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Characterization of P_2-purinoceptors in the smooth muscle of the rat tail artery: a comparison between contractile and electrophysiological responses

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1 The electrophysiological actions of the P_2-purinoceptor agonists, adenosine 5′-triphosphate (ATP), 2-methylthioATP (2-meSATP), α,β-methyleneATP (α,β-meATP) and uridine 5′-triphosphate (UTP) were studied under concentration and voltage-clamp conditions in acutely dissociated rat tail artery smooth muscle cells. For comparison, their actions as vasoconstrictors were studied in intact ring preparations.

2 Rapid application of ATP (100 nM–1 μM) via a U-tube superfusion system activated concentration-dependent inward currents with a latency to onset of less than 3 ms. The inward current decayed by more than 95% during a 2 s application of 300 nM and 1 μM ATP.

3 2-meSATP (100 nM–1 μM) and α,β-meATP (100 nM–1 μM) also evoked transient inward currents. The agonist order of potency was ATP = 2-meSATP ⩾ α,β-meATP. UTP (300 nM–1 μM) did not produce a change in the holding current.

4 A second application of ATP (300 nM and 1 μM) 10 min after the first, evoked currents which were one third of the initial amplitude. This decline was dependent upon activation of the P_2-purinoceptor. Similar results were seen with 2-meSATP and α,β-meATP (both 300 nM and 1 μM). Cross-desensitization was seen between ATP and 2-meSATP or α,β-meATP.

5 Inward currents evoked by ATP, 2-meSATP and α,β-meATP (all 1 μM) were abolished by the P_2-purinoceptor antagonist suramin (100 μM).

6 α,β-meATP (100 nM–30 μM), 2-meSATP (3 μM–100 μM), ATP (3 μM–1 mM) and UTP (3 μM–1 mM) produced concentration-dependent contractions of rat tail artery rings. When measured at a level equal to 50% of the maximum response to noradrenaline, the rank order of agonist potency was α,β-meATP >> 2-meSATP > UTP > ATP.

7 This study shows that the rank order of agonist potency at the P_{2X}-purinoceptor which mediates contractions of the rat isolated tail artery is very different from the potency order for evoking the inward current which initiates the contractions. It is concluded that this difference may be due to the relative absence of breakdown of some of the agonists in the single cell system compared with artery rings.

Keywords: Rat tail artery; smooth muscle; P_2-purinoceptors; ATP; UTP; suramin

Introduction

There is now considerable evidence that adenosine 5′-triphosphate (ATP) acts as a neurotransmitter in vascular (Burnstock, 1990; Evans & Surprenant, 1992; Kennedy, 1993a) and neuronal (Evans *et al.*, 1993; Edwards *et al.*, 1993) preparations. ATP produces its physiological and pharmacological actions through the activation of P_2-purinoceptors (Burnstock, 1978), which have been subdivided into at least five subtypes, P_{2X}, P_{2Y}, P_{2U}, P_{2T} and P_{2Z}, largely on the basis of the relative agonist potencies of ATP and a number of its structural analogues (Burnstock & Kennedy, 1985; Gordon, 1986; Kennedy, 1990; O'Connor *et al.*, 1991). The best characterized of these are the P_{2X}-, P_{2Y}- and P_{2U}-subtypes.

The P_{2Y}- and P_{2U}-purinoceptors were cloned recently and both belong to the G protein-coupled receptor superfamily (Lustig *et al.*, 1993; Webb *et al.*, 1993). In contrast, the P_{2X}-purinoceptor is thought to be a ligand-gated cation channel (Bean & Friel, 1990; Kennedy, 1990). In smooth muscle preparations the opening of this cation channel underlies membrane depolarization and contraction following P_2-purinoceptor activation (Benham *et al.*, 1987; Nakazawa & Matsuki, 1987; Inoue & Brading, 1990; Bean, 1992; Kennedy, 1993b).

The rank order of agonist potency at the P_{2X}-purinoceptor is α,β-methyleneATP (α,β-meATP) >> 2-methylthioATP (2-meSATP) ⩾ ATP, while the potency order at the P_{2Y}-purinoceptor is 2-meSATP >> ATP > α,β-meATP (Burnstock & Kennedy, 1985; Kennedy, 1990). Uridine 5′-triphosphate (UTP) has little or no action at these receptors, but is an agonist at the P_{2U}-purinoceptor (O'Connor *et al.*, 1991). ATP is also an agonist at the P_{2U}-purinoceptor, whereas 2-meSATP and α,β-meATP have little or no effect. One weakness with this classification is that it is based on results from whole tissue preparations in which the influence of ectonucleotidase activity could not be determined. ATP and 2-meSATP are susceptible to breakdown by ectonucleotidases, but α,β-meATP is relatively resistant (Gordon, 1986; Welford *et al.*, 1986; 1987).

In several studies where P_2-purinoceptors were examined in single acutely dissociated smooth muscle cells using electrophysiological techniques, the relative potency of ATP and its analogues did not correspond to that described in the whole tissues (Benham & Tsien, 1987; Friel, 1988; Inoue & Brading, 1990; Xiong *et al.*, 1991). The reason for this difference is uncertain, but it could simply reflect different enzymatic breakdown of ATP and its analogues in whole tissues and dissociated cells. However, the pharmacological profile of P_2-purinoceptors in single smooth muscle cells has not been well characterized.

Therefore, the aim of the present study was to compare the agonist order of potency of ATP and a number of its

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analogues in whole tissue and single cell preparations. Thus, we have determined the relative potency of ATP, 2-meSATP, α,β-meATP and UTP in evoking inward currents in acutely dissociated smooth muscle cells and contractions in isolated rings of the rat tail artery, a vessel in which ATP acts as an excitatory cotransmitter (Sneddon & Burnstock, 1984; Åstrand *et al.*, 1988) and which has a high density of P_{2X}-purinoceptors (Bo & Burnstock, 1993).

Methods

Single cell isolation and electrophysiological experiments

Rat tail artery smooth muscle cells were isolated by a modification of the method of overnight digestion with papain (Clapp & Gurney, 1991). Male Sprague Dawley rats (100–175 g) were killed by exposure to carbon dioxide followed by exsanguination. The tail artery was removed and placed in a 'low calcium' dissociation solution of the following composition (mM): NaCl 140, KCl 5, Na_2HPO_4 0.06, glucose 10, HEPES 10, EGTA 0.5, $MgCl_2$ 1.2, $CaCl_2$ 0.1, titrated to pH 7.3 with NaOH. The connective tissue was removed, the cleaned vessel opened along its longitudinal axis and the endothelial layer removed by gentle rubbing. The artery was then cut into small pieces 1–2 mm long, transferred to 5 ml of dissociation solution containing 5 mg ml^{-1} bovine serum albumin and 8.5 units ml^{-1} papain and incubated overnight (16–18 h) at 4–6°C. The following morning the tail artery pieces and enzyme solution were incubated at 37°C. Gentle trituration with a fire-polished Pasteur pipette (tip diameter 1 mm) 30–40 times at 10, 20 and 25 min yielded single, spindle shaped cells. The supernatant was centrifuged at 150 g for 5 min, the pellet resuspended in 1 ml of dissociation solution and stored at 4–6°C until the cells were plated. Cells were used within 8 h of dissociation.

Smooth muscle cells were plated onto glass coverslips and allowed to settle for 10–20 min before transfer to the recording chamber. Cells were superfused at 3–4 ml min^{-1} at room temperature with 'normal calcium' bathing solution of the following composition (mM): NaCl 140, KCl 5, Na_2HPO_4 0.06, glucose 10, HEPES 10, $MgCl_2$ 1.2, $CaCl_2$ 2.5, titrated to pH 7.3 with NaOH. In some experiments cells were initially bathed in the 'low calcium' dissociation solution, followed by the 'normal calcium' bathing solution once the whole cell recording mode was achieved. Only one cell per coverslip was tested for responses to drugs.

Whole cell currents were recorded by the patch clamp technique with an Axopatch 1D amplifier (Axon Instruments, U.S.A.). The resistance of the patch pipette was 2–5 MΩ when filled with internal solution of the following composition (mM): KCl 160, $MgCl_2$ 2, HEPES 10, EGTA 5, Na_2ATP 2, Na_2GTP 0.1, adjusted to pH 7.3 with KOH. The pipette tips were coated with beeswax to reduce capacitance artefacts. In all experiments the membrane potential was clamped at −60 mV. Data were collected on an IBM compatible PC using WCP software (J. Dempster, University of Strathclyde, Glasgow, Scotland) with a National Instruments Lab PC Plus interface.

Application of agonists

In initial studies, bath superfusion of 10 and 30 μM ATP for 10–20 s evoked small inward currents of 203 ± 63 pA ($n = 6$) and 176 ± 19 pA ($n = 6$) respectively. The currents developed slowly, with a 10–90% rise time of approximately 4–5 s and decayed by more than 95% during the continued application of ATP. A second application of ATP after 10 min washout evoked a current which was usually less than 10% of the initial test response. The inward currents were associated with an increase in membrane conductance in response to brief (200 ms) voltage jumps to +60 mV.

Due to the relatively slow exchange time of the bath solution (about 30 s) cells are exposed to a slow concentration-ramp rather than a rapid concentration-step and if, as seen here, there is desensitization of the inward current during ATP application, then it is very likely that the peak amplitude of the response to ATP is underestimated. Therefore, in order to minimize these problems, throughout this study ATP and other agonists were applied rapidly by use of a solenoid valve-controlled, U-tube superfusion system placed close (100–150 μm) to the cell (Fenwick *et al.*, 1982). The delay to onset of solution change by the U-tube and the equilibration time (calculated as the time between 10–90% of the maximum change) were measured by monitoring the change in resistance of a patch electrode when deionised water was applied from the U-tube. The delay between activation of the solenoid valve and onset of increased resistance was 20–25 ms and the equilibration time was 15–20 ms. The increase in resistance was maintained throughout a 2 s application of deionised water, indicating that the U-tube was providing an effective concentration-clamp. Agonists were routinely applied to cells 5 min after whole cell mode was achieved, in order to standardize any time-dependent effects of cell dialysis on the currents evoked.

Contraction experiments

Rings (5–10 mm) of the proximal tail artery (internal diameter ≈300 μm) from rats (100–175 g) were mounted horizontally under isometric conditions in 10 ml organ baths by inserting two stainless steel wires into the lumen according to the method of Bevan & Osher (1972). The tissues were bathed in the 'normal calcium' bathing solution described above, at 37°C and bubbled with 100% O_2. Preparations were allowed to equilibrate for at least 60 min under a resting tension of 0.5–0.75 g. Contractions of the smooth muscle were recorded with a Grass FT103 transducer. In all preparations the endothelium was removed by gently pulling a 20 mm length of platinum wire (250 μm diameter) through the lumen of the vessel. Following precontraction with 1 μM noradrenaline, 10 μM acetylcholine did not produce vasodilatation, confirming that the mechanical rubbing had successfully removed the endothelium.

ATP, 2-meSATP, α,β-meATP and UTP were added as single concentrations to give the required final bath concentrations, with at least 35 min allowed between additions in order to avoid desensitization. Control experiments showed that no change in sensitivity to the agonists was observed over the time course of the experiments. As a maximum response was not reached for any of the agonists, contractions are expressed as a percentage of the maximum response evoked by noradrenaline in each tissue. Relative agonist potency was measured at a level equal to 50% of the maximum response to noradrenaline.

Statistics

Data are expressed throughout as the mean ± s.e.mean or the mean with 95% confidence limits where indicated. Results were compared by Student's paired and unpaired t tests where appropriate and $P < 0.05$ was considered to be statistically significant.

Drugs

Stock solutions of acetylcholine chloride, adenosine 5′-triphosphate (sodium salt), α,β-methyleneATP (lithium salt), uridine 5′-triphosphate (sodium salt) (all Sigma), 2-methylthio ATP (Research Biochemicals Inc.) and suramin (Bayer, UK) were dissolved in deionised water. (−)-Noradrenaline bitartrate (Sigma) was dissolved in acid saline. All drugs were then diluted in 'normal calcium' bathing solution.

Results

Responses of single rat tail artery smooth muscle cells to rapid application of ATP

Isolated smooth muscle cells of the rat tail artery had a resting membrane potential of -43.8 ± 1.4 mV ($n = 70$), which is in the range of those recorded in other smooth muscle preparations (Nakazawa & Matsuki, 1987; Friel, 1988; Inoue & Brading, 1990; Clapp & Gurney, 1991). When applied rapidly for 2 s, ATP (100 nM–1 μM) evoked inward currents in cells held at -60 mV (Figure 1). The delay between application of ATP and the onset of the rise of the inward current was less than 3 ms, which indicates that the current was due to the direct activation of an ion channel and not through a G protein-coupled mechanism. The peak amplitude and time course of ATP responses were concentration-dependent (Figure 1, Table 1). There was a large variation in the peak current amplitude for a given concentration of ATP, which was not related to cell size and is reflected in the relatively large s.e.mean values.

ATP (100 nM) produced a relatively sustained inward current which decayed by less than 30% during 2 s application. ATP (300 nM and 1 μM) evoked transient inward currents which were larger and which reached a peak and decayed much more rapidly as concentration increased. These currents decayed by more than 95% during the 2 s application. The rise time of the response to 3 μM ATP ($n = 2$) was faster than that for solution changeover and so may underestimate the peak current amplitude. Therefore, concentrations of ATP greater than 1 μM were not applied.

Desensitization by repeated application of ATP

Initial studies showed that there was a marked diminution in peak current amplitude on repeated application of ATP. A second application of ATP (300 nM and 1 μM) 10 min after the first, evoked a response that was significantly smaller ($P < 0.05$), but which had a similar time course (Figure 2, Table 2). In three cells in which ATP was applied a third time, 10 min after the second, a further decrease in peak current amplitude was seen (Figure 2). If ATP was applied for a shorter period (500 ms) a similar degree of desensitization was seen (not shown).

The dialysis of the cell interior which occurs during whole cell recordings can lead to rundown of ionic currents. Therefore, in order to determine if this was involved in the decrease in peak current amplitude seen here, ATP was first applied 15 min after the whole cell recording mode was achieved, rather than 5 min after, as was standard throughout this study. In cells in which ATP (300 nM) was first applied after 15 min, the evoked currents (470 ± 160 pA, $n = 4$) were not significantly different from those recorded in cells where ATP was first applied after 5 min (686 ± 116 pA, $n = 20$). Thus, the decreased response to repeated application of ATP was not simply due to time-dependent rundown, but appears to depend upon ATP-induced activation of the P_2-purinoceptor.

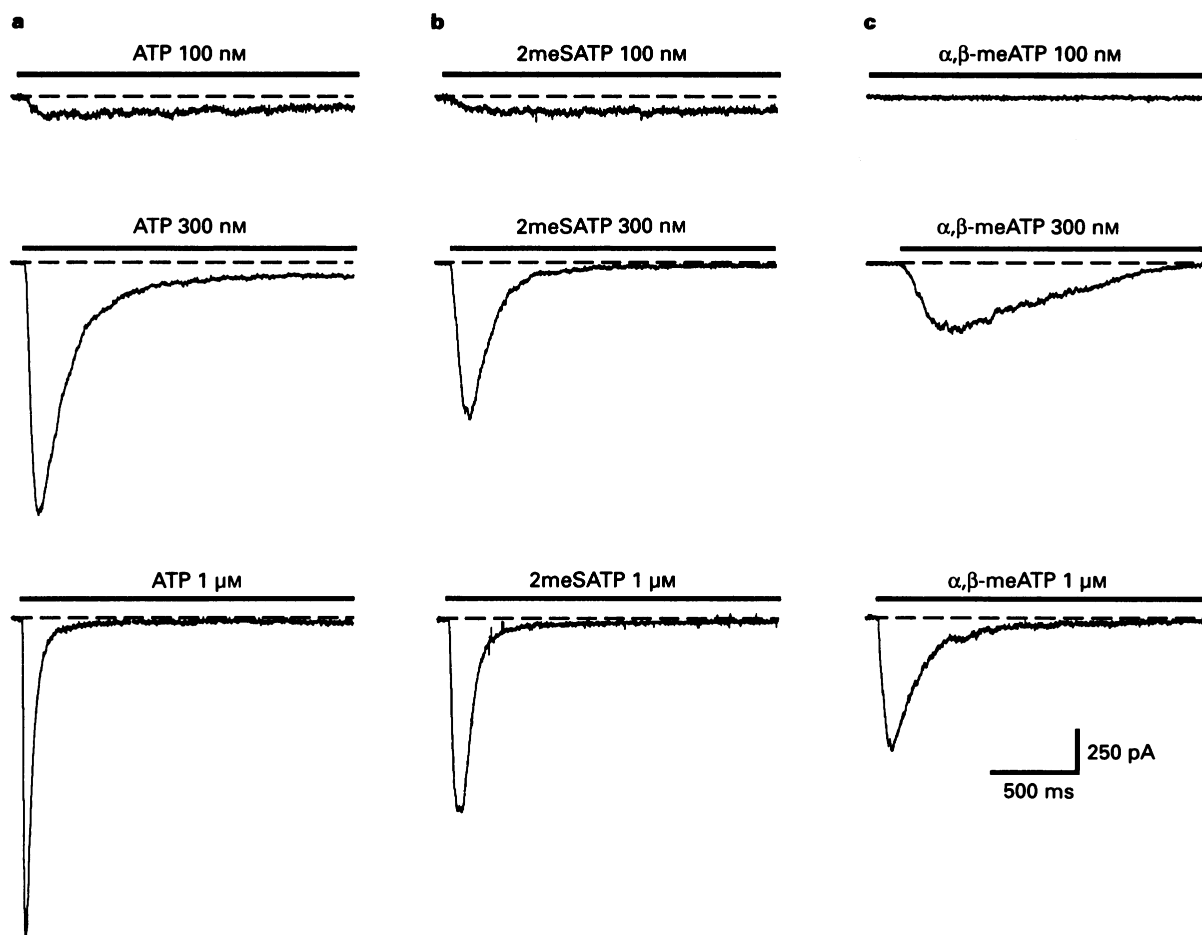

Figure 1 ATP, 2-meSATP and α,β-meATP evoke fast inward currents when rapidly applied to acutely dissociated smooth muscle cells from the rat tail artery: traces show the inward currents evoked by ATP (panel a), 2-meSATP (panel b) and α,β-meATP (panel c) (all 100 nM–1 μM) when applied rapidly for 2 s using a U-tube superfusion system, as indicated by the solid bars. The dashed lines represent zero current levels. The holding potential was -60 mV. Each record is from a different cell.

Responses of rat isolated tail artery smooth muscle cells to 2-meSATP and α,β-meATP

The lack of reproducible responses to ATP in any given cell meant that it was not possible to construct concentration-response curves or to compare the order of potency of ATP and its analogues in a single cell, so the responses were compared in a population of cells. As we were interested in agonist potency relative to ATP, alternate cells from a dissociation were exposed to ATP or to 2-meSATP, α,β-meATP or UTP.

2-meSATP and α,β-meATP evoked inward currents which, like those evoked by ATP, had a delay of less than 3 ms between drug application and onset of the response, indicating that they were due to the direct activation of a ligand-gated ion channel. The peak amplitude, rise time and 50% decay time of inward currents evoked by 2-meSATP (100 nM – 1 μM) were concentration-dependent and of similar amplitude and time course to those evoked by ATP (Figure 1, Table 1). A similar degree of desensitization was also seen on repeated application of 2-meSATP (300 nM and 1 μM) (Table 2).

α,β-meATP (100 nM) had no effect on the holding current of rat isolated tail artery smooth muscle cells (Figure 1, Table 1). In these cells, subsequent application of ATP (100 nM) evoked inward currents which were of similar magnitude to those recorded in control cells. Higher concentrations of α,β-meATP (300 nM and 1 μM) evoked inward currents, the peak amplitude, rise time and 50% decay time of which were concentration-dependent. At 300 nM the current was significantly smaller than that evoked by ATP ($P < 0.01$), while at both concentrations the rise time and rate of decay were significantly slower ($P < 0.05$) (Figure 1, Table 1). When compared at concentrations which evoked a current of similar mean amplitude (300 nM ATP and 2-meSATP, 1 μM α,β-meATP), the time-course of currents to α,β-meATP was similar to those to ATP and 2-meSATP (Table 1). Thus, α,β-meATP has a different interaction with the P_2-purinoceptor, but it not clear if this is due to a difference in affinity or efficacy or both. Desensitization was also seen on repeated application of α,β-meATP (300 nM and 1 μM) (Table 2).

Cross-desensitization

In all cells where ATP was applied 10 min after the same concentration of 2-meSATP ($n = 4$) or α,β-meATP ($n = 3$) had been applied, the peak current amplitude evoked by ATP was much smaller than the mean response to a single application of ATP in control cells. Similar cross-desensitization was seen when ATP was applied before 2-meSATP ($n = 3$). Although cross-desensitization was not studied in detail, this suggests that ATP, 2-meSATP and α,β-meATP act through a common site.

Responses of rat isolated tail artery smooth muscle cells to the pyrimidine UTP

UTP (300 nM, $n = 5$ or 1 μM, $n = 3$) applied for 2 s, had no effect on the holding current in rat tail artery smooth muscle cells (Figure 3). In many tissues it has been found that only a subpopulation of cells express P_2-purinoceptors (see Bean & Friel, 1990, and references therein). However, in all of the cells in which UTP was ineffective here, subsequent application of ATP (300 nM or 1 μM) evoked inward currents (Figure 3). This suggests that the inability of UTP to evoke currents was not due to the absence of P_2-purinoceptors.

Effects of suramin

In a number of vascular and neuronal preparations suramin has been shown to be an antagonist at P_2-purinoceptors (Kennedy, 1990; Dubyak & El-Moatassim, 1993). However, we could not determine the effects of ATP, 2-meSATP or α,β-meATP before and during application of suramin as we would have been unable to differentiate between an antagonistic effect of suramin and desensitization on the second application of agonist. In order to circumvent this problem we determined the effects of these agonists first in the

Table 1 Characteristics of inward currents evoked by ATP, 2-meSATP and α,β-meATP in acutely dissociated rat tail artery smooth muscle cells

	(n)	*Peak amplitude* (pA)	*10–90% rise time* (ms)	*50% decay time* (ms)
ATP				
100 nM	(9)	89 ± 68	84 ± 14	–
300 nM	(20)	686 ± 116	92 ± 7	199 ± 12
1 μM	(17)	904 ± 128	25 ± 3	79 ± 9
2-meSATP				
100 nM	(5)	45 ± 9	214 ± 74	–
300 nM	(9)	517 ± 129	65 ± 7	177 ± 22
1 μM	(9)	800 ± 368	35 ± 4	96 ± 10
α,β-meATP				
100 nM	(4)	0	–	–
300 nM	(9)	222 ± 40	206 ± 28	692 ± 121
1 μM	(9)	535 ± 160	71 ± 16	208 ± 25

Currents evoked by 100 nM ATP and 2-meSATP decayed by less than 30% during 2 s application.

Table 2 Desensitization evoked by repeated agonist application in acutely dissociated rat tail artery smooth muscle cells

	ATP		*2-meSATP*		*α,β-meATP*	
	300 nM	1 μM	300 nM	1 μM	300 nM	1 μM
% of initial peak amplitude	37 ± 5 (8)	31 ± 6 (7)	31 ± 6 (6)	31 ± 8 (4)	55 ± 7 (6)	36 ± 8 (4)

Values indicate the mean amplitude of the current evoked by a second application of the agonist expressed as a percentage of the response evoked by the initial application.
The number of cells is indicated in parentheses.

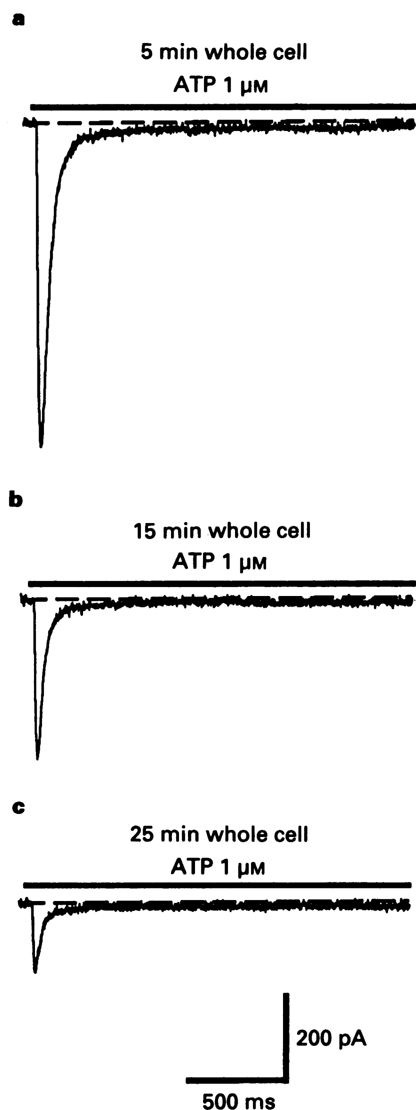

Figure 2 The peak inward current evoked by ATP in acutely dissociated rat tail artery smooth muscle cells decreases on repeated application. The records show the inward currents evoked by ATP (1 μM) in the same cell when applied rapidly for 2 s using a U-tube superfusion system, as indicated by the solid bars at (a) 5, (b) 15 and (c) 25 min after the whole cell mode was achieved. The dashed lines represent zero current levels. The holding potential was − 60 mV.

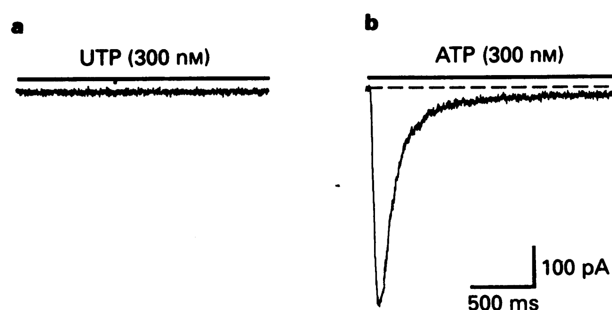

Figure 3 UTP has no effect on the holding current in acutely dissociated rat tail artery smooth muscle cells: (a) UTP (300 nM) has no effect on the holding current. (b) However, a transient inward current was evoked by ATP (300 nM) from the same cell 10 min later. ATP and UTP were applied for 2 s using a U-tube superfusion system as indicated by the solid bars. The dashed line represents the zero current level. The holding potential was − 60 mV.

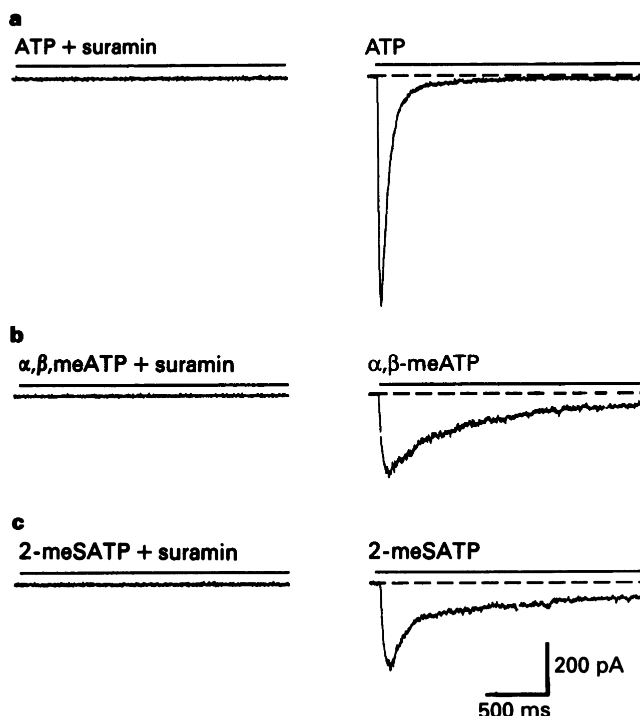

Figure 4 Suramin abolishes inward currents evoked by ATP, 2-meSATP and α,β-meATP in acutely dissociated rat tail artery smooth muscle cells: the left hand panel shows that after 5 min bath superfusion with suramin (100 μM), the concomitant application of (a) ATP (b) 2-meSATP or (c) α,β-meATP (all 1 μM) with suramin (100 μM) had no effect on the holding current. The right hand panel shows that after 10 min washout of suramin, inward currents were evoked by reapplication of these compounds in the same cells. ATP, 2-meSATP and α,β-meATP were applied for 2 s using a U-tube superfusion system as indicated by the solid bars. The dashed lines represent zero current levels. The holding potential was − 60 mV. Paired records in (a), (b) and (c) are from different cells.

presence of suramin and then 10 min later in the same cell in the absence of suramin.

Cells were exposed to suramin (100 μM) for 5 min, before concomitant application of one of the agonists plus suramin for 2 s. Under these conditions ATP, 2-meSATP and α,β-meATP (1 μM, $n = 4$ for each) did not evoke inward currents (Figure 4). Following washout of suramin for 10 min, ATP, 2-meSATP or α,β-meATP (1 μM, $n = 4$ for each) now evoked inward currents in all cells treated. The time course of the decay of the currents evoked by 2-meSATP and α,β-meATP appeared to be prolonged compared to those recorded in untreated cells which may reflect incomplete washout of suramin. This was not investigated further. Thus, it is concluded that ATP, 2-meSATP and α,β-meATP evoke inward currents through the activation of P$_2$-purinoceptors which are sensitive to suramin.

Contractions evoked by purines and UTP

α,β-meATP (100 nM–30 μM), 2-meSATP (3 μM–100 μM), ATP (3 μM–1 mM) and UTP (3 μM–1 mM) produced reproducible, concentration-dependent contractions of rat tail artery rings (Figure 5a,b,c). Contractions to α,β-meATP, 2-meSATP and ATP usually reached a peak within 1 min, but were not maintained, whereas those to UTP developed more slowly and were maintained during administration of the drug. Contractions reversed rapidly on washout of the drugs and could be inhibited by preincubation with suramin (100 μM) (not shown).

α,β-meATP was the most potent agonist. At a level equal to 50% of the maximum response to noradrenaline, the rank order of agonist potency was α,β-meATP >> 2-meSATP

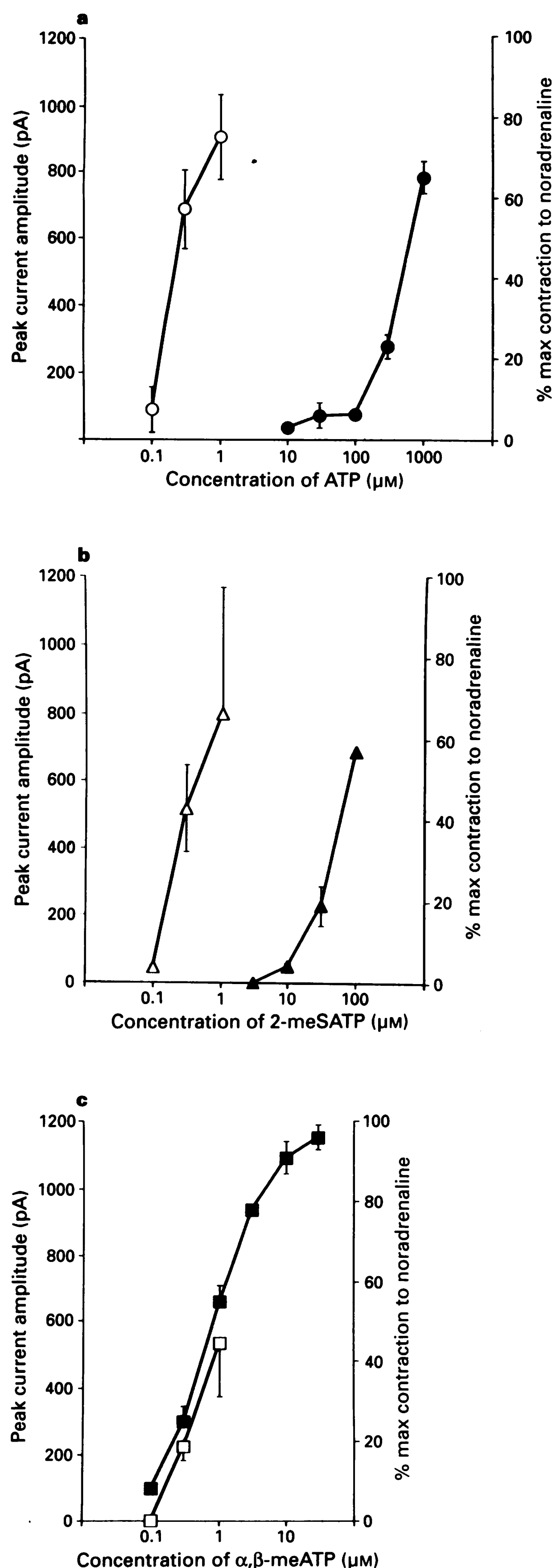

Figure 5 ATP, 2-meSATP and α,β-meATP evoke contractions of rat tail artery rings: comparison of the log concentration-response curves for contractions (solid symbols) ($n = 4$) and inward currents (pA) (open symbols) (n as indicated in Table 1) evoked by (a) ATP, (b) 2-meSATP and (c) α,β-meATP. Contractions are expressed as a percentage of the maximum response to noradrenaline. Mean values ± s.e.mean are shown.

> UTP > ATP (Table 3). This is very different from the rank order of agonist potency for eliciting inward currents in single cells. The concentrations of ATP required to evoke inward currents were three orders of magnitude lower than those required to evoke contraction (Figure 5a) and the difference for 2-meSATP was two orders of magnitude (Figure 5b). In contrast, similar concentrations of α,β-meATP were effective at inducing both responses (Figure 5c). This similar potency of α,β-meATP in the two types of preparation suggests that the dissociation procedure had no effect on the P_{2X}-purinoceptor *per se.*

Discussion

This study shows that the rank order of agonist potency for evoking contraction of the rat isolated tail artery is α,β-meATP >> 2-meSATP > ATP, which is characteristic of the P_{2X}-purinoceptor (Burnstock & Kennedy, 1985; Kennedy, 1990). However, when inward currents were evoked in acutely dissociated smooth muscle cells the potency order was ATP = 2-meSATP ⩾ α,β-meATP. This is clearly different and does not correspond pharmacologically to any of the P_2-purinoceptor subtypes identified in whole tissues. The inward currents and contractions were inhibited by the P_2-purinoceptor antagonist, suramin and agonist cross-desensitization was seen in single cells. This is consistent with ATP, 2-meSATP and α,β-meATP acting at a common site, but the possibility cannot be excluded that they acted at more than one suramin-sensitive site or that desensitization was heterologous.

UTP also evoked suramin-sensitive contractions of the rat tail artery, but did not activate an inward current. This suggests that UTP was acting via a P_{2U}-purinoceptor and agrees with a previous report that UTP acts at a different site from ATP to evoke contraction in this vessel (Saïag *et al.*, 1990). The P_{2U}-purinoceptor has been cloned and belongs to the G protein-coupled receptor superfamily (Lustig *et al.*, 1993).

In this study low concentrations of ATP evoked inward currents with a delay to onset of less than 3 ms, supporting the suggestion that the P_{2X}-purinoceptor is a ligand-gated ion channel. This is consistent with ATP acting as an excitatory cotransmitter in sympathetic nerves innervating smooth muscle, as stimulation of these nerves in the rat tail artery evokes excitatory junction potentials (Sneddon & Burnstock, 1984) and currents (Åstrand *et al.*, 1988) with a similar short latency. Responses to ATP showed marked desensitization in the present experiments, yet in the intact rat tail artery, excitatory junction potentials (Sneddon & Burnstock, 1984) and current (Åstrand *et al.*, 1988) can be evoked repetitively without any decrease in amplitude. Desensitization of P_{2X}-purinoceptors may be minimal *in vivo* because neurotransmission at individual sympathetic varicosities in the rat tail artery is brief and intermittent (Åstrand *et al.*, 1988).

One objection to the cotransmission hypothesis is that the concentrations of ATP calculated to be present in the neuroeffector junction during neurotransmission (1–2 μM) (see Sjöblom-Widfelt *et al.*, 1993), are much lower than those of exogenous ATP required to evoke contraction (10 μM–1 mM here). However, in the present study, the concentrations effective in single cells of the rat tail artery under concentration-clamp conditions (100 nM–1 μM), are close to the levels of ATP calculated to be released during sympathetic nerve stimulation.

The marked difference in agonist order of potency in rat tail artery rings and acutely dissociated cells is perhaps surprising, as opening of a ligand-gated cation channel is the initial step by which P_{2X}-purinoceptor agonists act to contract smooth muscle (Benham *et al.*, 1987; Nakazawa & Matsuki, 1987; Inoue & Brading, 1990; Bean, 1992; Kennedy, 1993b). The simplest explanation for this discrepancy is that ATP, 2-meSATP and α,β-meATP act at the same site (the

Table 3 Potency of purines and UTP in contracting rat tail artery rings

	EEC$_{50}$[1]	*95% CL*[2]	*Potency relative to ATP*
α,β-meATP	830 nM	(0.49–1.2 μM)	795 $P<0.01$
2-meSATP	81 μM	(70–91 μM)	8.1 $P<0.05$
UTP	350 μM	(150–550 μM)	1.9 $P<0.05$
ATP	660 μM	(310–1000 μM)	1.0

$n=4$ for each agonist.
[1]Equi-effective concentrations producing contraction equivalent to 50% of the maximum contraction to noradrenaline.
[2]95% confidence limits.

P_{2X}-purinoceptor) to evoke inward currents and contraction, but their relative potencies are determined by differences in their breakdown in intact muscles and dissociated cells. ATP and 2-meSATP are both susceptible to breakdown by ectonucleotidases, whereas α,β-meATP is relatively resistant (Welford *et al.*, 1986; 1987). In organ bath studies, drug equilibration in the bathing solution is relatively slow, so ectonucleotidases present in blood vessels in high levels (see Gordon, 1986), have more time to break down P_2-purinoceptor agonists which are also enzyme substrates. Furthermore, the enzymes which break down ATP to adenosine appear to be clustered together, allowing preferential delivery of substrates to the enzymes such that ATP is broken down extremely rapidly (Gordon *et al.*, 1989). In contrast, in single cells equilibration time was very short (less than 20 ms) and under concentration-clamp conditions the influence of ectonucleotidases on agonist action is likely to be substantially reduced or abolished. Thus, ATP and 2-meSATP were much more potent in single rat tail artery smooth muscle cells than in ring preparations, whereas α,β-meATP was of a similar potency. The similar potency of α,β-meATP also suggests that the dissociation procedure had no effect on the P_{2X}-purinoceptor *per se*.

To confirm this interpretation of the results it will be necessary to study contractions in ring preparations in the absence of agonist breakdown using the new ectonucleotidase inhibitor FPL 67156 (Leff *et al.*, 1994). However, similar results have been found in the guinea-pig urinary bladder, where α,β-meATP evokes inward currents and contraction over the same concentration-range and is more potent than ATP in contracting muscle strips, but less potent than ATP in evoking inward currents (Inoue & Brading, 1990). A similar shift in the potency of ATP has also been seen in rat mesenteric artery rings when a metabolically-stable compound, caged ATP, was used (Sjöblom-Widfelt *et al.*, 1993). When released rapidly from caged ATP by photolysis, ATP evoked concentration-dependent contractions and was 100 times more potent than when ATP itself was added to the bathing solution.

This explanation of the results means that a rank order of potency of α,β-meATP >> 2-meSATP > ATP can no longer be considered to be the pharmacological profile of the P_{2X}-purinoceptor. This study is not extensive enough to define the P_{2X}-purinoceptor in the absence of breakdown, but it is clear that ATP and 2-meSATP are more potent than α,β-meATP, although the difference in potency here was less than one order of magnitude. Nonetheless, α,β-meATP is still a useful tool for receptor characterization as it is the agonist most effective at producing desensitization of the P_{2X}-purinoceptor in whole tissues. In the present experiments in single cells, α,β-meATP, 2-meSATP and ATP induced a similar degree of desensitization, suggesting that the greater ability of α,β-meATP to produce desensitization in whole tissues is due to its resistance to breakdown.

ATP and 2-meSATP are also more potent than α,β-meATP at the P_{2Y}-purinoceptor, but the differences in potency are much larger, as 2-meSATP is approximately two orders of magnitude more potent than ATP, which in turn is an order of magnitude more potent than α,β-meATP (see Burnstock & Kennedy, 1985; Kennedy, 1990, for reviews). In a number of neuronal preparations, P_2-purinoceptor agonists also evoke a rapidly developing inward current via a ligand-gated cation channel (see Illes & Nörenberg, 1993). Since ATP and 2-meSATP were more potent than α,β-meATP, it was suggested that a P_{2Y}-purinoceptor was involved (Illes & Nörenberg, 1993). In view of the results presented here, it may be premature to characterize a response in single cells as being mediated via a P_{2Y}-purinoceptor simply because 2-meSATP and ATP are more potent than α,β-meATP.

In conclusion, this study shows that the rank order of agonist potency at the P_{2X}-purinoceptor which mediates contraction of the rat isolated tail artery is very different from the potency order for evoking the inward current which initiates the contractions. The difference may be due to greater breakdown of some of the agonists in artery rings compared with the single cell system. This has important consequences for the pharmacological characterization of P_2-purinoceptor subtypes.

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