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Pharmacological dissociation of UTP- and ATP-elicited contractions and relaxations in isolated rat aorta

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Abstract

Effects of UTP have been described in many tissues, but it is not clear whether these are due to purinoceptors. Specific receptors for UTP, 'pyrimidinoceptors', and 'nucleotide receptors' have also been proposed. We pharmacologically characterized the receptors involved in the ATP- and UTP-induced contraction under basal tone and the relaxation of raised tone elicited by noradrenaline in isolated rat aorta. The rank order of potency for the agonists for the contraction was α,β-methylene ATP > > ATP, and the desensitization by α,β-methylene ATP suggests that ATP contractions were mediated via P_{2X} purinoceptors which were located on the vascular smooth muscle. The rank order of potency of the agonists for relaxation was 2-methyl-thio ATP > > ATP, which is suggestive of a P_{2Y} purinoceptor. However, the relaxation seems to be unrelated to the classical P_{2Y} subtype and a heterogeneous population of purinoceptors might therefore exist. The evidence comes from the distinct location and the different pharmacological effect of reactive blue 2 on 2-methyl-thio ATP and ATP receptors. 2-Methyl-thio ATP produced an endothelium-dependent relaxation while ATP-induced relaxation was produced via endothelium-dependent and endothelium-independent mechanisms, unrelated to adenosine receptors. It is unlikely that UTP-induced contractions and the endothelium-dependent relaxation were produced via purinoceptors since the pharmacology is not consistent with that of the classical P_2 purinoceptors studied. Furthermore, UTP-sensitive receptors showed a pharmacological property that was also distinct from that of the 'nucleotide' or P_{2U} receptor reported. The results suggest the presence of a heterogeneous population of purinoceptors and pyrimidinoceptors pharmacologically different from the receptors for ATP.

Keywords: Nucleotide receptor; ATP receptor; UTP receptor; Aorta, rat; Endothelium; Smooth muscle, vascular

1. Introduction

Extracellular effects of purine nucleotides have been reported in many different tissues and species (Gordon, 1986; Williams, 1987). These effects are considered to be mediated via different subtypes of cell surface receptors, termed purinoceptors, P_1 and P_2 purinoceptors. These were based on the rank order of potency of the response to purine nucleotides and their structural analogs (Burnstock, 1978). Purine nucleotides have been identified as neurotransmitters, cotransmitters and neuromodulators (Su, 1983; Von Kügelgen and Starke, 1991; Illes and Nörenberg, 1993). However, despite this characterization the physiological and pathological significance of the nucleotides is still not yet clear.

UTP has been shown to be involved in many extracellular effects, such as in modulating the tone of different vascular smooth muscles, in which it is more effective in cerebral than in peripheral vessels (Urquilla, 1978). Although the biological significance of UTP is less well known than that of the purine nucleotides, it has been associated with some pathological conditions such as the contraction of cerebral vessels (Urquilla, 1978; Shirasawa et al., 1983), it is an effective chloride secretagogue in airway epithelium (Mason et al., 1991) and it is involved in many other biological actions (Seifert and Schultz, 1989).

The characteristics of UTP responses suggest that they are mediated through receptors. The fact that UTP can be more potent than ATP in eliciting some

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biological effects, and that the pharmacology of these receptors does not fit into the classification of P_2 purinoceptors (Burnstock and Kennedy, 1985), supports the idea of different types of receptors, a putative 'pyrimidinoceptor' (Seifert and Schultz, 1989; Saïag et al., 1990), or at least allows us to consider the possibility that the effects of UTP could be mediated via receptors distinct from the classic P_2 purinoceptors. However, the existence of specific receptors is not clear since some effects induced by UTP are elicited via purinoceptors (Von Kügelgen et al., 1989). The term 'nucleotide' receptor (Davidson et al., 1990) or P_{2U} purinoceptor (Dubyak, 1991) has been used to refer to this 'atypical' receptor, as it is not specific to the pyrimidine nucleotide and is also ATP-sensitive.

Evidence that UTP is more potent than ATP in releasing intracellular Ca^{2+} in vascular smooth muscle (Sánchez-Fernández et al., 1993) led to the study of the responses to both nucleotides in isolated rat aorta. The aim was to pharmacologically differentiate and characterize the receptors involved on the basis of the purposed classification of P_2 purinoceptors (Fredholm et al., 1994). The approach adopted was to perform a functional study, with isolated rat aorta, of the effects of agonists and antagonists of P_2 purinoceptors described in vascular smooth muscle, as no antagonist of the UTP receptor is yet known. In addition, the role of the endothelium on the contraction and relaxation was investigated in order to determine whether the receptors are located on smooth muscle or endothelial cells.

2. Materials and methods

2.1. Preparation of the tissues

2-month-old male Wistar rats were used. These were killed by decapitation and the descending thoracic aorta was carefully removed for further experimentation. The aorta was placed in refrigerated (4°C) Krebs-bicarbonate solution having the following composition (mM): NaCl, 118; KCl, 4.75; $CaCl_2$, 2.5; KPO_4H_2, 1.19; $NaCO_3H$, 25; $MgSO_4$, 1.2 and glucose, 11. The vessels were cleaned of adherent connective tissue and cut into helical strips (about 0.3×2 cm) with microscissors. The cutting angle was 45°. Special care was taken to avoid contact with the luminal surface of the blood vessel in order to preserve the endothelial layer. The strips were mounted in 6 ml organ baths containing Krebs solution at 37°C and bubbled continuously with 95% O_2 and 5% CO_2 mixture. The tissues were allowed to equilibrate for 120 min under basal tension of 2 g before experimentation. The bath solution was replaced every 30 min during the equilibration period. Isometric tension was recorded by force-displacement transducers (UF1) on an OmniScribe D-5000 polygraph. After the equilibration period, each vessel segment was contracted by 0.1 μM of noradrenaline to ensure stabilization of the smooth muscle. The tissues were maintained in fresh Krebs solution until the original baseline was reached. The vessels were contracted by 30 nM noradrenaline, then relaxed with acetylcholine, 30 nM to 10 μM. The preparations showing relaxation to acetylcholine were the only ones used as they had an intact endothelium (Furchgott and Zawadzki, 1980).

2.2. Experimental procedures

Concentration-response curves for contractions elicited by UTP (3 μM to 1 mM), ATP (0.1–1 mM) and α,β-methylene ATP (1–30 μM) were made and the following parameters were studied: desensitization, the effect of antagonists and the role of the endothelium. To study the possible desensitization to the agonists, single concentrations of nucleotides were added at 10-, 30- and 60-min intervals and, cumulative and non-cumulative dose-response curves were made. The antagonists were added 30 min before the second cumulative dose-response curve of UTP was made or single increasing concentrations were added before the non-cumulative curve of ATP was made. The endothelium was removed by mechanical rubbing of the endothelial layer of aorta strips in the organ bath. In this way, it is possible to remove the endothelium, as indicated by the absence of relaxation to acetylcholine, without causing functional damage to the vascular smooth muscle (Molina et al., 1992).

The relaxation elicited by the nucleotides and the analogs was studied in preparations with an increased tone (elicited by 30 nM of noradrenaline) in a cumulative way. The experimental procedure followed to study desensitization, antagonism and the role of the endothelium in the relaxation was the same as that used to study the contractions.

In some aorta strips the endothelium was removed before the tissue was mounted in the organ bath.

2.3. Drugs

UTP (uridine 5′-triphosphate, sodium salt), ATP (adenosine 5′-triphosphate, disodium salt), adenosine, reactive blue 2, noradrenaline (L-arterenol bitartrate) and acetylcholine (acetylcholine chloride) were purchased from Sigma; α,β-methylene ATP (dilithium salt), 2-methyl-thio ATP (tetrasodium salt) and 8-*p*-sulphophenyl theophylline were from Research Biochemicals Incorporated (RBI) and adenosine deaminase was from Boehringer Mannheim. All were dissolved in bidistilled water to prepare a stock solution. These stock solutions were preserved at −16°C. The appropriate dilutions were prepared daily and kept on ice during the experiment.

2.4. Statistical methods

The contractions elicited by UTP, ATP and α,β-methylene ATP were expressed as percentages of the maximal response to noradrenaline, while relaxation was expressed as a percentage of the maximal relaxation (100% relaxation when baseline was reached) induced in preparations treated with noradrenaline (30 nM). The maximum effect and the EC_{50} of UTP and ATP were not calculated since maximal contractions were not obtained. The results are expressed as means ± standard error of the means (±S.E.M.) for $n \geq 6$ experiments. Data were compared by analysis of the Student's t test – paired or unpaired – for individual comparison. $P < 0.05$ was considered significant.

3. Results

3.1. UTP and ATP contractile responses on basal vascular tone

Characteristics of the response

Although both UTP and ATP caused contraction, the responses were different (Fig. 1A). UTP caused a long-lasting sustained contractile response and produced oscillations in some cases at high concentrations. ATP caused a fast transient contraction, which decreased almost immediately even in the continued presence of the drug. The presence or absence of endothelium did not modify the characteristics of the responses to these nucleotides.

Dose-response curves

The maximum concentration tested for both nucleotides was 1 mM in order to avoid effects unrelated to receptor binding, although this concentration appeared to be submaximal for both nucleotides. At the range of assayed doses, UTP was more potent than ATP in inducing contractions (Fig. 1B). UTP (3 μM to 1 mM) contracted the isolated rat aorta in a dose-dependent way and the magnitude of the response was similar in cumulative or non-cumulative curves, being 62.25 ± 8% at the highest concentration assayed, in respect to the maximum contraction obtained with noradrenaline. With 10-min washout intervals between consecutive concentrations the response was reproducible (Table 1) and desensitization to UTP contractions and curves was not observed.

The effects of ATP (0.1–1 mM) on contraction were also studied. The magnitude of the response at the highest concentration assayed was 27.7 ± 2.9% of the maximum obtained with noradrenaline. It was not possible to obtain cumulative dose-response curves with this nucleotide due to the fact that the contraction was transient and because there was apparent desensitization. ATP (0.3 mM) was added every 10, 30 and 60 min. The response to ATP decreased following repeated exposure every 10 min (Table 1) and at 30 min (data not shown). With 60-min intervals between challenges, however, the response was reproducible. As a

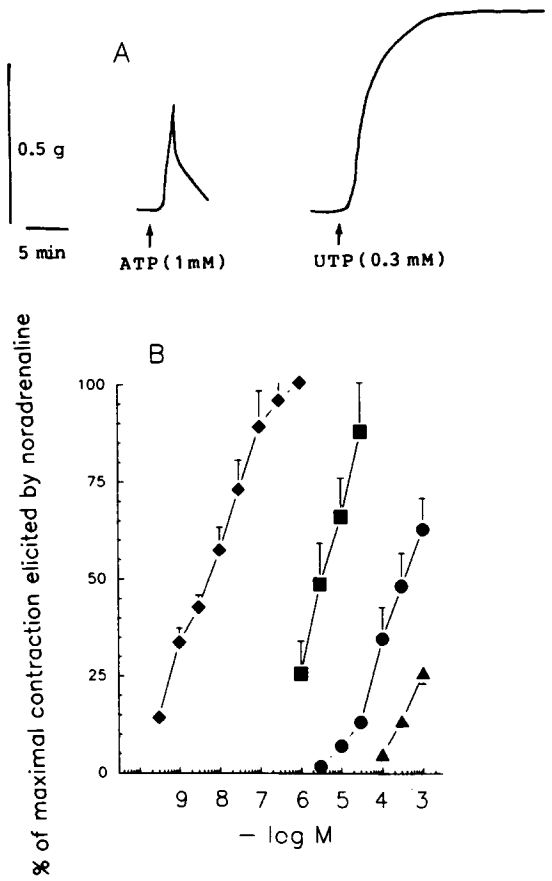

Fig. 1. (A) Isometric records of transient contractions in response to ATP (1 mM) and sustained contraction in response to UTP (0.3 mM) in isolated preparations of different rat aorta strips. Horizontal bar indicates 5 min, and vertical bar 0.5 g. (B) Cumulative concentration-response curves for noradrenaline (0.3 nM to 1 μM, ♦), α,β-methylene ATP (1–30 μM, ■), UTP (3 μM to 1 mM, ●) and non-cumulative concentration-response curve for ATP (0.1 to 1 mM, ▲). The values are expressed as percentages of the maximum contraction to noradrenaline. Vertical bars represent S.E.M. from at least eight experiments.

Table 1
Values of the magnitude of the contraction ± S.E.M. elicited by single concentrations (0.3 mM) of ATP and UTP in isolated rat aorta

Nucleotide	Dose	Exposure			
	mM	1st	2nd	3rd	4th
ATP ($n = 6$)	0.3	100	72.4 ± 10[a]	51.27 ± 14[b]	44.43 ± 13[b]
UTP ($n = 8$)	0.3	100	99.02 ± 11	114.25 ± 21	124.68 ± 29

The data are expressed in respect to those obtained for the first exposure (100%). There were 10-min intervals between exposures. [a]$P < 0.05$ and [b]$P < 0.01$ versus the first exposure.

result, the dose-response curves were made by adding the different concentrations of ATP in a non-cumulative way. Washout was followed, as soon as the maximum tension was reached, by 60-min intervals between each successive concentration in order to avoid desensitization.

Role of endothelium

In order to evaluate the location of the receptors the concentration-response curves for UTP and ATP were repeated in the same aorta strips after the endothelium was removed by mechanical rubbing. The magnitude of the contraction in response to UTP and ATP with intact endothelium was not significantly different when the endothelium was removed.

Pharmacological characterization of the receptor

To characterize the receptor involved in the UTP- and ATP-induced contractions, the effects of agonists and antagonists of the P_2 purinoceptors, P_{2X} and P_{2Y}, were studied. Thus, α,β-methylene ATP as a selective agonist of P_{2X} purinoceptors was used. This drug was also tested after prolonged exposure because it induces desensitization of P_{2X} purinoceptors. Reactive blue 2 was used as an antagonist of P_{2Y} purinoceptor.

α,β-Methylene ATP (1 to 30 μM) contracted the isolated rat aorta in a dose-dependent manner. Its potency was higher than that of UTP and ATP (Fig. 1B). The rank order of potency of the agonists was α,β-methylene ATP > > UTP > ATP. Desensitization of the response occurred after 1 h of exposure to α,β-methylene ATP (30 μM). Preincubation abolished subsequent responses to α,β-methylene ATP and to 0.1 and to 0.3 mM ATP (Fig. 2B). Contractile responses to 1 mM ATP were also significantly reduced. α,β-Methylene ATP dit not modify the contractile response induced by UTP (Fig. 2A).

Reactive blue 2 (100 μM) did not affect the dose-response curves for UTP, but significantly increased the contraction in response to ATP (1 mM) above the control (Fig. 3).

3.2. UTP- and ATP-induced relaxation in vascular raised tone

Dose-response curves

Noradrenaline 30 nM was used to raise the tone in isolated aorta strips and the relaxation effects of cumulative concentrations of UTP and ATP were studied. Both nucleotides caused a sustained dose-dependent relaxation of the vessel. Cumulative addition of both nucleotides UTP (3 μM to 0.3 mM) and ATP (1 μM to 0.3 mM) produced relaxation in a dose-dependent way. ATP at concentrations above 0.1 mM produced a small transient contraction that preceded the relaxation. UTP at concentrations above 0.3 mM induced tissue contractions. No significant differences were observed in the relaxation produced by these nucleotides, and the maximum relaxation obtained was similar to that of the acetylcholine (Fig. 5A). These dose-response curves were reproducible when there was a 1-h interval between the first and second curves.

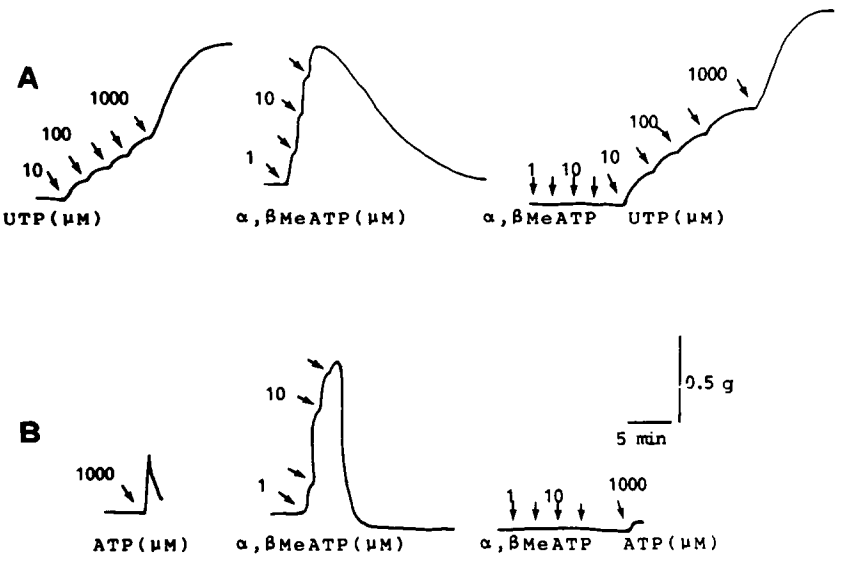

Fig. 2. (A) Isometric recordings of cumulative concentration-response curves for contractions induced by UTP (10–1000 μM) and α,β-methylene ATP (1–30 μM). As shown, the prolonged exposure to α,β-methylene ATP at the highest concentration (30 μM) after 1 h (and previous washout) abolished the response to a subsequent challenge with the same agent without affecting UTP-induced contractions. (B) The contraction elicited by ATP was markedly reduced by exposure to this analog of ATP in following the same experimental protocol. The recordings represent samples of four experiments with identical results.

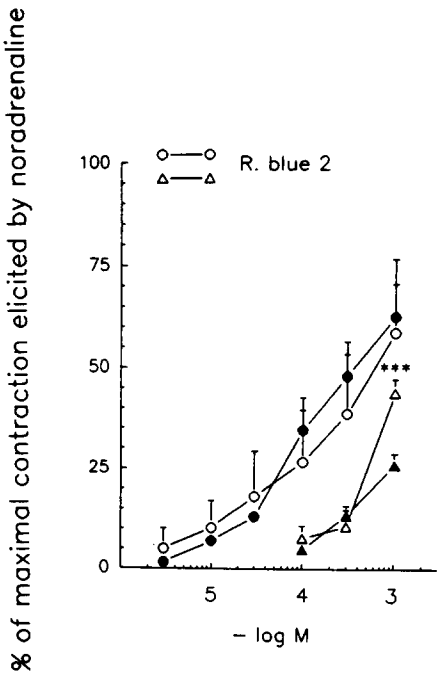

Fig. 3. Concentration-response curves for contractions induced by UTP (●) and ATP (▲), in isolated rat aorta strips, and in the presence of reactive blue 2 (100 μM) (△) by ATP and (○) by UTP. The values are expressed as percentages of contraction elicited by noradrenaline. Vertical bars represent S.E.M. from at least eight experiments. $^{***}P < 0.001$ using Student's t-test for paired data.

Role of endothelium

The role of the endothelium in the relaxation induced by UTP, ATP and 2-methyl-thio ATP (agonist of P_{2Y} purinoceptors) of rat aorta precontracted by noradrenaline was studied. The effects of the nucleotides were examined before and after mechanical removal of the endothelium.

In the presence of endothelium, UTP (3 μM to 0.3 mM), ATP (1 μM to 0.3 mM) (Fig. 4A) and 2-methyl-thio ATP (0.1–3 μM) (Fig. 5A) relaxed the rat aorta strips. In the absence of endothelium, which was removed by mechanical rubbing, the relaxation in response to UTP (Fig. 4B) and 2-methyl-thio ATP was abolished. However, ATP produced an endothelium-independent relaxation (Fig. 4B), although the concentration range in which ATP was potent (0.1–10 mM) was different from the one observed in the presence of endothelium. Thus, the dose-response curve was shifted to the right (Fig. 5B).

Receptor characterization

To characterize the endothelial receptor involved in the relaxation elicited by UTP and ATP, the effects of 2-methyl-thio ATP, reactive blue 2 (agonist and antagonist of P_{2Y} purinoceptors, respectively) and α,β-methylene ATP (on P_{2X} purinoceptors) were studied. Furthermore, the pharmacology of the endothelium-independent receptor for ATP was also studied.

In rat aorta strips with an intact endothelium, 2-methyl thio ATP (0.1–3 μM) was equally effective as acetylcholine in inducing relaxation. The rank order of potencies for the nucleotides was 2-methyl-thio ATP > > UTP = ATP.

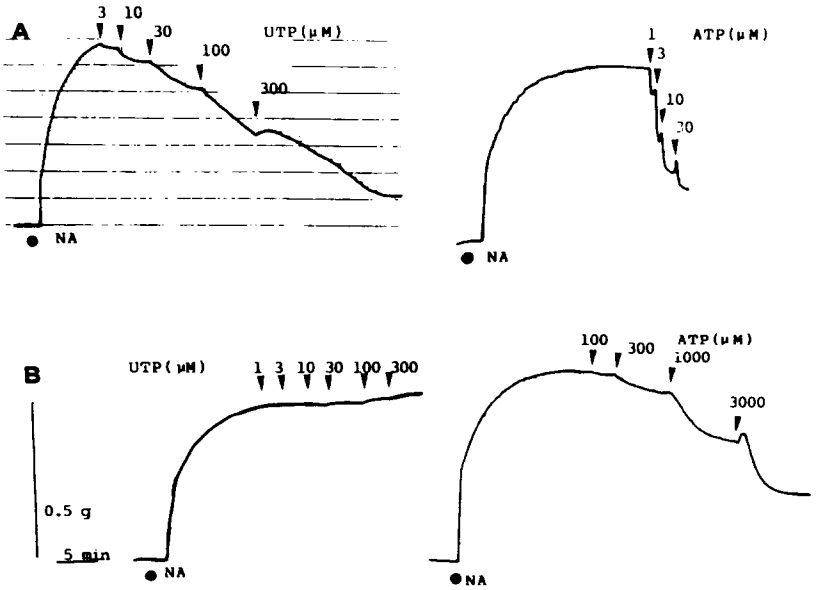

Fig. 4. Recordings of contraction and raised tension of isolated strip preparations of rat aorta by noradrenaline (30 nM). (A) In the presence of endothelium, UTP and ATP elicited relaxation in a dose-dependent way. (B) The absence of endothelium abolished the UTP-induced relaxation. However, ATP induced an endothelium-independent relaxation. The recordings represent samples of at least six experiments with identical results made with different rat aorta strips.

α,β-Methylene ATP (1–10 μM) induced oscillations in rat aorta strips with raised tension induced by noradrenaline but failed to induce relaxation and to modify (after 1 h exposure at 30 μM) the relaxation induced by ATP and UTP. However, the transient contractions induced by ATP, which preceded the relaxation, were abolished (data not shown).

In the presence of reactive blue 2 (100 μM) the endothelium-dependent relaxation curve for ATP was significantly shifted to the right, whereas the UTP, 2-methyl-thio ATP and ATP endothelium-independent relaxation curves were unaffected by reactive blue 2 (Fig. 5B, data for UTP and 2-methylthio ATP not shown).

To investigate the possibility that ATP acted via adenosine receptors, we first characterized the adenosine receptor in the rat aorta. Adenosine (3–100 μM) induced a dose-dependent relaxation which was inhibited by 8-*p*-sulphophenyl theophylline (10 μM) (antagonist of P_1 purinoceptor) and adenosine deaminase (10 U/ml) (which eliminates adenosine by transforming to inosine), whereas the relaxation was unaffected by reactive blue 2 (100 μM). In the presence of endothelium, the ATP-induced relaxation curve was shifted to the right by the adenosine antagonist, 8-*p*-sulphophenyl theophylline (10 μM), and adenosine deaminase (10 U/ml) (Fig. 5B). However, in the absence of endothelium, neither 8-*p*-sulphophenyl theophylline nor adenosine deaminase produced an effect on the endothelium-independent relaxation elicited by ATP (Fig. 5B).

4. Discussion

4.1. Contractile effects of nucleotides

As described in other species and vascular beds, extracellular UTP and ATP caused concentration-dependent contractions in resting isolated rat aorta. UTP was more potent than ATP in producing contractions. Our results strongly suggest the existence of different receptors mediating the contractions elicited by UTP and ATP. Evidence for this comes from the fact that the shape of the contractions was different for both nucleotides and the effects of agonists and antagonists on UTP and ATP contractions were also different. The fast and transient characteristics of ATP-elicited contractions have also been described in other vascular and non-vascular tissues (Muramatsu et al., 1980; Sneddon and Westfall, 1984; Saïag et al., 1990). The sustained morphology of UTP-induced contractions was similar to the description of Urquilla (1978) in human and canine middle cerebral arteries, and later descriptions in different cerebral arteries (Urquilla et al., 1978; Lee, 1982; Hardebo et al., 1983; Shirasawa et al., 1983) and in other vascular territories (Saïag et al., 1990).

The rank order of potency of purinergic agonists, α,β-methylene ATP > > ATP, suggests that the P_{2X} purinoceptor mediates the contraction caused by ATP. Furthermore, P_{2X} purinoceptor desensitization (Kasakov and Burnstock, 1983; Mathieson and Burnstock, 1985) was confirmed by the fact that the prolonged

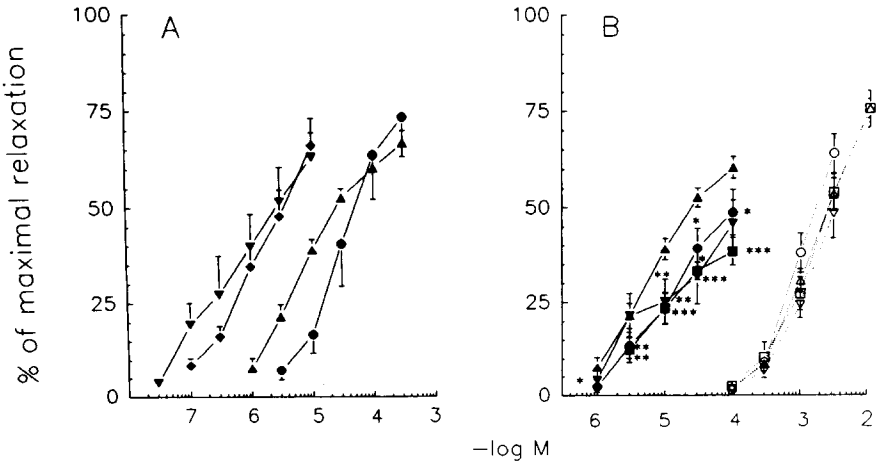

Fig. 5. (A) Cumulative concentration-response curves for relaxation (in the presence of endothelium) elicited by acetylcholine (30 nM to 10 μM, ▼), 2-methylthio ATP (0.1–10 μM, ◆), UTP (3–300 μM, ●) and ATP (3–300 μM, ▲). (B) Filled symbols and continuous line, effect of reactive blue 2 (100 μM, ●), adenosine deaminase (10 U/ml, ▼) and 8-*p*-sulphophenyl theophylline (10 μM, ■) on ATP-induced relaxation (3–300 μM, ▲), in the presence of endothelium. Open symbols and discontinuous line, effect of reactive blue 2 (100 μM, ○), adenosine deaminase (10 U/ml, ▽) and 8-*p*-sulphophenyl theophylline (10 μM, □) on ATP-induced relaxation (3–300 μM, △), in the absence of endothelium. The values are expressed as a percentage of the maximal relaxation. Vertical bars represent S.E.M. from at least eight experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ using Student's *t*-test for paired data.

exposure to α,β-methylene ATP selectively abolished its further response and also reduced the contraction to ATP. This suggests that the contractions to both purinergic agonists are produced via the same receptor. On the other hand, α,β-methylene ATP did not modify the contraction to UTP.

Considering that reactive blue 2 inhibits selective responses mediated via the P_{2Y} purinoceptor (Burnstock and Warland, 1987), this receptor could modulate the contraction to ATP. In its presence a significant increase in the contraction elicited by ATP was observed. Thus, ATP may simultaneously act as an agonist at the two subtypes of receptors, inducing contraction via P_{2X} receptors and attenuating contraction via P_{2Y} receptors.

The removal of the endothelial layer did not modify the characteristics of the contraction nor the dose-response curve for ATP. This is in agreement with other studies reporting that P_{2X} purinoceptors are located on vascular smooth muscle (Kennedy and Burnstock, 1985; Ralevic and Burnstock, 1988) and that the contraction is not endothelium-dependent (Mathieson and Burnstock, 1985). Our results also ruled out the possibility that ATP elicited endothelium-dependent contractions in isolated aorta as observed by Dominiczak et al. (1991).

Purinoceptors are unlikely to be involved in the UTP contraction. A clear dissociation of receptors exists. Neither P_{2X} nor P_{2Y} purinoceptors seem to be related to the contraction or modulation of UTP contraction as observed with ATP. UTP responses were not affected by desensitization of P_{2X} purinoceptors by prolonged exposure to α,β-methylene ATP (which markedly diminished the contraction to ATP) nor by reactive blue 2 (antagonist of P_{2Y} purinoceptors). These differences in pharmacological behaviour support evidence for the existence of distinct receptors which mediate UTP and ATP effects. Receptors for UTP distinct from P_2 purinoceptors have been proposed as mediators of vasoconstriction in ear (Von Kügelgen et al., 1987) and basilar rabbit arteries (Von Kügelgen and Starke, 1990), and in other arterial and venous smooth muscle (Saïag et al., 1990), suggesting that there is a 'pyrimidinergic' receptor. The term 'nucleotide' receptor (Davidson et al., 1990) or P_{2U} purinoceptor (Dubyak, 1991) has also been proposed to refer some receptors to nucleotides whose pharmacology does not fit that of the P_{2X}, P_{2Y}, P_{2T}, P_{2Z} or P_{2D} purinoceptors (Fredholm et al., 1994). Furthermore, UTP and ATP may act as agonists at this receptor. It is unlikely in isolated rat aorta that UTP and ATP can elicit contractions via this P_{2U} purinoceptor, as it seems to be clear that the characteristic of the response is different for both nucleotides and the pharmacology, as mentioned above, is distinct from that of ATP and UTP. This suggests that there is a receptor for UTP that is pharmacologically unrelated to the purinoceptors.

Endothelium-dependent factors are not involved in the UTP contraction, which indicates that the receptors are located on the smooth muscle.

4.2. Relaxant effects of nucleotides

Both ATP and UTP elicited relaxation in isolated rat aorta precontracted by noradrenaline. In endothelium-intact tissues, both nucleotides exhibited similar potency and maximum responses. ATP caused a transient contraction which preceded the relaxation elicited by ATP and this is similar to the responses produced in other arteries with raised tone (Mathieson and Burnstock, 1985; Ferrell and Khoshbaten, 1990; Juul et al., 1993). According to our results, the ATP relaxation is produced by an endothelium-dependent and -independent mechanism with a different pharmacology. The endothelium-dependent relaxation induced by ATP is partially produced via adenosine receptors since 8-*p*-sulphophenyl theophylline and adenosine deaminase significantly shifted the ATP relaxation curve to the right. These effects are compatible with the catabolism of ATP to adenosine via endothelial ectonucleotidase (Pearson and Gordon, 1985). Thus the relaxation could partially be due to adenosine. The relative order of agonist potencies (2-methylthio ATP > > ATP = UTP) is consistent with the fact that the relaxation could be mediated via P_{2Y} purinoceptors. As expected, it is possible to exclude the P_{2X} receptors since the exposure to α,β-methylene ATP (which presumably desensitized the P_{2X} purinoceptor) did not alter the relaxation attributed to ATP, but did abolish the transient contractions that preceded each relaxation. This proved the selective desensitization by α,β-methylene ATP of P_{2X} purinoceptors. The P_{2Y} purinoceptor has been related with many neuronal (Illes and Nörenberg, 1993) and non-neuronal actions of ATP. These include endothelium-dependent vasodilation via prostacyclin and nitric oxide release from vascular endothelial cells (Boeynaems and Pearson, 1990). However, the pharmacology of ATP relaxation did not fit with that described in other vascular territories. The discrepancies arose from the fact that reactive blue 2 shifted the endothelium-dependent relaxation curve of ATP to the right, but did not affect the relaxation elicited by 2-methylthio ATP. This suggests that the receptors involved in the relaxation evoked by ATP and 2-methylthio ATP must be different.

Furthermore, contrary to most reported studies on rat aorta where ATP induces an endothelium-dependent relaxation (Rose'Meyer and Hope, 1990; Schini and Vanhoutte, 1992), ATP also induced an endothelium-independent relaxation in our preparation via re-

ceptors located on smooth muscle. In the absence of endothelium, the rate of catabolism of ATP to adenosine is functionally unimportant as 8-*p*-sulphophenyl theophylline and adenosine deaminase did not modify ATP relaxation. However, this ATP effect is mediated through receptors different to those located on the endothelium because reactive blue 2, contrary to what happens in the presence of endothelium, did not modify the relaxation curve. An endothelium-independent relaxation has also been described in rat and guinea-pig aorta, rabbit portal vein (Kennedy and Burnstock, 1985; Brizzolara et al., 1993) and rabbit mesenteric artery (Mathieson and Burnstock, 1985). According to our results, it is difficult to believe that ATP could mediate the relaxation via the classical P_{2Y} purinoceptor (or via adenosine receptors) and the existence of more than one purinoceptor or subtypes which mediate their relaxation, as postulated by O'Connor et al. (1991) and Barnard et al. (1994), is likely.

The relaxation elicited by UTP is mediated via receptors different from the classical P_2 purinoceptors characterized in the ATP effects. Neither α,β-methylene ATP nor reactive blue 2 affected the UTP relaxation in isolated rat aorta, which ruled out the possibility of a P_{2X} or P_{2Y} purinoceptor being involved in its effects. Furthermore, the location of the UTP receptors is at the endothelium level, because removal of the endothelium abolished the relaxation. According to these results, UTP and ATP do not act at the same receptor and it is possible to exclude an interaction of UTP at ATP receptors. In some tissues, purines and pyrimidines have been pharmacologically characterized as acting on a common receptor distinct from the classic P_2 purinoceptors (P_{2X} and P_{2Y}). They are referred to as 'nucleotide' receptors (Davidson et al., 1990) or P_{2U} purinoceptors (Dubyak, 1991) and could coexist with purinergic receptors in rat mesenteric artery (Ralevic and Burnstock, 1991) and bovine aortic endothelial cells (Wilkinson et al., 1993) which mediate nucleotide relaxation. The agonist potency order for these receptors is: UTP > ATP (Davidson et al., 1990; O'Connor et al., 1991). These do not seem to be the receptors associated with the UTP relaxation in our preparation, and it is unlikely that they were functionally present.

In conclusion, the findings of this study are in agreement with previous investigations which demonstrate the existence of mixed heterogeneous receptor populations that mediate the effects of UTP and ATP (Seifert and Schultz, 1989; Motte et al., 1993). ATP responses were mediated via P_2 purinoceptors located on the endothelium and smooth muscle. The UTP effects do not fit into the pharmacology of either the P_2 purinoceptors investigated, the hypothetical 'nucleotide' receptor, or the P_{2U} purinoceptor. This dissociation of responses led us to believe in the existence of a 'pyrimidinoceptor' sensitive to UTP but not to ATP in isolated rat aorta.

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