British Journal of Pharmacology (1996) 119, 1628-1634

Inhibition of ecto-ATPase by PPADS, suramin and reactive blue in endothelial cells, C_6 glioma cells and RAW 264.7 macrophages

Bin C. Chen, *Chii-M. Lee & [1]Wan-W. Lin

Departments of Pharmacology, and *Internal Medicine, College of Medicine, National Taiwan University, Taipei, Taiwan

1 Previous studies have shown that bovine pulmonary artery endothelium (CPAE) has P_{2Y} and P_{2U} purinoceptors, rat C_6 glioma cells have P_{2U} purinoceptors and mouse RAW 264.7 cells have pyrimidinoceptors, all of which are coupled to phosphoinositide-specific phospholipase C (PI-PLC). The dual actions of PPADS, suramin and reactive blue as antagonists of receptor subtypes and ecto-ATPase inhibitors were studied in these three cell types.

2 In CPAE, suramin, at 3-100 μM, competitively inhibited the PI responses induced by 2MeSATP and UTP, with pA_2 values of 5.5 ± 0.3 and 4.4 ± 0.4, respectively. Reactive blue, at 1-3 μM, produced shifts to the right of the 2MeSATP and UTP curves, but no further right shift at 10 μM. PPADS, at 10 μM, caused a 3 fold right shift of the 2MeSATP curve, but no further shift at concentrations up to 100 μM. In contrast, a dose-dependent shift to the left of the UTP curve and a weak inhibition of the ATP response were seen with PPADS.

3 In RAW 264.7 cells, suramin and reactive blue, but not PPADS, competitively inhibited the UTP response, with pA_2 values of 4.8 ± 0.5 and 5.8 ± 0.7, respectively.

4 In C_6 glioma cells, although suramin and reactive blue inhibited the ATP response, a potentiation effect on ATP and UTP responses was seen with PPADS.

5 The ecto-ATPase inhibitory activity of these three receptor antagonists were determined. All three inhibited ecto-ATPase present in CPAE, C_6 and RAW 264.7 cells, with IC_{50} values of 4, 4.8 and 4.7 for PPADS, 4, 4.4 and $>>4$ for suramin, and 4.5, 4.7 and 4.7 for reactive blue.

6 This study indicates that PPADS, suramin and reactive blue are ecto-ATPase inhibitors. This property, combined with their antagonistic selectivity for receptor subtypes, can result in inhibition of, potentiation of, or lack of effect on agonist-mediated PI responses. Reactive blue is a more potent antagonist than suramin on P_{2Y}, P_{2U} and pyrimidinoceptors, and PPADS is a weak antagonist for P_{2Y} receptors.

Keywords: PPADS; reactive blue; suramin; ecto-ATPase; receptor antagonist; P_{2Y}-purinoceptor; P_{2U}-purinoceptor; pyrimidinoceptor

Introduction

On the basis of the relative potency of a range of agonist analogues, P_2 purinoceptors, which mediate the physiological functions of extracellular ATP, have been classified into five subtypes (P_{2X}, P_{2Y}, P_{2U}, P_{2Z} and P_{2T}) (Cusack, 1993; Fredholm *et al.*, 1994; Harden *et al.*, 1995). A more systemic classification is hindered by the lack of potent selective antagonists and the susceptibility of nucleotide analogues to degradation by ecto-nucleotides, plasma membrane-associated enzymes which dephosphorylate extracellular nucleotide analogues. Although such ecto-nucleotidase breakdown of nucleotide analogues and the subsequent reduction of agonist potency has been clearly demonstrated (Welford *et al.*, 1987), its importance in the pharmacological classification of P_2 purinoceptors has been emphasised by recent studies on the P_{2X} purinoceptors (Kennedy & Leff, 1995).

The P_{2X} purinoceptor was originally defined as having a rank agonist potency of α, β-methylene ATP $>>$ ATP $\geq$ 2-methylthio-ATP (2MeSATP) in intact tissues (Burnstock & Kennedy, 1985), but ATP = 2MeSATP $>$ α, β-methylene ATP in isolated cells from the same tissues and in cells transfected with P_{2X}-purinoceptor cDNA (Inoue & Brading, 1990; Evans & Kennedy, 1994; Valera *et al.*, 1994). By using suramin, a non-selective P_2 antagonist, several reports showed that, under conditions in which the P_{2X} response to α, β-methylene ATP (a poorly hydrolysable ATP analogue) were blocked, the effects of ATP could be unaffected, be slightly inhibited or even be potentiated by suramin (von Kugelgen *et al.*, 1990; von Kugelgen & Starke, 1991; Bailey & Hourani, 1994; Crack *et al.*, 1994). This effect was shown to be due to the ability of suramin to act as a non-competitive inhibitor of ecto-ATPase with micromolar affinity (Hourani & Chown, 1989; Crack *et al.*, 1994; Beukers *et al.*, 1995). Subsequently, the discovery of a novel synthetic ATP analogue, FPL67156, pharmacologically and biochemically identified as a selective ecto-ATPase inhibitor, provided further support for the influence of ecto-ATPases on agonist potency and purinoceptor classification (Crack *et al.*, 1995).

Pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS), reactive blue and suramin are three commonly used P_2 antagonists with different antagonist selectivities for P_2 receptor subtypes. Suramin, mentioned above, is known to inhibit ecto-ATPase and thus change agonist potency at P_{2X} purinoceptors, but the actions of other two antagonists on ATP degradation have not been examined; it therefore seemed of interest to investigate their ecto-ATPase inhibitory capacities and to correlate this with their antagonist affinities for G protein-coupled P_2 purinoceptors (P_{2U}, P_{2Y} and pyrimidinoceptors).

Bovine pulmonary artery endothelium (CPAE), rat C_6 glioma and mouse RAW 264.7 macrophages were used in the study, since we have previously shown the presence of P_{2Y} and P_{2U} receptors in CPAE (Chen *et al.*, 1996), P_{2U} receptors in C_6 glioma (Lin & Chuang, 1994) and pyrimidinoceptors in RAW 264.7 macrophages (Lin & Lee, 1996). All these receptor subtypes are coupled to G proteins and regulate the inositol lipid via phospholipase C (PLC).

[1] Author for correspondence.

Materials and methods

Cell culture

CPAE, RAW 264.7 and C_6 glioma cells were obtained from ATCC (Rockville, MO, U.S.A.) and cultured in 35 mm Petri dishes at 37°C in a humidified atmosphere of 95% air and 5% CO_2. CPAE was grown in Minimum Essential medium supplemented with 10% FCS, while RAW 264.7 and C_6 glioma cells were grown in Dulbecco's modified Eagle's medium, containing 10% FCS, 100 u ml^{-1} penicillin and 100 μg ml^{-1} streptomycin.

Measurement of PI turnover

PI hydrolysis was measured by the accumulation of inositol phosphates (IP) in the presence of 10 mM LiCl, as described previously (Lin & Chuang, 1994). Confluent cells on 35 mm Petri dishes were labelled for 24 h with [^{3}H]-*myo*inositol (2.5 μCi ml^{-1} per dish) in growth medium. The cells were then washed with physiological saline solution (PSS), composition mM: NaCl 118, KCl 4.7, $CaCl_2$ 1.8, $MgCl_2$ 1.2, KH_2PO_4 1.2, glucose 11 and HEPES 20 containing 10 mM LiCl, and incubated at 37°C for 20 min. After this preincubation stage, the indicated drugs were added and incubation continued for another 30 min. The reaction was then terminated by aspiration of the reaction solution and addition of ice-cold methanol. The cells were scraped off and the [^{3}H]-IP isolated on an AG 1X8 column and eluted with 0.2 M ammonium formate/0.1 M formic acid. When P_2 antagonists were tested, the cells were pretreated with antagonist for 20 min before the addition of agonist. [^{3}H]-IP formation was expressed as a percentage of control (basal IP formation without agonist treatment).

Ecto-ATPase assay

The degradation of [γ-^{32}P]-ATP by ecto-ATPase present in three cell lines was measured as described previously (Crack *et al.*, 1994). Cells in 1 ml of PSS were pretreated with vehicle or the indicated antagonist for 20 min at 37°C prior to the addition of 10 μM ATP (0.1 μCi $nmol^{-1}$). After incubation at 37°C for the indicated period, the medium was rapidly transferred to an Eppendorff tube containing activated charcoal (40 mg ml^{-1} charcoal in 0.1 M HCl), then centrifuged at 15,000 *g* for 5 min. The supernatant samples were mixed with scintillation cocktail and the radioactivity measured in a Beckman LS 6000 β-counter. The specific activity of the ecto-ATPase was expressed as pmol mg^{-1} protein. Inhibitory effcts of antagonists were expressed as a percentage of the degradation seen in the absence of the antagonists.

Materials

Culture medium, foetal calf serum (FCS), 0.25% trypsin/EDTA, penicillin and streptomycin were obtained from Gibco BRL (Grand Island, NY, U.S.A.). [^{3}H]-*myo*inositol (20 Ci $mmol^{-1}$) and [γ-^{32}P]-ATP (6000 Ci $mmol^{-1}$) were purchased from New England Nuclear (Boston, MA, U.S.A.). UTP and ATP were purchased from Sigma (St. Louis, MO, U.S.A.). 2MeSATP, reactive blue, suramin and PPADS were obtained from Research Biochemicals Inc. (Natick, MA, U.S.A.). Dowex AG 1X8 (formate form, 100–200 mesh) was from Bio-Rad Laboratories.

Statistical analysis

Each experiment was performed in duplicate several times (*n* refers to independent experiments). The data are the mean ± s.e.mean. Student's *t* test was used to evaluate the statistical differences with $P<0.05$ being considered significant. Error bars were omitted when they fell within the symbol representing the mean value. The agonist concentration at which agonist could induce 50% of the maximal response (EC_{50}) was determined from the dose-response curve which reached its maximal response. In the experiments with 2MeSATP where maximal responses were not achieved, the agonist response at the maximal concentration used (i.e. 100 μM) and without antagonist pretreatment was regarded as 100%. The antagonist effects of suramin, reactive blue and PPADS were examined according to Arunlakshana-Schild analysis (Arunlakshana & Schild, 1959). The pA_2 values were determined from the *x* intercepts and calculated by least squares regression analysis of the Schild plots, where the log of the dose-ratios DR-1 is plotted as a function of the antagonist concentration.

Results

Figure 1 shows the effect of suramin on 2MeSATP- and UTP-stimulated IP formation in CPAE. Over the concentration-range of 3–100 μM, suramin competitively and dose-dependently inhibited the effect of both agonists. Although suramin at 10–100 μM produced shifts to the right of the UTP curve

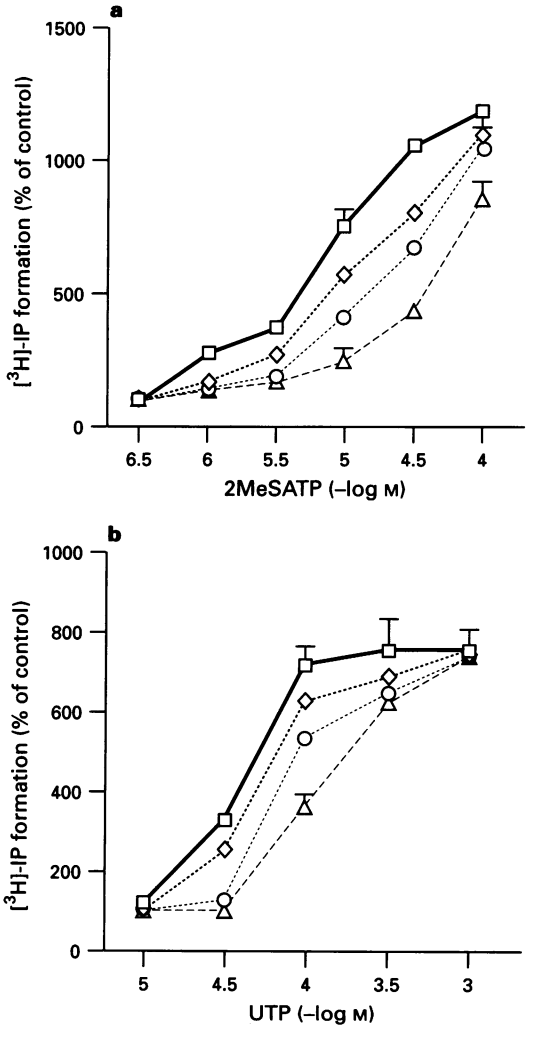

Figure 1 Inhibition of 2MeSATP- or UTP-stimulated IP formation by suramin in CPAE. [^{3}H]-inositol-labelled cells were preincubated for 20 min without (□) or with 3 μM (◇), 10 μM (○), 30 μM (△) or 100 μM (▽) suramin and challenged with 2MeSATP (a) or UTP (b) for an additional 30 min. The results are plotted as the percentage of IP accumulation in the treated cells compared with the untreated control cells. The data represent the mean ± s.e.mean of results obtained in three independent experiments. The value of basal (100%) [^{3}H]-IP formation was 156 ± 20 c.p.m./dish.

but no further right shift at 300 μM (data not shown). The pA_2 values were 5.5 ± 0.3 ($n=3$) for 2MeSATP and 4.4 ± 0.4 ($n=4$) for UTP. Reactive blue, which shows the same selective antagonism for P_{2Y} purinoceptors, shifted the dose-response curves of 2MeSATP and UTP at 1 and 3 μM to the right with pA_2 values of 6.3 ± 0.6 ($n=3$) and 5.7 ± 0.5 ($n=3$) for 2MeSATP and UTP, respectively (Figure 2). No further inhibition was observed at 10 μM.

The effect of PPADS on agonist-induced IP formation in CPAE is shown in Figure 3. Although the 2MeSATP curve (Figure 3a) was shifted to the right by a factor of 3 at 10 μM PPADS, no further effect was seen at concentrations up to 100 μM. However, with UTP, PPADS at 10–100 μM caused a dose-dependent enhancement of the PI response (Figure 3b). Because of these opposite effects of PPADS on the responses to P_{2Y} and P_{2U} agonists, ATP-induced PI turnover was determined. As shown in Figure 3c, PPADS, at the same concentration-range, had essentially no effect, other than that the response to 30 μM ATP was slightly inhibited by 3 μM PPADS or slightly potentiated by 30 or 100 μM PPADS.

Figure 4 shows the effects of three P_2 antagonists on UTP-induced PI signalling in RAW 264.7 cells, in which a non-P_2 purinoceptor, possibly a pyrimidinoceptor, with a 3 orders of magnitude higher specificity for UTP than ATP with respect to PI turnover and PLA_2 activation has been demonstrated (Lin & Lee, 1996). When cells were pretreated with suramin (10 or 30 μM) or reactive blue (1–30 μM), the dose-response curve for UTP was shifted to the right. The competitive inhibition curves

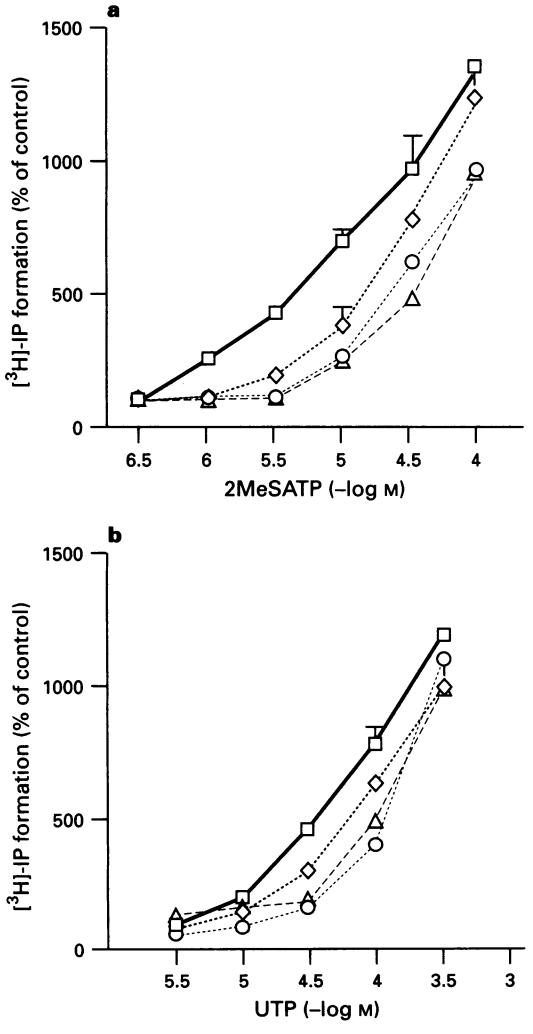

Figure 2 Inhibition of 2MeSATP- or UTP-stimulated IP formation by reactive blue in CPAE. The cells were preincubated for 20 min without (□) or with 1 μM (◇), 3 μM (○) or 10 μM (△) reactive blue before challenge with 2MeSATP (a) or UTP (b). The data are expressed as the percentage of untreated control values and are the mean ± s.e.mean from three independent experiments.

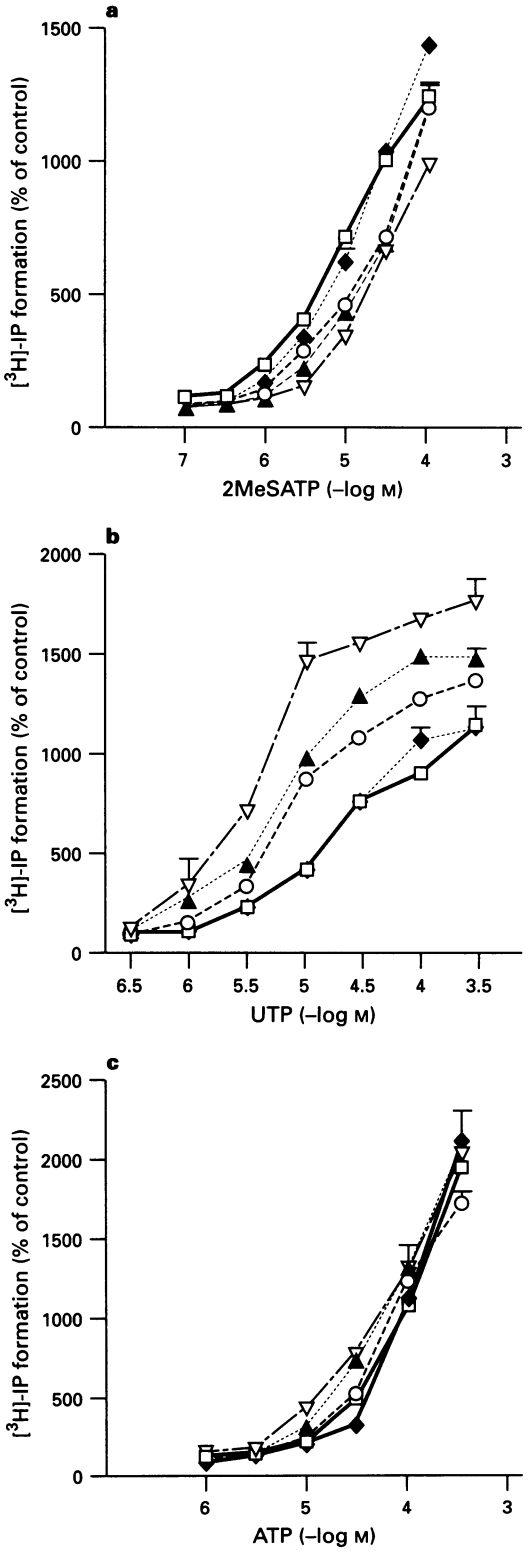

Figure 3 Effects of PPADS on 2MeSATP-, UTP- or ATP-stimulated IP formation in CPAE. The cells were either unpretreated (□) or pretreated with 3 μM (◆), 10 μM (○), 30 μM (▲) or 100 μM (▽) PPADS 20 min prior to stimulation of PI turnover by 2MeSATP (a), UTP (b), or ATP (c). The data are expressed as the percentage of untreated control values and are the mean ± s.e.mean from triplicate determinations which are representative of those obtained in three separate experiments.

seen for suramin (10 and 30 μM) and reactive blue (1–30 μM) gave pA_2 values of 4.8±0.5 (n=3) and 5.8±0.7 (n=3), respectively. In contrast, the PI response to UTP was essentially unchanged, except that the response to lower concentrations (1 and 3 μM) was approximately inhibited about 50% by the highest concentration of PPADS used.

We have previously demonstrated the presence in C_6 glioma cells of a P_{2U} purinoceptor-mediated PI response inhibitable by suramin and reactive blue (Lin & Chuang, 1994). The effect of PPADS on ATP- or UTP-induced P_{2U} signalling in these cells is shown in Figure 5. The responses to lower concentrations of ATP (10 μM) or UTP (10–30 μM) were significantly enhanced by PPADS at 3–100 μM, although the enhancement was not dose-dependent. In contrast to the increased UTP (300 μM) response seen with CPAE, PPADS had no effect on the responses induced by 100–300 μM UTP or 30–300 μM ATP.

Figure 6 shows the effect of PPADS on the time-course of [γ-^{32}P]-ATP degradation by CPAE, C_6 or RAW 264.7 cells. The rate of degradation was different in the three cell types, which had ecto-ATPase specific activities of the order of 4,673, 113 and 99 pmol mg^{-1} min^{-1} for CPAE, C_6 and RAW 264.7 cells, respectively. Pretreatment of the cells with 30 μM PPADS significantly slowed the degradation rates of [γ–^{32}P]-ATP, the effect being greater with C_6 and RAW 264.7 cells.

To test if P_2 antagonists were able to inhibit ecto-ATPase, and thus mask inhibition of agonist-induced PI turnover, the

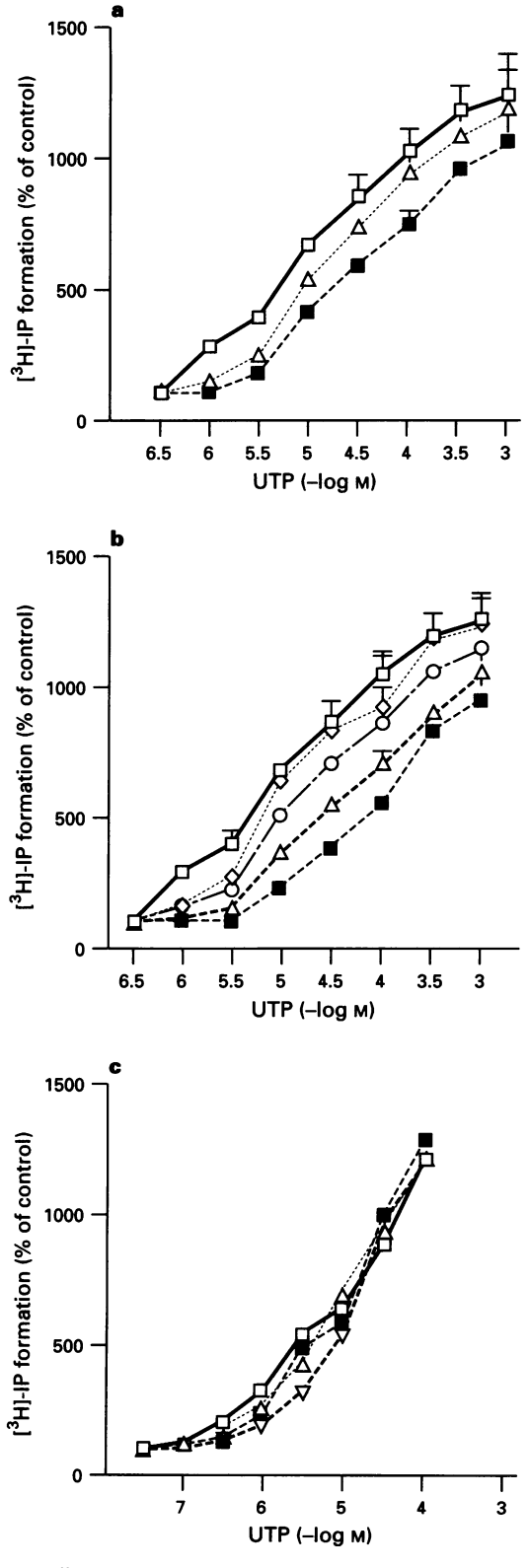

Figure 4 Effects of suramin, reactive blue and PPADS on UTP-induced PI turnover in RAW 264.7 cells. The cells were preincubated for 20 min without (□) or with suramin (a), reactive blue (b), or PPADS (c) at 1 μM (◇), 3 μM (○), 10 μM (△), 30 μM (■), or 100 μM (▽), and challenged with UTP for additional 30 min. The data are expressed as the percentage of untreated control values and are the mean±s.e.mean from three independent experiments. The value of basal (100%) [^{3}H]-IP formation was 202±15 c.p.m. per dish.

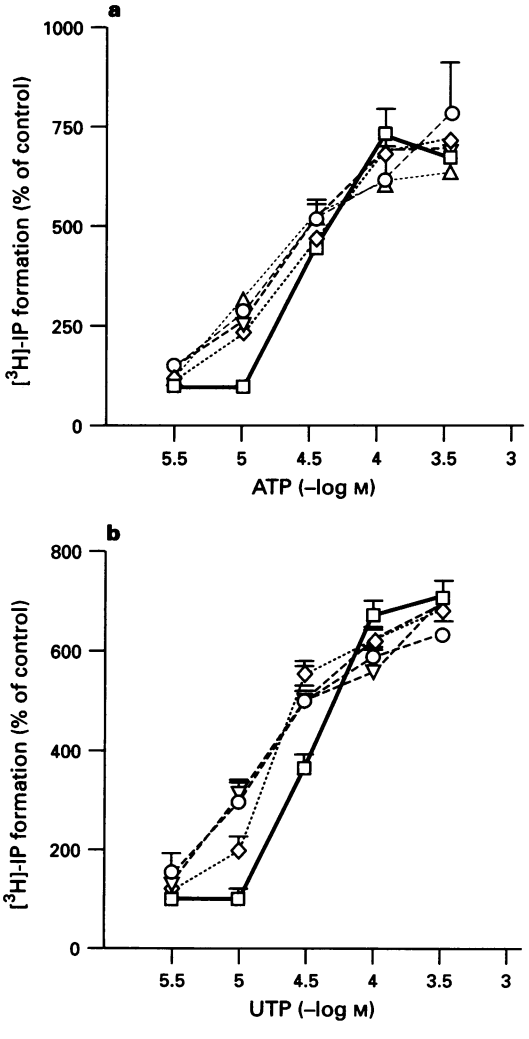

Figure 5 Effects of PPADS on ATP- or UTP-induced PI turnover in C_6 glioma cells. The cells were preincubated for 20 min without (□) or with 3 μM (◇), 10 μM (○), 30 μM (△) or 100 μM (▽) PPADS and challenged with ATP (a) or UTP (b) for an additional 30 min. The data are the mean±s.e.mean from three different experiments. The value of basal (100%) [^{3}H]-IP formation was 174±20 c.p.m. per dish.

dose-dependent inhibition of ecto-ATPase in these three cell types by PPADS, suramin and reactive blue were determined. Cells were loaded with 10 μM [$\gamma-^{32}$P]-ATP and the ^{32}P released within 3 min (CPAE) or 10 min (C_6 and RAW 264.7) was measured in the presence or absence of the inhibitor. As shown in Figure 7, all three antagonists, over the same concentration-

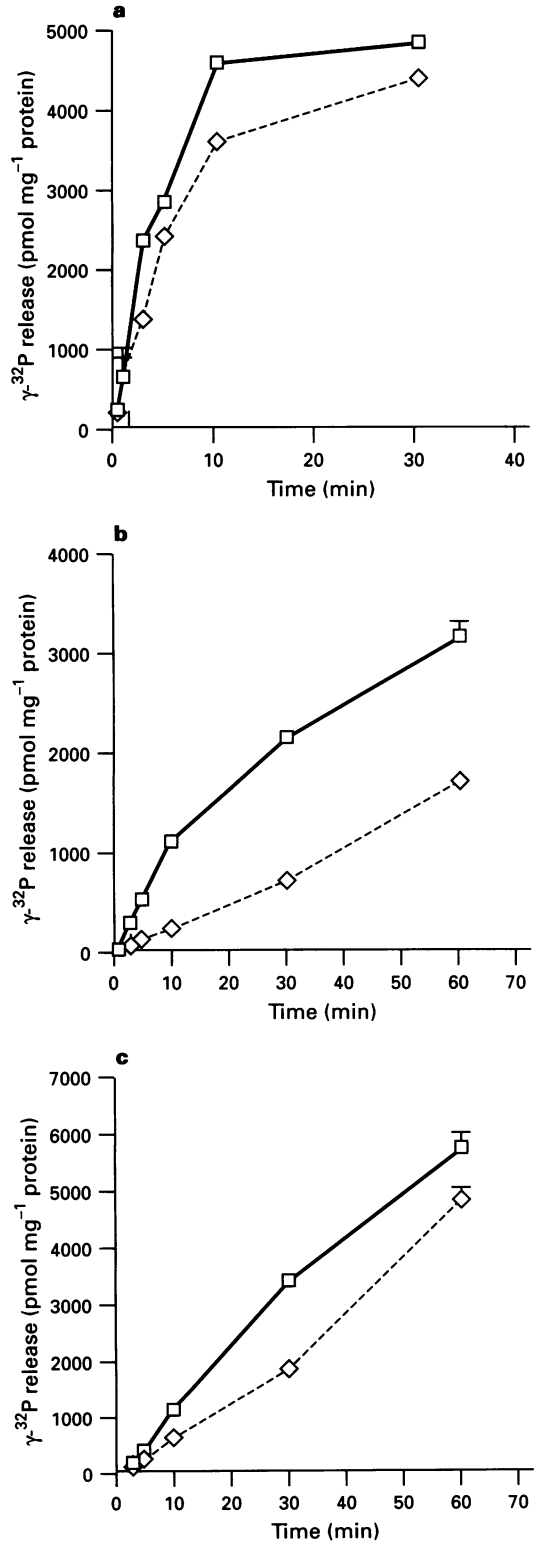

Figure 6 Effects of PPADS on ecto-ATPase activity in CPAE (a), C_6 glioma (b) or RAW 264.7 (c) cells. The cells were preincubated for 20 min without (□) or with 30 μM PPADS (◇), then 10 μM ATP containing [$\gamma-^{32}$P]-ATP was added. Time-dependent [$\gamma-^{32}$P]-ATP dephosphorylation by ecto-ATPase in three cell lines was measured as described in Methods. The data are the mean ± s.e.mean from triplicate determinations which are representative of results from three different experiments.

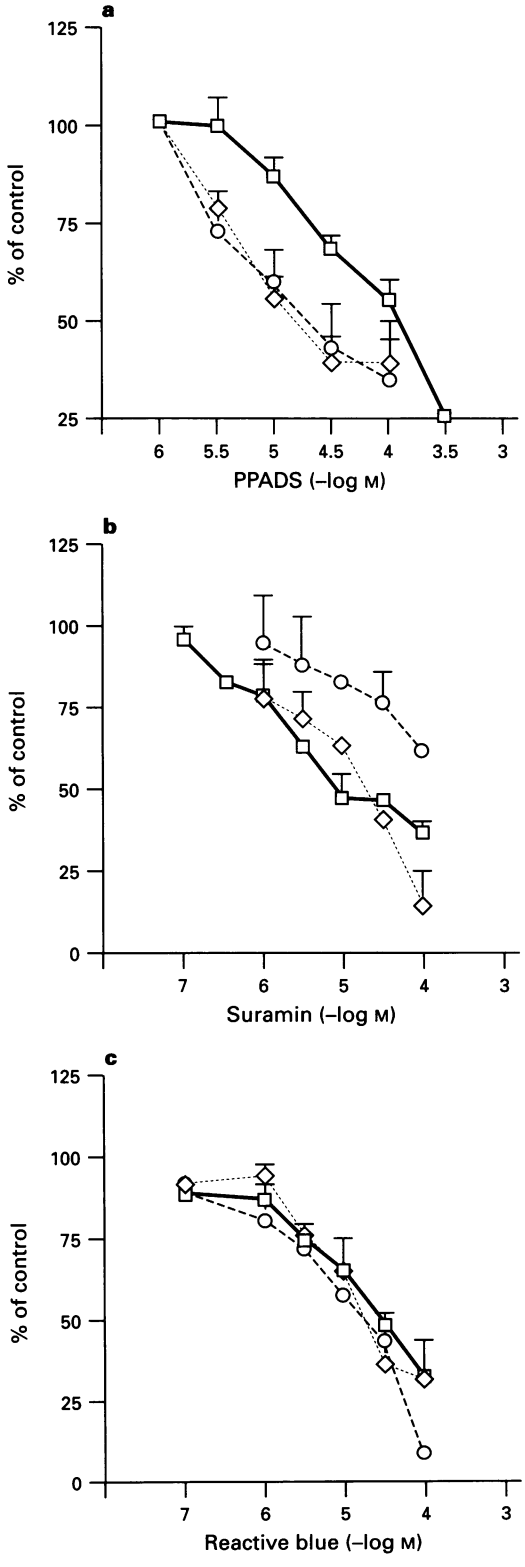

Figure 7 Effects of suramin, reactive blue and PPADS on ATP degradation in three cell lines. Inhibition curves are shown for PPADS (a), suramin (b) and reactive blue (c) on [$\gamma-^{32}$P]-ATP dephosphorylation by ecto-ATPase. Cells were pretreated for 20 min, with or without the indicated concentrations of antagonists, and then γ^{32}P release was measured 3, 10 and 10 min (CPAE, RAW 264.7 and C_6 glioma cells) after the addition of [$\gamma-^{32}$P]ATP. The data are expressed as the percentages of γ-^{32}P release in unpretreated control cells over the period indicated and are the mean ± s.e.mean from at least three independent experiments.

range influencing agonist responses (as above) caused dose-dependent inhibition of ecto-ATPase. The pIC_{50} value (IC_{50}: concentrations inducing 50% inhibition of ATP breakdown) were approximately 4.0, 4.8 and 4.7 for PPADS; 4.0, 4.4 and $>>4$ for suramin and 4.5, 4.7 and 4.7 for reactive blue in CPAE, C_6 glioma and RAW 264.7 cells, respectively.

Discussion

The present study shows that PPADS, suramin and reactive blue, over the same concentration-range, produce both P_2-purinoceptor/pyrimidinoceptor blockade and inhibition of ecto-ATPase in the three cell lines tested. CPAE has P_{2Y} and P_{2U} purinoceptors, the respective selective agonists for which are 2MeSATP and UTP (Chen *et al.*, 1996). In contrast, ATP is a non-selective agonist for both P_2 purinoceptor subtypes. RAW 264.7 cells have a pyrimidinoceptor with a 3 orders of magnitude higher affinity for UTP and UDP than for ATP (Lin & Lee, 1996), with ATP being incapable of inducing PI turnover at concentrations less than 300 μM. Rat C_6 glioma cells have a P_{2U} purinoceptor for which ATP has a 3 fold higher potency than UTP (Lin & Chuang, 1994). These three cell lines therefore provide useful systems for pharmacological characterization, including signalling cascades and selective antagonists for P_{2Y}, P_{2U} and pyrimidinoceptors. However, at the present time, due to the lack of specific ligands, it is still impossible to quantify these receptors. In addition, the present study also demonstrated the higher specific activity of ecto-ATPase (at least 45 fold) in CPAE than in C_6 and RAW 264.7 cells. This finding is consistent with the high levels of ecto-ATPase present in the artery biophase which could thus efficiently hydrolyse agonists that are enzyme substrates (Gordon *et al.*, 1989).

PPADS, in the concentration-range of 0.3 – 10 μM, has been described as a selective antagonist of P_{2X} purinoceptors in various preparations (Lambrecht *et al.*, 1992; Ziganshin *et al.*, 1993; Windscheif *et al.*, 1994). However, PPADS has also been reported to antagonize P_{2Y}-mediated responses with different potencies. For example, the classical P_{2Y}-mediated PLC activation seen in turkey erythrocytes is competitively inhibited by PPADS with a pA_2 of 5.9, while atypical P_{2Y}-mediated adenyl cyclase inhibition in C_6 glioma cells is not affected at concentrations of PPADS up to 100 μM (Boyer *et al.*, 1994). P_{2Y}-mediated relaxation of the rabbit urinary bladder (Ziganshin *et al.*, 1993) and rat mesenteric artery (Winscheif *et al.*, 1994; Ralevic & Burnstock, 1996) is antagonized by 10 μM PPADS. In agreement with the results for the vascular bed, PPADS inhibited the P_{2Y} purinoceptor-mediated PI response in CPAE at a concentration of 10 μM. However, increasing the concentration to 30 – 100 μM, PPADS produced no increased effect. The antagonistic effect on P_{2U} purinoceptors has not been previously studied. The UTP-induced PI response was unexpectedly and significantly potentiated by PPADS (3 – 100 μM) in a dose-dependent manner. These contradictory actions of PPADS on P_{2Y} and P_{2U} purinoceptors were further supported by the complete lack of any effect on the response to ATP, which is known to act on both P_{2Y} and P_{2U} purinoceptors in CPAE (Chen *et al.*, 1996). These results in CPAE may possibly explain why PPADS slightly antagonizes 2MeSATP-induced vasodilatation but is ineffective on UTP-induced vasodilatation in the rat perfused mesenteric arterial bed (Windscheif *et al.*, 1994).

The results with PPADS in C_6 glioma cells are consistent with those in CPAE; PPADS producing a decrease in the threshold concentrations of ATP (30 μM to 10 μM) and UTP (30 μM to 10 μM) and an increase in the PI response to 30 μM UTP. However, unlike the increased maximal response to UTP in CPAE, PPADS did not change the maximal response mediated by P_{2U} receptors in C_6 cells. In the case of pyrimidinoceptors, PPADS caused only a slight decrease in the response at lower concentrations of UTP in RAW 264.7 cells. From the totality of these effects of PPADS on the various receptor types and its inhibition of ecto-ATPase, we conclude that PPADS is a much more selective antagonist for P_{2Y} receptors than pyrimidinoceptors. Nonetheless, we cannot confidently rule out any antagonistic effect on P_{2U} purinoceptors, but, if present, the effect on P_{2U} receptors is much weaker than that on P_{2Y} receptors.

In terms of the IC_{50} for inhibition of ATP degradation, PPADS was demonstrated to be about 10 fold more potent in C_6 and RAW 264.7 cells than in CPAE. However, the potentiation of P_{2U} responses, and even its maximal response in the case of CPAE, was much greater than that in C_6 glioma. Several possible mechanisms, including receptor density and efficiency of signal coupling, could be involved. We suggest that the higher efficiency of enzymatic degradation in CPAE, compared with RAW 264.7 and C_6 cells, plays a more significant role in regulating agonist function, and even though partially obscured by PPADS would markedly elevate the biophasic concentrations of agonist for signalling. On the other hand, the limited degree of inhibition of the 2MeSATP and UTP responses in CPAE and RAW 264.7 cells, respectively, might result from a balance between receptor blockade and inhibition of agonist degradation.

Reactive blue has been claimed to be selective for P_{2Y} purinoceptors on the basis of studies in which the effect of enzymatic degradation of P_2 agonists was not taken into account (Fredholm *et al.*, 1994). However, in the present study, we found that reactive blue is also an inhibitor of ecto-ATPase over the same concentration-range used to test for receptor antagonism. In CPAE, the pA_2 values for reactive blue for the P_{2Y} (6.3) and P_{2U} (5.7) receptors suggest that it is approximately 4 fold more selective for P_{2Y} antagonism, assuming that 2MeSATP and UTP are equally susceptible to ecto-ATPase. The limited rightward shifts of the dose-response curves for 2MeSATP and UTP seen in the presence of 10 μM reactive blue might be attributed to the relative ability of reactive blue to inhibit the degradation of nucleotide analogues. In addition, reactive blue (1 – 30 μM) can also inhibit the UTP-induced PI response in RAW 264.7 cells with a pA_2 value of 5.8 ± 0.7, again suggesting its nonselective action on pyrimidinoceptors. Similar results were obtained with suramin, which is about 10 fold more selective for P_{2Y} (pA_2: 5.5) receptors than P_{2U} (pA_2: 4.4) receptors in CPAE, and is also a competitive inhibitor (pA_2: 4.8) for pyrimidinoceptors in RAW 264.7 cells.

In order to determine the potency order of these three antagonists on each receptor subtype, their effects on the agonist-induced PI response, and their degree of ecto-ATPase inhibition were compared. For the P_{2Y} receptors in CPAE, the percentage inhibition of ATP degradation at their respective pA_2 values was approximately 30% (pA_2: 5.5), 13% (pA_2: 6.3) and 15% (pA_2: 5) for suramin, reactive blue and PPADS respectively, suggesting an antagonistic potency order of reactive blue $\geqslant$ suramin > PPADS. On the other hand, for the P_{2U} receptors in CPAE, the values were 43% (pA_2: 4.4) and 22% (pA_2: 5.7) for suramin and reactive blue, respectively, indicating a potency order of reactive blue > suramin. With CPAE P_{2U} receptors, PPADS possibly lacks the antagonist effect based on the parallel leftward shift of UTP curves and the ecto-ATPase inhibition at 10 – 100 μM. For the pyrimidinoceptors in RAW 264.7 cells, the values were 15% (pA_2: 4.8) and 20% (pA_2: 5.8) for suramin and reactive blue; reactive blue therefore being about 10 fold more selective than suramin. Since PPADS showed weak inhibition of the UTP response, accompanied by greater enzyme inhibition, it is difficult to speculate on its antagonist affinity for pyrimidinoceptors. Furthermore, the inhibition of ATP degradation induced by these antagonists differs between cell types and we are not yet able to explain this.

In summary, PPADS, suramin and reactive blue have the dual properties of causing receptor blockade and of acting as ecto-ATPase inhibitors. The balance between these properties can lead to inhibition, potentiation or no effect on P_{2Y}-, P_{2U}- and pyrimidinoceptor-mediated PI responses. Of these, re-

active blue shows the highest antagonist affinity for these receptor subtypes, and PPADS is a weak antagonist of P_{2Y} receptors.

The study was supported by grants NSC86-2314-B002-304 from the National Science Council, Taiwan, and CRC85-T04 from IBMS.

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(Received November 20, 1995
Revised July 16, 1996
Accepted September 4, 1996)