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European Journal of Pharmacology 351 (1998) 139–144

ejp

Comparison of the actions of ATP and UTP at P_{2X1} receptors in smooth muscle of the rat tail artery

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Received 1 April 1998; accepted 7 April 1998

Abstract

The actions of ATP and uridine 5′-triphosphate (UTP) were compared at P_{2X1} receptors in acutely dissociated smooth muscles cells of the rat tail artery. ATP (30 nM–100 μM) and UTP (1 μM–1 mM) elicited concentration-dependent inward currents. ATP was approximately 100-fold more potent than UTP. In both cases, currents were activated within 3 ms of agonist application and had similar time-courses of activation and inactivation. The decay of responses for both agonists was concentration-dependent and in most cells could be fitted by two exponentials. The P_{2X} receptor antagonists suramin (100 μM) and pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS, 5 μM) inhibited responses to both ATP and UTP. An action of UTP at P_{2X1} receptors has not previously been reported. However, since the responses to ATP and UTP had similar time-courses and as PPADS and suramin inhibited both agonists, it is concluded that ATP and UTP are acting at the same site in these cells, the P_{2X1} receptor.

Keywords: ATP; UTP; P_{2X1} receptor; Suramin; PPADS (pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid); Tail artery, rat

1. Introduction

P_{2X} receptors are ligand-gated ion channels which mediate the excitatory actions of extracellular ATP in many tissues. Seven subtypes of P_{2X} receptor have been characterised ($P2X_1$–$P2X_7$, Buell et al., 1996). The $P2X_1$-subtype, cloned from the rat vas deferens and human urinary bladder (Valera et al., 1994, 1995), is the predominant P_{2X} receptor found in smooth muscle and it mediates the neurotransmitter actions of ATP in vascular and visceral smooth muscle (see Burnstock, 1996; Sneddon et al., 1996). P_{2X1} receptor agonists such as α,β-methyleneATP and 2-methylthioATP mimic neurogenic responses, while the P_2-receptor antagonists suramin and pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS) inhibit contractions to exogenous P_{2X1}-agonists and nerve stimulation (Kennedy, 1990; Lambrecht et al., 1992; Bao and Stjärne, 1993; Zignashin et al., 1993, 1994; Khakh et al., 1994) and also inhibit the depolarisations which underlie these contractions (Sneddon, 1992; McLaren et al., 1994; McLaren et al., 1995b).

We have previously described the basic biophysical and pharmacological properties of P_{2X1} receptors in acutely dissociated smooth muscle cells of the rat tail artery (Evans and Kennedy, 1994). ATP and its analogues elicited rapidly activating and inactivating inward currents in these cells, with a rank order of potency of 2-methylthioATP ≅ ATP > α,β-methylene ATP. Suramin inhibited the responses to each agonist. These pharmacological and biophysical properties are consistent with activation of a P_{2X1} receptor. In the same study, uridine 5′-triphosphate (UTP) was inactive at concentrations up to and including 1 μM. However, higher concentrations of UTP causes contractions of rings of rat tail artery smooth muscle (Saïag et al., 1990). This effect of UTP may be mediated through a P_{2Y}-receptor (a G-protein coupled receptor), but this is not clear.

The aim of this study was to examine more fully the interaction of ATP and UTP with P_{2X1} receptors in smooth muscle cells of the rat tail artery. First, higher concentrations of ATP and UTP than previously used, were applied. The ability of suramin and PPADS to inhibit responses to ATP and UTP was then compared. A preliminary account has been published (McLaren et al., 1995a).

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0014-2999/98/$19.00

2. Materials and methods

2.1. Isolation of single vascular smooth muscle cells

Rat tail artery smooth muscle cells were isolated as described previously (Evans and Kennedy, 1994). Male Sprague–Dawley rats (100–175 g) were killed by exposure to CO_2 followed by exsanguination. The artery was dissected out, cleaned of connective tissue, cut longitudinally to reveal the lumen and the endothelium rubbed off. The artery was then cut into small pieces and suspended 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 6.9 with NaOH, containing papain (8.5 units ml^{-1}) and bovine serum albumin (5 mg ml^{-1}).

The pieces of artery were stored overnight (16–18 h) at 4°C, to allow the papain to equilibrate through the tissue. The following morning the solution was heated to 37°C and at regular intervals the suspension was gently triturated using a glass pipette until characteristically spindle shaped smooth muscle cells appeared. The suspension was then micro-centrifuged at 150 g for 5 min and the supernatant removed. The pellet containing the cells was resuspended in the extracellular solution described below and stored at 4°C. For recording, the cell suspension was plated out in 100 μl aliquots on glass coverslips and left for at least 30 min to allow the cells to adhere to the coverslip. Dissociated cells were used within 8 h.

2.2. Electrophysiological recording

Patch clamp recording was carried out in the whole-cell configuration, using an Axopatch 1D amplifier (Axon Instruments, USA). The resistance of the patch pipettes was 2–5 MΩ when filled with a 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 or Sigmacote (Sigma) to reduce capacitance artefacts. The liquid junction potential was nulled using the d.c. offset on the amplifier. Series resistance was not compensated for. In all experiments the membrane potential was voltage clamped at -60 mV. Data were collected, stored and analysed on an IBM compatible PC using WCP software (Dr. J. Dempster, University of Strathclyde, Glasgow, Scotland) with a National Instruments Lab PC Plus interface at a sampling frequency of 1 kHz.

In the recording chamber, cells were perfused at 3–5 ml min^{-1} with an extracellular 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. Once a stable whole-cell recording was achieved, cells were left for 5 min before an agonist was applied, to standardise any effect of cell dialysis. ATP and UTP were applied to single smooth muscle cells in concentration steps lasting 1 s using a solenoid activated U-tube fast application system (see Evans and Kennedy, 1994). Using this system, agonists reached a steady-state 'concentration clamp' within 10 ms. Due to the tachyphylaxis of responses it was not possible to construct agonist concentration–response curves in individual cells. Therefore, these were constructed by calculating the mean response to each concentration of ATP and UTP in a number of individual cells. Only one cell was used per coverslip.

2.3. Antagonist studies

Because of the profound tachyphylaxis produced by a single application of agonist (see Evans and Kennedy, 1994), it was not possible to obtain control agonist responses before addition of an antagonist. Therefore, once the whole cell recording mode was achieved, the cell was perfused for 5 min with extracellular solution containing suramin or PPADS. ATP or UTP, plus the appropriate antagonist, were then applied via the U-tube. The cell was then perfused with antagonist-free solution for 10 min, before ATP or UTP were reapplied in the absence of antagonist.

2.4. Drugs

Stock solutions of ATP (sodium salt), UTP (sodium salt) (both Sigma), PPADS (Cooksons) and suramin (Bayer) were dissolved in deionised water and diluted in extracellular solution on the day of use. High performance lipid chromatography (HPLC) analysis of the stock UTP solution showed that no other nucleotides were present (see Robertson et al., 1996).

2.5. Statistics

Data are expressed as mean $\pm$ S.E.M., where indicated. Concentration–response curves were fitted to the data by logistic (Hill equation), nonlinear regression analysis (Graphpad Prism, Graphpad Software).

3. Results

3.1. Currents evoked by ATP and UTP

In smooth muscle cells clamped at -60 mV, ATP (30 nM–100 μM) evoked inward currents in a concentration-dependent manner (Fig. 1a). The time to current onset was only several milliseconds, consistent with the activation of a ligand-gated cation channel. The threshold concentration of ATP for current activation was around 30 nM and the EC_{50} for these responses was 1.8 μM (95% confidence limits = 1.2 μM–2.6 μM, $n = 3$–9), with a Hill slope of 1.02 (Fig. 1b). UTP (1 μM–1 mM) also evoked rapidly developing inward currents in a concentration-dependent

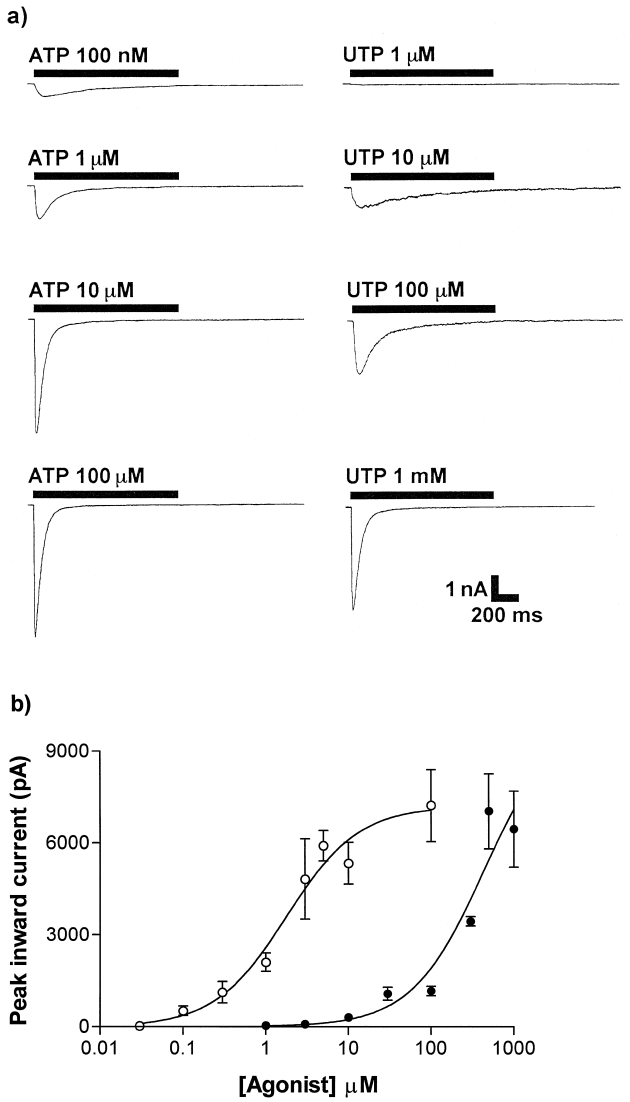

Fig. 1. Inward currents evoked by ATP and UTP in single vascular smooth muscle cells of the rat tail artery. (a) Original responses to increasing concentrations of ATP and UTP, when rapidly applied for 1 s as indicated by black bars. Each trace was obtained in a separate cell. (b) The concentration-dependence of the peak amplitude of responses evoked by ATP (○) and UTP (●). Mean values ± S.E.M. are shown, $n = 3–9$ for each point.

manner (Fig. 1a). Threshold for current activation was around 1 μM, but no clear maximum to the concentration–response curve was observed and so EC_{50} and Hill slope values could not be calculated (Fig. 1b). Nonetheless, it is clear that ATP was approximately two orders of magnitude more potent than UTP.

3.2. Current kinetics

As the concentration of ATP increased, so the evoked currents reached a peak response more rapidly (Fig. 2a). For example, the time to peak was reduced from 72 ± 7 ms ($n = 5$) for 100 nM ATP to 8 ± 1 ms for 100 μM ($n = 6$). The time to peak response for currents evoked by UTP showed a similar dependence on concentration (Fig. 2a).

The inward currents evoked by ATP and UTP were not maintained and demonstrated rapid decay in the continued presence of agonist (Fig. 1a). At low concentrations, the time constant of decay of responses to ATP and UTP were fitted by one or two exponentials. For example, at 1 μM ATP and 30 μM UTP, the decay of responses was best fitted by a single exponential in 3/6 and 2/9 cells, respectively. In the remaining cells, two exponentials were required. At higher concentrations of ATP and UTP, the decay phase of currents could be best fitted by two exponentials in all cells.

The time constant of the fast component of current decay decreased with increasing agonist concentration (Fig. 2b). For example, it was reduced from 437 ± 108 ms ($n = 5$) at 100 nM ATP to 55 ± 4 ms at 100 μM ATP ($n = 6$). The fast time constant of decay for currents evoked by UTP showed a similar dependence on concentration (Fig. 2b). In general, the time constant of the slower component of decay for both ATP and UTP was much

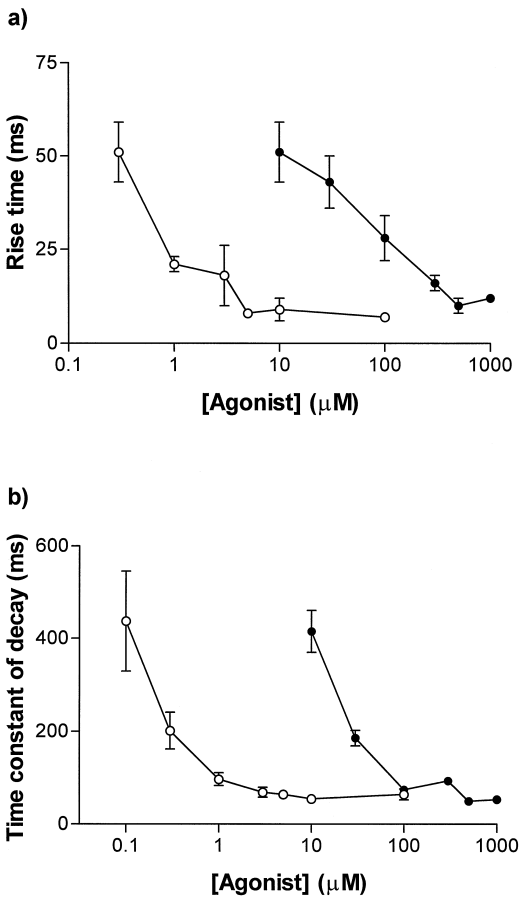

Fig. 2. The concentration-dependent activation and inactivation of responses to ATP (○) and UTP (●). (a) Shows the rise time of responses (time for currents to increase from 10–90% of the maximum amplitude), and (b) the time constant of the fast phase of current decay, plotted against agonist concentration. Mean values ± S.E.M. mean are shown, $n = 3–9$ for each point.

more variable and often tended to be greater than the period of drug application and so could not be accurately measured. Thus, the currents activated by ATP and UTP display similar kinetics.

3.3. Antagonist studies

Due to the tachyphylaxis observed on repeated administration of agonists, ATP and UTP were first applied to cells which had been incubated with an antagonist (see Section 2). In the presence of suramin (100 μM), ATP (300 nM) was ineffective, but 10 min after washout of suramin, ATP evoked a large inward current (Fig. 3a; -1421 pA, $n = 1$). This confirms the finding of Evans and Kennedy (1994). Similarly, in the presence of suramin (100 μM), UTP (30 μM) did not evoke a response, but after 10 min perfusion with suramin-free bathing solution, UTP evoked substantial inward currents (Fig. 3b; mean = -1348 ± 614 pA, $n = 4$).

In the presence of PPADS (5 μM), ATP (300 nM) evoked only small inward currents in 3 of 4 cells (Fig. 3c; mean = -96 ± 16 pA) and had no effect in the fourth, but 10 min after washout of PPADS, ATP elicited large inward currents (mean = -443 ± 267 pA). Likewise, in the presence of PPADS (5 μM), UTP (30 μM) elicited small inward currents in 4 of 4 cells (Fig. 3d; mean = -73 ± 17 pA), but 10 min after washout of PPADS, UTP evoked large inward currents (mean = -1389 ± 493 pA). Therefore, these results show the channels activated by ATP and UTP in these cells are sensitive to the P_2-receptor antagonists PPADS and suramin.

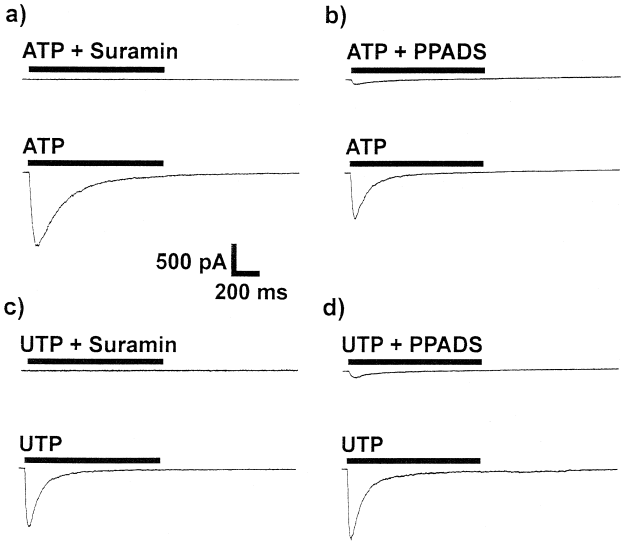

Fig. 3. The inhibition of currents elicited by ATP (300 nM) (panels a and b) and UTP (30 μM) (panels c and d) by suramin (100 μM) and PPADS (5 μM). Antagonists were applied to the bath for 5 min before application of agonist (upper trace of each pair) for 1 s, as indicated by black bars. After perfusing the cell with antagonist-free solution for 10 min, the agonist was reapplied (lower traces). Each pair of records was obtained from a separate cell.

4. Discussion

4.1. Activity of ATP and UTP

In the present study, ATP and UTP elicited concentration-dependent inward currents in acutely dissociated smooth muscle cells of the rat tail artery. These currents activated within 3 ms of agonist application, consistent with an action at a ligand-gated cation channel. P_{2X1} receptors are the only ligand-gated ion channels known to be expressed in smooth muscle cells and are most likely to be mediating the actions of ATP and UTP. Other P_{2X} receptor subtypes are not involved, as a previous study in the same preparation (Evans and Kennedy, 1994) showed that α,β-methylene ATP, which is only active at the P_{2X1}- and P_{2X3}-subtypes (Buell et al., 1996), was a potent agonist. The P_{2X3}-receptor is very unlikely to be involved as it is only expressed at high levels in sensory neurones (Chen et al., 1995).

The threshold for current activation was around 30 nM for ATP and 1 μM for UTP. The EC_{50} for ATP was 1.8 μM, but the concentration–response curve for UTP did not reach a maximum. Nonetheless, UTP was clearly at least 2 orders of magnitude less potent than ATP. ATP elicits similar inward currents with comparable potency in the smooth muscle cells of guinea-pig urinary bladder (Inoue and Brading, 1990) and rat vas deferens (Friel, 1988; Khakh et al., 1995) and at the cloned P_{2X1} receptor (Valera et al., 1994, 1995). However, this is the first report of a rapidly-activated inward current in response to UTP. UTP (200 μM) was ineffective at the native P_{2X1} receptor in smooth muscle cells of the rat vas deferens (Friel, 1988), while at 1–200 μM it was either inactive or virtually inactive at the P_{2X1} receptor cloned from the rat vas deferens (Valera et al., 1994) and human urinary bladder (Evans et al., 1995). UTP (10 μM) elicits a rapidly desensitising depolarisation in rabbit aorta smooth muscle, but this may occur via a G-protein coupled P_{2Y}-receptor (Pavenstädt et al., 1991). Thus, while the potency of ATP at eliciting inward currents in smooth muscle cells isolated from the rat tail artery is similar to that of ATP in other visceral smooth muscle preparations, the ability of UTP to elicit these responses in the present study is novel.

4.2. Antagonist studies

In the present study, suramin abolished responses to ATP and UTP, similar to the antagonist action of suramin at native P_{2X1} receptors in the rat vas deferens (Khakh et al., 1995) and at cloned P_{2X1} receptors (Valera et al., 1994). Likewise, PPADS inhibited responses to ATP and UTP, similar to the antagonist action of PPADS at the P_{2X1} receptor cloned from the rat vas deferens (Valera et al., 1994) and human urinary bladder (Evans et al., 1995). Both antagonists also inhibit the neurotransmitter actions

of ATP via P_{2X1} receptors in visceral and vascular smooth muscle (for review, see Sneddon et al., 1996).

Neither antagonist is selective for P_{2X1} receptors, but PPADS does display more selectivity than suramin. PPADS was originally characterised as a P_{2X1}-antagonist (Lambrecht et al., 1992; McLaren et al., 1994), but has since been found to also potently antagonise P_{2X2}-, P_{2X3}-, and P_{2X5}-receptors (Buell et al., 1996), as well as native (Boyer et al., 1994; Windscheif et al., 1994; Brown et al., 1995; Ho et al., 1995) and cloned P_{2Y1}-receptors (Charlton et al., 1996a; Brown et al., 1997). However, PPADS is ineffective at cloned P_{2Y2}- and P_{2Y4}-receptors, at which UTP is a potent agonist, (Brown et al., 1995; Charlton et al., 1996a,b; Vigne et al., 1996).

4.3. Current kinetics

In this study, the currents activated by ATP and UTP were transient, resembling the purinergic excitatory junction potentials and currents seen in the intact rat tail artery (Bao and Stjärne, 1993; McLaren et al., 1995b). The rate of current activation and decay increased with increasing agonist concentration. A similar concentration-dependence of the time course of current activation by ATP has been reported in smooth muscle cells of the rat vas deferens (Khakh et al., 1995) and guinea-pig urinary bladder (Inoue and Brading, 1990).

In the present study, the decay phase of responses to both ATP and UTP could be fitted by two exponentials at most agonist concentrations. Also, the rate of the fast phase of current decay increased with increasing agonist concentration. This is in contrast to ATP-activated inward currents in smooth muscle cells of the rat vas deferens (Khakh et al., 1995), where not only was the decay of current responses best fitted by a single exponential, but was also concentration-independent. Indeed, studies on the P_{2X1} receptor cloned from the rat vas deferens (Valera et al., 1994) and human urinary bladder (Evans et al., 1995) also demonstrated a concentration-independent, mono-exponential decay to applied agonists (see Buell et al., 1996). The reasons for these differences between P_{2X1} receptors in vascular and visceral smooth muscle is not known.

5. Conclusions

In conclusion, while UTP is not generally considered to be an agonist at P_{2X1} receptors, the similarity of the time-course of responses to UTP and ATP and the inhibition of both agonists by suramin and PPADS, suggests that ATP and UTP were acting at the same site, the P_{2X1} receptor. Thus, the P_{2X1} receptor in smooth muscle cells of the rat tail artery appears to have different properties from the cloned P_{2X1} receptor.

Acknowledgements

This work was supported by grants from the Medical Research Council and the Wellcome Trust.

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