outflow of NA and its metabolites, a segment of the ear artery (4–4·5 cm long) was mounted between a pair of Ag–AgCl wires (0·5 mm diameter, 5 cm long) which were fixed vertically in parallel, at a distance of 1·0–1·2 mm (Mishima *et al.* 1984). The Krebs solution (35 °C) was allowed to drip onto the tissue, at a constant rate of 1 ml min^{-1}, using a perfusion pump (Tokyo Rikakikai, MP-101). The solutions so perfused were collected in a conical test tube at the bottom of the tissue, usually 5 min before and after nerve stimulation. Electrical stimulation was applied through the pair of Ag–AgCl wires. Square currents of 0·2 ms duration, 50 V intensity and 10 Hz frequency were sufficient to stimulate perivascular nerves selectively (Miyahara & Suzuki, 1985). To the collected solution was added 50 μl perchloric acid (60%) and the preparation was stored in a freezer at −20 °C until assay (usually the assay of catecholamine was done the following day). At the end of the experiments the tissue was blotted and weighed.

NA and its metabolites in the samples were analysed by the alumina adsorption method (Oishi, Mishima & Kuriyama, 1983), and 50 μl aliquots of the extracted samples were injected onto a high-performance liquid chromatography column (Yanagimoto-MGF, L-2000L). The content of catecholamines was expressed as ng g^{-1} wet weight of tissue.

The Krebs solution was of the following ionic composition (mM); Na^+, 137·4; K^+, 5·9; Mg^{2+}, 1·2; Ca^{2+}, 2·5; HCO_3^-, 15·5; $H_2PO_4^-$, 1·2; Cl^-, 137; glucose, 1·5. The solution was bubbled with O_2 containing 3% CO_2, and the pH of the solution was maintained at 7·2–7·3.

Drugs used were L-noradrenaline HCl (NA), adenosine 5′-triphosphate disodium salt (ATP), α,β-methylene-adenosine 5′-triphosphate lithium salt (mATP), 5′-adenylylimidodiphosphate tetralithium salt (AMP-PNP) (Sigma), guanethidine sulphate (Tokyo Kasei), phentolamine mesylate (CIBA-Geigy) and tetrodotoxin (Sankyo).

The values were expressed by the mean ±S.D., and statistical significances were determined using paired and unpaired Student's *t* tests. When the paired *t* test was used, the parameters observed in the same tissues or cells before and after applied experimental conditions were compared. Probabilities of less than 5% ($P < 0.05$) were considered significant.

RESULTS

Effects of ATP, AMP-PNP or mATP on smooth muscle membrane

Membrane potentials of smooth muscle cells of the rabbit ear artery were measured by successive impalements of the micro-electrode into different cells during a 40 min period when ATP (10^{-5} M), AMP-PNP (10^{-5} M) or mATP (10^{-7} M) were present in the superfusate. As shown in Fig. 1*A*, these substances transiently depolarized the

smooth muscle membrane by about 20 mV from the resting membrane potential (about −68 mV), and the membrane then gradually repolarized to a level specific for each substance; the ATP-induced depolarization ceased within 20 min, while the AMP-PNP or depolarization induced by AMP-PNP or mATP reached a steady

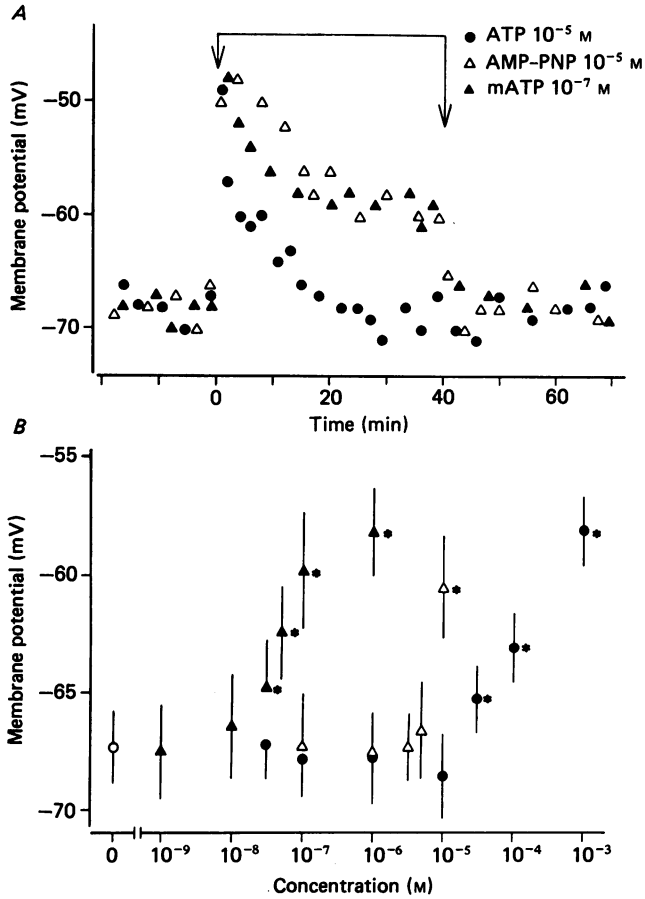

Fig. 1. Effects of ATP, AMP-PNP or mATP on membrane potentials of smooth muscle cells in the rabbit ear artery. Guanethidine (5×10^{-6} M) and tetrodotoxin (3×10^{-7} M) were present throughout. *A*, changes in membrane potential during application of 10^{-5} M-ATP (●), 10^{-5} M-AMP-PNP (△) or 10^{-7} M-mATP (▲) for 40 min. Membrane potentials were measured by successive penetration of the micro-electrode into different cells. *B*, concentration–response relationship of the effects of 20–40 min application of ATP, AMP-PNP or mATP on membrane potential. Mean ± S.D. (n = 12–36 from three to eight tissues). *, significant difference from the resting membrane potential (○).

membrane potential of about −60 mV. ATP is rapidly broken down to adenosine by ectonucleotidase (Zimmermann, 1982); therefore in separate experiments, freshly prepared ATP-containing solutions were successively applied every 10 min. The membrane potential changes produced by these ATP solutions were identical to those produced by continuous application of the solution with previously dissolved ATP, i.e. the diminished amplitude of depolarization during application of ATP may

be not due to decreases in the concentration of ATP or to inhibitory effects of adenosine, a compound which hyperpolarizes the smooth muscle membrane of the rabbit ear artery (Suzuki, 1985).

When the transient depolarizations produced by these substances were recorded in single cells, ATP produced one or occasionally several spike potentials during development of the depolarization, as has already been reported (Suzuki, 1985), while AMP-PNP or mATP depolarized the membrane without generating spike potentials.

Fig. 1*B* shows the effects of ATP, AMP-PNP or mATP on membrane potential of smooth muscle cells of the rabbit ear artery, in which the membrane potentials were measured after exposure of the artery to the substances for 20 min, i.e. at a time when steady-state responses had been reached. Threshold concentrations of these substances for membrane depolarization were: ATP, 3×10^{-5} M; AMP-PNP, 5×10^{-6} M; mATP, 3×10^{-8} M. At higher concentrations, the membrane was depolarized in a concentration-dependent manner.

In the presence of phentolamine (10^{-6} M), membrane depolarizations produced by ATP, AMP-PNP or mATP remained unchanged, indicating that these depolarizations were not due to stimulation of α-adrenoceptors.

In smooth muscle of the rabbit ear artery, the amplitude of electrotonic potential was measured during application of ATP, AMP-PNP or mATP, in the presence of guanethidine (5×10^{-6} M) and phentolamine (10^{-6} M). The electrotonic potentials produced by inward current pulses (0·5 V cm^{-1}, 1·5 s) were recorded before (V) and during 20–30 min application of these substances (V'), from smooth muscle cells located in close proximity to the stimulating electrode (less than 0·2 mm). This distance was short enough compared to the electrical length constant of muscular arteries (0·9–1·6 mm, Hirst & Neild, 1978; Holman & Surprenant, 1979; Kuriyama & Suzuki, 1981). This set-up allowed for estimation of the change in membrane resistance from the $(V'\ V^{-1})^2$ values (Hodgkin & Rushton, 1946). Fig. 2 shows the relationship between $(V'\ V^{-1})^2$ values and concentrations of ATP, AMP-PNP or mATP. The membrane resistance was decreased with all the substances, in a concentration-dependent manner. Decreases in membrane resistance induced by AMP-PNP or mATP were observed at concentrations which depolarized the smooth muscle membrane (i.e. AMP-PNP, above 5×10^{-6} M; mATP, above 3×10^{-8} M) (Fig. 1*B*). ATP (10^{-5} M) decreased the membrane resistance, with no change in the membrane potential.

In the presence of 10·1 mM-K_o^+, the membrane was depolarized to a similar extent as seen with 10^{-4} M-ATP, 10^{-7} M-mATP or 10^{-5} M-AMP-PNP (Fig. 1*B*) (i.e. $-58{\cdot}3 \pm 2{\cdot}1$ mV, $n = 28$). In the presence of high-K_o^+ solution, the $(V'\ V^{-1})^2$ value was decreased to $0{\cdot}50 \pm 0{\cdot}1$ ($n = 10$) times the control.

Effects of ATP, AMP-PNP or mATP on junction potentials

Effects of ATP, AMP-PNP, and mATP on the e.j.p. and slow depolarization produced by perivascular nerve stimulation (0·03–0·1 ms duration, 15–50 V intensity) in the rabbit ear artery were then observed. Fig. 3*A* shows e.j.p.s and slow depolarizations produced by five stimuli at 0·5 Hz. ATP (10^{-5} M) transiently decreased the amplitude of both the e.j.p.s and the slow depolarization with an associated depolarization of the membrane. During continued application of ATP, the am-

plitudes of these nerve-mediated electrical responses were increased over the control, at a time associated with repolarization of the membrane. These effects of ATP on the e.j.p. were also observed in the presence of phentolamine (10^{-6} M) which blocked generation of the slow depolarization (Fig. 3*B*). The effects of ATP on the e.j.p. and slow depolarization were reversible, and 10–15 min washing was required for recovery.

The time course of changes in the amplitude of e.j.p. and slow depolarization during 37 min application of ATP (10^{-6} M) is shown in Fig. 3*C*. The amplitudes of both electrical responses were increased during 15 min superfusion with ATP.

Application of AMP-PNP (3×10^{-6} M) for 15 min enhanced the amplitudes of e.j.p.s and slow depolarization, with no detectable change in the resting membrane

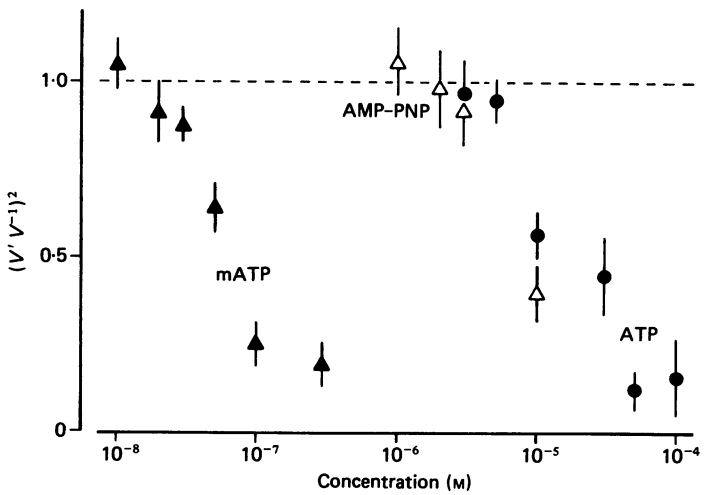

Fig. 2. Relationship between concentrations of ATP (●), AMP-PNP (△) or mATP (▲) and the $(V'.V^{-1})^2$ values. Electrotonic potentials were produced by the constant intensity (0·5 V cm^{-1}) and duration (1·5 s) of square-current pulses, before (V) and during (V') application of these compounds for 20–30 min. Mean ±S.D. (n=3–7).

potential (Fig. 4*A*). With application of mATP (10^{-8} M), the amplitude of both the e.j.p.s and slow depolarization transiently (3–5 min) decreased together with depolarization of the membrane, and after repolarization of the membrane to about −66 mV by continued application of mATP the amplitudes of these electrical responses were smaller than the control (Fig. 4*B*).

The relationship between the amplitude of the e.j.p. (Fig. 5*A*) or slow depolarization (Fig. 5*B*) produced by perivascular nerve stimulation (five stimuli at 0·5 Hz frequency) and the concentrations of ATP, AMP-PNP or mATP, showed that mATP (above 10^{-8} M) decreased and ATP (10^{-6}–10^{-5} M) or AMP-PNP (10^{-6}–5×10^{-6} M) increased the amplitude of the e.j.p. and slow depolarization. High concentrations of ATP (10^{-4} M) or AMP-PNP (3×10^{-5} M) decreased the amplitudes of the e.j.p. and slow depolarization.

Effects of post-junctional membrane depolarization on the e.j.p. and slow depolarization were examined by applying external K^+ (K_o^+) at a concentration of

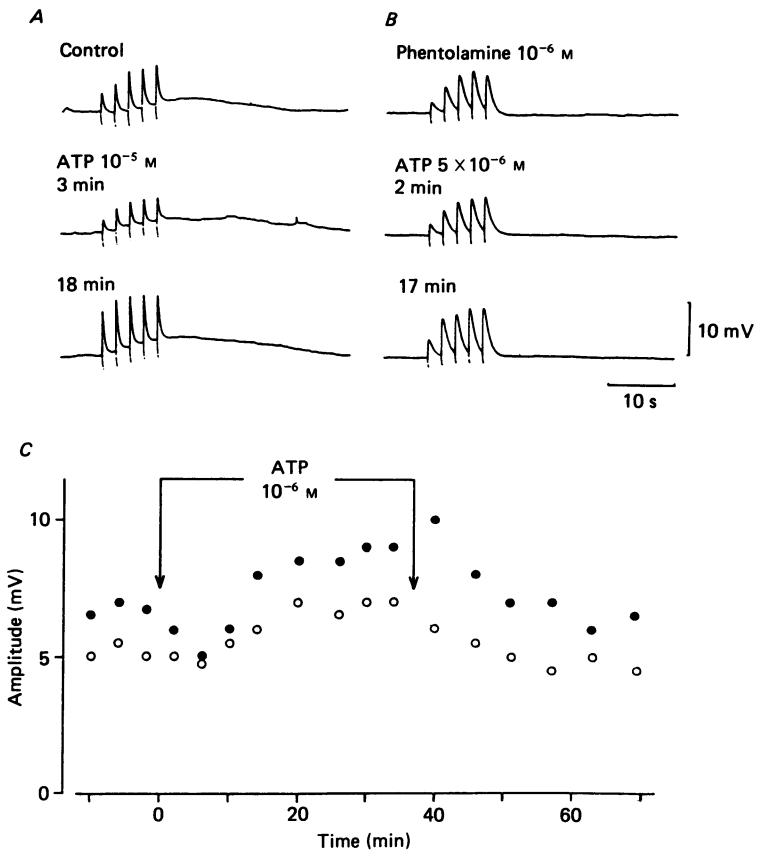

Fig. 3. Effects of ATP on e.j.p. and slow depolarization in the rabbit ear artery. Perivascular nerves were stimulated five times by square-current pulses (0·05 ms duration, 20 V intensity) at 0·5 Hz. *A*, e.j.p.s and slow depolarizations evoked before (control) and during application of 10^{-5} M-ATP (3 and 18 min). *B*, e.j.p.s and slow depolarizations evoked before and during application of 5×10^{-6} M-ATP (2 and 17 min), in the presence of 10^{-6} M-phentolamine. Responses in each panel were recorded in single smooth-muscle cells from different tissues. *C*, changes in amplitude of e.j.p. (●) and slow depolarization (○) during application of 10^{-6} M-ATP (between arrows). Amplitudes of the largest e.j.p. and the peak of the slow depolarization evoked by five stimuli at 0·5 Hz frequency were plotted. All the points represent data on the same cell.

10·1 mM solution, and the results were compared with those produced during application of 10^{-7} M-mATP. Fig. 6*A* shows that when the membrane was depolarized by about 8 mV with either 10^{-7} M-mATP or 10·1 mM-K_o^+ solution, the amplitude of the e.j.p.s and the slow depolarization decreased; the e.j.p. amplitude was decreased by mATP more than by 10·1 mM-K_o^+ solution.

The effects of mATP or 10·1 mM-K_o^+ solution on the e.j.p. were quantified by plotting the actual (Fig. 6*B*) and the relative amplitude (Fig. 6*C*) of e.j.p.s produced by the initial five stimuli of the train of stimuli, under each condition. Both mATP and 10·1 mM-K_o^+ solution decreased the e.j.p. amplitude, but the facilitation process of the e.j.p.s remained unchanged (Fig. 6 *C*).

Relationship between e.j.p. and slow depolarization

Perivascular nerves were stimulated five times at 10 Hz, with intensities sufficient to evoke e.j.p.s but not spike potentials (28–40 V). These stimulations evoked a summed e.j.p. followed by a slow depolarization. When the amplitude of the e.j.p. was plotted against that of the slow depolarization there was a linear relationship. Application of ATP (10^{-6}–10^{-4} M) decreased the slope of this relationship, in a

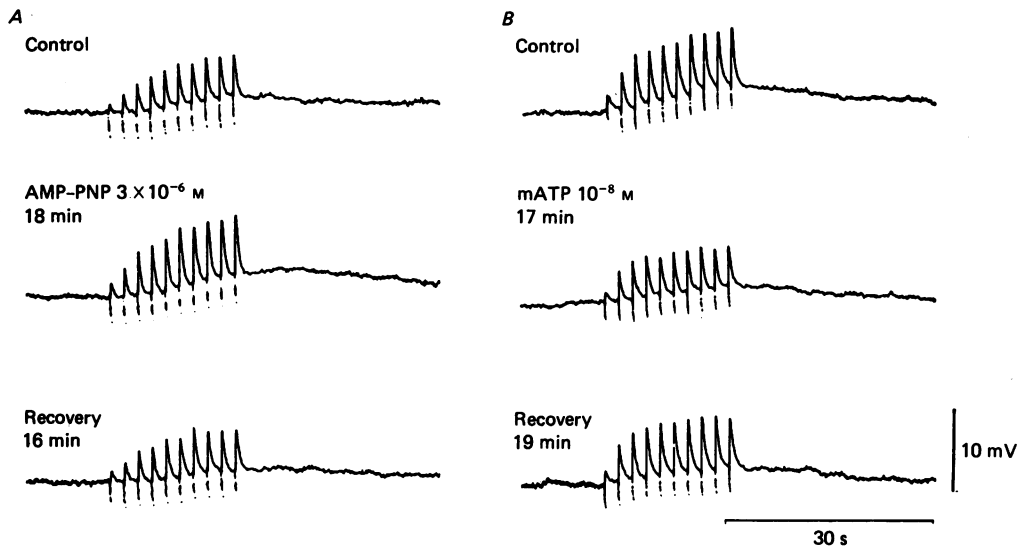

Fig. 4. Effects of 3×10^{-6} M-AMP-PNP (*A*) and 10^{-8} M-mATP (*B*) on e.j.p.s and slow depolarization produced by perivascular nerve stimulation (0·03 ms duration, 21 V intensity, 0·5 Hz frequency, ten stimuli). Responses in each panel were recorded in single smooth-muscle cells from different tissue specimens.

concentration-dependent manner (Fig. 7*A*), and with 10^{-4} M-ATP the slope was decreased to $0{\cdot}46 \pm 0{\cdot}04$ times the control ($n = 7$). The decrease in the slope of the relationship was also observed during application of mATP (5×10^{-8}–10^{-6} M) for over 20 min. With application of 10^{-7} M-mATP, the slope was decreased to $0{\cdot}42 \pm 0{\cdot}09$ times the control ($n = 5$, Fig. 7*B*). During depolarization of the smooth muscle membrane by 10·1 mM-K_o^+ solution, the slope remained unchanged ($0{\cdot}97 \pm 0{\cdot}07$ times the control, $n = 3$, $P > 0{\cdot}1$, Fig. 7*C*).

Effects of mATP on ATP-induced enhancement of e.j.p.

Effects of ATP on the e.j.p. were observed in tissues pre-treated with mATP. Experiments were carried out in the presence of phentolamine (10^{-6} M) in order to block the slow depolarization.

As shown in Fig. 8*A*, application of 3×10^{-8} M-mATP for more than 20 min reduced the amplitude of e.j.p.s to about half that of the control. Additional application of ATP (10^{-5} M) transiently (2–3 min) depolarized the membrane by about 10 mV and decreased the amplitude of e.j.p.s. Continued application of ATP together

with mATP increased the amplitude of the e.j.p.s within 10 min to a steady amplitude larger than that evoked before the application of ATP (i.e. in the presence of mATP alone). Removal of ATP and mATP from the superfusate restored the amplitude of e.j.p.s to the control value within 20 min.

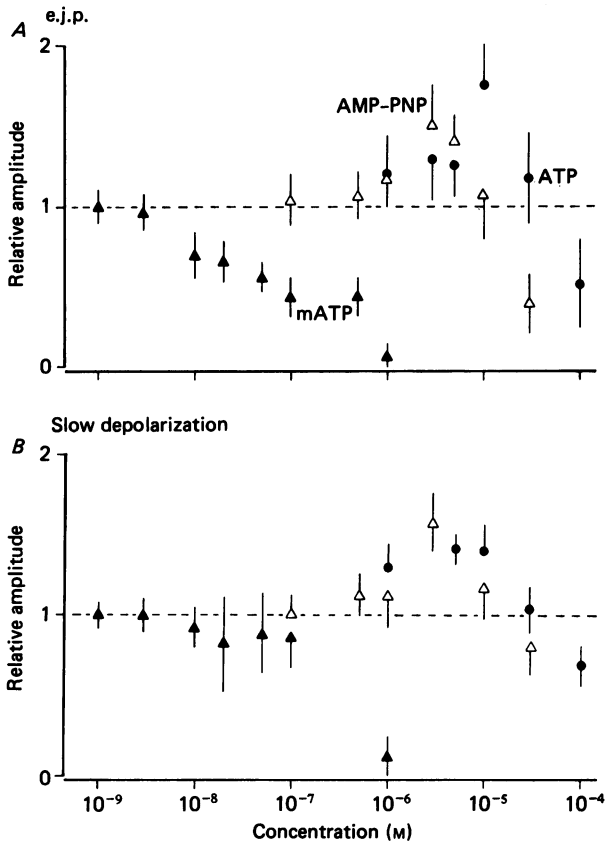

Fig. 5. Concentration–response relationship of the effects of ATP (●), AMP-PNP (△) or mATP (▲) on amplitude of e.j.p. (*A*) and slow depolarization (*B*). A train of perivascular nerve stimulation (0·03 ms duration, 18–25 V intensity, 0·5 Hz frequency, five stimuli) was applied every 3–5 min, while ATP, AMP-PNP or mATP was applied for 30 min. Amplitudes of the largest e.j.p. in each stimulation train and the peak of the slow depolarization obtained at 20–30 min were expressed relative to those before application of these compounds. Mean ±S.D. (n = 7–15).

Fig. 8*B* shows the effects of ATP (10^{-5} M) on the amplitude of e.j.p.s, after pre-treatment with various concentrations of mATP (10^{-8}–10^{-7} M) for over 20 min. The maximum amplitude of e.j.p.s produced by five stimuli at 0·5 Hz was expressed relative to that evoked before application of ATP. Pre-treatment with mATP reduced the amplitude of the e.j.p. in a concentration-dependent manner (Fig. 5*A*), and the ATP-induced enhancement of the e.j.p. was also reduced with increasing concentrations of mATP. At 10^{-7} M-mATP, ATP (10^{-5} M) neither increased the amplitude of the e.j.p. nor produced any transient depolarization of the membrane.

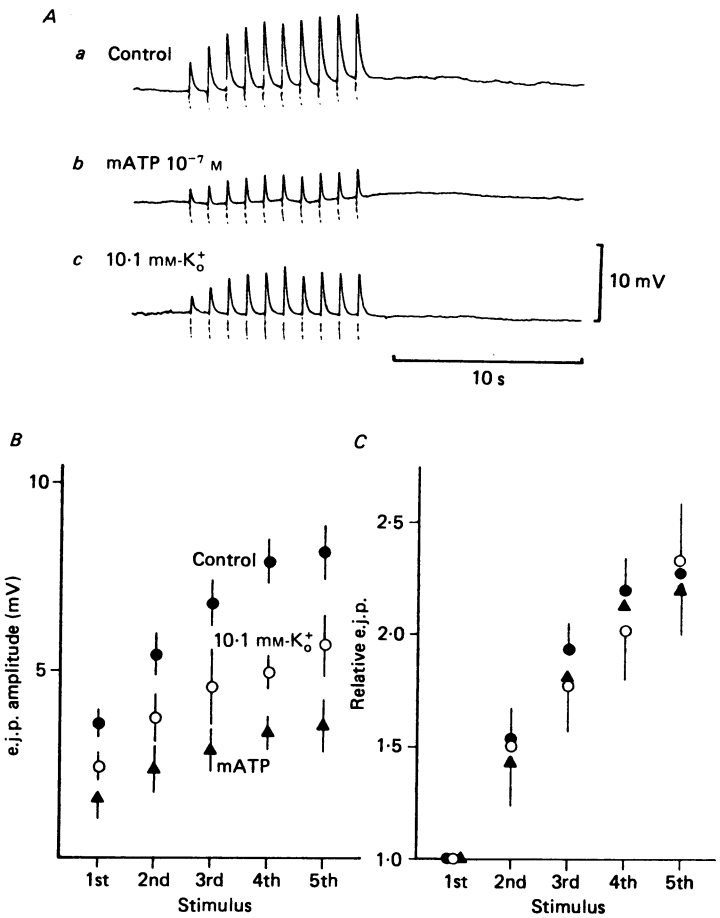

Fig. 6. Comparison of the effects of mATP and 10·1 mM-K_o^+ solution on e.j.p. and slow depolarization. *A*, e.j.p.s and slow depolarizations evoked before (*a*) and during 25 min application of 10^{-7} M-mATP (*b*) or 10 min application of 10·1 mM-K_o^+ solution (*c*). Nerve stimulation: 0·05 ms duration, 20 V intensity (ten stimuli at 0·5 Hz frequency). *B*, amplitude of e.j.p.s evoked by the initial five stimuli of a train stimulation at 0·5 Hz in the frequency, before (●) and during application of 10^{-7} M-mATP (▲) or 10·1 mM-K_o^+ solution (○). Mean ±S.D., $n = 10$ (control), 7 (mATP) and 8 (10·1 mM-K_o^+). *C*, relative amplitude of e.j.p.s calculated from the results shown in *B*. The e.j.p.s were expressed relative to the first of each train of stimulation. Mean ±S.D.

Effects of ATP, AMP-PNP or mATP on NA outflow

The outflow of NA or DOPEG from the rabbit ear artery into perfusate was much the same as reported by Miyahara & Suzuki (1985); briefly, at rest the outflow of NA or DOPEG was below detectable or 1–5 ng g^{-1} wet weight of tissue, respectively. With perivascular nerve stimulation (600 stimuli at 10 Hz frequency), the outflow of NA or DOPEG increased to 10–15 ng g^{-1} or 5–10 ng g^{-1}, respectively.

With no nerve stimulation, the outflow of NA or DOPEG was below detectable levels or 1–5 ng g^{-1}, respectively, during the application of ATP (10^{-7}–10^{-4} M),

AMP-PNP (10^{-7}–10^{-5} M), mATP (10^{-8}–10^{-5} M) or 10·1 mM-K_o^+ solution, for over 1 h.

Repeated perivascular nerve stimulation (six times at 25 min interval) gradually decreased the outflow of NA and DOPEG, to reach a constant value of about 80 and 90%, respectively, of the amount produced by the first stimulus, over the third stimulation period. ATP (10^{-7}–10^{-4} M), AMP-PNP (10^{-7}–10^{-5} M) or mATP (10^{-8}–10^{-5} M) was applied during the third and fourth stimulation periods, and the outflow

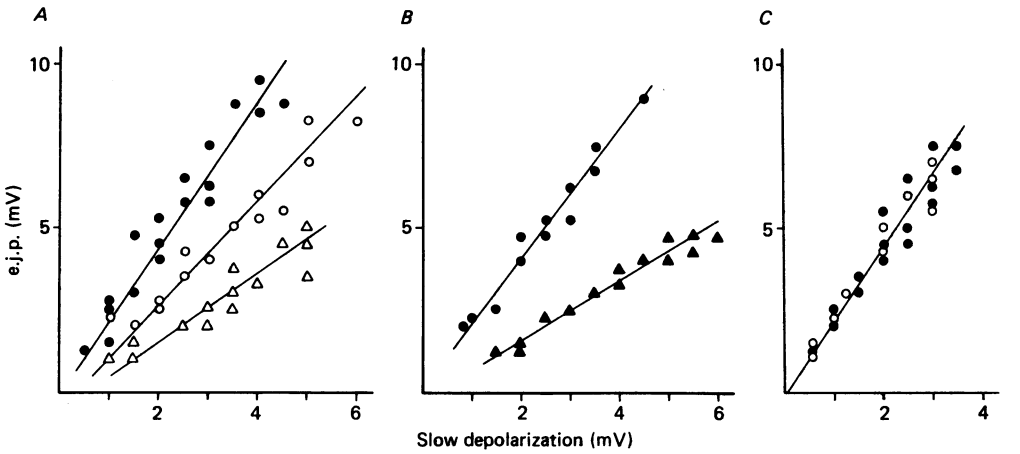

Fig. 7. Modulation of the relationship between slow depolarization and e.j.p. amplitudes in the rabbit ear artery. Perivascular nerves were stimulated by five pulses (0·05 ms duration, 10–30 V intensity) at 10 Hz frequency. *A*, responses before (●) and during 20–60 min application of ATP (○, 10^{-5} M; △, 10^{-4} M) were plotted. Regression lines calculated by the least-squares method are: control, $Y = 4{\cdot}5\ X - 0{\cdot}2$; ATP 10^{-5} M, $Y = 3{\cdot}7\ X - 1{\cdot}1$; ATP 10^{-4} M, $Y = 2{\cdot}1\ X - 1{\cdot}1$; ($Y$ = e.j.p.; X = slow depolarization). *B*, responses before (●) and during 20–50 min application of 10^{-7} M-mATP (▲). Regression lines are: control, $Y = 4{\cdot}0\ X - 0{\cdot}1$; mATP 10^{-7} M, $Y = 1{\cdot}8\ X - 0{\cdot}5$; *C*, responses before (●) and during 10–30 min application of 10·1 mM-K_o^+ solution (○). Regression lines are: control, $Y = 4{\cdot}5\ X - 0{\cdot}2$; 10·1 mM-$K_o^+$, $Y = 4{,}3\ X - 0{\cdot}1$ (not shown).

of NA and DOPEG was significantly increased by comparison with that in the absence of these compounds. Fig. 9 shows the relationship between concentrations of ATP, AMP-PNP or mATP and outflow of NA and DOPEG measured at the fourth stimulation period. The outflow of NA and DOPEG is shown relative to the outflow produced by the first stimulation. The NA outflow was significantly increased by ATP (above 10^{-6} M) or AMP-PNP (10^{-5} M), while the DOPEG outflow was increased by ATP (above 10^{-5} M) or AMP-PNP (10^{-5} M). The outflow of NA and DOPEG induced by nerve stimulation was not significantly changed by mATP.

In separate experiments, application of 10·1 mM-K_o^+ solution during the third and fourth stimulation period did not change the outflow of NA and DOPEG (outflow of NA at the fourth stimulation, control $80{\cdot}6 \pm 3{\cdot}6\%$, $n = 4$; in 10·1 mM-K_o^+ $83{\cdot}2 \pm 6{\cdot}7\%$, $n = 4$, $P > 0{\cdot}5$; DOPEG, control $92{\cdot}6 \pm 12{\cdot}0\%$, $n = 4$; in 10·1 mM-K_o^+, $86{\cdot}7 \pm 8{\cdot}9\%$, $n = 4$, $P > 0{\cdot}5$).

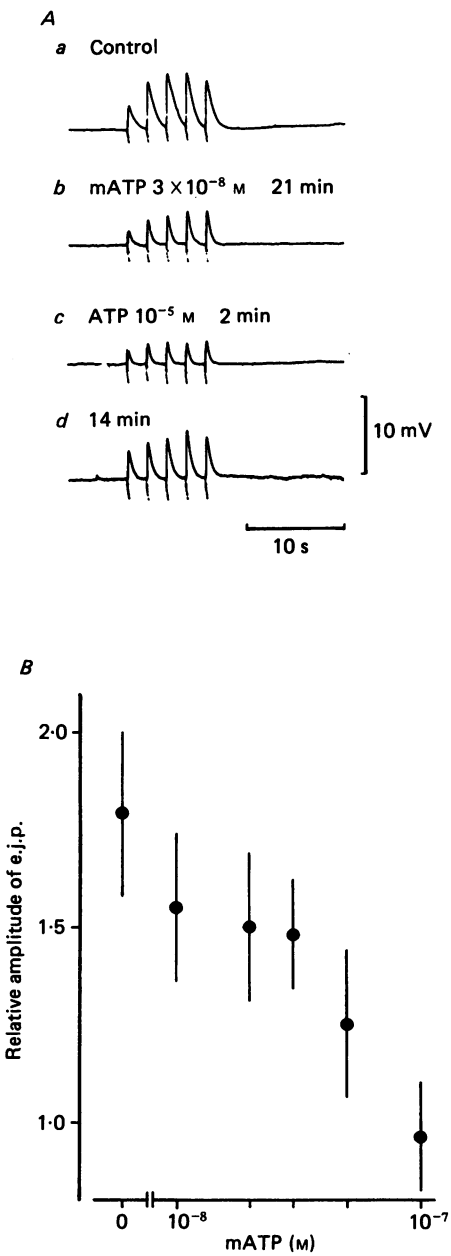

Fig. 8. Effects of pre-treatment with mATP on the ATP-induced enhancement of e.j.p.s in the rabbit ear artery. Phentolamine (10^{-6} M) was present throughout. Nerve stimulation: 0·05 ms duration, 25 V intensity, five stimuli at 0·5 Hz frequency. *A*, e.j.p.s evoked before (*a*), during application of 3×10^{-8} M-mATP for 21 min (*b*), and additional application of 10^{-5} M-ATP (*c*, 2 min; *d*, 14 min). Membrane potentials at which the e.j.p.s were recorded were *a*, −68 mV; *b*, −65 mV; *c*, −60 mV; and *d*, −66 mV. *B*, concentration–response relationship of the effects of pre-treatment with mATP on 10^{-5} M-ATP-induced enhancement of e.j.p.s. Amplitude of e.j.p.s in the presence of ATP (20–30 min) is plotted relative to that before ATP. Mean ±S.D. ($n = 5$–7).

The effects of ATP on the outflow of NA and DOPEG induced by perivascular nerve stimulation were studied in the presence of mATP. Tissues were perfused with solution containing 10^{-7} M-mATP and a train of perivascular nerve stimulation (600 stimuli at 10 Hz frequency) was applied six times at 25 min intervals. The time course of the decrease in outflow of NA and DOPEG during successive nerve stimulations remained unchanged in the presence or absence of mATP. ATP (10^{-5} M) applied during the third and fourth stimulation period did not significantly increase the outflow of NA and DOPEG (Fig. 9).

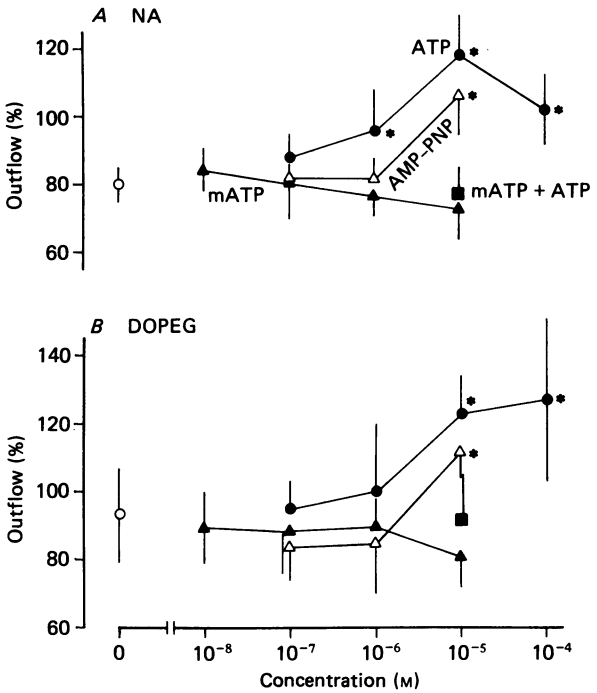

Fig. 9. Concentration–response relationship of the effects of ATP (●), AMP-PNP (△) or mATP (▲) on outflows of NA (*A*) and DOPEG (*B*) in the rabbit ear artery. Outflows of NA and DOPEG produced by the fourth stimulus train are expressed relative to the first stimulus. ○, control. ■, pre-treated (90 min) with 10^{-7} M-mATP. Mean ±S.D. (n = 4–6). *, significant difference from the control.

DISCUSSION

In the rabbit ear artery, ATP increased the release of transmitter substances during perivascular nerve stimulation. This action of ATP was not due to energy liberated from hydrolysis of the phosphate bond, since AMP-PNP showed actions similar to ATP in this artery. In the absence of nerve stimulation, ATP did not modify the basal outflow of NA and DOPEG, thereby indicating that the increase in NA outflow by ATP may be linked with excitation–secretion coupling mechanisms at the nerve terminals.

These actions of ATP in facilitating release of NA in the rabbit ear artery contrasted with findings in the portal vein (Enero & Saidman, 1977; Su, 1978) or the mesenteric artery of rabbits (Ishikawa, 1985), in which ATP inhibited the release of NA during perivascular nerve stimulation. When the extent of NA uptake into the nerve terminals is estimated from the amount of outflow of DOPEG (Mishima *et al.* 1984), the increase in the NA outflow by ATP may be not due to inhibition of the uptake mechanism of the released NA, unlike that in the case of cocaine or procaine which increases the NA outflow and decreases the DOPEG outflow (Fujii *et al.* 1985), possibly due to inhibition of the re-uptake mechanisms of NA (Paton, 1979). However, this may not be the case in the rabbit mesenteric artery, in which ATP decreases the NA outflow without altering the DOPEG outflow (Ishikawa, 1985), thereby suggesting that ATP accelerates the re-uptake of NA and consequently maintains a constant level of DOPEG outflow. The inhibitory actions of ATP reported by Su (1978) in the portal vein might possibly be due to actions of ATP metabolites such as adenosine.

Application of ATP, AMP-PNP or mATP produced a transient or sustained depolarization of the smooth muscle membrane and a reduction in the membrane resistance, as estimated by changes in the amplitude of electrotonic potential. These post-junctional membrane changes would partly explain the decrease in amplitude of e.j.p. during application of mATP or 10·1 mM-K_o^+ solution. However, in the case of ATP or AMP-PNP the amplitude of e.j.p. was increased, under conditions at which the membrane was depolarized and the membrane resistance was decreased, until the smooth muscle membrane was depolarized beyond -60 mV by application of very high concentrations of ATP (over 10^{-4} M) or AMP-PNP (over 3×10^{-5} M). This also indicates that ATP or AMP-PNP increases the release of transmitter substances from perivascular nerves, although the effects were underestimated due to change in post-junctional membrane properties.

The action of mATP in decreasing the e.j.p. was only in part due to the depolarization it caused, suggesting that the remainder may have been due to inhibition of responses produced by substances released from nerves. In the rat tail artery and the rabbit mesenteric artery, mATP blocks membrane deplolarizations produced by exogenously applied ATP but not by NA (Sneddon & Burnstock, 1985; Ishikawa, 1985). These observations would support the concept that the e.j.p. is generated by ATP released from perivascular adrenergic nerves (Sneddon *et al.* 1982; Sneddon & Westfall, 1984; Sneddon & Burnstock, 1984, 1985; Ishikawa, 1985). However, in the rat basilar artery membrane depolarizations produced by local application of both NA and ATP are blocked by mATP (Byrne & Large, 1986). Smooth muscle cell membranes of the basilar arteries are less sensitive to NA, and concentrations which are sufficient to depolarize the membrane of systemic vascular smooth mucles (10^{-6} –10^{-5} M) do not depolarize the membrane of the basilar arteries, while concentrations of ATP required for the membrane depolarization are much the same in both the basilar and systemic vessels (10^{-6}–10^{-5} M: Karashima & Kuriyama, 1981; Fujiwara, Itoh & Suzuki, 1982; Suzuki & Fujiwara, 1982; Nagao, Suzuki & Kuriyama, 1986). Moreover, in the basilar artery field stimulation, which is generally used for perivascular nerve excitation in systemic vessels (0·05–0·1 ms duration, 10–100 V intensity), evokes fast and slow depolarizations, both of which are resistant to tetrodotoxin or guanethidine, i.e. these electrical responses may be non-neuronal in

origin (Yamamoto & Hotta, 1986; Nagao *et al.* 1986). Thus, properties of smooth muscle cells are different between the basilar and the systemic vessels. It remains unclear whether the discrepancy in the effects of mATP on NA actions between these two vessels is due to tissue specificities such as an involvement of mATP actions on NA receptors only in the cerebral arteries.

The electrical or mechanical responses of smooth muscle tissues, including blood vessels, to purine compounds are mediated by 'purinergic' receptors, and ATP is an agonist of the P_2-purinoceptor (Burnstock, 1981). Long periods of application of mATP desensitize the P_2-purinoceptor, thus reducing the responses to ATP (Hedlund, Fandriks, Delbro & Fasth, 1983; Meldrum & Burnstock, 1983). In the rabbit ear artery, direct effects of mATP appeared only on the post-junctional membrane, as depolarization of the smooth muscle membrane, and the release of NA during perivascular nerve stimulation was not modified by mATP, as was noted in the rabbit mesenteric artery (Ishikawa, 1985; Kugelgen & Starke, 1985). On the other hand, ATP depolarized the smooth muscle membrane and increased the release of NA and DOPEG during perivascular nerve stimulation. Thus, in the rabbit ear artery, the nature of the P_2-purinoceptor differs between the pre- and the post-junctional membranes. The ATP-induced increase in NA release was antagonized by mATP, as estimated from changes in the amplitude of the e.j.p. and also from the outflow of NA and DOPEG. This suggests that mATP has agonistic actions on the post-junctional P_2-receptors and antagonistic actions to the prejunctional P_2-receptors.

In the rabbit ear artery, mATP did not modify the outflow of NA and DOPEG during perivascular nerve stimulation, thereby suggesting that the released ATP does not directly act prejunctionally for regulation of transmitter release. In this artery, the α-autoinhibition mechanism (Langer, 1977; Starke, 1977) operating during adrenergic transmission may also have minor roles in regulation of transmitter release (Suzuki & Kou, 1983; Miyahara & Suzuki, 1985). However, ATP released from noradrenergic nerves is hydrolysed to adenosine and this substance may stimulate prejunctional adenosine receptors to inhibit the subsequent release of transmitter substances (Vanhoutte *et al.* 1981).

The decrease in membrane resistance during application of AMP-PNP or mATP was accompanied by depolarization of the membrane. In vascular smooth muscle cells, the membrane shows rectification to outward current, and depolarization decreases the membrane resistance (Mekata, 1976; Casteels, Kitamura, Kuriyama & Suzuki, 1977; Kuriyama & Suzuki, 1981), thereby indicating that the decrease in membrane resistance by AMP-PNP or mATP involves passive components which are the result of depolarization of the membrane. In the case of ATP, however, the membrane resistance was decreased, with no change in the membrane potential at a certain concentration in the range (i.e. 10^{-5} M). Although the nature of this phenomenon is obscure, similar observations were made in the rabbit mesenteric artery (Ishikawa, 1985). These effects of ATP are likely to be complicated by an inhibitory action exerted by endothelium (Furchgott, 1983; Bolton, Lang & Takewaki, 1984).

It is concluded that in the rabbit ear artery, ATP possesses excitatory actions on both the pre- and post-junctional membrane, i.e. increase in the release of transmitter and depolarization of smooth muscle membrane, respectively. The ATP-induced

increase in the release of transmitter may be related to the generation of the nerve action potential. The receptor mechanisms of ATP seem to differ between the pre- and post-junctional membranes, in that mATP excites only the post-junctional receptor mechanisms yet desensitizes both pre- and post-junctional ATP receptor mechanisms. The results are consistent with the concept that the e.j.p. is generated by ATP released together with NA from perivascular adrenergic nerves.

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