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P_{2Y} Purinoceptor and Nucleotide Receptor-Induced Relaxation of Precontracted Bovine Aortic Collateral Artery Rings: Differential Sensitivity to Suramin and Indomethacin[1]

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ABSTRACT

We have examined a series of adenine nucleotides and UTP for their ability to cause relaxation of precontracted bovine aortic collateral artery rings. The overall rank order of agonist potency for relaxation was 2-methylthioadenosine 5′-triphosphate (2MeSATP) > adenosine 5′-O-(3-thiotriphosphate) (ATPγS) > UTP > ADP > ATP. These responses were endothelium-dependent. Interaction studies showed that responses to the selective P_{2Y} purinoceptor agonist 2MeSATP, and to ADP, were mediated by different receptors from those mediating responses to UTP. Suramin, a P_2 purinoceptor antagonist that binds to diverse sites for ATP, produced a concentration-dependent shift in the agonist concentration-effect curve to 2MeSATP, with a pK_B of 5.45 ± 0.15 and a slope of 0.94 ± 0.09. Suramin produced a small, nonsignificant shift in the UTP response curve and had little effect on responses to ATP. Indomethacin (2.8×10^{-6} M) had no effect on concentration-effect curves to UTP but almost abolished the relaxations produced by 2MeSATP and ADP. The concentration-effect curves to ATP and ATPγS showed a significant ($P < .05$) rightward shift in the presence of indomethacin. These results suggest the presence of separate P_{2Y} purinoceptor and nucleotide receptors mediating endothelium-dependent relaxations of bovine aortic collateral artery smooth muscle. ATP acts at both receptors, whereas ADP acts at only one (P_{2Y}). The effects of indomethacin show that these receptors differentially modulate the release of cyclooxygenase-derived mediators of relaxation.

The adenine nucleotides ATP and ADP have been shown to exert effects *via* P_2 purinoceptors in a wide variety of cells and tissue systems. Because of the lack of potent and selective antagonists, the classification of P_2 purinoceptors has been carried out with rank orders of agonist potency with analogues of ATP and ADP. Responses with the rank order α,β-methylene ATP = β,γ-methylene ATP > ATP = 2MeSATP characterize P_{2X} receptors, whereas the rank order 2MeSATP > ATP > α,β-methylene ATP = β,γ-methylene ATP is characteristic of P_{2Y} receptors (Burnstock and Kennedy, 1985).

Recently this classification has been broadened to include responses where the pyrimidine nucleotide UTP is as effective as or more potent than ATP, and where both 2MeSATP and α,β-methylene ATP are ineffective. These responses are mediated *via* so-called nucleotide receptors (Davidson *et al.*, 1990; Murrin and Boarder, 1992; O'Connor *et al.*, 1991; Pfeilschifter, 1990). Both P_{2Y} and nucleotide receptors are linked to the generation of inositol phosphates and to an increase in cellular calcium concentration *via* activation of phospholipase C (Allsup and Boarder, 1990; Boyer *et al.*, 1989; Fine *et al.*, 1989; Pirotton *et al.*, 1987). They are also linked to phospholipase D activation (Martin and Michaelis, 1989; Pirotton *et al.*, 1990; Purkiss *et al.*, 1993a; Purkiss and Boarder, 1992). In both porcine and canine major blood vessels, ATP has been shown to cause an endothelium-dependent relaxation of the associated smooth muscle through P_{2Y} purinoceptors (Houston *et al.*, 1987; Martin *et al.*, 1985).

Cultured endothelial cells have been shown to release both PGI_2 and EDRF by P_2 purinoceptor activation, which can produce relaxation of the underlying smooth muscle (De Nucci *et al.*, 1988; Mitchell *et al.*, 1992; Needham *et al.*, 1987). We and others have shown that cultured BAECs possess co-existing populations of P_{2Y} purinoceptor and nucleotide receptors linked to phospholipase C activation (Motte *et al.*, 1993; Wilkinson and Boarder, 1992; Wilkinson *et al.*, 1993). We have also shown that these receptors are differentially sensitive to the nonspecific P_2 purinoceptor antagonist suramin. That is, responses to

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ABBREVIATIONS: BAECs, bovine aortic endothelial cells; (EDRF), endothelium-derived relaxing factor; (PGI_2), prostacyclin; (2MeSATP), 2-methylthioadenosine 5′-triphosphate; (ATPγS), adenosine 5′-O-(3-thiotriphosphate); (8-SPT), 8-sulphophenyltheophylline; UTP, uridine 5′-triphosphate.

P_{2Y} stimulation are shifted in a concentration-dependent manner by suramin, whereas responses to nucleotide stimulation are not (Wilkinson *et al.*, 1993). These experiments confirm that in cultured BAECs, 2MeSATP and UTP are selective agonists at P_{2Y} purinoceptor and nucleotide receptors, respectively.

In the study reported here, we have extended the receptor subclassification from BAECs to an *in vitro* tissue preparation using the bovine aortic collateral artery. These arteries branch from the aorta and supply the intercostal muscles of the rib. This tissue was chosen because it was from the same species used to generate aortic endothelial cell cultures, and the endothelial cell layer and underlying smooth muscle were continuous with those of the aorta from which they branched. We have studied the effects of adenine nucleotides and UTP in producing endothelium-dependent relaxations of precontracted collateral artery rings, and we have investigated the tissue sensitivity to antagonism by suramin and the effects of the cyclo-oxygenase inhibitor indomethacin.

Materials and Methods

Tissue preparation. Fresh bovine aortas were collected from a local abattoir and transported on ice maintained in Krebs solution of the following composition (mM): NaCl, 117.56; NaH_2PO_4, 0.89; $NaHCO_3$, 25.0; $MgSO_4$, 1.18; D-glucose, 11.1; KCl, 5.36; $CaCl_2$, 2.55. The aortas were dissected free of fat and connective tissue, and the collateral arteries were then removed. The collateral artery was cut into 5-mm rings, and each ring was mounted horizontally on wire hooks in a 10-ml organ bath for recording under isometric conditions. The tissue was maintained in Krebs solution of the above composition at 37°C and gassed for the duration of the experiment with 95% O_2/5% CO_2. Tissues were set with an initial tension of 3 g and were allowed to equilibrate for 1 hr.

Experimental procedure. A number of preliminary experiments were carried out to establish the optimal conditions for the tissue's use in this study. a) Length-tension experiments were performed by contracting collateral artery rings with 80 mM KCl under varying loads to determine the optimal isometric tension to place the tissue under for subsequent experiments. b) Cumulative concentration-effect curves were carried out to the contracting agonist, phenylephrine, to determine a concentration that gave an approximately EC_{50-75} value. To establish whether the agonist sensitivity to phenylephrine remained unchanged over the course of the intended protocol, three consecutive cumulative concentration-effect curves were carried out at 90-min intervals. After each curve was completed, the agonist was washed out of the organ bath with three changes of the bathing Krebs solution. c) To establish the agonist stability of the relaxant response over the time of the intended protocol, three consecutive cumulative concentration-effect curves to ATP were carried out at 90-min intervals on rings precontracted with phenylephrine. d) To eliminate any possible interference due to hydrolysis of adenine nucleotides to adenosine, the selective P_1-receptor antagonist 8-SPT (Gustaffson, 1984) was included in all experiments. Studies were carried out to ensure that the concentration of 8-SPT chosen (3×10^{-4} M) did not influence the concentration-effect curves to ATP. e) The endothelium dependence of the relaxant response was tested by denudation with gentle rubbing of the intima to remove the endothelium. Relaxation responses to ATP, sodium nitroprusside and glycerol trinitrate were tested before and after endothelial denudation.

Analysis of the mechanism of agonist action. The following experimental protocol was used to determine the potencies and mechanisms of action of a number of purinergic agonists and UTP. After equilibration, the tissue was contracted with 80 mM KCl until the contraction stabilized. After washing and a 20-min re-equilibration period, the tissue was incubated with 3×10^{-4} M 8-SPT for 45 min. The tissue was then contracted with phenylephrine (3×10^{-6} M), and after the contraction had stabilized, a cumulative concentration-effect curve to ATP was constructed in 0.5 $\log_{10}$-unit increments. The tissue was washed and 8-SPT was readded, and after 90 min, a second cumulative concentration-effect curve to a test agonist was carried out. After the tissue was washed again and 8-SPT was once more readded, a third concentration-effect curve was performed either to the test agonist or to ATP. To determine whether there was any interaction between P_{2Y} and nucleotide receptor agonists, interaction experiments were carried out between UTP and either 2MeSATP or ADP. After the initial concentration-effect curve to ATP, a test curve to UTP was carried out. A third curve to UTP was then performed after the relaxation produced by a maximal concentration of either 2MeSATP or ADP, *i.e.*, 10^{-4} M. A series of experiments was conducted to assess the degree of antagonism of the relaxant response by suramin, a nonspecific P_2 purinoceptor antagonist. The first concentration-effect curve was to the test agonist, and subsequent curves were to the test agonist in the presence of suramin (3×10^{-5} M and 10^{-4} M), which was added to the organ bath 45 min before construction of the curve. To establish what proportion of the relaxation to each agonist was due to the products of cyclo-oxygenase, such as prostacyclin (PGI_2), the final curve to each test agonist was performed in the presence of indomethacin (2.8×10^{-6} M, a concentration found to be maximally effective in related studies), which was added to the organ bath at the same time as the last addition of 8-SPT.

Materials. The following drugs and chemicals were obtained from the sources indicated: 2-methylthio ATP (Research Biochemicals, Semat, Herts, U.K.), suramin (Bayer, U.K.), other chemicals (Sigma, Poole, or Fisons, Loughborough, U.K.). Indomethacin was initially made up in Na_2CO_3 (0.35 M) and was subsequently diluted with Krebs solution.

Data Analysis. Relaxations to each agonist were expressed as a percentage of the contraction produced by phenylephrine before the construction of each relaxant concentration-effect curve, *i.e.*, the distance measured between the plateau produced by the contraction and the base-line value. Data were analyzed using the Graph-Pad curve-fitting program, which fitted each curve to a logistic function of the form

$$E = \alpha[A]^n/[A_{50}]^n + [A]^n$$

in which α, $[A_{50}]$ and n are the asymptote, location and slope parameters, respectively, E is effect and [A] is agonist concentration.

Analysis of antagonism. In experiments where suramin was shown to produce parallel shifts, dose ratios (r) were calculated from the $[A_{50}]$ estimates from each curve and fitted to the equation (Arunlakshana and Schild, 1959)

$$\log_{10}(r - 1) = n \log_{10}[B] + pK_B$$

Accordance with simple competition was tested by comparing the Schild slope parameter n with unity by Student's t test.

Statistical analysis. Mean pA_{50} values were derived by taking the mean of the individual values from single tissues each from a different animal, where n = number of animals. A one-way analysis of variance was used to test for time and treatment effects on the computed estimates of α and n. Where differences were found, they were analyzed by Student's t test with $P < .05$ considered to be significant.

Results

Preliminary Studies. a) Bovine collateral artery rings were contracted with 80 mM KCl under increasing tension. The optimal contraction was obtained under a 3-g load, and in all subsequent experiments, tissues were set up under this initiation tension. b) Cumulative concentration-effect curves to the contracting agonist phenylephrine repeated at 90-min intervals did not significantly change over the experimental time course. The values of pA_{50} for the three consecutive curves were 5.85

± 0.11, 5.77 ± 0.15 and 5.53 ± 0.04, n = 3. c) Cumulative concentration-effect curves to ATP at 90-min intervals after phenylephrine precontraction showed no significant variation in the pA_{50} values, which were 4.39 ± 0.05, 4.29 ± 0.09 and 4.38 ± 0.11, n = 3. d) When 8-SPT was added, the pA_{50} values for consecutive ATP concentration-effect curves were 4.38 ± 0.11 (control) and 4.04 ± 0.09 (with 3×10^{-4} M 8-SPT), n = 3. e) Denudation of the endothelium did not affect relaxation responses to either glycerol trinitrate or sodium nitroprusside; however, responses to ATP were almost completely lost (data not shown).

Analysis of the mechanism of agonist action. Figure 1 shows a typical concentration-effect curve for relaxation to ATP after the vessel had been precontracted with 3×10^{-6} M phenylephrine. Figure 2 shows a series of concentration-effect curves for the individual agonists used in this study. Each agonist produced a sigmoidal curve with a calculated slope value of approximately unity. Both 2MeSATP and ADP were unable to produce full relaxation in this system when compared with the relaxations produced by ATP. Mean values for maximal relaxation (α) and pA_{50} and a comparison of relative agonist potencies are shown in table 1. The overall rank order of agonist potency for relaxation was 2MeSATP > ATPγS = UTP > ADP > ATP. The selective P_{2X} purinoceptor agonist β,γ-methylene ATP was ineffective, producing no relaxation (data not shown).

Figure 3 shows concentration-effect curves for UTP in the absence and presence of 2MeSATP (fig. 3A) and ADP (fig. 3B). The pA_{50} for the UTP control curve was 5.11 ± 0.09 (n = 18). A second concentration-effect curve was then constructed to

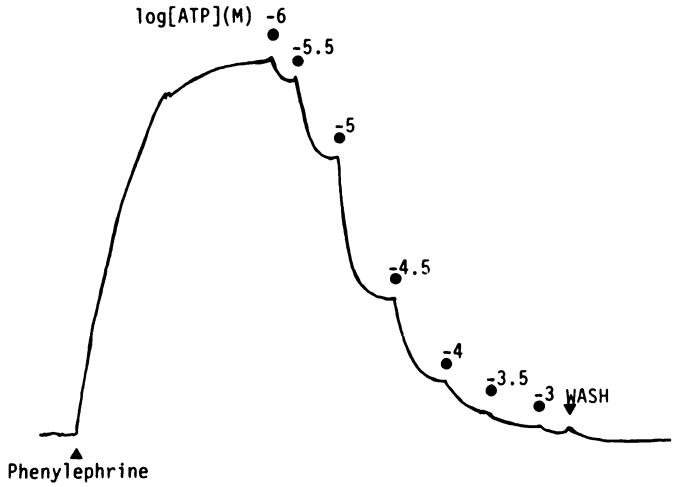

Fig. 1. Representation of a trace showing contraction to phenylephrine (3×10^{-6} M) and subsequent relaxation response to ATP in isolated endothelial intact bovine aortic collateral artery ring. This is a typical example from which the pooled response curves are derived.

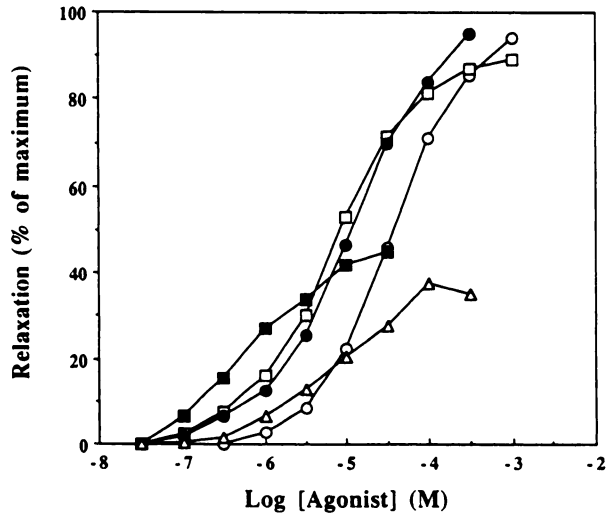

Fig. 2. Agonist concentration-effect curves for relaxation of precontracted bovine aortic collateral artery rings. Relaxation is expressed as a percentage of the maximal value, where 100% is the base-line value before contraction with phenylephrine. The data are generated from 6 to 18 separate experiments for each data point. Error bars are omitted for clarity; standard errors were less than 10% of the mean values. (■) 2MeSATP; (●) ATPγS; (□) UTP; (△) ADP; (○) ATP. See text for full names.

TABLE 1

Potencies of a number of P_{2Y} purinoceptor agonists and UTP in causing relaxation of phenylephrine-precontracted bovine aortic collateral artery rings

Agonist	n	pA_{50}	α^a	Relative Potency
2MeSATP	10	5.99 ± 0.08	45.0 ± 5.0	46
ATPγS	13	5.16 ± 0.09	95.0 ± 0.5	7
UTP	18	5.11 ± 0.09	89.0 ± 3.0	6
ADP	6	5.05 ± 0.07	37.5 ± 5.5	5
ATP	11	4.33 ± 0.08	94.5 ± 2.0	1

[a] Maximum responses (α) are expressed as a percentage of the initial contraction to phenylephrine (with 100% = maximal relaxation) for each individual E/[A] curve and represent the ability of an agonist to cause relaxation.

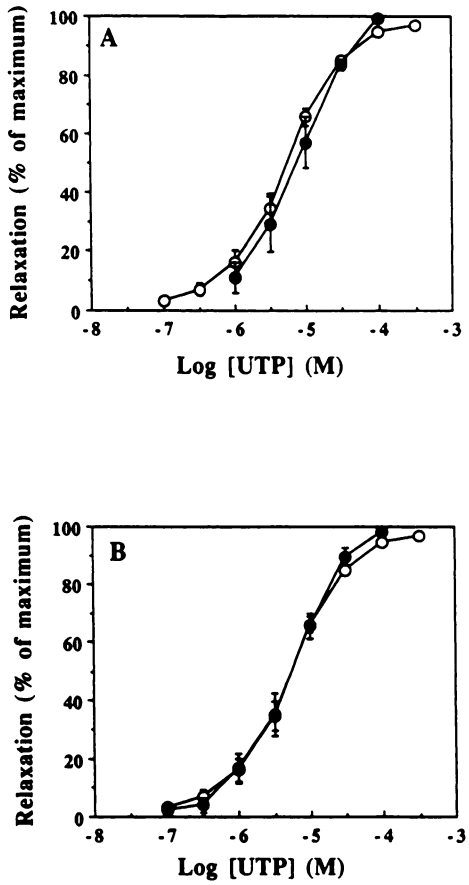

Fig. 3. Concentration-effect curves for UTP in the presence (●) or absence (○) of 10^{-4} M 2MeSATP (A) or 10^{-4} M ADP (B). Data points are the mean ± S.E.M. from five separate experiments.

UTP after the relaxation produced by the addition of a maximal concentration of either 2MeSATP or ADP (both at 10^{-4} M). There was no significant difference between the pA_{50} values for UTP in the absence and in the presence of either 2MeSATP or ADP (P = .05). The pA_{50} in the presence of 2MeSATP was 5.03 ± 0.10 (n = 5) and in the presence of ADP was 5.26 ± 0.08 (n = 5).

The effects of suramin on responses to 2MeSATP, ATP and UTP are shown in figure 4. Suramin produced a concentration-dependent shift in concentration-effect curves to 2MeSATP (fig. 4A). The pA_{50} values changing from 6.01 ± 0.11 (n = 5) to 5.04 ± 0.08 (in the presence of 3×10^{-5} M suramin, n = 5, P = .01) and to 4.50 ± 0.12 (in the presence of 10^{-4} M, suramin, n = 5, P < .01). Schild analysis of this data gave a pK_B for suramin of 5.45 ± 0.15 and a slope of 0.94 ± 0.09 (n = 5). The concentration-effect curves to ATP (fig. 4B) and UTP (fig. 4C) both showed small, nonsignificant rightward shifts caused by increasing concentrations of suramin. For ATP the pA_{50} values were 4.33 ± 0.08 (control, n = 11), 4.36 ± 0.19 (3×10^{-5} M suramin, n = 4) and 4.35 ± 0.13 (10^{-4} M suramin, n = 5). For UTP, the pA_{50} values were 5.08 ± 0.18 (control, n = 6), 4.79 ± 0.21 (3×10^{-5} M suramin, n = 5) and 4.64 ± 0.06 (10^{-4} M suramin, n = 5).

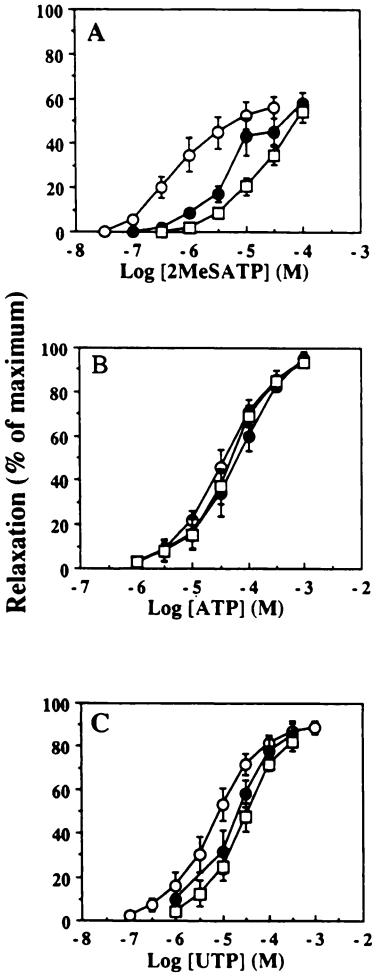

Fig. 4. Effects of suramin on relaxant responses to 2MeSATP (A), ATP (B) and UTP (C). Concentration-response curves to each agonist were constructed in the presence of zero (○), 3×10^{-5} M (●) or 10^{-4} M (□) suramin. Data points are the mean ± S.E.M. from four to five separate experiments.

Construction of concentration-effect curves after incubation of the tissue with indomethacin (2.8×10^{-6} M) showed clear differences between the agonists. The responses to both 2MeSATP and ADP were virtually abolished in the presence of indomethacin (figs. 5A and B), whereas the response to UTP was unaffected (fig. 6A). The pA_{50} values for both ATP (fig. 6B) and ATPγS (fig. 6C) were significantly shifted to the right in the presence of indomethacin. The pA_{50} value for ATP changed from 4.33 ± 0.08 (n = 11) to 3.95 ± 0.10 (n = 11; P = .01), and the pA_{50} value for ATPγS changed from 5.16 ± 0.09 (n = 13) to 4.78 ± 0.07 (n = 8; P < .05).

Discussion

Adenine nucleotides have been shown to release both EDRF and prostacyclin from cultured endothelial cells by stimulating P_{2Y} purinoceptors (Forstermann *et al.*, 1991; Mitchell *et al.*, 1992; Needham *et al.*, 1987). These agonists have also been shown to produce endothelium-dependent relaxations in a number of vascular smooth preparations (Dainty *et al.*, 1991; Houston *et al.*, 1987; Martin *et al.*, 1985). In previous studies we have demonstrated the presence of phospholipase C-linked P_{2Y} purinoceptors and nucleotide receptors on vascular endothelial and neuronal cells in culture (Murrin and Boarder, 1992; Purkiss *et al.*, 1993a; Wilkinson *et al.*, 1993). In particular, we and others have shown that cultured bovine aortic endothelial cells possess coexisting populations of these receptors (Motte *et al.*, 1993; Wilkinson and Boarder, 1992; Wilkinson *et al.*,

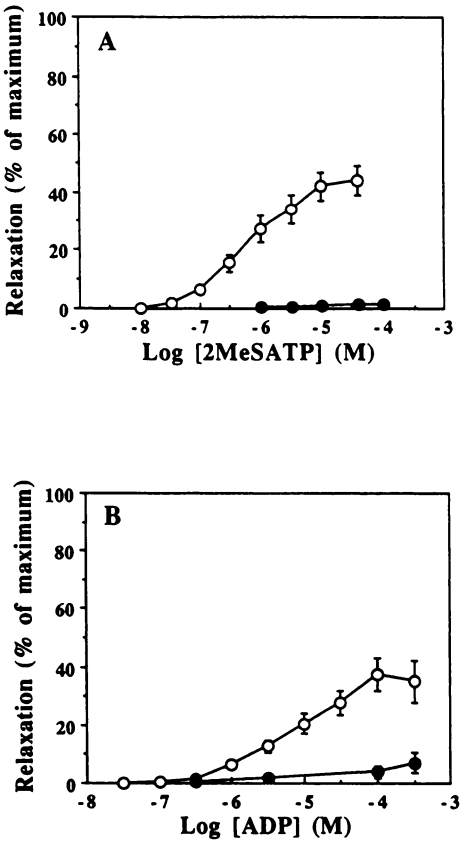

Fig. 5. Effect of indomethacin on relaxant responses to 2MeSATP (A) and ADP (B). Concentration-response curves to each agonist were constructed in the absence (○) or presence (●) of indomethacin (2.8×10^{-6} M). Data points are the mean ± S.E.M. from four to five separate experiments.

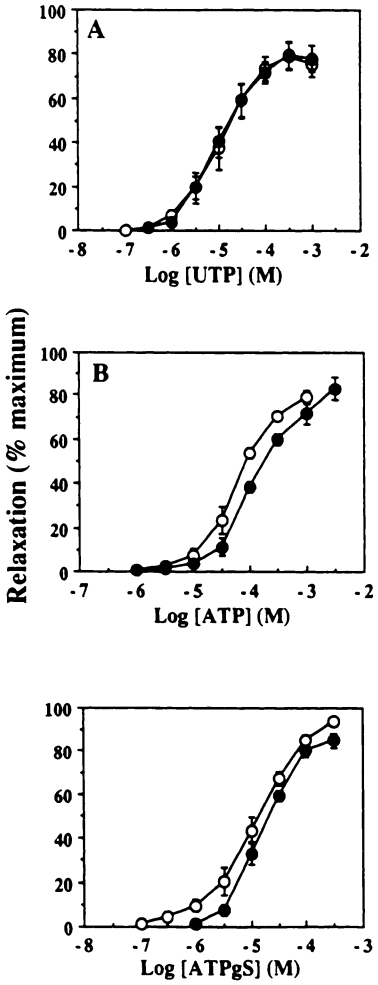

Fig. 6. Effect of indomethacin on relaxant responses to UTP (A), ATP (B) and ATPγS (C). Concentration-response curves to each agonist were constructed in the absence (○) or presence (●) of indomethacin (2.8×10^{-6} M). Data points are the mean ± S.E.M. of 8 to 13 separate experiments.

1993). In the present study we have extended this characterization to an *in vitro* tissue preparation.

The results reported here show that bovine aortic collateral artery rings that had been precontracted with the α_1-adrenoceptor agonist phenylephrine underwent endothelium-dependent relaxations when stimulated with both adenine nucleotides and the pyrimidine UTP. The agonist profile of this relaxation is consistent with the presence of both P_{2Y} purinoceptors and nucleotide receptors, which are responsive to both UTP and ATP (O'Connor *et al.*, 1991). The specific P_{2Y} purinoceptor agonist 2MeSATP, although it is the most potent agonist tested, did not cause so large a relaxation as ATP. This property of 2MeSATP, *i.e.*, low maximal response (α) but high potency, has been observed in a number of other cases, such as in stimulating prostacyclin production in cultured pig aortic endothelial cells (Needham *et al.*, 1987) and causing relaxations of precontracted rat aortic rings (Dainty *et al.*, 1991; O'Connor *et al.*, 1991). This may be indicative of the presence of a heterogeneous population of ATP-sensitive receptors—that is, P_{2Y} purinoceptors and nucleotide receptors. An alternative explanation is that 2MeSATP was acting as a partial agonist at a common receptor. We have previously reported experiments that measured total [^{3}H] inositol (poly)phosphate accumulation in BAECs and addressed this issue. We found that responses to UTP in the presence of 2MeSATP were additive over the range of the concentration-effect curve to UTP. This indicated that their responses were mediated *via* separate receptor populations (Wilkinson and Boarder, 1992; Wilkinson *et al.*, 1993), a result consistent with a report from others (Motte *et al.*, 1993). In the present report, we have shown that there was no interaction between the relaxations caused by 2MeSATP and those caused by UTP. A concentration-effect curve to UTP was constructed after the relaxation produced by a concentration of 2MeSATP that should have produced a 100-fold shift if both were acting at the same receptor. There was no alteration in either the slope or the pA_{50} value of the UTP curve. This confirms that the responses to these two agonists were mediated *via* separate receptors and that 2MeSATP was not acting as a partial agonist through a single receptor population in this system. A similar situation was observed with ADP. The presence of 10^{-4} M ADP had no effect on the concentration-effect curves for UTP. This indicates that ADP was not acting at the same receptor population as UTP.

We have also observed in BAECs that there was no additivity between the maximal concentration of ATPγS and UTP, showing that these agonists are stimulating total [^{3}H]inositol (poly)phosphate accumulation through a common receptor population, *i.e.*, a nucleotide receptor. Similar observations have also been described in BAECs for the interaction of maximal concentrations of ATP and UTP (Motte *et al.*, 1993). These experiments were not possible in this instance because UTP, ATP and ATPγS all produced full relaxations.

The conclusion that 2MeSATP and UTP were acting at separate receptors to generate the relaxation response was further investigated by use of the nonspecific P_2 purinoceptor antagonist suramin (Dunn and Blakely, 1988). Suramin has been shown to antagonize both P_{2X} and P_{2Y} responses in vascular smooth muscle (Hoyle *et al.*, 1990; Leff *et al.*, 1990). In BAECs we have shown suramin to have a differential effect on responses mediated by P_{2Y} purinoceptor and nucleotide receptor stimulation of phospholipase C. That is, suramin produced a concentration-dependent rightward shift of concentration-effect curves to 2MeSATP but not to UTP. In BAECs suramin produced a significant rightward shift of concentration-effect curves to 2MeSATP ($pK_B = 5.6 \pm 0.4$) but not to UTP (Wilkinson *et al.*, 1993). In this study we have described identical effects of suramin to those observed in BAECs. To be specific, suramin produced a significant concentration-dependent shift of concentration-effect curves to 2MeSATP ($pK_B = 5.45 \pm 0.15$) but not of such curves to UTP. Concentration-effect curves to ATP were also unaffected by treatment with suramin. One possible explanation is that the effects of ATP in this tissue occurred predominantly *via* the nucleotide receptor. However, it is also possible that ATP is acting at both receptor populations, but that the effects of suramin on the portion of the ATP response mediated *via* the P_{2Y} receptor are too small to be reliably seen as a shift in the curve.

Endothelium-dependent relaxations of vascular smooth muscle have been shown to be caused by the release of mediators upon agonist stimulation. These include EDRF and prostanoids such as prostacyclin (PGI_2). Both cause smooth muscle relaxation (Vane *et al.*, 1987). We have modulated the relaxations produced by P_{2Y} purinoceptor and nucleotide agonists by preincubating with indomethacin, an agent that inhibits cyclo-oxygenase and hence prostacyclin formation. In the presence of

indomethacin, responses to both 2MeSATP and ADP were almost totally inhibited, whereas responses to UTP were unaffected. These results suggest that the relaxations elicited by P_{2Y} purinoceptor stimulation were largely linked to cyclo-oxygenase products such as prostacyclin. This is apparently not so for the relaxations elicited by UTP *via* the nucleotide receptor, which may be predominantly caused by EDRF formation. Significantly, the actions of ATP and ATPγS were partially affected by indomethacin; their E/[A] curves were shifted to the right, which is consistent with earlier evidence that these agonists produce both prostacyclin and EDRF (De Nucci *et al.*, 1988) and hence interact with both receptor populations. This is an important point. It indicates that both ATP and UTP were able to interact with a common receptor and that this is, therefore, a nucleotide receptor (O'Connor *et al.*, 1991).

The conclusion that the relaxations produced by ADP were predominantly indomethacin-sensitive might appear to be in conflict with the observations of Mitchell *et al.* (1992), who have shown that in cultured BAECs, ADP causes a larger stimulation of EDRF than does bradykinin, whereas both stimulate prostacyclin production. However, they do not report a comparison between P_{2Y} and nucleotide agonists with respect to their ability to stimulate EDRF and prostacyclin production. Furthermore, on luminal perfusion of intact rabbit aortas, ADP was able to elicit only a small release of EDRF compared with acetylcholine (Mitchell *et al.*, 1992). It is also pointed out by De Nucci *et al.*, (1988) that the process of collagenase digestion to produce endothelial cell cultures may effect receptor expression. It is possible that in our experiments, ADP does indeed lead to EDRF production (consistent with Mitchell *et al.*, 1992) but that this EDRF production is small and relatively ineffectual in causing relaxation, compared to that elicited by UTP.

Two lines of evidence presented here are consistent with the conclusion that ADP produces its relaxant response by acting at the P_{2Y} receptor and not at the nucleotide receptor. First, ADP failed to shift the concentration-response curve for UTP. Second, the effect of ADP, like that of 2MeSATP but unlike that of UTP, was inhibited by indomethacin. This conclusion, that ADP act selectively at P_{2Y} receptors, was consistent with our earlier report that ADP was similarly selective in stimulating inositol 1,4,5-trisphosphate formation in cultured BAECs (Purkiss *et al.*, 1993b). Combined with our earlier report on BAECs (Wilkinson *et al.*, 1993) this body of work shows that ADP and ATP have different profiles of action on aortic endothelium.

In conclusion, these results show that the relaxations produced by adenine nucleotides and UTP of precontracted bovine aortic collateral artery rings are mediated *via* two coexisting receptor populations (P_{2Y} and nucleotide receptors), both activated by ATP but only one (P_{2Y} receptors) activated by ADP. These are the same receptors that have been described on BAECs in culture (Wilkinson and Boarder, 1992; Wilkinson *et al.*, 1993; Motte *et al.*, 1993). This subdivision is further confirmed by the differential effects of suramin and of indomethacin. The effects of indomethacin may indicate separate coupling of these receptors to their effector mechanisms. That is, the relaxation caused by activation of P_{2Y} purinoceptors may be principally mediated by products of cyclo-oxygenase metabolism, whereas the nucleotide response may be caused mainly by EDRF.

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