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P_2-purinoceptors mediating spasm of the isolated uterus of the non-pregnant guinea-pig

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1 The isolated uterus of the non-pregnant guinea-pig has been suggested to contain P_1-, and possibly P_2-purinoceptors mediating spasm. The presence of P_1-purinoceptors has been confirmed and these receptors have been further characterized.

2 In the presence of the adenosine uptake inhibitor, S-(4-nitrobenzyl)-6-thioinosine (NBTI, 300 nM) and a pA_{100} concentration of the P_1-purinoceptor antagonist 8-sulphophenyltheophylline (140 μM), the potency order of agonists as spasmogens was: 2 methylthio ATP>>α,β methylene ATP=UTP=ATP>> β,γ methylene ATP. This order is not consistent with any single recognised P_2-purinoceptor subtype.

3 Indomethacin (1 μM) treatment abolished responses to 2 methylthio ATP, α,β methylene ATP and UTP, while spasm to ATP was significantly inhibited. When the endometrial and circular smooth muscle cell layers were removed, spasmogenic responses to ATP, 2 methylthio ATP, α,β methylene ATP and UTP were significantly reduced.

4 2 methylthio ATP was able to cause desensitization to itself, but not to UTP, indicating that these agonists act at different receptor sites.

5 The P_2-purinoceptor antagonist, suramin antagonized 2 methylthio ATP with a pA_2 of 5.9 ± 0.3. Suramin was also an antagonist of ATP and UTP. In the case of ATP, the antagonism was not dependent on suramin concentration, while for UTP the interaction appeared to be non-equilibrium. Pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS, 10 μM) had no effect on spasm to ATP, UTP or 2 methythio ATP.

6 In the presence of indomethacin, responses to ATP were unaffected by 8-sulphophenyltheophylline (140 μM) or by suramin (100 μM), but PPADS (10 μM) antagonized ATP.

7 These results suggest that the isolated uterus of the non-pregnant guinea-pig contains a mixture of P_2-purinoceptors. P_{2U}- (or UTP-selective pyrimidinoceptors) and P_{2Y}-purinoceptors appear to be present, probably mainly located on the endometrial or circular smooth muscle layer. Activation of these receptors leads to spasm via increases in prostanoid generation. There appears also to be a third class of non-P_{2X}-, non P_{2Y}-purinoceptor present, at which ATP is an agonist and PPADS is an antagonist, located on the longitudinal smooth muscle, activation of which causes spasm independent of changes in prostanoids.

Keywords: Guinea-pig isolated uterus; P_{2Y}-purinoceptors; P_{2U}-purinoceptors; adenosine 5'-triphosphate (ATP); suramin; pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS)

Introduction

Receptors for adenosine 5'-triphosphate (ATP, P_2-purinoceptors; Burnstock, 1978) have been described in a wide variety of tissues. P_2-purinoceptors have been divided into P_{2X}-, P_{2Y}-, P_{2U}, P_{2T}-, and P_{2Z}- subtypes based mainly on different agonist potency orders (Fredholm *et al.*, 1994). Recent molecular biology studies have shown that the P_{2X}-purinoceptor is a ligand-gated ion channel (Brake *et al.*, 1994; Valera *et al.*, 1994) while both the P_{2Y}- and P_{2U}-classes belong to the G-protein coupled receptor superfamily (Webb *et al.*, 1993; Parr *et al.*, 1994).

The presence of purinoceptors in the isolated uterus of the guinea-pig was first demonstrated by Moritoki *et al.* (1979). They found that adenosine, ATP and ATP analogues produced spasm of the intact uterus, which was inhibited by indomethacin but did not identify the type of purinoceptor. A later study characterized the P_1-purinoceptor as being of the A_1 subtype (Smith *et al.*, 1988) and provided some evidence for the existence of separate P_2-purinoceptors. There are conflicting reports as to whether prostanoid synthesis may be involved in spasm mediated via the A_1 receptor. Schiemann *et al.* (1991) demonstrated that agonist interaction with A_1 receptors led to the formation of a cyclo-oxygenase product which was responsible for spasm, while a later report suggested that cyclo-oxygenase was not involved, at least in myometrial cells (Bradley *et al.*, 1992). Whole cell voltage clamp experiments using isolated smooth muscle cells from myometrium of the pregnant rat in primary culture demonstrated that ATP and α,β methylene ATP rapidly activated an inward cation current (Honoré *et al.*, 1989) which would be characteristic of a P_{2X}-purinoceptor. However, the current produced by ATP in myometrial cells did not undergo desensitization, as observed with the P_{2X}-purinoceptor cloned from PC12 cells (Brake *et al.*, 1994) but in contrast to desensitization found with inward current produced by activation of cloned P_{2X}-purinoceptors from rat vas deferens (Valera *et al.*, 1994) or P_{2X}-purinoceptors in cells from rat vas deferens (Khakh *et al.*, 1995).

As the studies of Honoré *et al.* (1989) appeared to describe a class of myometrial P_2-purinoceptors with novel electrophysiological properties, the aims of the present study were to

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determine if P_2-purinoceptors are present in guinea-pig isolated uterus, and if so, to characterize them by determining the agonist potency order and sensitivity to antagonists. Suramin was used as an antagonist at P_2-purinoceptors, displaying inhibitory activity equally at both P_{2X}- and P_{2Y}-purinoceptors (Dunn & Blakely, 1988; Den Hertog *et al.*, 1989a,b; Hoyle *et al.*, 1990; Leff *et al.*, 1990; Von Kügelgen *et al.*, 1990), while pyridoxalphosphate-6-azophenyl-2′, 4′-disulphonic acid (PPADS) has been reported to be an antagonist selective for P_{2X}-purinoceptors (Lambrecht *et al.*, 1992; Ziganshin *et al.*, 1993; 1994; McLaren *et al.*, 1994; Piper & Hollingsworth, 1995). As it is known that this tissue contains A_1 receptors, all experiments were carried out in the presence of a pA_{100} concentration (Piper & Hollingsworth, 1995) of the selective P_1-purinoceptor antagonist, 8-sulphophenyltheophylline (Gustafsson, 1984; Collis *et al.*, 1987; Hourani *et al.*, 1991). The isolated uterus of the guinea-pig is believed to possess a selective adenosine uptake mechanism (Smith *et al.*, 1988). As it has been shown previously that ATP can be potentiated in this tissue by adenosine uptake inhibition (Smith *et al.*, 1988), a maximal concentration (Piper & Hollingsworth, 1995) of the selective adenosine uptake inhibitor S-(4-nitrobenzyl)-6-thioinosine (NBTI) (Clanachan *et al.*, 1987) was also present. Experiments were carried out using the cyclo-oxygenase inhibitor indomethacin (Vane, 1971) to determine if prostanoid synthesis was responsible for the spasm to ATP and analogues as has been observed in other smooth muscle e.g. rat fundus (Matharu & Hollingsworth, 1992). To ascertain the location of the purinoceptors involved in spasm, studies were carried out on tissues in which the endometrial layer had been removed to leave strips of longitudinal myometrium. Preliminary results have been presented to the British Pharmacological Society (Kelley & Hollingsworth, 1994).

Methods

Tissue preparation

Female tricolour non-pregnant guinea-pigs (350–800 g), unselected for the stage of the oestrous cycle, were stunned and bled. Each uterine horn was isolated and placed in a physiological salt solution (PSS) of the following composition (mM): NaCl 118, KCl 4.75, $CaCl_2.6H_2O$ 2.55, $MgSO_4.7H_2O$ 1.2, KH_2PO_4 1.19, $NaHCO_3$ 25 and glucose 11. The ovarian half of each uterine horn was divided in two and each piece of tissue was mounted for isometric tension recording in 10 or 20 ml tissue baths where they were gassed with 95% O_2 and 5% CO_2 and maintained at 37°C. The preparations were initially placed under a resting tension of 1 g, left to equilibrate for 1 h and washed at 15 min intervals. NBTI (300 nM) was present in the PSS in all experiments, while 8-sulphophenyltheophylline (140 μM) was present in all except the set of experiments where the indomethacin-resistant component of the spasm to ATP was investigated.

Preparation of longitudinal myometrial smooth muscle strips

A 1–2 cm segment of uterus was cut longitudinally, pinned to a cork board, and the endometrium together with the underlying circular smooth muscle layer stripped away. Control tissues were taken from an adjacent segment of the same uterine horn.

Non-cumulative agonist concentration-effect curves

After determination of the response to 3 repeated additions of KCl (50 mM), a non-cumulative agonist concentration-effect curve was constructed for each tissue. Increasing agonist concentrations were added at 10 min intervals and left in contact with the uterus for 3 min before washing. Tissues were then incubated for 30–90 min with antagonists or other modifying agents before the concentration-effect curve was repeated. Experiments were designed such that each test tissue was matched with a vehicle-treated tissue from the same uterine horn.

Determination of agonist potency order

An initial concentration-effect curve to ATP was constructed in each tissue. After washing and a further 30 min, a second agonist concentration response curve was constructed to either α,β methylene ATP, β,γ methylene ATP, 2-methylthio ATP or uridine 5′-triphosphate (UTP). The pD_2 of ATP in repeated concentration-effect curves did not differ from that in initial curves (data not shown).

Studies of antagonism by suramin

After an initial concentration-effect curve was obtained to either 2 methylthio ATP, UTP or ATP, four preparations from the same animal were exposed to suramin (3 μM, 10 μM or 30 μM in the case of 2 methylthio ATP and UTP; 10 μM, 100 μM and 1 mM in the case of ATP) or vehicle equivalent to the highest concentration of suramin used. As suramin (100 μM) has been reported to require 90 min to reach equilibrium (Leff *et al.*, 1990), tissues were incubated with suramin for 90 min before construction of a second agonist concentration-effect curve.

Studies of antagonism by PPADS

After the construction of a control curve to either 2 methylthio ATP, ATP or UTP, tissues were exposed to either vehicle or the P_{2X}-purinoceptor antagonist PPADS (10 μM) for 30 min and the agonist curve repeated.

Determination of the potency of UTP in the presence of 2 methylthio ATP

A control concentration-effect curve was constructed to either 2 methylthio ATP or UTP. After 30 min, a second curve was constructed to these agonists in the presence of 2 methylthio ATP. A maximal concentration of 2 methylthio ATP (1 μM), or the equivalent vehicle in time-matched control tissues, was added to the tissue bath for 5 min and washed out immediately before addition of each concentration of agonist.

Characterization of the indomethacin-resistant component of the spasm to ATP

Tissues were exposed to indomethacin (1 μM) throughout in these experiments. After the construction of a control concentration-effect curve to ATP, tissues were incubated with either the P_{2X}-purinoceptor antagonist, PPADS (10 μM), the P_2-purinoceptor antagonist, suramin (100 μM), the P_1-purinoceptor antagonist, 8-sulphophenyltheophylline (140 μM) or the appropriate vehicle for 30 min (90 min for suramin) and the agonist curve repeated.

Drugs and solutions

Drugs used in these studies were: indomethacin (Sigma), carbachol (Sigma), NBTI (Research Biochemicals Inc.), 8-sulphophenyltheophylline (Research Biochemicals Inc.), ATP (sodium salt, Sigma), α,β-methylene ATP (lithium salt, Sigma), β,γ-methylene ATP (sodium salt, Sigma), 2 methylthio ATP (tetrasodium salt, Research Biochemicals Inc.), UTP (sodium salt, Sigma), suramin (Bayer) and PPADS (Cooksons). Indomethacin was prepared at a concentration of 10 mM in 95% ethanol, while NBTI (1 mM) was dissolved in 100% dimethylsulphoxide (Sigma). Stock solutions of all other compounds (1–10 mM) were dissolved in distilled water and diluted when necessary with 0.9% saline.

Statistical analysis of results

Data are expressed as mean ± standard error of the mean. The potency of agonists was calculated as pD_2 ($-\log_{10} EC_{50}$ where EC_{50} was the molar concentration of agonist that produced 50% of the maximum spasm) providing there was no significant difference in the maximum spasm recorded to each agonist. pD_2 was determined by linear regression of % spasm against $\log_{10}$ agonist concentration. Antagonism was measured as log concentration ratio (CR) and log (CR − 1) values were calculated (when possible) from the difference in pD_2 values between the initial and second concentration-effect curves for each tissue. In experimental designs where the maximum was not reached, e.g. sometimes in the presence of antagonists, % depressions of responses were compared. Statistical analysis was carried out by Student's paired or unpaired t test, with a probability level of $P<0.05$ being considered significant. Where it was necessary to make multiple comparisons one way analysis of variance (ANOVA) was used. A supplementary Student's t test was then carried out to define the boundaries around the control mean outside of which any treated group mean would be significant at the 5% level (Wardlaw, 1989).

Analysis of antagonism of ATP, 2 methylthio ATP and UTP by suramin was carried out according to the method of Arunlakshana & Schild (1959). Schild plots of log (CR − 1) versus antagonist concentration were prepared for uteri obtained from each animal and used to calculate the mean pA_2 for suramin.

Results

Potency order for ATP and analogues

Addition of ATP to tissues caused spasm with maximum tension reached after approximately 2 min, then tension fell even in the continued presence of ATP. Spasmogenic responses were typically all or nothing, giving very steep concentration-effect curves in individual tissues. The pD_2 of ATP was 4.9 ± 0.1 ($n=24$) and the maximum response was 114.0 ± 2.3% of the spasm to KCl (50 mM, Figure 1). The time course and maximum responses evoked by UTP, 2 methylthio ATP, α,β methylene ATP and β,γ methylene ATP were similar to those produced by ATP (Figure 1). 2 Methylthio ATP was significantly more potent than ATP ($pD_2=7.5\pm0.3$; relative potency 490; $P<0.05$; $n=6$), while α,β methylene ATP was equipotent with ATP ($pD_2=5.0\pm0.1$; relative potency 1.05; $P>0.05$; $n=5$). UTP was also equipotent with ATP ($pD_2=5.0\pm0.1$; relative potency 1.3; $P>0.05$; $n=6$). Addition of β,γ methylene ATP did produce spasm, but the maximum response recorded was significantly less than for the other agonists. For this reason no pD_2 was calculated.

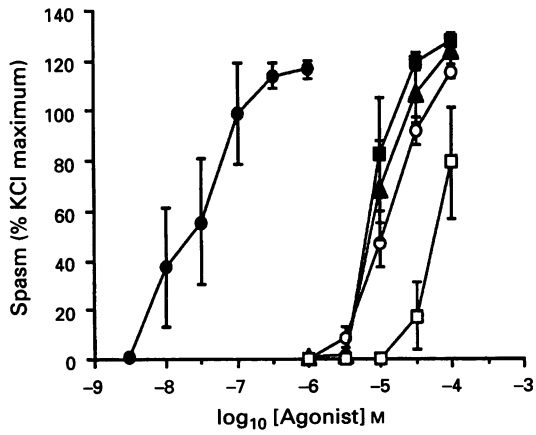

Figure 1 Potency order for ATP and analogues causing spasm of the isolated uterus of the guinea-pig: (●) 2 methylthio ATP; (■) α,β methylene ATP; (▲) UTP; (○) ATP and (□) β,γ methylene ATP. Spasmogenic responses are expressed as the percentage of KCl (50 mM)-induced spasm. Points represent mean values ± s.e.mean ($n=6$ except for ATP where $n=24$).

Effect of indomethacin (1 μM) on responses to ATP and analogues

Indomethacin (1 μM) significantly reduced spasmogenic responses to ATP (Figure 2a), virtually abolished responses to 2 methylthio ATP and α,β methylene ATP (Figure 2b,c) and completely abolished responses to UTP (Figure 2d). The vehicle for indomethacin had no effect on spasm to ATP (control: initial $pD_2=4.2\pm0.3$; + vehicle: $pD_2=4.4\pm0.1$; $n=4$, $P>0.05$) or on spasmogenic responses to α,β methylene ATP (control: initial $pD_2=4.9\pm0.2$; + vehicle: $pD_2=4.8\pm0.2$; $n=6$, $P>0.05$), UTP (control: initial $pD_2=4.9\pm0.2$; + vehicle: $pD_2=4.6\pm0.2$; $n=6$, $P>0.05$) or 2 methylthio ATP (control: initial $pD_2=7.0\pm0.1$; + vehicle: $pD_2=7.3\pm0.3$; $n=6$, $P>0.05$).

Effect of removal of the endometrium and circular muscle layer on spasmogenic responses

The maximum tension developed to KCl (50 mM) in longitudinal muscle strips (2.9 ± 0.4 g, $n=20$) was significantly less than in tissues taken from adjacent intact segments of the same uterine horns (4.1 ± 0.4 g, $n=20$, $P<0.05$). Spasmogenic responses to agonists recorded in each strip were thus expressed as a % of the response to KCl (50 mM) in that strip. In the case of ATP, endometrium and circular muscle layer removal caused a significant depression in the spasm recorded to the three highest concentrations tested (10, 30 and 100 μM, Figure 3a). Removal of the endometrium and circular muscle layer significantly reduced spasmogenic responses to α,β methylene ATP, 2 methylthio ATP and UTP (Figure 3b,c,d).

Effect of 2 methylthio ATP on responses to 2 methylthio ATP and UTP

In order to determine whether 2 methylthio ATP and UTP act at the same receptor, 2 methylthio ATP was tested for its ability to cause desensitization to itself and also to UTP. Pretreatment for 5 min with 2 methylthio ATP (1 μM) virtually abolished responses to subsequent addition of 2 methylthio ATP (Figure 4a), while the vehicle for 2 methylthio ATP had no effect (control: initial $pD_2=7.3\pm0.2$; + vehicle $pD_2=7.4\pm0.1$; $n=6$, $P>0.05$). However, a prior addition of 2 methylthio ATP did not reduce spasmogenic responses to UTP (test: initial $pD_2=5.3\pm0.1$; + 2 methylthio ATP (1 μM); $pD_2=4.9\pm0.2$, $n=5$, $P>0.05$; control: initial $pD_2=5.1\pm0.1$; + vehicle: $pD_2=4.8\pm0.1$, $n=6$, $P>0.05$; Figure 4b).

Effect of suramin on responses to ATP, 2 methylthio ATP and UTP

Figure 5a illustrates the effect of increasing concentrations of suramin (3, 10 and 30 μM) on a concentration-effect curve to 2 methylthio ATP. The two higher concentrations of suramin produced a significant shift in the 2 methylthio ATP concentration-effect curve (vehicle: $\log_{10}$ CR = −0.7 ± 0.3; 3 μM: $\log_{10}$ CR = 0.4 ± 0.3; 10 μM: $\log_{10}$ CR = 1.1 ± 0.2, $P<0.05$; 30 μM: $\log_{10}$ CR = 1.5 ± 0.1, $P<0.05$. $n=6$). The Schild plot obtained from these data is shown in Figure 5b. The slope of this plot was not significantly greater than 1 (1.5 ± 0.4, $n=6$, $P>0.05$) giving a pA_2 of 5.9 ± 0.3.

Suramin also antagonized ATP (Figure 6a). However, the only suramin concentration that produced a significant shift in the $\log_{10}$ concentration-effect curve was 100 μM (vehicle: $\log_{10}$ CR = −0.4 ± 0.2; 10 μM; $\log_{10}$ CR = 0.3 ± 0.2; 100 μM: $\log_{10}$ CR = 0.9 ± 0.4, $P<0.05$; 1 mM: $\log_{10}$ CR = 0.6 ± 0.3, $n=6$). As the antagonism of ATP by suramin was not concentration-dependent, no pA_2 was calculated. Suramin was also an antagonist of UTP (Figure 6b). However, the interaction did not appear to be competitive as the maximum response to UTP

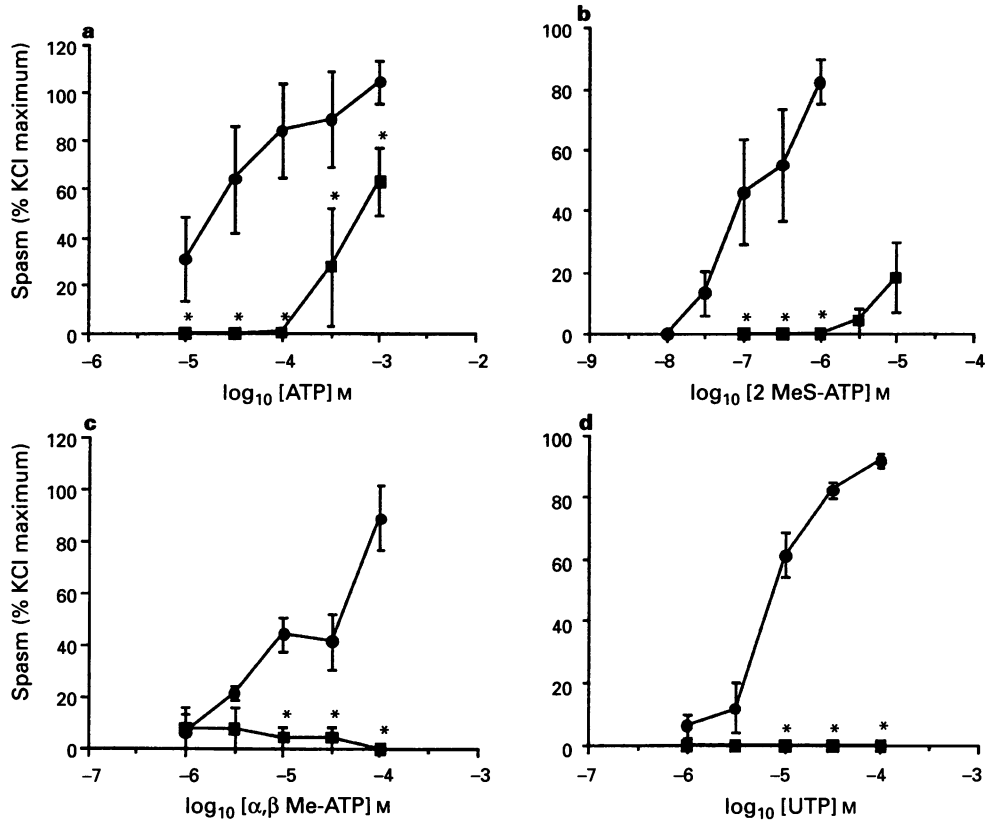

Figure 2 Effect of indomethacin on spasm induced by ATP, 2 methylthio ATP, α,β methylene ATP and UTP in the isolated uterus of the guinea-pig. Shown are (a) responses to ATP, (b) responses to 2 methylthio ATP (2 MeS-ATP), (c) responses to α,β methylene ATP (α,β Me ATP) and (d) responses to UTP in the absence (●) and in the presence of indomethacin (1 μM, ■) after 30 min equilibration. Spasmogenic responses are expressed as the percentage of KCl (50 mM)-induced spasm. Points represent mean values ± s.e.mean ($n=4-6$). *Significant difference from initial curve ($P<0.05$, paired t test).

was reduced in the presence of suramin concentrations higher than 3 μM (10 μM: initial curve: $E_{max}=108.8\pm6.5\%$; + suramin: $E_{max}=51.1\pm31.9\%$, $n=6$; 30 μM: initial curve: $E_{max}=110.1\pm19.5\%$, + suramin: $E_{max}=65.9\pm35\%$, $n=6$). For this reason, no pA_2 was calculated. Suramin (10 and 30 μM) produced similar antagonism of UTP.

Effect of PPADS on responses to ATP, 2 methylthio ATP and UTP

PPADS (10 μM) had no effect on spasm to UTP (test tissues: initial $pD_2=4.9\pm0.1$; + PPADS: $pD_2=4.7\pm0.2$; $n=4$, $P>0.05$; control tissues: initial $pD_2=4.9\pm0.2$; + vehicle: $pD_2=4.7\pm0.2$; $n=4$, $P>0.05$) or 2 methylthio ATP (test tissues: initial $pD_2=7.6\pm0.2$; + PPADS: $pD_2=7.1\pm0.0$; $n=4$, $P>0.05$; control tissues: initial $pD_2=7.6\pm0.1$; + vehicle: $pD_2=7.2\pm0.1$; $n=4$, $P>0.05$). Under the same experimental conditions, PPADS (10 μM) had no effect on spasm to ATP in the non-pregnant guinea-pig isolated uterus (test tissues: initial $pD_2=4.7\pm0.1$; + PPADS: $pD_2=4.5\pm0.1$; $n=5$, $P>0.05$; control tissues: initial $pD_2=4.9\pm0.1$; + vehicle: $pD_2=4.8\pm0.1$; $n=5$, $P>0.05$).

Nature of the indomethacin-resistant component of the spasm recorded to ATP

It has been shown above that indomethacin (1 μM) reduced spasmogenic responses to ATP while responses to UTP, 2 methylthio ATP and α,β methylene ATP were virtually or completely abolished. Further experiments were, therefore, conducted to determine the nature of the indomethacin-resistant spasm to ATP. In the presence of indomethacin (1 μM), responses to ATP were unaffected by the P_1-purinoceptor antagonist 8-sulphophenyltheophylline (140 μM; Figure 7a) (test tissues: initial $pD_2=3.5\pm0.1$; + 8-sulphophenyltheophylline: $pD_2=3.4\pm0.1$; $n=5$, $P>0.05$; control tissues: initial $pD_2=3.5\pm0.1$; + vehicle: $pD_2=3.6\pm0.2$; $n=4$, $P>0.05$) or by the P_2-purinoceptor antagonist suramin (100 μM; Figure 7b) (test tissues: initial $pD_2=4.1\pm0.4$; + suramin: $pD_2=4.1\pm0.3$; $n=6$, $P>0.05$; control tissues: initial $pD_2=4.4\pm0.4$; + vehicle: $pD_2=3.8\pm0.2$; $n=6$, $P>0.05$). However PPADS (10 μM) significantly reduced responses to ATP (Figure 7c), while the vehicle for PPADS had no effect (control tissues: initial $pD_2=3.9\pm0.4$; + vehicle: $pD_2=3.5\pm0.2$; $n=6$, $P>0.05$).

Discussion

We have demonstrated that ATP and analogues can produce spasm of the isolated uterus of the non-pregnant guinea-pig. Based on data from the use of rank order of agonist potencies, antagonists and desensitization, we suggest that several P_2-purinoceptors are present.

Which P_2-purinoceptor subtypes are present in guinea-pig isolated uterus?

The potency order for spasm in the isolated uterus [2 methylthio ATP > > α,β methylene ATP = UTP = ATP > > β,γ methylene ATP] is not consistent with any single P_2-purinoceptor subtype. As 2 methylthio ATP was by far the most potent agonist and this agonist is normally reasonably selective for the P_{2Y}-purinoceptor subtype (Fredholm *et al.*, 1994), the data suggests that P_{2Y}-purinoceptors are present. However, UTP was equipotent with ATP and α,β methylene ATP. UTP normally has significant activity only at P_{2U}-purinoceptors (Fredholm *et al.*, 1994) or UTP-selective pyrimidinoceptors (Lazarowski & Harden, 1994). It is, therefore, likely that P_{2U}-purinoceptors or pyrimidine receptors are also present. There

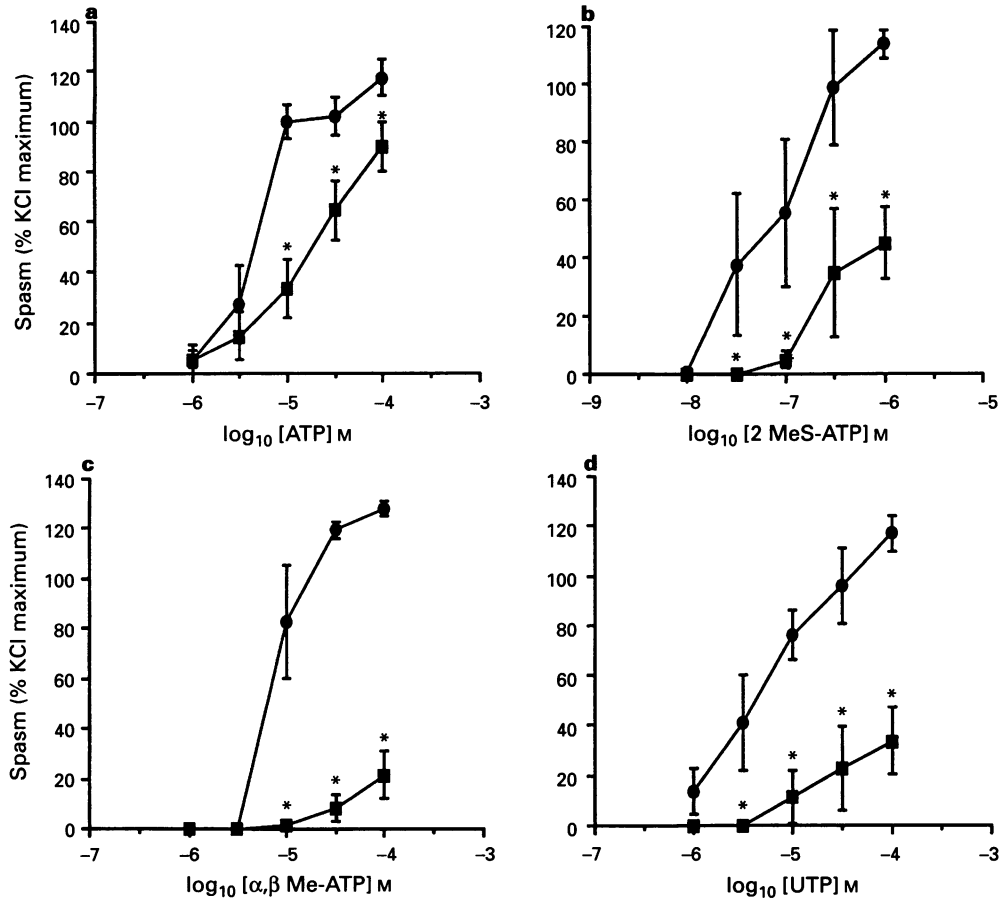

Figure 3 Effect of endometrium and circular muscle layer removal on spasm induced by ATP, 2 methylthio ATP, α,β methylene ATP and UTP in the isolated uterus of the guinea-pig. Shown are responses to (a) ATP, (b) 2 MeS-ATP, (c) α,β Me ATP and (d) responses to UTP in intact strips (●) and longitudinal muscle layer (■). Spasmogenic responses are expressed as the percentage of KCl (50 mM)-induced spasm. Points represent mean values ± s.e.mean ($n=6$). *Significant difference from intact tissues from the same uterine horn ($P<0.05$, unpaired t test).

have been reports of P_{2Y}- and P_{2U}-purinoceptors co-existing in bovine aortic endothelial cells (Wilkinson *et al.*, 1994a,b), C6 glioma cells (Lin & Chuang, 1994) and phaeochromocytoma PC12 cells (Nikodijevic *et al.*, 1994). Strong evidence for the existence of both P_{2Y}- and P_{2U}-purinoceptors in the isolated uterus of the guinea-pig was indicated by the finding that pretreatment with 2 methylthio ATP caused desensitization to 2 methylthio ATP, but not to UTP. The data do not provide evidence for the presence of P_{2X}-purinoceptors.

Effect of suramin on responses to 2 methylthio ATP, UTP and ATP

Suramin antagonized 2 methylthio ATP. The antagonism of 2 methylthio ATP was concentration-dependent and the interaction may have been competitive as the slope of the resultant Schild plot was not significantly different from 1. Suramin had a pA_2 of 5.9, which is similar to the value obtained at P_{2Y}-purinoceptors in the taenia caeci of the guinea-pig (5.1; Piper & Hollingsworth, 1995). Suramin also antagonized ATP. In this case the degree of antagonism did not vary with the concentration of suramin. Suramin is known to be an inhibitor of ecto-nucleotidases responsible for the metabolism of ATP and 2 methylthio ATP (Hourani & Chown, 1989; Ziganshin *et al.*, 1995) and this property may have been responsible for the anomalous results seen here. At high concentrations of suramin, inhibition of ATP metabolism and thus potentiation may overcome any antagonism. Alternatively, the equilibration time for suramin at P_{2X}-purinoceptors has been shown to be inversely proportional to concentration (Leff *et al.*, 1990) and thus incomplete equilibration could explain the data here.

The antagonism of UTP by suramin did not appear to be competitive. Suramin has been demonstrated to be an antagonist at P_{2U}-purinoceptors in PC12 cells (Murrin & Boarder, 1992) and at the pyrimidinoceptor described by Lazarowski & Harden (1994), but it did not inhibit UTP-induced relaxation of bovine aorta (Wilkinson *et al.*, 1994b). It is possible that these differences reflect the existence of different subtypes of P_{2U}-purinoceptor, one of which may be the UTP-selective pyrimidinoceptor (Lazarowski & Harden, 1994). Further molecular biology studies may shed some light on this hypothesis.

Are cyclo-oxygenase products involved in mediating spasm to ATP and analogues?

Spasm to ATP was significantly reduced by indomethacin treatment, while spasm to 2 methylthio ATP, α,β methylene ATP and UTP was virtually or completely abolished. In the isolated uterus of the non-pregnant guinea-pig the endometrium is the major site of prostanoid synthesis (Poyser, 1972). Removal of the endometrium and circular muscle layer had an effect similar to that of indomethacin. Based on these two pieces of evidence, it is possible that agonist interaction with P_2-purinoceptors in these layers leads to prostanoid synthesis. It has been shown that addition of phospholipase C to the isolated uterus of the guinea-pig caused a significant increase in the synthesis of the spasmogenic prostanoid prostaglandin E_2 (Poyser, 1987). It has also been shown in a variety of systems that P_{2Y}-, P_{2U}- and pyrimidinoceptors may be

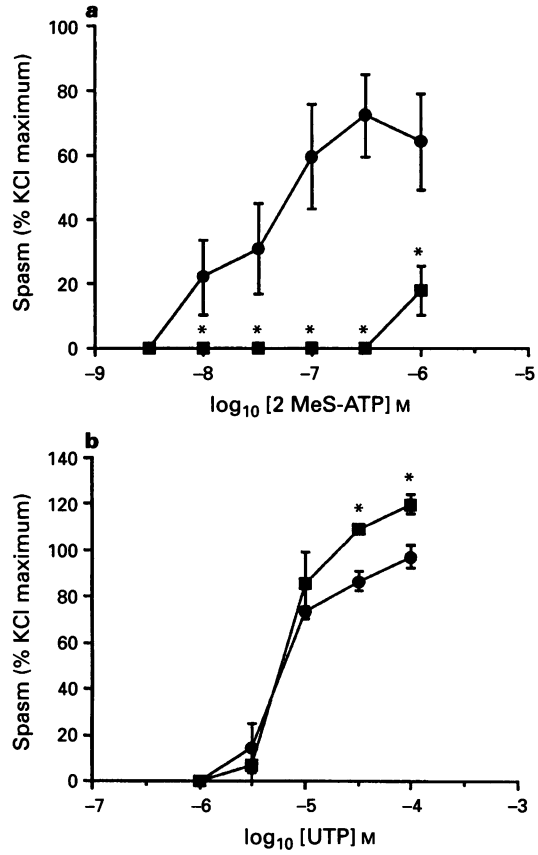

Figure 4 Effect of 2 methylthio ATP on spasm to 2 methylthio ATP and UTP in the isolated uterus of the guinea-pig. Shown are (a) responses to 2 methylthio ATP (2 MeS-ATP) and (b) responses to UTP in the absence (●) and after 5 min incubation with 2 methylthio ATP (1 μM, ■). Spasmogenic responses are expressed as the percentage of KCl (50 mM)-induced spasm. Points represent mean values ± s.e.mean ($n=6$). *Significant difference from initial curve ($P<0.05$, paired t test).

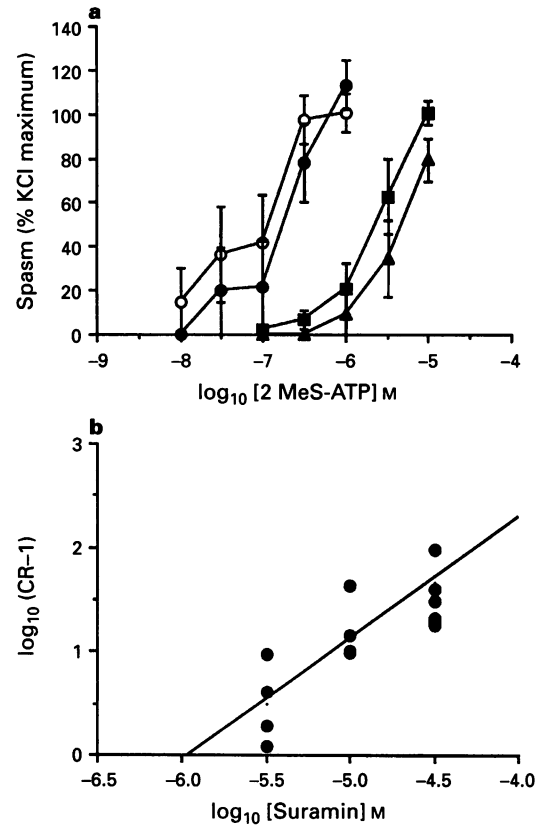

Figure 5 Antagonism of 2 methylthio ATP by suramin in the isolated uterus of the guinea-pig. Shown are (a) the $\log_{10}$ concentration-effect curves to 2 methylthio ATP in the presence of vehicle (○) or suramin (3 μM ●, 10 μM ■ and 30 μM ▲) after 90 min equilibration and (b) the Schild plot derived from these data (see text). Spasmogenic responses are expressed as the percentage of KCl (50 mM)-induced spasm. Points represent mean values ± s.e.mean ($n=6$).

coupled via GTP-binding proteins to phospholipase C (P_{2Y}-, turkey erythrocytes: Berrie *et al.*, 1988; P_{2U}-, human airways: Parr *et al.*, 1994; pyrimidinoceptors, Lazarowski & Harden, 1994; P_{2Y}- and P_{2U}, bovine aortic endothelial cells: Wilkinson *et al.*, 1994b; for a more detailed review, see Dubyak & El Moatassim, 1993) so it is possible that interaction with either P_{2Y}- or P_{2U}-purinoceptors could lead to increases in prostanoid synthesis. Direct evidence to support this hypothesis could be obtained by biochemical measurement of prostanoid production.

Alternatively, it is possible that endogenous prostanoids normally present in the isolated uterus of the guinea-pig sensitize the myometrium to ATP and analogues, thus enhancing their potency. A similar phenomenon has been described for 5-HT-mediated spasm of guinea-pig isolated iliac artery (Sahin-Erdemli *et al.*, 1991). In the tissue, spasm to 5-HT was abolished in the presence of indomethacin, but could be restored with the addition of a low concentration of prostaglandin E_2. Regardless of the exact mechanism, it is clear that the presence of prostanoids is crucial for the spasmogenic action of ATP and analogues.

Nature of the indomethacin-resistant spasm to ATP

Surprisingly, while indomethacin abolished spasmogenic responses to α,β methylene ATP, 2-methylthio ATP and UTP, a major component of the spasm to ATP remained. Also, removal of the endometrium and circular muscle layer appeared to have caused less inhibition of spasm to ATP than to the other agonists (Figure 3). This suggests that there was a component of the ATP-induced spasm that did not involve prostanoids and did not require the endometrium or the circular muscle layer to be present, i.e. ATP acted directly on the longitudinal myometrium. Experiments carried out in the presence of indomethacin were designed to determine of this direct action was mediated by purinoceptors. The indomethacin-resistant ATP-induced spasm was not inhibited by the non-selective P_1-purinoceptor antagonist 8-sulphophenyltheophylline (140 μM), suggesting that P_1-purinoceptors were not involved. In the presence of indomethacin, ATP was not antagonized by suramin (100 μM) in contrast to the profound antagonism of ATP by suramin in the absence of indomethacin. If antagonism by suramin is taken to indicate the involvement of P_2-purinoceptors, then the current data suggest the presence of a non P_{2X}-, non P_{2Y}-purinoceptor. Alternatively, it is possible that in the presence of indomethacin, inhibition of ectonucleotidases by suramin and hence prevention of ATP metabolism, is so marked that antagonism of ATP by suramin was not seen. Surprisingly, PPADS (10 μM) did cause a significant depression in ATP-induced spasm in the presence of indomethacin, but not in its absence. However, if multiple purinoceptor subtypes are indeed present in non-pregnant guinea-pig isolated uterus, ATP could produce spasm in the absence of indomethacin via both prostanoid-dependent and prostanoid-independent mechanisms. As PPADS caused inhibition of prostanoid-independent spasm only, it is possible that the part of the ATP-induced spasm via prostanoid-dependent mechanisms would be still operative and so no antagonism would be seen. In the presence of indomethacin, ATP may produce spasm only via a non-prostanoid-dependent

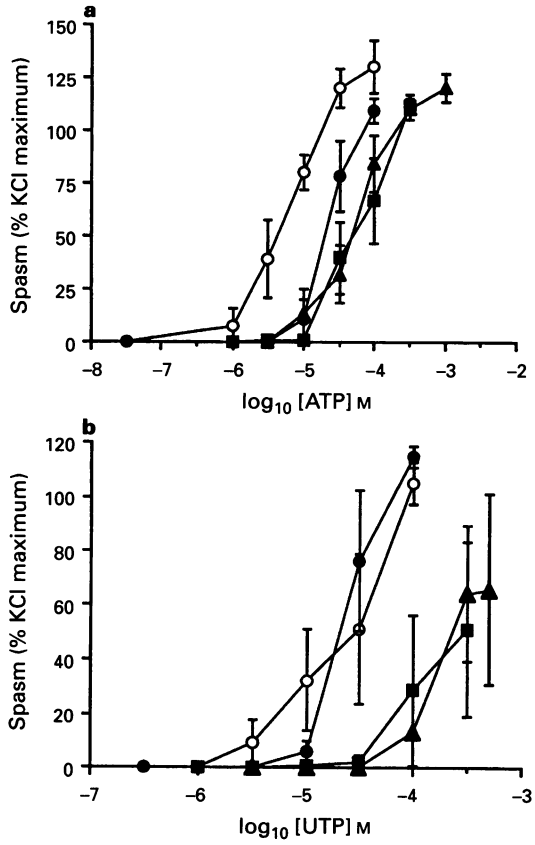

Figure 6 Antagonism of ATP and UTP by suramin in the isolated uterus of the guinea-pig. Shown in (a) are the $\log_{10}$ concentration-effect curves to ATP in the presence of vehicle (○) or suramin (10 μM, ●, 100 μM ■ and 1 mM ▲) after 90 min equilibration. Shown in (b) are the $\log_{10}$ concentration-effect curves to UTP in the presence of vehicle (○) or suramin (3 μM ●, 10 μM ■ and 30 μM ▲) after 90 min equilibration. Spasmogenic responses are expressed as the percentage of KCl (50 mM)-induced spasm. Points represent mean values ± s.e.mean ($n = 6$).

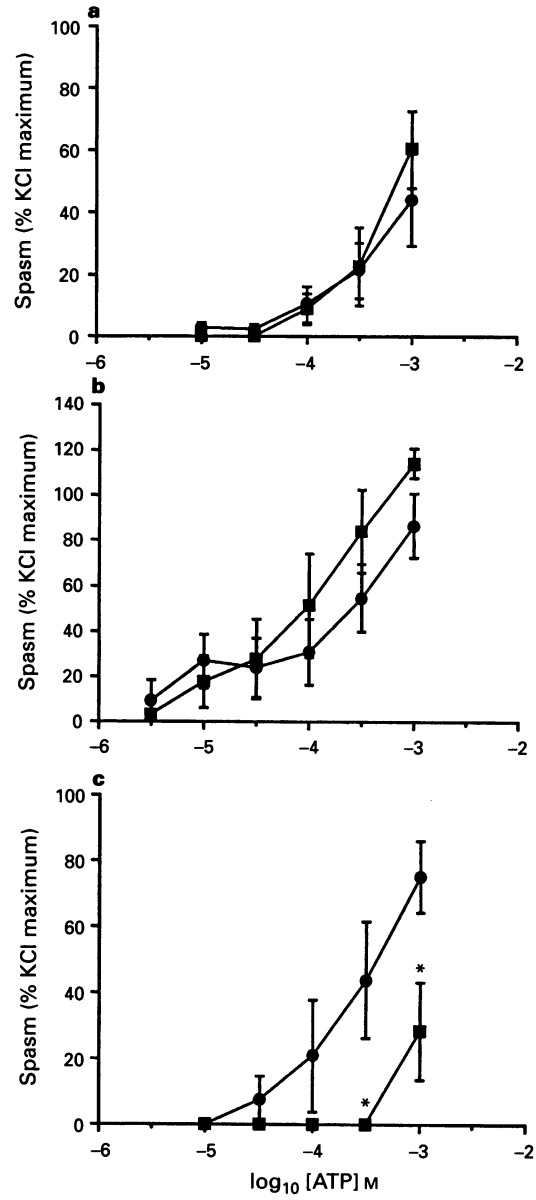

Figure 7 Effect of 8-sulphophenyltheophylline, suramin and PPADS on spasm to ATP in the presence of indomethacin (1 μM) in the isolated uterus of the guinea-pig. Shown are (a) responses to ATP in the absence (●) and in the presence of 8-sulphophenyltheophylline (140 μM ■); (b) in the absence (●) and in the presence of suramin (100 μM ■) and (c) in the absence (●) and in the presence of PPADS (10 μM ■). Spasmogenic responses are expressed as the percentage of KCl (50 mM)-induced spasm. Points represent mean values ± s.e.mean ($n = 6$). *Significant difference from initial curve ($P < 0.05$, paired t test).

mechanism which is sensitive to inhibition by PPADS. Purinoceptors activated by ATP and insensitive to suramin have been reported in mouse (Von Kügelgen *et al.*, 1990) and rat vas deferens (Bültmann & Starke, 1994). In the rat vas deferens, non-P_{2X}, non-P_{2Y}-purinoceptors activated by ATP (suramin-insensitive) at which PPADS was an antagonist have been described (Bültmann & Starke, 1994). If such receptors were present in the myometrium of guinea-pig isolated uterus, they might be responsible for the indomethacin-resistant spasm recorded on addition of ATP.

In conclusion, these studies have shown that the isolated uterus of the non-pregnant guinea-pig probably contains a mixture of P_2-purinoceptors, activation of any subtype leading to spasm. Co-existing P_{2U}- or pyrimidinoceptors and P_{2Y}-purinoceptors are present, probably predominantly within the endometrium or circular smooth muscle layer. Activation of these purinoceptors leads to spasm dependent on prostanoid(s). Suramin is an antagonist at both the P_{2Y}- and P_{2U}-purinoceptors. A third type of purinoceptor activated by ATP is found on the myometrial cells and spasm mediated via this purinoceptor is not dependent on prostanoid synthesis. While PPADS was an antagonist at the myometrical purinoceptors, suramin was not. The nature of these latter purinoceptors, which do not conform to any recognised P_2-purinoceptor subtypes, requires further study.

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