Effects of ATP on cultured smooth muscle cells from rat aorta

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1 Membrane ionic currents provoked by externally applied ATP were studied by patch-clamp techniques in cultured aortic smooth muscle cells of the rat.

2 Using standard bath and pipette solutions and whole-cell voltage-clamp, ATP evoked an inward current when the cell membrane potential was held at -50 mV and an outward current when the potential was held at 30 mV, with a reversal potential near -10 mV.

3 Application of ATPyS gave results similar to those obtained with ATP, while adenosine, AMP and α,β -methylene ATP were ineffective. The ATP-activated current was inhibited by suramin, $100 \mu\text{M}$.

4 ATP also induced a biphasic rise in internal free Ca levels as shown directly by Fura-2 measurements and by the increase in Ca-dependent K single-channel activity in cell-attached patches.

5 With outward current through K channels blocked by internal Cs and TEA, modification of the ionic composition of bath and pipette solutions revealed that the reversal potential for the ATP-induced whole-cell current closely followed E_{Cl} , the chloride equilibrium potential, and was insensitive to manipulations of the monovalent cation gradient.

6 These results indicate that in rat cultured aortic smooth muscle cells, ATP binding to P_2 -purinoceptors produces increases of internal free Ca levels and subsequent activation of both Ca-dependent K and Cl currents.

Keywords: Vascular smooth muscle; ATP; purinoceptor; internal calcium; Ca-dependent K channel; Ca-dependent Cl channel

Introduction

Adenosine 5'-triphosphate (ATP) is considered to be one of the key physiological regulators of vascular tone (for review, see Burnstock & Kennedy, 1986). For example, ATP is an endothelium-dependent vasodilator but is also co-released with noradrenaline at sympathetic nerve terminals present in smooth muscle, thereby directly inducing vasoconstriction. Application of exogenous ATP causes membrane depolarization in many smooth muscle cells, for example, from ear artery (Suzuki, 1985; Benham *et al.*, 1987), vas deferens (Friel, 1988) and urinary bladder (Fuji, 1988). The patch-clamp technique has been used in a variety of smooth muscle preparations to investigate the membrane mechanism(s) underlying the ATP-induced depolarization. In smooth muscle cells from the rabbit ear artery and also in the rat vas deferens, ATP directly activates a Ca-permeable, non-selective cationic channel (Benham & Tsien, 1987a,b; Benham *et al.*, 1987; Nakazawa & Matsuki, 1987; Friel, 1988; Benham, 1989; Declerck *et al.*, 1989). Ca influx through this channel results in increases in internal free Ca levels in normal physiological solutions (Benham, 1989). On the other hand, in smooth muscle cells from the rat portal vein, ATP-induced depolarization was attributed to an increase in Na and Ca conductances together with the subsequent activation of Ca-dependent Cl channels (Karashima & Takata 1979; Xiong *et al.*, 1991). Furthermore, it has also been demonstrated in primary culture of rat aortic smooth muscle cells that ATP activates P_2 -purinoceptors and induces the release of Ca from

IP_3 -sensitive intracellular stores (Phaneuf *et al.*, 1987; Tawada *et al.*, 1987). Similar actions of ATP were described in primary cultures of pig aortic smooth muscle cells, where cell membrane depolarization resulted from the activation of Ca-dependent Cl channels (Droogmans *et al.*, 1991).

Because of these apparent discrepancies concerning the effects of ATP on various types of vascular smooth muscular cells, we investigated the actions of exogenously applied ATP on membrane currents and on internal Ca levels in cultured aortic smooth muscle cells of the rat which retain some characteristics associated with a contractile or differentiated phenotype (Bodin *et al.*, 1987; 1991; Serebryakov & Takeda 1992).

Methods

Cell culture

Primary cultures of smooth muscle cells were prepared from aortae of 10 week-old male Wistar-Kyoto rats as previously described in detail (Bodin *et al.*, 1987; 1991; Serebryakov & Takeda, 1992). Briefly, following isolation and enzymatic digestion with collagenase and elastase of aortic tissue, dispersed cells were plated at $1500\text{--}2000$ cells cm^{-2} onto collagen-coated glass coverslips in 35 mm Petri dishes in a defined serum-free culture medium (Eagle's MEM/Ham's F-10 (1:1 vol/vol; Eurobio), supplemented with 2% (vol/vol) synthetic serum substitutes Ultrosor SF/Ultrosor G (3:1 v/v; IBF), 100 u ml^{-1} penicillin G, 0.05 mM vitamin C and 2 mM glutamine) and kept in a 37°C incubator gassed with 5% $\text{CO}_2/95\%$ air.

Electrophysiology

Single cells were studied usually 7–15 days after plating by standard patch-clamp techniques (Hamill *et al.*, 1981). In some cases, cells from first passages were also used. Patch pipettes were made from thin-wall (Vitrex BRI/E) or thick-

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wall (Hilgenberg) borosilicate glass capillaries and had resistances of 3–8 M Ω when filled with normal internal KCl solution (Table 1). To minimize associated capacitance, pipettes were coated with beeswax and dipped in Sigmacote (Sigma). The bath (≈ 1.5 ml) was usually superfused with normal external NaCl solution (0.5 – 1.0 ml min $^{-1}$) to remove applied compounds and when different external solutions were used. ATP and other agonists (adenosine, ATP γ S, α , β -methylene ATP, AMP; Sigma) were locally microperfused (5 – 10 s) by use of pressurized (≈ 10 kPa) puffer pipettes located about 50 μ m downstream (relative to the bath superfusion) from the cell under study. Control application of external NaCl solution alone had no effect. Suramin (Bayer) was added directly to the bath. The ionic composition of the various external bath and pipette internal solutions used is given in Table 1. Data were recorded on FM or videotape and analysed after appropriate filtering and acquisition with a CED 1401 interface and software from CED and a laboratory personal computer (Goupil 386DX). Compensation for series resistance and capacitance was made using the inbuilt circuitry of the patch-clamp amplifier (List EPC-7). Voltage ramp commands (usually 1 s in duration) were used to obtain quasi steady-state current-voltage (I - V) curves with and without agonists. In some cases several responses were averaged, and net agonist I - V curves were obtained by subtraction of control I - V s from those in the presence of drug. Voltage-dependent Ca channel currents were recorded as previously described (Serebryakov & Takeda, 1992). Data are presented as mean $\pm$ s.e.mean for n cells where appropriate.

Measurement of intracellular calcium

The effects of ATP on internal free Ca levels were studied in Fura-2 AM loaded cells as previously described (André *et al.*, 1990). Briefly, confluent rat aortic myocytes plated on glass coverslips without collagen were washed of culture medium 3 times with 3 ml of Hank's balanced salt solution (HBSS; Gibco; composition in mM: NaCl 135, KCl 5, CaCl $_2$ 1.25, MgCl $_2$ 2, glucose 10, HEPES 20, pH 7.5). The cells were placed in a quartz spectrophotometric cuvette for 20 min at 37°C in Ca-free HBSS containing 5 μ M Fura-2 AM (Molecular Probes). The cells were then washed 3 times with HBSS and left for an additional 10 min at 37°C to ensure complete hydrolysis of the Fura-2 AM. Internal Ca measurements were carried out at 37°C using a SPEX 1681 spectrofluorometer with excitation at 340 and 380 nm and emission measured at 510 nm as previously reported (André *et al.*, 1990). Calculation of internal Ca concentrations was made following Grynkiewicz *et al.* (1985), after calibration using 20 μ M ionomycin (Calbiochem) and 5 mM Ca in HBSS followed by 20 mM EGTA.

Results

ATP-induced whole-cell current

The whole-cell membrane response to externally applied ATP (100 μ M) is shown in Figure 1a from a single smooth muscle cell. The cell was voltage-clamped at a holding potential of -50 mV and step depolarizations to 30 mV were given regularly. Standard external NaCl and pipette internal KCl solutions (Table 1) were used. After a latency of a few seconds, a transitory ATP-induced inward current developed at -50 mV, and simultaneously, the outward current observed at 30 mV increased with much the same time course. Whole-cell, quasi steady-state I - V relationships in the absence and at the peak of the ATP-induced response were obtained by applying 1 s long voltage-ramp commands between -50 and 180 mV (Figure 1b). The control I - V was fairly linear, while the I - V in the presence of ATP showed outward rectification at potentials more depolarized than 100 mV. The net ATP-activated current was obtained by subtraction of the

Figure 1 Responses of rat aortic smooth muscle cells under whole-cell voltage-clamp to microperfusion of ATP (100 μ M). (a) ATP evoked an inward current at -50 mV and an outward current at 30 mV. Holding potential was -50 mV and 80 mV depolarizing voltage pulses 300 ms in duration were applied every 600 ms. (b) Current-voltage (I - V) relationships in control and with ATP (100 μ M) were obtained using a 1 s voltage ramp from -50 to 180 mV in another cell. (c) Net ATP-activated current obtained by subtraction of the control response from that measured in the presence of ATP for the cell in (b). The reversal potential of the ATP-activated current was -18 mV in this cell. Note the outward rectification at positive potentials. (Standard NaCl bath and KCl pipette solutions).

control I - V curve from that observed in the presence of ATP (Figure 1c), and had a reversal potential of -18 mV in this cell. The mean value of the reversal potential of the net ATP-induced current under these conditions was -10.3 ± 10.1 mV ($n = 5$). Because this value is close to 0 mV, it may be that ATP activates either a non-selective cationic current or a Cl current, as the equilibrium potentials for both Cl and for monovalent cations is ≈ 0 mV with the solutions used. Furthermore, as ATP is known to cause increases in internal free Ca levels, it is very likely that at least part of the outward current at positive potentials results from activation of Ca-dependent K channels, as suggested from the observed outward rectification at positive potentials. Reversal potentials intermediate between 0 mV and E_K , which was usually -85 mV, could be explained by simultaneous activation of several conductances.

When calcium was absent from the external solution (Na/ 0 Ca solution; Table 1) and when 10 mM BAPTA was used instead of 0.1 mM EGTA in the pipette internal KCl solution, ATP-activated currents were observed in 5 of 9 cells tested. In 2 of the 5 cells, responses similar to those in Figure 1 were observed, while in the 3 others, only outward currents at positive potentials were seen (results not shown). It was possible to obtain only a single response to ATP from each

Table 1 Ionic composition (in mM) of the solutions used

	External (bath) solutions					Internal (pipette) solutions					
	NaCl	Na/TEA	Na/0 Ca	Na/0 Cl	Low Na	20 Ba	Choline	KCl	Cs/TEA	Cs/Low Cl	Choline
NaCl	140	125	140	—	40	130	—	—	—	—	—
KCl	5	—	5	—	—	—	—	135	—	—	—
CsCl	—	—	—	—	20	—	—	—	140	20	—
Na glutamate	—	—	—	140	—	—	—	—	—	—	—
Cs glutamate	—	—	—	—	—	—	—	—	—	100	—
CaCl ₂	2	2	—	2	2	—	2	—	—	—	—
MgCl ₂	2	2	2	2	2	2	2	2	2	2	2
BaCl ₂	—	—	—	—	—	20	—	—	—	—	—
TEA Cl	—	20	—	—	20	—	—	—	20	20	—
Choline Cl	—	—	—	—	65	—	140	—	—	—	140
Mg ATP	—	—	—	—	—	—	—	5	5	5	5
Li GTP	—	—	—	—	—	—	—	0.2	0.2	0.2	0.2
EGTA	—	—	—	—	—	—	—	0.1	0.1	0.1	0.1
Glucose	10	10	10	10	10	10	10	—	—	—	—
HEPES	10	10	10	10	10	10	10	10	10	10	10

The pH was adjusted to 7.3 with NaOH and KOH or CsOH for external and internal solutions respectively. In some internal solutions, 10 mM BAPTA was used instead of EGTA.

cell with these solutions. Note that this predominately outward current response was also observed for a few cells under the same experimental conditions as in Figure 1.

Several related nucleotides and adenosine were tested (all at 100 μ M, in at least 4 cells) in order to characterize the purinoceptor type responsible for the whole-cell currents evoked by ATP (Figure 2a). With the same voltage-clamp protocol and solutions as in Figure 1a, only ATPyS, a non-hydrolysable ATP analogue, was as effective as ATP, while α,β -methylene ATP, AMP or adenosine had no marked action. The observed rank order of potency was ATP = ATPyS $\gg$ AMP = α,β -methylene ATP = adenosine = 0. When 100 μ M suramin, a potent blocker of P₂ purinoceptors (Dunn & Blakeley, 1988; Nakazawa *et al.*, 1990a) was added to the bath, the currents activated by 100 μ M ATPyS were inhibited partially after 15 min and totally after 20 min (Figure 2b; $n = 3$). A similar blocking action of suramin was found for currents induced by ATP ($n = 2$).

Effect of ATP on single-channel activity

When recordings from cell-attached patches were made from single cells bathed in standard external NaCl solution using a patch pipette containing standard internal KCl solution without MgATP, outward single-channel currents having an elementary conductance of 176 ± 10 pS were observed at pipette potentials depolarized with respect to the cell resting potential (Figure 3b; $n = 4$). These currents had an interpolated reversal potential close to 0 mV (assuming a cell resting potential of ≈ -45 mV) and are thus probably due to the opening of large-conductance Ca-activated K channels (Bregestovski *et al.*, 1988; 1989). Upon application of 100 μ M ATP to the cell, a large increase in the activity of this channel type in the patch was observed (Figure 3a). As many as 10 channels were simultaneously open at the peak of the response compared to basal levels, where 1 or rarely 2 channels were open on average. This increase in activity was transient, with a return to basal levels of channel openings ≈ 10 s after the end of ATP application (Figure 3c). The activation of Ca-sensitive K channels in the patch following ATP application to the cell exterior would be accounted for by an ATP-induced rise in cellular free Ca levels. The same effect was observed in 4 different cells.

Ionic nature of the ATP-activated current

In standard external NaCl and pipette internal KCl solutions, the reversal potential of ATP-activated current was close to 0 mV (Figure 1c). As indicated above, activation of

either a Cl conductance or a non-selective cationic conductance would account for the ATP-induced current. In a first set of experiments, the contribution of monovalent cations was examined, firstly by substituting choline Cl for NaCl and KCl in the external and internal solutions respectively (Figure 4a–c), and secondly, by decreasing external NaCl by replacement with choline Cl and tetraethylammonium (TEA) Cl and by replacing internal KCl by CsCl and TEA Cl (Figure 4d–f). Under both of these conditions, the reversal potentials of the net ATP-activated current was close 0 mV, the expected value of E_{Cl} . When the internal solution contained Cs and TEA to block K currents and the Cl gradient was altered, either by decreasing external or internal Cl by substitution with glutamate (Figure 5), the reversal potentials of the ATP-induced currents followed the expected values of E_{Cl} . In symmetrical Cl solutions with K conductance blocked (Figure 5a), the reversal potential was somewhat closer to 0 mV (-4.5 ± 4.8 mV; $n = 4$), than in Figure 1. When the external NaCl was replaced by Na glutamate, the reversal potential shifted rightwards to 63.0 ± 2.3 mV ($n = 3$), close to the calculated E_{Cl} of 73 mV (Figure 5b). When the internal Cl was decreased, the observed reversal potential was -21.4 ± 1.8 mV ($n = 10$) and the calculated E_{Cl} was -34 mV (Figure 5c). These data argue strongly that the ATP-induced current reversing at ≈ 0 mV in our normal external and internal solutions arises in large part from an increase in Cl conductance and not from an increase in a non-selective cationic conductance.

Effect of ATP on internal free calcium concentration

ATP (10 μ M) produced biphasic increases in internal free calcium levels (Figure 6a), with an initial transient peak followed by a smaller maintained plateau of elevated Ca ($n = 8$ preparations). This second plateau phase depended on the presence of extracellular Ca (not shown), consistent with the commonly held view that the initial peak arises mostly from release of Ca from internal stores while the maintained phase requires Ca influx. Application of 100 μ M Mn during the plateau phase caused a rapid decrease in Ca (Figure 6b; $n = 5$), presumably because of Mn permeation through an ATP-associated conductance (cf. Hallam *et al.*, 1988), thereupon resulting in a quenching of the intracellular Fura-2 Ca signal.

Effect of ATP on voltage-activated Ca current

As previously reported (Serebryakov & Takeda, 1992), depolarization-activated whole-cell inward Ba currents having a

Figure 2 (a) Comparison of whole-cell current responses elicited by ATP γ S, α,β -methylene ATP ($\alpha\beta$ MeATP), AMP and adenosine. Drugs were tested at 100 μ M on different cells. Current scale is 0.5 nA except for AMP, where it is 0.25 nA. (b) ATP γ S-activated current before (upper trace), 15 min (middle trace) and 20 min (lower trace) after bath application of suramin (100 μ M). Voltage-clamp protocol as in Figure 1a. (Standard NaCl bath and KCl pipette solutions).

fast onset and a rapid time course of inactivation were observed with external 20 mM Ba bath and internal Cs/TEA pipette solutions. These currents have properties consistent with those of T-type or low-voltage-activated Ca channels: rapid inactivation during depolarizing steps, high sensitivity to block by Ni but not to Cd and steady-state half-activation and half-inactivation voltages of -48 and -41 mV, respectively (Serebryakov & Takeda, 1992). They were essentially unaffected by the dihydropyridine agonists (–)-Bay K 8644 and S(+)-202 791 at 1 μ M, while the dihydropyridine antagonist nicardipine was effective at 5 and 10 μ M, but not at 0.5 μ M (Serebryakov & Takeda, 1992). Maximal Ca channel current amplitude occurred between -10 and -20 mV with a -70 mV holding potential. When 100 μ M ATP was applied by local microperfusion, peak Ca channel current was decreased with a rapid onset and in a reversible fashion (Figure 7a). The average decrease was $60.5 \pm 13.0\%$ with a recovery to $79.1 \pm 12.3\%$ of control values ($n = 5$). Figure 7b shows that 100 μ M adenosine, which did not activate any current in our cells (cf. Figure 2a), had negligibly small effects on peak Ca channel current ($n = 3$).

Figure 3 (a) Single Ca-activated K channel activity from a cell-attached patch was greatly increased by microperfusion of the cell with ATP (100 μ M). Pipette potential was clamped 60 mV positive to the cell resting potential. (b,c) Higher time resolution traces before and after ATP application for the indicated times in the recording in (a). (Standard NaCl bath solution; MgATP was absent from the KCl pipette solution).

Figure 4 ATP-activated whole-cell current is not sensitive to changes in the monovalent cation gradient. (a–c) Currents evoked by ATP ($100\ \mu\text{M}$) in symmetrical $140\ \text{mM}$ choline Cl bath and pipette solutions. (d–f) Currents evoked by ATP ($100\ \mu\text{M}$) in low monovalent cation bath solution and Cs/TEA pipette solution. (a,d) Voltage ramps $3\ \text{s}$ long from -50 to $20\ \text{mV}$ were applied periodically. (b,e) I - V curves were obtained before and at peak of the ATP-activated current. (c,f) Net ATP-activated I - V relationships calculated by subtracting control responses from those in the presence of ATP had reversal potentials very close to $0\ \text{mV}$.

Figure 5 The reversal potential of the ATP-activated current is sensitive to the Cl gradient. Left-hand panels show I - V curves in control and in the presence of ATP while right-hand panels illustrate the net ATP-activated current. (a) In symmetrical Cl solutions, the ATP-activated current reversed close to $-6\ \text{mV}$. (b) When external Cl was replaced by glutamate, the reversal potential shifted rightwards to $63\ \text{mV}$. (c) When the Cl concentration in pipette solution was decreased to $40\ \text{mM}$, the reversal potential shifted leftwards to $-25\ \text{mV}$. Voltage-clamp protocol as in Figure 4a; range was from -50 to $120\ \text{mV}$ (a,b) or to $20\ \text{mV}$ (c). Cs/TEA was present in the pipette solutions to block K currents.

Figure 6 (a) ATP induced a biphasic increase in internal free Ca levels in Fura-2 loaded aortic smooth muscle cell monolayers. Basal Ca concentration initially was $105\ \text{nM}$. (b) The second plateau phase of elevated Ca was rapidly inhibited by Mn ($100\ \mu\text{M}$). Basal Ca concentration initially was $179\ \text{nM}$.

Figure 7 (a) Reversible inhibition of peak inward Ca channel current induced by ATP ($100\ \mu\text{M}$). (b) Adenosine ($100\ \mu\text{M}$) had negligibly small effects on peak Ca channel current. Bath solution contained *inter alia* $20\ \text{mM}$ Ba and the pipette solution Cs/TEA. Holding potential $-70\ \text{mV}$, test potential $-10\ \text{mV}$. Note the different current amplitude scale bars in (a) and (b).

Discussion

In the present study, ATP-activated membrane currents were observed using whole-cell patch-clamp techniques in rat aortic myocytes that retain some aspects of a contractile or differentiated phenotype in culture (Serebryakov & Takeda, 1992). A similar action of ATP has already been reported in other mammalian smooth muscle cells (Karashima & Takata, 1979; Benham & Tsien 1987a,b; Benham *et al.*, 1987; Nakazawa & Matsuki, 1987; Friel, 1988; Declerck *et al.*, 1989; Droogmans *et al.*, 1991; Schneider *et al.*, 1991; Xiong *et al.*, 1991). Given that this response was elicited by ATP and ATP γ S but not by AMP or adenosine, it may be inferred from the classification proposed by Burnstock & Kennedy (1985), that the P₂ subclass of purinoceptors mediates the responses described here. This is supported by the inhibition of the ATP-induced current caused by suramin, a P₂-receptor antagonist. The absence of effect of α,β -methylene ATP is therefore surprising, since it is generally considered to be a P₂-receptor agonist (cf. Burnstock & Kennedy, 1985; Benham & Tsien, 1987b). However, such a finding has already been described in smooth muscle cells from rat vas deferens (Friel, 1988). Similarly, in rat cultured aortic smooth muscle cells, α,β -methylene ATP produced only a relatively weak increase in internal free Ca compared to ATP (Tawada *et al.*, 1987). It was shown that the major part of the increase in internal free Ca was due to Ca release from IP₃-sensitive intracellular stores (Tawada *et al.*, 1987). In the present study, application of ATP caused a transient increase in internal free Ca levels in Fura-2 loaded aortic smooth muscle cells, followed by a sustained plateau phase which depended on the presence of extracellular Ca and which diminished rapidly following application of external Mn, presumably due to quenching of fluorescence following Mn entry (Figure 6). This ATP-induced rise in free Ca could also be demonstrated by the strong increase in activity of Ca-activated K channels recorded from cell-attached patches. The second plateau phase of elevated internal Ca levels is usually taken as evidence for an influx of Ca (e.g. Hallam *et al.*, 1988). Note that in non voltage-clamped cells, this Ca influx may arise secondarily to the opening of voltage-sensitive Ca channels produced by depolarizing ATP-activated current, as previously proposed (Droogmans *et al.*, 1991; Pacaud *et al.*, 1991).

In whole-cell recordings using our standard external and internal solutions, the ATP-activated current had a reversal potential around -10 mV and approached 0 mV when K conductances were minimized. With Ca-activated K currents blocked by internal Cs and TEA, the remaining ATP-induced current could either be due to a non-selective cationic current or to a chloride current. In smooth muscle cells from rabbit ear artery (Benham *et al.*, 1987; Benham & Tsien, 1987a,b; Benham, 1989; Declerck *et al.*, 1989) and portal vein (Xiong *et al.*, 1991) and in rat vas deferens (Nakazawa & Matsuki, 1987; Friel, 1988), it was shown that ATP activated a non-selective cationic conductance. Furthermore, the ATP-induced inward current in rabbit ear artery cells was insensitive to changes in the Cl gradient and to Cl channel blockers (Benham *et al.*, 1987). However, here we were unable to demonstrate convincingly the involvement of such non-selective cationic channels, since neither replacement of monovalent cations by choline nor a decrease in external monovalent cation concentration diminished the ATP-induced current amplitude or changed its reversal potential. On the other hand, it may well be that there is activation of a non-selective cationic, Ca-permeable channel by ATP, but that both its single-channel conductance and the resulting whole-cell current are very small and thus masked by the activation of other currents. Such a non-selective cationic conductance would provide a pathway for Ca influx, as can be inferred from our Fura-2 experiments. Nevertheless, the effects of changing the Cl concentration gradient on the reversal potential of the ATP-activated current were clearly consistent with the idea that an increase in Cl conductance

was produced by ATP. Our results are indeed compatible with those obtained in cultured smooth muscle cells from pig aorta where ATP induced a Ca-activated Cl current (Droogmans *et al.*, 1991). This current would produce membrane depolarization and presumably lead to a Ca entry via voltage-dependent Ca channels in non voltage-clamped cells (as used in the Fura-2 studies and *in situ*). We do not have any explanation as to why there is an apparent absence of ATP-activated non-selective cation currents and presence of Cl currents in our cells, other than the obvious differences between the tissue or species origins of the various smooth muscle cells studied.

Ca-activated Cl currents have been described in several other types of smooth muscle cells, including those from rat anococcygeus (Byrne & Large, 1987), rabbit (Byrne & Large, 1988) and rat (Pacaud *et al.*, 1989) portal vein, guinea-pig mesenteric artery (van Helden, 1988), and rabbit ear artery (Amédée *et al.*, 1990). In these smooth muscle cells, the Ca-activated Cl current was produced notably by adrenoceptor stimulation and consecutively, to a rise in internal free Ca levels. Similarly, in smooth muscle cells from porcine coronary and human mesenteric arteries, endothelin produced an increase in internal Ca and opening of Ca-activated Cl channels (Klößner & Isenberg, 1991). This current probably contributes in a major fashion to agonist-associated membrane depolarization and cell contraction.

That ATP produces multiple types of current responses has been well-documented in many other cell types (for review see, Bean & Friel, 1990). For example, ATP activates non-selective cationic currents in rat sensory neurones (Krishtal *et al.*, 1983), in chick myotubes, (Hume & Honig, 1986), in PC12 cells, a pheochromocytoma cell line (Nakazawa *et al.*, 1990a,b), and in cells from mouse (Sasaki & Gallacher, 1990) and rat (Vincent, 1992) lacrimal glands. Recently, ATP was reported to activate depolarizing Ca-dependent Cl currents in FRTL-5 cells, a rat thyroid cell line (Martin, 1992), in a small proportion of C3H10T1/2 cells, a mouse mesodermal stem cell line (Kubo, 1991), and in guinea-pig atrial cells (Matsuura & Ehara, 1992). In FRTL-5 cells, ATP acted via P₂-receptors, as adenosine and AMP were unable to mimic the responses produced by ATP. Based on the extracellular Ca-dependence of the ATP-induced currents, it was further concluded that in these cells, ATP also opens a Ca-influx pathway (Martin, 1992). Interestingly, in guinea-pig atrial cells, an atypical purinoceptor pharmacology was found, with ATP, ADP and adenosine being almost equipotent (Matsuura & Ehara, 1992). In bovine cultured aortic endothelial cells, ATP also produces rises in internal free Ca concomitant with activation of Ca-dependent K and Cl channels (Snetkov *et al.*, 1992).

A reversible decrease in voltage-dependent Ca channel current was caused by ATP but not adenosine. A similar decrease was produced with noradrenaline and adrenaline in the same cells (Serebryakov & Takeda, 1992) and in smooth muscle cells from rabbit ear artery (Droogmans *et al.*, 1987). In smooth muscle cells from the rabbit portal vein (Xiong *et al.*, 1991), a consistent inhibition of voltage-dependent Ca current was observed with 3 mM ATP, while a transient increase in Ca current was seen with 30 μ M ATP. It was suggested that the action of ATP on voltage-sensitive Ca channels involved both GTP-binding proteins and a Ca-mediated inactivation process. A similar mechanism involving Ca-mediated inactivation was also proposed by Schneider *et al.* (1991) who described an ATP-induced inhibition of Ca current in myocytes of guinea-pig urinary bladder.

In summary, the data presented here demonstrate that in smooth muscle cells cultured from rat aorta, external ATP acting on P₂-purinoceptors causes increases in internal free Ca levels, leading to activation of Ca-dependent K and Cl channels. No convincing evidence was obtained for an ATP-induced non-selective cationic conductance in our cells. While it has been clearly shown that in other types of smooth muscle cells, a non-selective cationic current is activated by

ATP, it is not at all evident why there should be such heterogeneity especially between vascular myocytes obtained from large vessels.

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