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# ACTION OF EXTERNALLY APPLIED ADENOSINE TRIPHOSPHATE O' ' SINGLE SMOOTH MUSCLE CELLS DISPERSED FROM RABBIT EAR ARTERY

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SUMMARY

1. Adenosine triphosphate (ATP), applied in the bathing solution or ionophoretically, depolarized freshly dispersed single arterial smooth muscle cells obtained by collagenase and elastase treatment of the rabbit ear artery.

2. Ionophoretic application of ATP evoked an inward current with a latency of about 70 ms and a time to peak of about 230 ms in cells held under voltage clamp using whole-cell patch-pipette techniques.

3. Bath application of 10 $\mu$M-ATP evoked a transient inward current at negative holding potentials. The amplitude of the ATP-induced current was linearly related to the clamp potential with a reversal potential near 0 mV. Removal of extracellular calcium, buffering intracellular calcium with high EGTA concentration, or depleting calcium stores with caffeine or noradrenaline treatment did not affect the ATP-evoked current.

4. Changing the chloride concentration gradient by decreasing extracellular or intracellular chloride concentration, or using the chloride channel blocker, frusemide, had no effect on the currents.

5. Replacing sodium with Tris shifted the reversal potential to more negative potentials. The reversal potential was not affected by exchanging intracellular potassium for caesium or sodium. Replacing extracellular sodium with 89 mM-barium also had little effect on the reversal potential.

6. These results are consistent with ATP activating a conductance that is cation selective but allows both monovalent and divalent cations to pass across the membrane.

## INTRODUCTION

It is well established that in many arteries stimulation of the motor sympathetic nerves produces an excitatory junction potential (e.j.p.) which is resistant to $\alpha$-adrenoreceptor antagonists (see Bolton & Large, 1986). It has been suggested that

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the e.j.p. results from the action of noradrenaline on a specialized junctional receptor termed the $\gamma$-adrenoreceptor (Hirst & Neild, 1980; Hirst, Neild & Silverberg, 1982). Another explanation is that the e.j.p. is caused by the action of adenosine triphosphate (ATP) on a purinoreceptor. Thus it has been suggested that ATP (or a closely related analogue) is released with noradrenaline from sympathetic nerves in the dog basilar artery (Muramatsu, Fujiwara, Miura & Sakakibara, 1981), rat tail artery (Sneddon & Burnstock, 1984) and in the mesenteric artery of the rabbit (Ishikawa, 1985; Kügelgen & Starke, 1985) and the dog (Muramatsu, 1986).

In the rabbit ear artery, perivascular stimulation also produces an e.j.p. that persists in the presence of $\alpha$-receptor antagonists (Holman & Surprenant, 1980; Suzuki & Kou, 1983). Moreover, the ionophoretic application of ATP evoked electrical responses similar to the e.j.p. (Suzuki, 1985). In the present investigation we have studied the action of ATP using patch-pipette recording on single smooth muscle cells freshly dispersed from the rabbit ear artery. Using this technique it is possible to control the membrane potential under voltage clamp and to dialyse the interior of the cell. The main purpose of the study was to investigate the membrane mechanism underlying the depolarization which occurs in response to ATP.

## METHODS

Adult rabbits (1–2 kg) of either sex were killed by cervical dislocation. The ear artery was dissected free of connective tissue and single cells were obtained using a dispersal procedure similar to that described previously (Benham & Bolton, 1986). Small pieces of artery were incubated at 37 °C for three successive periods of 30 min in a modified physiological salt solution containing low calcium (10 $\mu$M added), bovine serum albumin (2 mg/ml) and various concentrations of collagenase and elastase. The concentrations of collagenase and elastase were respectively: 0·6 mg/ml and 1·7 u/ml; 0·5 mg/ml and 2·1 u/ml; 0·4 mg/ml and 2·5 u/ml in the three consecutive 30 min incubation periods. Subsequently the cells were stored on cover-slips in a physiological salt solution containing 0·75 mM-calcium at 4 °C and were used on the same day.

Whole-cell membrane currents were measured using standard patch-clamp techniques (Hamill, Marty, Neher, Sakmann & Sigworth, 1981). Patch pipettes had resistances of 2–5 MΩ. Data recording and illustration was carried out as described previously (Benham & Bolton, 1986).

Since one of the main objectives of the study was to investigate the ionic mechanism underlying the action of ATP, various extracellular and pipette solutions were used as shown in Table 1. Solutions 1 and A (Table 1) were the standard extracellular and pipette solutions used except where indicated in the text. ATP was not included in the pipette solution as solution leaking out of the pipette before making a seal tended to desensitize the cells. All solutions were adjusted to pH 7·2 and experiments were carried out at room temperature (20–23 °C). When using non-physiological intracellular solutions at least 4 min was allowed to elapse following commencement of whole-cell recording before applying ATP. This has been shown to be sufficient time to allow dialysis of the cells with small cations in the pipette (Benham & Bolton, 1986).

Under voltage clamp the membrane potential of the cells was usually set at a holding potential between −30 and −50 mV by passing a small amount of inward or outward current (less than 20 pA).

*Series resistance errors*

In the presence of ATP cell input resistance fell from about 2 GΩ to about 180 MΩ (using the values of 265 pA mean current at 47·5 mV from the reversal potential. Pipette resistances were 2–4 MΩ and when attached to the cell in whole-cell recording mode series resistances of 5–8 MΩ were measured. Thus even at the peak of the ATP-evoked response errors in potential measurement were less than 5% due to series resistance. For reversal potential measurements close to 0 mV the series resistance error will be less than 0·5 mV. Thus no correction was applied to these values. ATP

TABLE 1. Summary of solutions used

| Extracellular (bath) solution | Concentration (mM) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Sodium | Potassium | Calcium | Magnesium | Barium | Tris | Chloride | Isethionate | EGTA |
| 1 | 125 | 6 | 1·5 | 1·2 | — | — | 131 | — | — |
| 2 | 125 | 6 | 1·5 | 1·2 | — | — | 11 | 120 | — |
| 3 | 5 | 6 | 1·5 | 1·2 | — | 120 | 131 | — | — |
| 4 | — | 6 | — | — | — | 125 | 131 | — | 1 |
| 5 | 5 | — | — | — | 89 | — | 178 | — | — |

| Intracellular (pipette) solution | Concentration (mM) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Sodium | Potassium | Magnesium | Chloride | EGTA | Caesium | Gluconate | $H_2PO_4^-$ | $SO_4^{2-}$ |
| A | 5 | 126 | 1·2 | 128 | 1 | — | — | — | — |
| *B | 30 | 126 | 1·2 | 128 | 10 | — | — | — | — |
| *C | 30 | 127 | 1·2 | 2 | 10 | — | 124 | 1·0 | 1·2 |
| D | 5 | — | 1·2 | 128 | 1 | 126 | — | — | — |
| E | 126 | 6 | 1·2 | 128 | 1 | — | — | — | — |

All solutions contained 11 mM-glucose and 10 mM-HEPES except for solution 4 (no HEPES) and solution 5 (5 mM-HEPES).
* More $Na^+$ due to extra buffering required for the increased EGTA concentration.

was applied usually by bath perfusion but in some experiments ATP was ionophoresed (Byrne & Large, 1984) to study the time course of the responses. The ionophoretic electrode was placed within 5 $\mu$m of the cell.

The values given in the text and in Table 2 are the mean $\pm$ S.E. of mean.

Chemicals used were: adenosine 5′-triphosphate (sodium salt), bovine serum albumin, caffeine, elastase (all Sigma), collagenase (Cooper Biomedical) and frusemide (Hoechst).

## RESULTS

### *Ionophoresis of ATP*

With the membrane potential set at about $-50$ mV using the 'current-clamp' mode of recording a brief ionophoretic pulse of ATP (5 ms, 100 nA) evoked depolarization (Fig. 1*A*). Increasing the ionophoretic charge (10 ms, 100 nA) produced a larger response (Fig. 1*B*) and depolarizations of up to 35 mV in amplitude were obtained using ionophoretic parameters of 100 nA for 5–100 ms. Under voltage clamp ATP (100 nA, 5 ms) evoked an inward current of about 40 pA (Fig. 1*C*) and responses of up to 500 pA were obtained. The responses to ATP were fast in onset and had a short time to peak. In ten cells the latency (interval between start of ionophoretic pulse and onset of inward current) and time to peak were respectively $71 \pm 8{\cdot}4$ and $229 \pm 29$ ms. The minimum latency recorded was 38 ms. It was possible usually to obtain several responses to ATP in a cell but the amplitude of the responses declined. Thus it did not seem feasible to study how changes in experimental conditions influenced responses in a single cell.

### *Bath application of ATP*

At negative holding potentials, bath application of ATP produced an inward current which declined in the presence of the drug (e.g. Fig. 2 *A* and *B*). In fifteen cells at a holding potential of $-50$ mV, 10 $\mu$M-ATP produced a peak inward current of $265 \pm 44$ pA with a rise time of $2{\cdot}0 \pm 0{\cdot}24$ s. The slow time course of the response induced by bath application compared to ionophoresis of ATP was caused by the slower rise of concentration of ATP due to the perfusion system which required about 1 s to give one change of bathing solution. Responses to ATP were obtained in about 90% of the cells tested but as with ionophoresis of ATP the amplitude of the peak inward current declined on repeated application. In segments of the intact artery the amplitude of the depolarizations to ATP decline on repeated application (Suzuki, 1985) but not to the extent observed in the present experiments. The transient nature of the responses may be related to the use of patch pipettes on single dispersed cells as this phenomenon also exists for muscarinic receptor activation in rat lacrimal cells (Marty, Tan & Trautmann, 1984) and for $\alpha$-adrenoreceptor activation in isolated vascular smooth muscle cells (C. D. Benham, T. B. Bolton, N. G. Byrne & W. A. Large, in preparation). Consequently, in the present study a single response to ATP was obtained from a cell and then new cells not previously exposed to ATP were substituted.

In experiments where either depolarizing or hyperpolarizing voltage steps were applied when holding at $-40$ or $-50$ mV it was apparent that there was a large increase in membrane conductance during the inward current induced by ATP (Fig. 2*B*). Moreover it can be seen that at $+5$ mV the ATP current is outward. We

investigated the influence of clamp potential on the ATP-induced current. Fig. 3 illustrates the results of an experiment in which the holding potential was −60 mV and the membrane potential was jumped for 50 ms to test potentials of −40, −20 and +20 mV. During the action of ATP it can be seen that the current is inward at −40 and −20 mV but outward at +20 mV. Fig. 3*B* shows that the amplitude of the ATP-induced current is linearly related to the clamp potential and the reversal

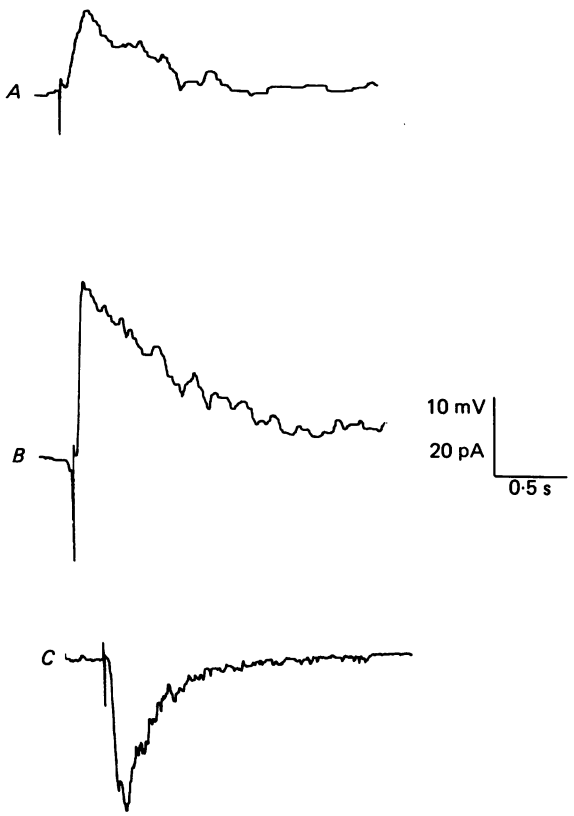


Fig. 1. Responses of the membrane potential of isolated rabbit ear artery cells to ionophoresis of ATP. *A* and *B* were recorded with the membrane potential set at −51 mV by passing current. *C* was recorded in the same cell under voltage clamp at a holding potential of −50 mV. Ionophoretic current was 100 nA and was applied for 5, 10 or 5 ms (*A*, *B* or *C*, respectively).

potential was about −5 mV. In ion substitution experiments (see below) we used a single test potential to near 0 mV for convenience and the reversal potential was estimated by extrapolation or interpolation. The accuracy of this method was sufficient for present purposes. Estimates of the ATP equilibrium potential were obtained from the instantaneous currents to voltage steps from records such as those in Fig. 2*B*. In normal conditions (extracellular solution 1, pipette solution A) the reversal potential of ATP was −2·5±2·5 mV (nine cells, Table 2). Taking this value for the reversal potential with a current activated by ATP at −50 mV of 265 pA gives an increase in conductance of 5·6 nS.

Depolarization of dispersed rabbit ear artery cells activated spontaneous transient outward currents which are at first enhanced and then inhibited by noradrenaline and which are thought to be triggered by release of calcium from intracellular stores (Benham & Bolton, 1986). Fig. 2*A* illustrates an experiment in which spontaneous transient outward currents were observed at a holding potential of

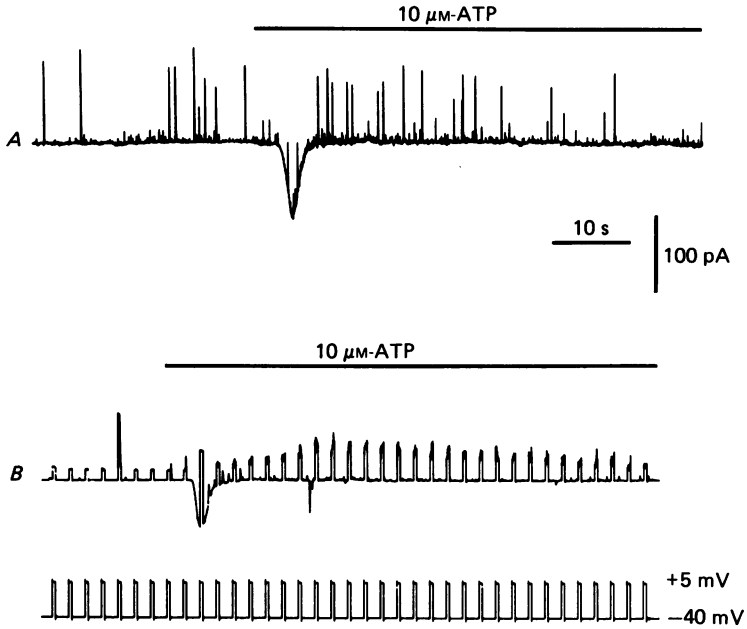


Fig. 2. Effect of bath application of 10 μM-ATP. *A*, holding potential, −30 mV; *B*, holding potential, −40 mV. Voltage jumps of 500 ms to +5 mV were applied every 2 s. ATP was present throughout the period indicated by the horizontal bar. Note that ATP did not affect the spontaneous transient outward currents (*A*). After the inward current an outward current developed slowly in ATP (*B*). This had a time course of about 30 s and was transient. Transient capacitative currents in response to voltage jumps have been removed by hand on the records for clarity.

−30 mV. Application of 10 μM-ATP evoked an inward current but there was little if any change in either the amplitude or frequency of the spontaneous transient outward currents although in some experiments the frequency did increase transiently following ATP application. Spontaneous transient outward currents were not seen in all cells held at −40 mV (e.g. Fig. 2*B*).

In some records (e.g. Fig. 2*B*) it appeared that after the inward current had declined a small, slower, outward current persisted for a short while at depolarized membrane potentials. It is possible that this represents a calcium-activated outward current as it was never observed when 10 mM-EGTA was included in the patch pipette (e.g. Fig. 4*B*). Its time course did not appear to overlap with that of the inward current as the reversal potential of the inward current was the same with 1 or 10 mM-EGTA in the pipette solution. This late outward current was not observed when ATP responses were evoked with no extracellular calcium suggesting that the current may be dependent on calcium influx.

*ATP-induced inward current is not calcium dependent*

Fig. 4*A* shows a response to ATP in an experiment where calcium was omitted from the extracellular solution. The application of 10 $\mu$M-ATP produced an inward current (holding potential of −50 mV) which was accompanied by an increase in membrane conductance. When 10 mM-EGTA was included in the patch pipette,

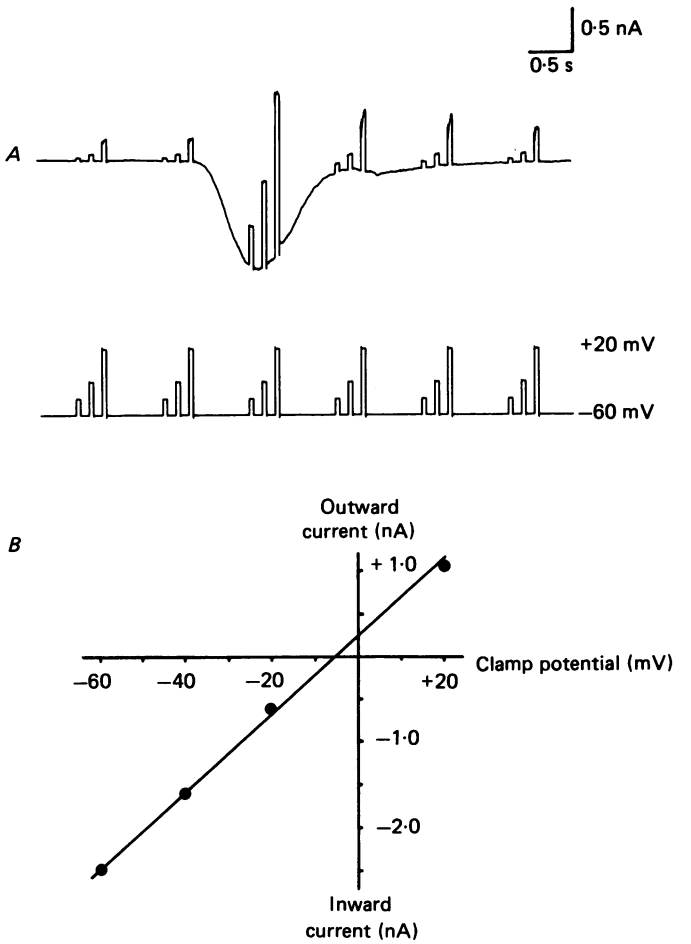


Fig. 3. Relationship between amplitude of ATP-induced current and clamp potential. *A*, the holding potential was −60 mV and the test potentials were −40, −20 and +20 mV. Duration of test pulses was 50 ms and trains were applied every second. *B*, amplitude of ATP-induced current is plotted against clamp potential. Experiment done in collaboration with Dr S. P. Lim.

10 $\mu$M-ATP produced an inward current (Fig. 4*B*) and an increase in membrane conductance with an equilibrium potential of $-3{\cdot}2 \pm 1{\cdot}2$ mV (seven cells, solutions 1 and B in Table 2). In this latter situation the calcium-activated potassium current evoked by noradrenaline in the rabbit ear artery is greatly attenuated (C. D. Benham, T. B. Bolton, N. G. Byrne & W. A. Large, unpublished).

TABLE 2. Equilibrium potentials of the ATP response in various ionic solutions

| Experiment | Solutions (see Table 1) | ATP equilibrium potential (mV) ($n$ experiments) |
|---|---|---|
| 'Standard' solutions | 1 and A | $-2{\cdot}5 \pm 2{\cdot}5$ (9) |
| High intracellular EGTA | 1 and B | $-3{\cdot}2 \pm 1{\cdot}2$ (7) |
| Low extracellular chloride | 2 and B | $-6{\cdot}2 \pm 1{\cdot}1$ (4) |
| Low extracellular sodium | 3 and A | $-21{\cdot}0 \pm 0{\cdot}5$ (5) |
| Zero extracellular sodium | 4 and B | $-35{\cdot}0 \pm 1{\cdot}5$ (4) |
| Caesium intracellular solution | 1 and D | $-2{\cdot}0 \pm 2{\cdot}6$ (3) |
| Sodium intracellular solution | 1 and E | $-0{\cdot}7 \pm 1{\cdot}0$ (3) |
| High extracellular barium | 5 and C | $-3{\cdot}0 \pm 2{\cdot}5$ (3) |

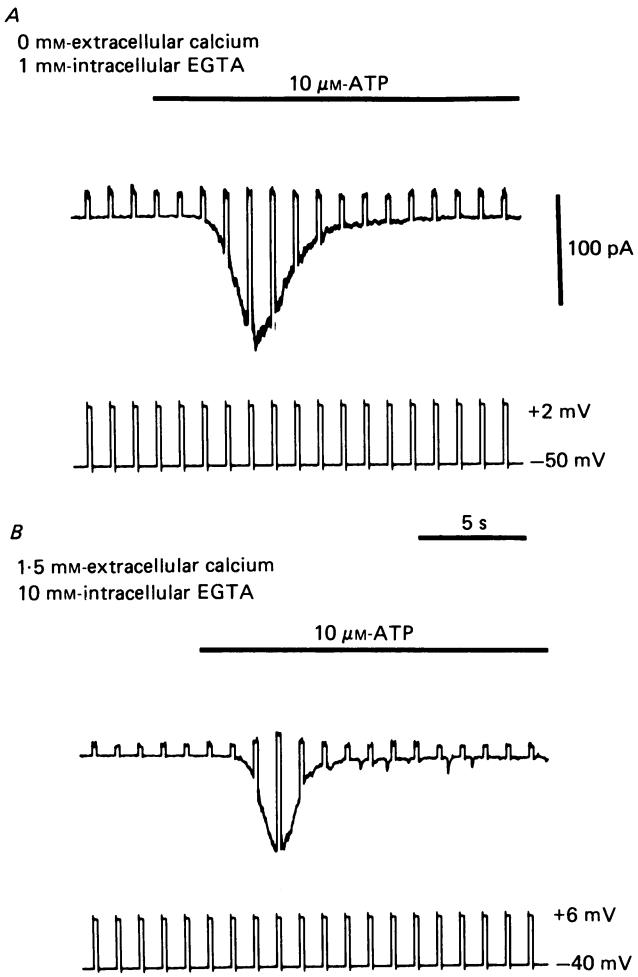


Fig. 4. The ATP-induced inward current is not dependent on the presence of calcium in the extra- or intracellular solutions. In *A*, calcium was omitted from the external solution which also contained 1 mM-EGTA. Holding potential, −50 mV; test potential, +2 mV. *B*, pipette solution contained 10 mM-EGTA. Holding potential, −40 mV; test potential, +6 mV.

Caffeine displaces calcium from intracellular stores and it was of interest to investigate whether the response to ATP was altered in cells treated with caffeine. Caffeine evokes complex membrane responses in these cells depending on holding potential and on the extent of intracellular calcium buffering. Application of 20 mM-caffeine evoked an inward current at −50 mV with 10 mM-EGTA in the pipette which was followed by a small outward current at −50 mV and larger

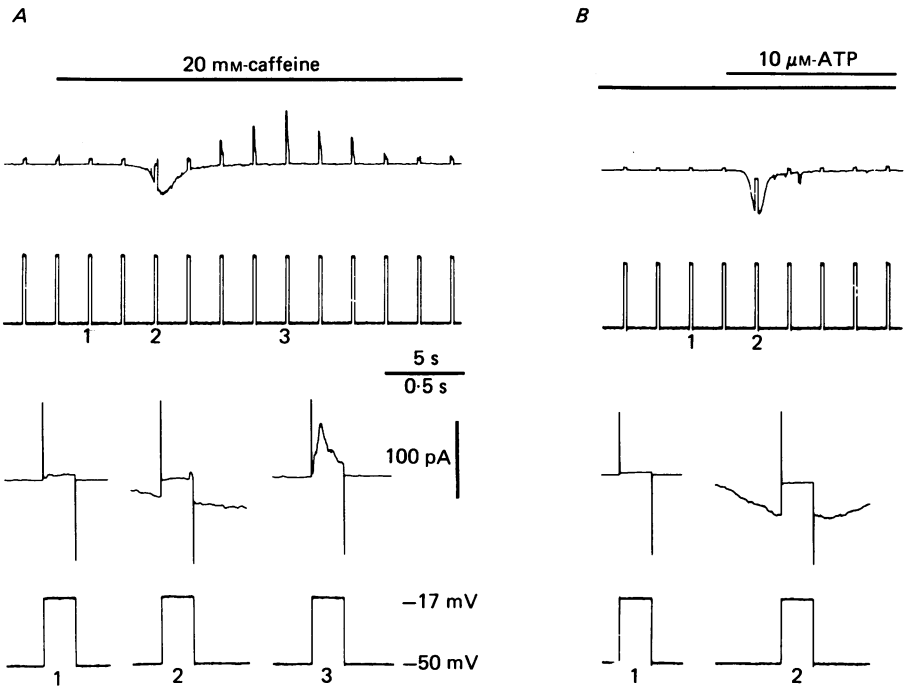


Fig. 5. The effect of caffeine treatment on the response to ATP. *A* and *B* were from the same cell with an interval of about 2 min between application of caffeine and ATP. Holding potential, −50 mV; test potential, −17 mV. 10 mM-EGTA was included in the pipette. The lower records illustrate on a faster time base the voltage jumps numbered in the upper traces. Note the outward current elicited by the depolarizing jump in the presence of caffeine (labelled 3 in *A*).

outward currents during depolarizing jumps (Fig. 5*A*). On other occasions caffeine evoked an outward current prior to an inward current or an outward current alone as described previously (Benham & Bolton, 1986). Irrespective of the nature of the caffeine response, subsequent exposure of the same cell to 10 μM-ATP always produced an inward current and increase in conductance (Fig. 5*B*). Pre-treating cells with 1 μM-noradrenaline, which also releases calcium stores, had no effect on subsequent ATP-induced current. It appears that the inward current produced by ATP does not rely on the release of calcium from intracellular stores or depend on the presence of calcium in the extracellular solution.

In standard solutions the reversal potential of the ATP-induced current was close to 0 mV (solutions 1 and A in Table 2). There would appear to be two likely explanations for the ionic mechanism underlying the response to ATP; ATP may

produce an increase in membrane conductance to cations or ATP may increase the chloride conductance. In solutions 1 and A the chloride equilibrium potential is about 0 mV. We tested the latter possibility first.

*Ionic mechanism of the ATP-induced inward current*

*Chloride substitution.* We substituted most of the extracellular chloride with isethionate (an impermeant anion, solution 2). With this chloride gradient (solutions 2 and B) the chloride equilibrium potential was about +62 mV. However, in these

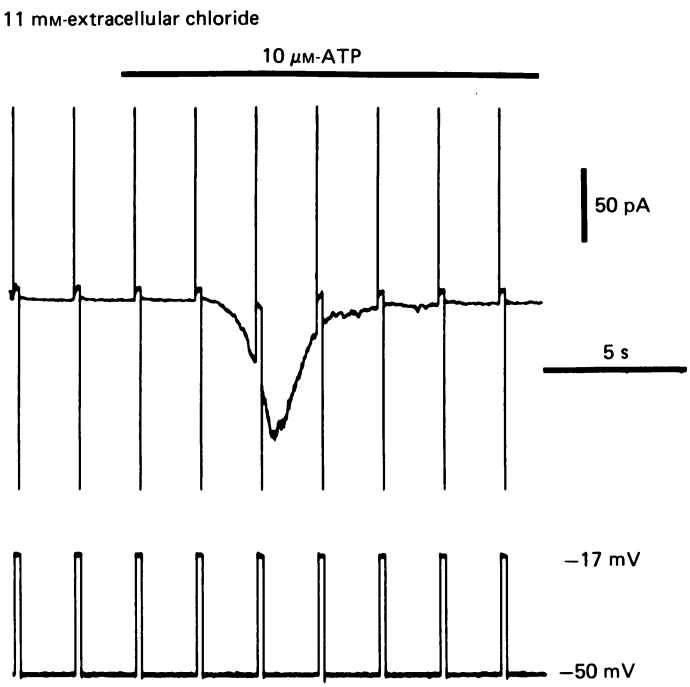


Fig. 6. Lack of effect of reduction of the chloride concentration in the extracellular solution on the response to ATP. Holding potential, −50 mV; test potential, −17 mV. 10 mM-EGTA was included in the pipette solution.

conditions ATP still produced an inward current (Fig. 6) with an equilibrium potential of $-6{\cdot}2 \pm 1{\cdot}1$ mV (Table 2), close to the control value of $-3{\cdot}2 \pm 1{\cdot}2$ mV for solutions 1 and B.

In rat lacrimal glands Evans, Marty, Tan & Trautmann (1986) have shown that frusemide (1 mM) reduces the acetylcholine-induced inward chloride current by over 80 %. We tested the effect of frusemide on the responses to ATP in thirteen cells from a single batch to reduce variation between control and frusemide-treated cells. The cell capacitance of each cell was measured by integrating the capacitative transient current following a small hyperpolarizing potential jump. The membrane capacitance of this group of cells was $49{\cdot}3 \pm 5{\cdot}3$ pF ($n = 13$) and currents evoked by ATP were corrected for the 'standard' 50 pF cell to compensate for variation in cell size. In normal solutions (1 and A) the 'corrected' current induced by 10 μM-ATP at −50 mV was $141 \pm 36$ pA (five cells). Addition of 1 mM-frusemide did not alter the

response to ATP (Fig. 7*A* and *B*) and the 'corrected' peak current was 146±25 pA (eight cells). We also studied the effect of replacing all chloride in the pipette solution with gluconate which should result in a very negative chloride equilibrium potential. This manipulation also had no effect on the reversal potential of the ATP-evoked response. From these data it would seem unlikely that the outward movement of chloride is involved in the ATP-induced inward current.

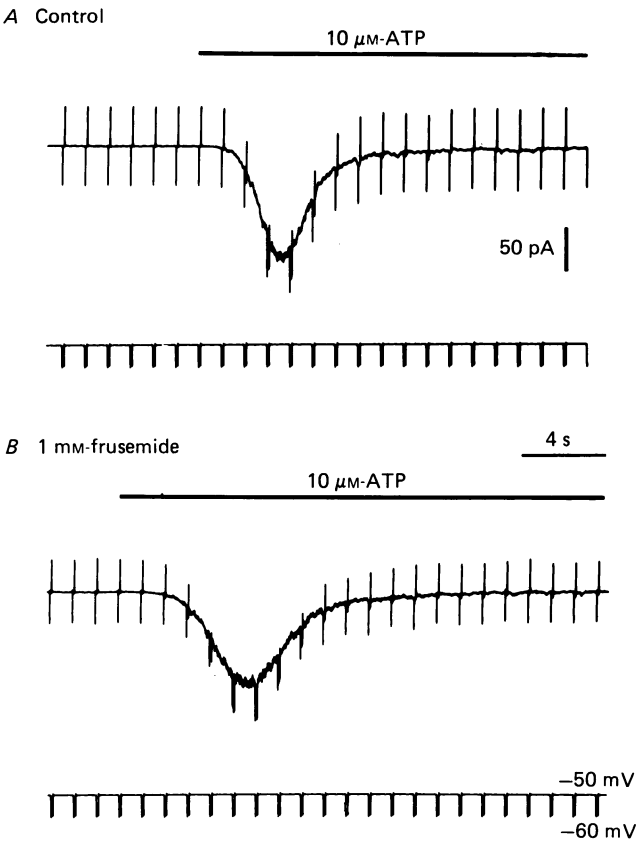


Fig. 7. The effect of 1 mM-frusemide on the response to ATP. Holding potential, −50 mV; test potential, −60 mV. *A*, control response. *B*, response in another cell bathed in the same extracellular solution but containing 1 mM-frusemide.

*Cation substitution*. When most of the external sodium was replaced by Tris the ATP-induced inward current (at −50 mV) was reduced in amplitude and the equilibrium potential was moved to more negative potentials (Fig. 8). Thus, with 5 mM-sodium and 120 mM-Tris in the extracellular solution (solutions 3 and A) the ATP-induced current was reduced in amplitude at −50 mV and the reversal potential was −21·0±0·5 mV (Table 2). Moreover, complete removal of sodium from the extracellular solution made the reversal potential even more negative, being −35·0±1·5 mV (solutions 4 and B in Table 2). These results suggest that the channels are permeable to sodium and less permeable to Tris. We then tested the selectivity

of the ATP-activated conductance to sodium, caesium and potassium by replacing the KCl in the intracellular solution A with CsCl or NaCl. With intracellular caesium the reversal potential was $-2{\cdot}0 \pm 2{\cdot}6$ ($n = 3$; Fig. 9; solutions 1 and D, Table 2) and with intracellular sodium the reversal potential was $-0{\cdot}7 \pm 1{\cdot}0$ mV ($n = 3$; solutions 1 and E, Table 2). These values are not significantly different from those obtained with intracellular KCl ($-2{\cdot}5 \pm 2{\cdot}5$ mV), suggesting that the channels are almost equally permeable to sodium, potassium or caesium.

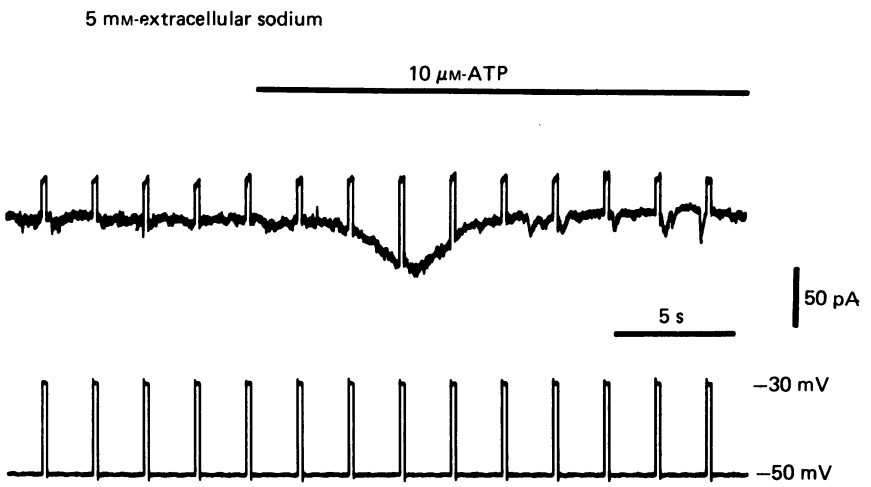


Fig. 8. The effect of reduction of the extracellular sodium concentration (Tris substitution) on the response to ATP. Holding potential, −50 mV; test potential, −30 mV.

The late outward current appeared to be dependent on extracellular calcium, an observation consistent with the current being activated by calcium entry. The divalent cation permeability of the ATP-activated channels was tested by replacing most of the extracellular cations with barium (89 mM). When most of the external sodium was replaced by barium (solutions 5 and C) ATP evoked a response with an equilibrium potential of $-3{\cdot}0 \pm 2{\cdot}5$ mV. In these experiments the pipette solution contained gluconate (impermeant anion) rather than chloride.

## DISCUSSION

The present study shows that in dispersed cells of the rabbit ear artery ATP evokes depolarization which is caused by an inward current. Under certain conditions following the inward current there appeared to be a small slower outward current. This may be due to the opening of calcium-activated potassium channels, but was distinct from the major excitatory response of ATP in these isolated vascular smooth muscle cells.

The inward current evoked by ATP recorded at −50 mV seemed to reflect an increased permeability to cations. With standard intra- and extracellular solutions the ATP reversal potential was close to 0 mV, similar to the value of the acetylcholine (nicotinic) reversal potential at the mammalian skeletal neuromuscular junction (e.g.

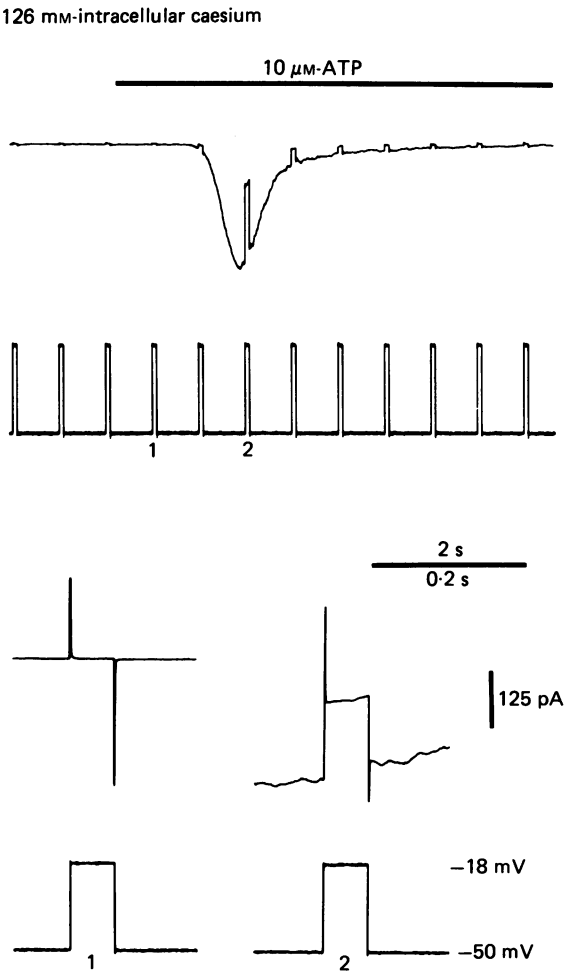


Fig. 9. The effect of replacement of the intracellular potassium by caesium on the responses to ATP. Holding potential, −50 mV; test potential, −18 mV. 126 mM-caesium present in the pipette solution. The lower records illustrate on a faster time base the voltage steps numbered in the upper trace and show the large increase in conductance produced by ATP.

Colquhoun, Large & Rang, 1977) or the reversal potential for the depolarization in jejunal smooth muscle to acetylcholine (muscarinic; Benham, Bolton & Lang, 1985). By analogy this may suggest that the channel opened by ATP in vascular smooth muscle may be selective to sodium and potassium ions as in the present experiments the equilibrium potentials in normal solutions (1 and A) for sodium and potassium were +81 and −77 mV, respectively. However, there was evidence that ions other than sodium and potassium can pass through the channel opened by ATP. When sodium was replaced by Tris in the extracellular solution the value of the ATP reversal potential changed from −2·5 to −35·0 mV; it may have been expected that the reversal potential should be more negative as the potassium equilibrium potential was −77 mV and there was an outward sodium gradient. This result indicates that

either Tris and/or divalent cations can pass through the channels since experiments indicated that chloride did not readily permeate them. Tris can cross the acetylcholine channel at the frog end-plate (Dwyer, Adams & Hille, 1980) and in cultured rat myotubes (Ritchie & Fambrough, 1975). Moreover both calcium and magnesium can permeate the acetylcholine-activated channels at the frog end-plate (Takeuchi, 1963; Dwyer *et al.* 1980) and in *Aplysia* neurones (Ascher, Marty & Neild, 1978). It appears that the channel opened by glutamate at the *Drosophila* larval neuromuscular junction is highly permeable to magnesium ions (Jan & Jan, 1976). In cultured brain neurones the channels activated by *N*-methyl-D-aspartate (NMDA) have recently been shown to have an appreciable permeability to calcium (Ascher & Nowak, 1986; MacDermott, Mayer, Westbrook, Smith & Barker, 1986). MacDermott *et al.* have used the indicator dye Arsenazo III to show that cytoplasmic calcium rises following NMDA application. Interestingly the rise in cytoplasmic calcium lagged behind the peak of the cation current flow and showed a similar time course to the development of the outward current in our experiments that we believe is calcium activated.

Further evidence that the ATP-induced current can carry divalent cations was provided by the experiments with solutions containing high extracellular barium concentrations where the ATP-induced current had a reversal potential of −3·0 mV. This result would suggest that the calcium permeability may lie somewhere between that of the frog neuromuscular junction where the reversal potential is shifted in a negative direction by increasing extracellular divalent cations (Lewis, 1979) and the NMDA channel where the reversal potential is shifted to positive potentials by the same manipulation (Ascher & Nowak, 1986). Our data is thus consistent with the channel being permeable to divalent cations and to a lesser extent to Tris and it is concluded that it is likely that the inward current evoked by ATP in isolated rabbit ear artery cells is produced by a non-selective increase in cation permeability.

It has been suggested that calcium may enter smooth muscle cells not only through voltage-dependent channels but also through receptor-operated channels (Bolton, 1979; van Breemen, Aaronson & Loutzenhiser, 1979). The non-selective nature of the channels opened by ATP may result in some calcium entry through these along with other, larger, movements of monovalent cations. The channels opened by ATP would thus constitute the receptor-operated portal of entry for calcium ions which has been postulated.

In ionophoretic experiments it was apparent that the action of ATP was rapid in onset. The mean latency of the inward current was about 72 ms. The minimum latency was 38 ms which presumably represents the upper limit of the onset of action of ATP. In whole tissue preparations of the rabbit ear artery ATP also has a rapid onset of action (Suzuki, 1985). In the rat anococcygeus muscle the latency to ionophoretically applied ATP is very brief and an upper limit of 30 ms was observed by Byrne & Large (1984). This is in contrast to the relatively slow onset of responses to noradrenaline which are mediated by $\alpha$-adrenoreceptors. Thus in the mouse and rat anococcygeus muscle at room temperature the latency of the depolarization to noradrenaline is several hundred milliseconds (Large, 1982; Byrne & Large, 1984). Also, in dispersed cells of the guinea-pig pulmonary artery the mean latency of the inward current induced by noradrenaline was 823 ms (Byrne & Large, 1986), an order of magnitude larger than the value found for ATP in the present study. The

ATP-induced inward current did not require the presence of calcium compared to some other autonomic responses (e.g. channels opened by the muscarinic action of ACh in the rat lacrimal gland; Marty *et al.* 1984). It appears that calcium dependency of receptor-operated channels is associated with a significant lag period between receptor binding and the beginning of the response. The speed of onset of the depolarization to ATP suggests that the receptor may be linked more closely to the ionic channel. The suggestion that the current can carry calcium into the cell provides a calcium entry pathway that would be resistant to calcium-entry blockers and to $\alpha$-blockers. In this vascular muscle 34% of the nerve-mediated contraction was resistant to a combination of nicardipine and prazosin (Suzuki & Kou, 1983). Activation of this conductance pathway by ATP or a related substance following sympathetic discharge may thus be one of the mechanisms for maintaining vascular tone.

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