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Pharmacological evidence for a receptor mediating sustained nucleotide-evoked contractions of rat aorta in the presence of UTP

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Abstract

The contractile effect of ATP given alone or in the presence of other nucleotides was studied in rat aortic strips. A sustained contraction in response to ATP (30 μM to 10 mM) was observed during UTP exposure instead of the fast transient contraction produced via P_{2X} purinoceptor activation in the absence of UTP, and contrary to the relaxation elicited when the tone had been raised by noradrenaline and KCl. This sustained ATP effect was produced in the smooth muscle and not via the same mechanism through which UTP elicited contraction, since the contractions in response to UTP and ATP were additive. They were also coupled to different transduction pathways: the effect of UTP but not that of ATP was pertussis toxin-sensitive. In contrast to the fast transient ATP contraction during basal tone, the sustained response was not desensitized by α,β-methylene ATP exposure (30 μM), but was inhibited by reactive blue 2 (10 and 30 μM). Among the nucleotides assayed, UDP and ATPγS also enabled ATP to elicit a sustained contraction. ADP, AMP, dATP, 2-methylthio ATP, α,β-methylene ATP, GTP, GDP, GMP, CTP and ITP also induced a sustained contraction in the presence of UTP. However, adenosine (1 mM) and adenine (0.3 to 3 mM) induced relaxation when the tone had been raised by UTP. According to these results a non-selective nucleotide receptor, different from the P_2 purinoceptors functionally characterized so far, seems to mediate sustained contractions in rat aortic strips in the presence of UTP, UDP or ATPγS.

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

1. Introduction

Extracellular nucleotides act as neurotransmitters and neuromodulators in the central and peripheral nervous system (Von Kügelgen and Starke, 1991; Illes and Nörenberg, 1993). These actions are mediated via activation of specific membrane receptors, termed P_2 purinoceptors (Burnstock, 1978). In functional studies, based on the rank order of agonist potencies, these receptors were classified into several types (Fredholm et al., 1994) coupled to different transduction mechanisms (Burnstock, 1990). The cloning of purinoceptors has confirmed that P_{2X} subtype is a ligand-gated ion channel (Brake et al., 1994; Valera et al., 1994) and that P_{2Y} and P_{2U} purinoceptors are members of the G-protein-coupled superfamily of receptors (Lustig et al., 1993; Webb et al., 1993; Barnard et al., 1994).

In blood vessels, activation of P_2 purinoceptors produces dual effects: contraction during basal tone and relaxation when the tension is raised by noradrenaline. In rat aortic strips, the ATP-induced contraction is mediated via P_{2X} purinoceptors. A different receptor seems to be involved in the contraction induced by UTP. In addition, in this preparation, ATP induces endothelium-dependent as well as endothelium-independent relaxation, whereas UTP induces an endothelium-dependent relaxation via receptors distinct from those for ATP (García-Velasco et al., 1995). This suggest the existence of a mixed receptor population.

It is known that ATP and UTP are co-stored in vesicles of blood platelets (Goetz et al., 1971) and are released from them upon aggregation (Gordon, 1986). Various nucleotides might also be released from other cells, for example under conditions of tissue injury or when cells are dying. The interaction of nucleotides in blood vessels, therefore, seemed of interest. For this reason, the characteristics of the vascular response to nucleotides taken as a

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whole were compared to the effect of nucleotides, added alone, on rat aortic strips.

2. Materials and methods

2.1. Preparation of tissues

Two-month-old male Wistar rats (University of Oviedo, number 3304-13A) were killed by decapitation under diethyl ether anesthesia. The aorta was placed in cold (4°C) Krebs-bicarbonate solution having the following composition (mM): NaCl, 118; KCl, 4.75; $CaCl_2$, 2.5; KH_2PO_4, 1.19; $NaHCO_3$, 25; $MgSO_4$, 1.2 and glucose, 11. It was cleaned of adherent connective tissue and cut into helical strips (about 0.2×3 cm) with microscissors. The cutting angle was 45 degrees. Special care was taken to avoid contact with the luminal surface in order to preserve the endothelium. The strips were mounted in 6 ml organ baths containing Krebs solution at 37°C and bubbled continuously with a 95% O_2 and 5% CO_2 mixture. The tissues were allowed to equilibrate for 120 min under 2 g basal tension 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 functionality of the preparation. After washout of noradrenaline the segment was kept in fresh Krebs solution until the original baseline was reached, and subsequently all segments were contracted with cumulative concentrations of noradrenaline (0.3 nM to 3 μM) to reach the maximal effect (E_{max}). The E_{max} to noradrenaline was 1.09 ± 0.03 g.

2.2. Experimental procedures

When the effects of different nucleotides, nucleosides and purine and pyrimidine bases were studied under basal tone conditions, cumulative (e.g., UTP) or non-cumulative (e.g., ATP) concentrations, or single concentrations were added to the bath. The interval between cumulative concentration-response curves for UTP, between single concentrations to perform the concentration-response curve for ATP (see García-Velasco et al., 1995) and single concentrations of all other drugs was 1 h.

When the modifications of the contractile effect of ATP caused by the presence of nucleotides, nucleosides and purine and pyrimidine bases in the organ bath were studied, cumulative concentration-response curves for ATP (30 μM to 10 mM) were made in the presence of single concentrations of UTP, UDP or ATPγS. At 1 h intervals, the concentration-response curves for ATP in the presence of other nucleotides, but in the absence of antagonists, were reproducible. Thus, this was the interval chosen to test the influence of antagonists. The antagonists were added 15 min before the second cumulative concentration-response curve for ATP. Cumulative concentration–response curves for ATP in the presence of different concentrations of UTP, were also made after desensitization of P_{2X} purinoceptors by 1 h exposure to α,β-methylene ATP.

In order to study the structure-activity relationship of nucleotides and nucleotides that allowed a sustained response, a contraction in response to ATP (1 mM) was elicited and one h later a second contraction was elicited in the presence of the drug under study. This second contraction with ATP was elicited either when the tone was still increased by the other nucleotide (or nucleoside) or 5 min later, when the contractile effect was not observed. In addition, cumulative concentration-response curves or contractions induced by single concentrations of nucleotides, nucleosides or purine and pyrimidine bases were made with aortic strips after the tension had been raised by UTP (0.3 mM) to study the structure-activity relationship of compounds that elicited a sustained response.

In order to demonstrate the presence of endothelium, each aortic strip was contracted with 30 nM noradrenaline at the beginning of the experiment, and then relaxed with acetylcholine (30 nM to 1 μM). The strips which were relaxed by more than 50% (100% relaxation was defined as relaxation to the baseline level) were considered to have an intact endothelium and were used in the study. Additional experiments were carried out with tissues that were endothelium-denuded by mechanical rubbing before being placed in the organ bath. This procedure did not cause functional damage to the vascular smooth muscle and the segments were not relaxed by acetylcholine (Molina et al., 1992).

The effect of pertussis toxin, an inhibitor of pertussis-toxin-sensitive G-proteins, was examined. The experiments were carried out with lyophilized pertussis toxin, which was dissolved in bidistilled water on the day of the experiment. A concentration-response curve was determined and then, after washout, the preparations were incubated for 3 h with 2 μg/ml of pertussis toxin (Abebe et al., 1995) and a second concentration-response curve was made. The incubation medium was not changed during this time. Additional experiments were done with the same protocol of a 3 h interval between successive concentration–response curves for ATP, in the absence of pertussis toxin and without changing of the Krebs solution, and no differences were observed.

A combination of a maximum of two nucleotides was tested on each aortic strip, except in experiments performed to desensitize P_{2X} purinoceptor with α,β-methylene ATP, in which case three nucleotides were present at the same time in the organ bath.

The possibility of changes in pH as a factor in the effect of ATP was considered. Triphosphate and diphosphate nucleotides at high concentrations reduced the pH of the medium. However, this change was not responsible for the

sustained contractions induced by ATP in the presence of UTP. The change in pH was the same when ATP was added alone or in the presence of UTP. Furthermore, when contractions were induced by noradrenaline or KCl, ATP produced a relaxation instead of a contraction, despite a similar change in pH. Also, the nucleotide monophosphates did not reduce the pH but also elicited sustained contractions in the presence of UTP.

2.3. Drugs

ATP (adenosine 5′-triphosphate, disodium salt), ADP (adenosine 5′-diphosphate, disodium salt), AMP (adenosine 5′-monophosphate, disodium salt), adenosine, adenine, ATPγS (adenosine 5′-*O*-(3-thiotriphosphate), tetralithium salt), UTP (uridine 5′-triphosphate, sodium salt), UDP (uridine 5′-diphosphate, sodium salt), UMP (uridine 5′-monophosphate, disodium salt), uridine, uracil, GTP (guanosine 5′-triphosphate, sodium salt), GDP (guanosine 5′-diphosphate, sodium salt), GMP (guanosine 5′-monophosphate, disodium salt), ITP (inosine 5′-triphosphate, sodium salt), dTTP (thymidine 5′-triphosphate, sodium salt), CTP (cytidine 5′-triphosphate, sodium salt), reactive blue 2, (−)noradrenaline bitartrate and acetylcholine chloride were purchased from Sigma; α,β-methylene ATP (dilithium salt), 2-methyl-thio ATP (tetrasodium salt), suramin hexasodium and pertussis toxin were purchased from Research Biochemicals (RBI); dATP (2′-deoxyadenosine 5′-triphosphate) from Pharmacia. All compounds were dissolved in bidistilled water to prepare a stock solution. The appropriate dilutions were prepared daily and kept on ice during the experiment.

2.4. Statistical methods

The sizes of responses elicited by the agonists were normalized as percentages of the maximal response to noradrenaline for each aortic strip. The results are expressed as the means ± S.E.M. for $n \geq 6$ experiments. Data were compared by Student's *t*-test—paired or unpaired—for individual comparison and by analysis of variance (ANOVA). Differences between group mean data were considered significant at $P < 0.05$. All analyses were done with the computer program, PHARM/PCS (Tallarida and Murray, 1987).

3. Results

3.1. Response of rat aortic strips to nucleotides, nucleosides and purine and pyrimidine bases given alone

Among the compounds studied, ATP, ADP, 2-methylthio ATP, GTP, GDP, dTTP and ITP ≥ 0.1 mM and α,β-methylene ATP ≥ 1 μ M induced a fast transient contraction from basal tone. UTP and UDP > 1 μ M induced a sustained contraction. Depending on the concentration used, ATPγS induced a fast transient contraction

Table 1
Effect of nucleotides, nucleosides and purine and pyrimidine bases on rat aortic strips during basal tone and tone increased by another nucleotide

	Contraction at basal tone		Compounds that enabled ATP to elicit tonic responses	Compound that elicited tonic response in presence of UTP
	Fast-transient	Sustained		
ATP	+	−	−	+
ADP	+	−	−	+
AMP	−	−	−	+
dATP	−	−	−	+
α,β-methylene ATP	+	−	−	+
2-methylthio ATP	+	−	−	+
ATPγ S	+(< 0.1 mM)	+(> 0.1 mM)	+	
UTP	−	+	+	
UDP	−	+	+	
UMP	−	−	−	−
GTP	+	−	−	+
GDP	+	−	−	+
GMP	−	−	−	+
dTTP	+	−	−	?
CTP	−	−	−	+
ITP	+	−	−	+
Adenine	−	−	−	−
Adenosine	−	−	−	−
Uridine	−	−	−	−
Uracil	−	−	−	−

+ = Effect; − = No effect; ? = Not assayed.

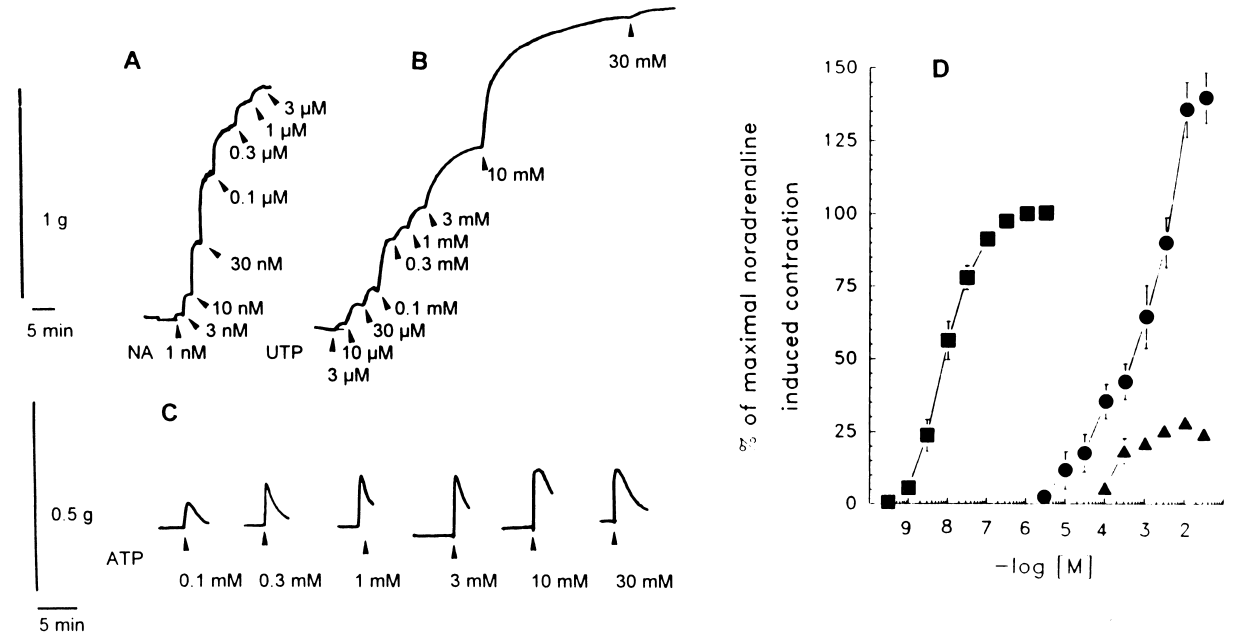

Fig. 1. Recordings of cumulative concentration–response curves for noradrenaline (NA) (A) and UTP (B), and non-cumulative concentration-response curve for ATP (1 h interval between successive concentrations) (C). The recordings are representative of at least 12 similar experiments. (D) Concentration-response curves for noradrenaline (■), UTP (●) and ATP (▲). The values are expressed as percentages of the maximal contraction induced by noradrenaline and each point is the mean ± S.E.M., $n \geq 12$.

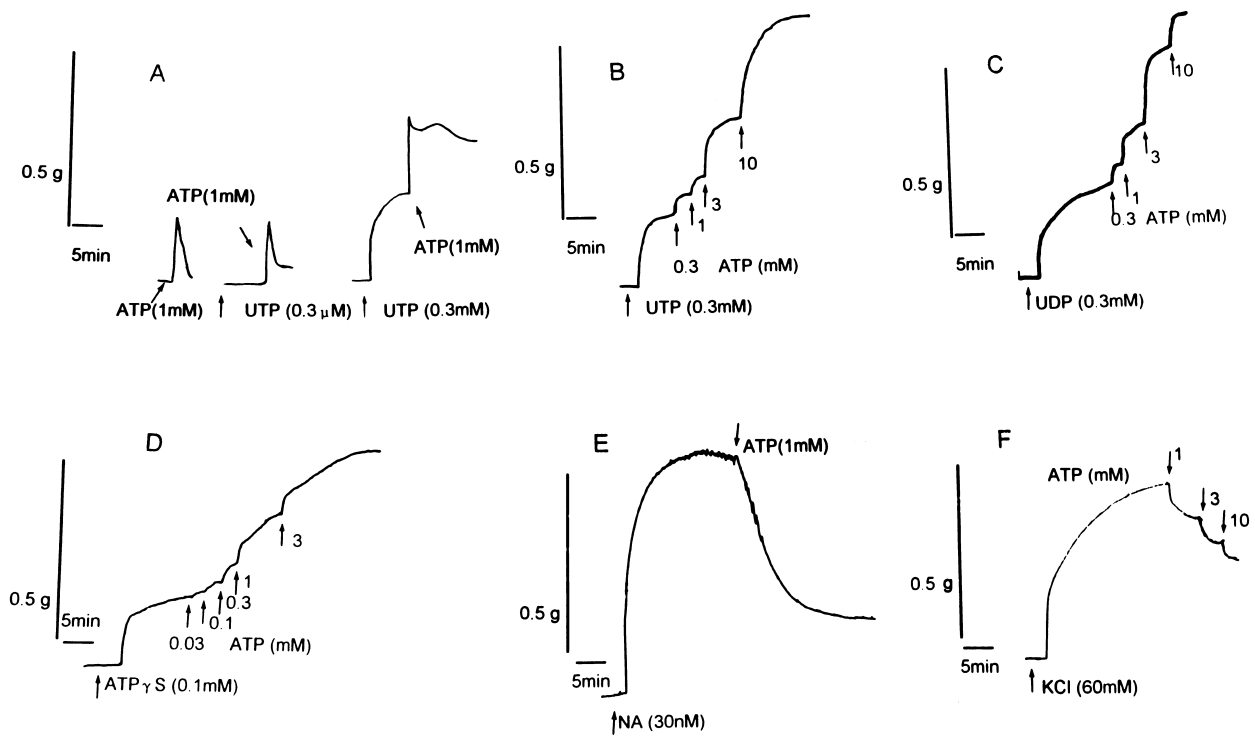

Fig. 2. Recordings of contraction in response to a single concentration of ATP 1 mM in the absence and the presence of UTP 0.3 μM and 0.3 mM in the same aortic strip (A), and cumulative concentration-response curves for ATP in the presence of UTP (0.3 mM) (B), UDP (0.3 mM) (C) or ATPγS (0.1 mM) (D). Relaxant effect elicited by ATP in aortic strips with tone raised by noradrenaline (NA, 30 nM) (E) and KCl (60 mM) (F). The recordings are representative of at least four similar experiments.

(≥ 1 μ M to < 0.1 mM) or a sustained response (≥ 0.1 mM) similar to the response to UTP. No contractile effect was observed with other compounds studied, AMP, dATP, UMP, GMP, CTP, adenine, adenosine, uridine and uracil, in concentrations up to 1 mM (Table 1).

UTP (3 μM to 30 mM) contracted the isolated rat aorta in a concentration-dependent way (Fig. 1B and D) and the magnitude of the response was similar in cumulative or non-cumulative curves. Cumulative concentration–response curves were reproducible, and desensitization to UTP was not observed at 1 h intervals between successive curves. Due to the fact that the contraction in response to ATP was fast and transient, it was not possible to perform cumulative concentration–response curves for ATP. At 1 h intervals between challenges, the response to ATP was reproducible. The concentration-response curves for ATP (0.1 to 30 mM) were therefore made by adding the different concentrations non-cumulatively (García-Velasco et al., 1995).

UTP induced larger contractions than did ATP, $138.9 \pm 8.7\%$ versus $25.1 \pm 2.4\%$ of the maximal contraction induced by noradrenaline (Fig. 1C and D).

3.2. Response to ATP in the presence of UTP

In the presence of UTP, the response to ATP (1 mM) changed from a monophasic, transient one to a biphasic one: when the tension had been raised by UTP (≥ 0.1 mM), ATP induced a fast contraction followed by a sustained response (Fig. 2A). In contrast, when the tension had been raised by noradrenaline or potassium chloride, ATP 1 mM caused relaxation (Fig. 2E, F). As ATP elicited a sustained contraction, it was possible to make cumulative concentration–response curves. The fast component of the biphasic response to ATP was not observed in these curves (Fig. 2B). The concentration–response curves (30 μM to 10 mM) were reproducible at 1 h intervals. At 30 mM, ATP induced relaxation in the presence of UTP. Moreover, the magnitude of contraction was different when ATP (10 mM) was added in the presence of UTP (0.3 mM), $48.8 \pm 6\%$ (considering the tone raised by UTP as the basal level for the sustained contraction induced by ATP) versus $25.1 \pm 2.4\%$ with ATP added during basal tone ($P < 0.001$; Fig. 3).

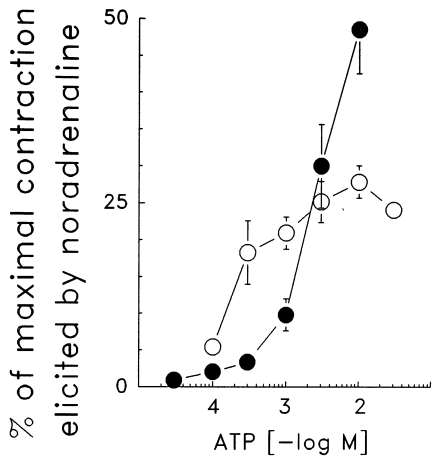

Fig. 3. Non-cumulative concentration–response curve for ATP during basal tone (1 h interval between successive concentrations) (○), and cumulative concentration–response curve for ATP elicited from tone raised by UTP (0.3 mM) (●). In the latter case the ATP-induced contraction was measured from the level of the tone raised in the presence of UTP. Each point is the mean ± S.E.M. of the contraction for each agonist concentration ($n \geq 6$).

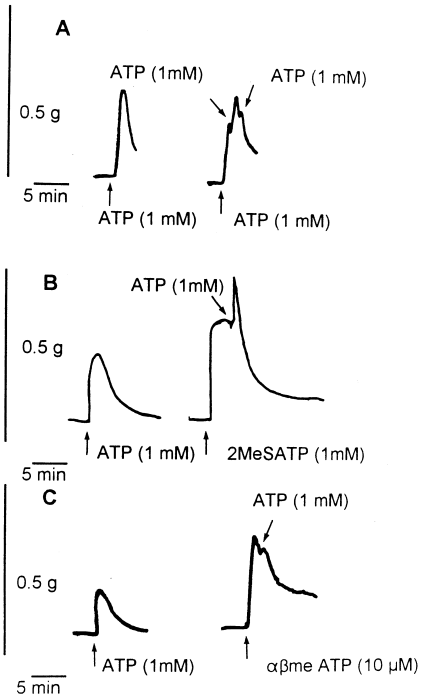

Fig. 4. Recordings of contraction in response to a single concentration of 1 mM ATP during basal tone and after precontractions by ATP (1 mM) (A), 2-methyl thio ATP (2MeSATP, 1 mM) (B) and α,β-methylene ATP (α,β me ATP, 10 μM) (C). The recordings are representative of at least four similar experiments.

3.3. Pharmacological characterization of the receptor site enabling ATP to elicit a sustained effect

The response to ATP in the presence of other nucleotides, nucleosides (ATP, ADP, AMP, dATP, α,β-methylene ATP, 2-methylthio ATP, ATPγS, UDP, UMP, GTP, GDP, GMP, dTTP, CTP, ITP) and adenine, adenosine, uridine and uracil in the organ bath was then investigated (all in concentrations up to 1 mM).

Besides UTP (Fig. 2B), the same characteristic sustained contraction in response to ATP was observed after a rise in tone induced by UDP (0.3 and 1 mM) (Fig. 2C) and ATPγS (10 μM to 0.1 mM) (Fig. 2D). The presence of other compounds did not lead to the sustained effect of ATP (Table 1, Fig. 4).

To study the effect of UTP which enables the production of a long-lasting response to ATP, different single concentrations of UTP (0.1 μ M to 3 mM) were added to the organ bath before ATP challenge. No significant difference was observed between the concentration–response curves for ATP (30 μ M to 3 mM) in the presence of the four different concentrations of UTP (0.1 to 3 mM) (Fig. 5A). These curves were calculated with the tone raised by UTP (Fig. 5B) taken as the basal level for the sustained contraction induced by ATP. As mentioned above, the same behavior was observed with a previous exposure to ATPγS (10 to 100 μM) (Fig. 5C, D) and UDP (0.3 and 1 mM) (Fig. 2C). In order to find the minimum amount of UTP needed to allow a sustained contraction in response to ATP, the components of the biphasic response to ATP were dissociated. To this end, the fast response to ATP was abolished by 1 h exposure to α,β-methylene ATP (30 μM). Under these conditions, UTP $\geq$ 0.1 μM was required to enable ATP to elicit a sustained contraction, and the concentration–response curves for ATP differed, depending on the concentration of UTP ($P < 0.01$ by ANOVA) (Fig. 5E). Even concentrations of UTP (0.1 and 0.3 μM) that did not induce contraction of rat aortic strips (Fig. 5F) allowed sustained contractions in response to ATP. No significant difference was observed in the concentration–response curves for ATP (30 μM to 3 mM) in the presence of UTP (0.3 mM) with α,β-methylene ATP (see Fig. 5A and E) present or absent.

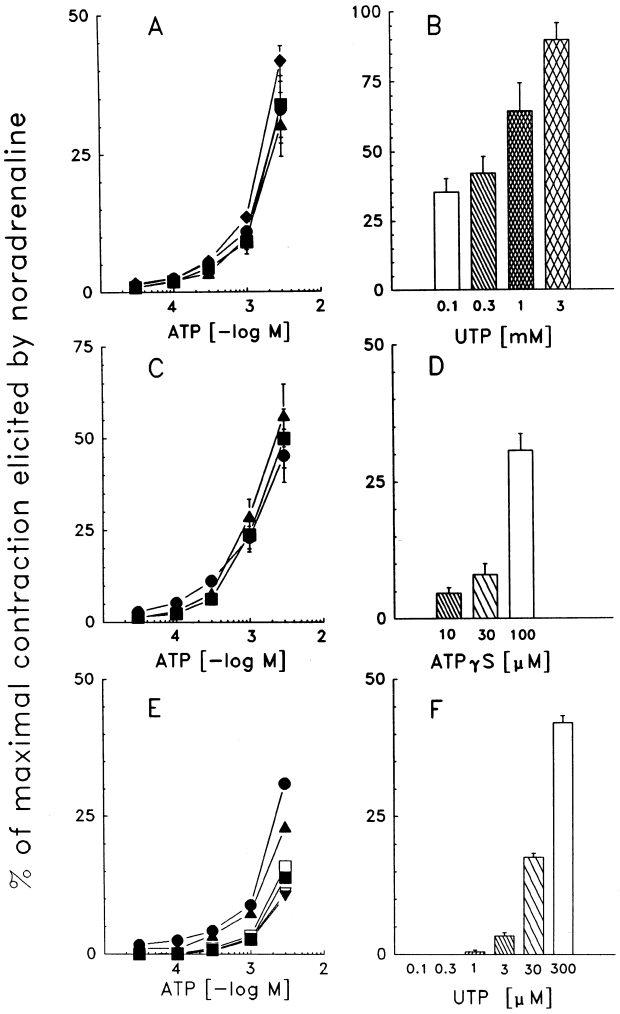

Fig. 5. Cumulative concentration–response curves for ATP (30 μM to 3 mM) determined in the presence of different concentrations of UTP, 0.1 mM (●), 0.3 mM (▲), 1 mM (■) and 3 mM (♦) (A) and ATPγS, 10 μM (●), 30 μM (■) and 100 μM (▲) (C). Histograms represent the contraction induced by UTP (B) and ATPγS (D). (E) Cumulative concentration–response curves for ATP (30 μM to 3 mM) made in the presence of different concentrations of UTP, 0.1 μM (▼), 0.3 μM (∇), 1 μM (■), 3 μM (□), 30 μM (▲) and 300 μM (●), after desensitization of P_{2X} purinoceptors by 1 h of exposure to α,β-methylene ATP (30 μM). There are significant differences between curves, $P < 0.01$ by ANOVA. (F) Histograms represent the contraction induced by UTP (0.1 to 300 μM). The magnitude of sustained contractions in response to ATP was determined by considering the tone raised by UTP as the basal level. Each point is the mean ± S.E.M., $n \geq 6$.

3.4. Pharmacological characterization of the receptor site mediating the sustained contractile effect

Besides the effects of ATP, those of different nucleotides (ADP, AMP, dATP, α,β-methylene ATP, 2-methylthio ATP, ATPγS, UMP, GTP, GDP, GMP, CTP and ITP), adenine, adenosine, uridine and uracil on preparations exposed to UTP (0.3 mM) were studied. All except UMP, adenine, adenosine, uridine and uracil induced sustained contractions similar to those elicited by ATP in rat aortic strips in the presence of UTP (0.3 mM) (Table 1). The concentration–response curves for ADP, AMP, GTP, GDP and ITP were similar to that for ATP (0.3 to 10 mM) (Fig. 6A). As an example, sustained contractions induced by dATP (0.3 mM) and CTP (1 mM) are shown in Fig. 6B. Adenosine (1 mM) and adenine (0.3 to 3 mM) induced weak relaxation in the presence of UTP (data not shown).

3.5. Effect of endothelium removal

To study the endothelium dependence of the effect of ATP, additional experiments were carried out with endothelium-denuded tissues. The characteristics of the response and the concentration–response curves elicited by ATP given alone (García-Velasco et al., 1995) and in the presence of UTP (0.3 mM) were the same in the presence or absence of endothelium (Fig. 7A). Absence of the endothelium also did not modify the contractile effect of UTP (0.3 mM) and ATP (1 mM) during basal tone (Fig. 7B).

3.6. Effect of pertussis toxin

Incubation with 2 μg/ml pertussis toxin for 3 h did not modify the response to ATP (1 mM) added alone. It also did not change the concentration–response curve for ATP

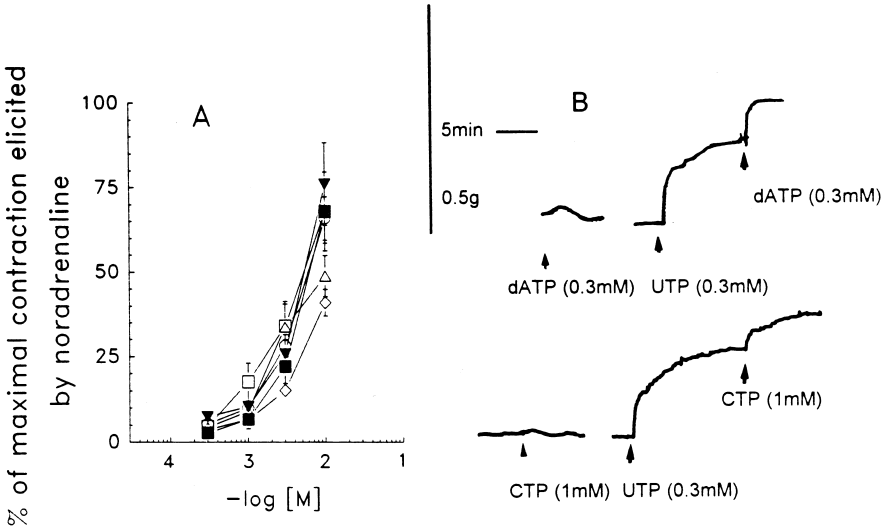

Fig. 6. (A) Cumulative concentration–response curves for different nucleotides (0.3 to 10 mM), ATP (△), ADP (○), AMP (□), GTP (▼), GDP (■) and ITP (◇), under conditions of UTP (0.3 mM)-raised tone. The magnitude of sustained contractions with the nucleotides was determined by considering the tone raised by UTP as the basal level and each point is the mean ± S.E.M. ($n \geq 6$). (B) Recordings of dATP (0.3 mM) and CTP (1 mM) effect in the absence and the presence of UTP (0.3 mM) exposure in rat aortic strips. Recordings shown are representative of four similar experiments.

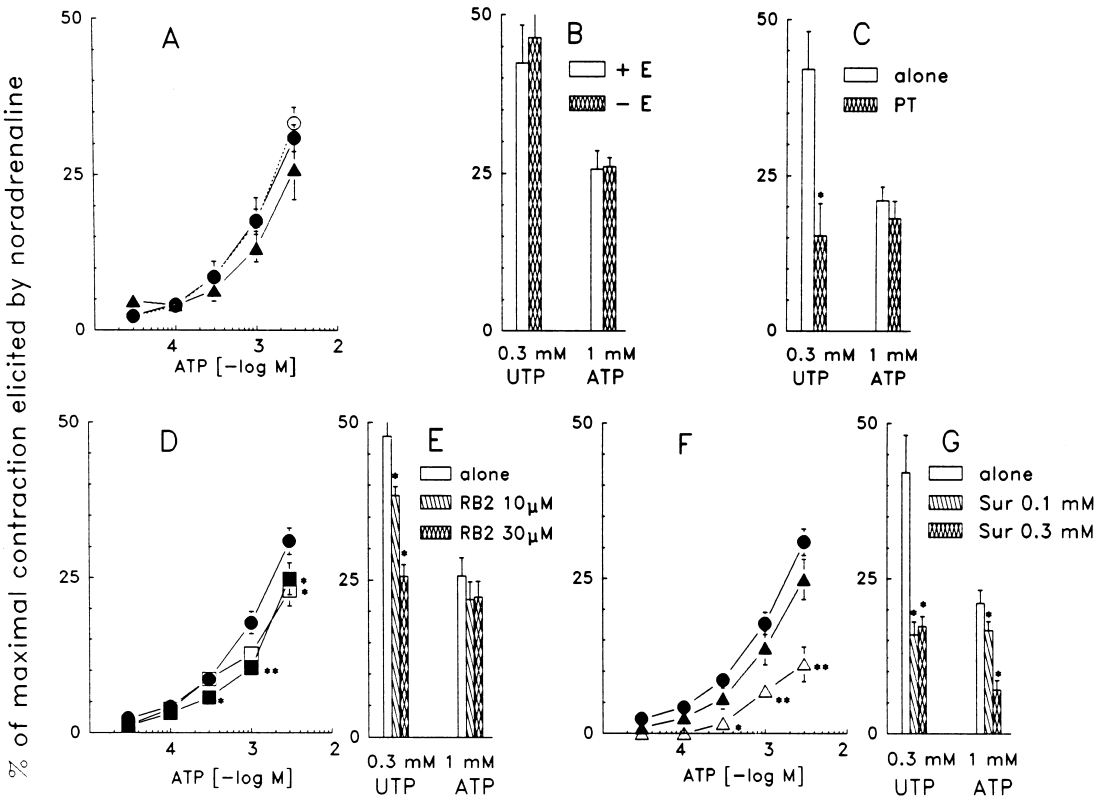

Fig. 7. (A) Cumulative concentration–response curves for ATP (30 μM to 3 mM) in the presence of UTP (0.3 mM) and in the presence (●) or absence (○, dashed line) of endothelium, and after exposure to pertussis toxin (2 μg/ml for 3 h) (▲). Histograms represent the contractile effect of UTP (0.3 mM) and ATP (1 mM) during basal tone, with (+E) and without (−E) endothelium (B), and with and without pertussis toxin pretreatment (PT) (C). Effect of reactive blue 2 (RB2), 10 (■) and 30 μM (□), on the cumulative concentration–response curves for ATP (0.3 to 30 mM) (●) in the presence of UTP (0.3 mM) (D). Effect of reactive blue 2, 10 and 30 μM on the contractile effect of UTP (0.3 mM) and ATP (1 mM) at basal tone (E). Effect of suramin 0.1 (▲) and 0.3 mM (△) on the cumulative concentration–response curves for ATP (0.3 to 30 mM) (●) in the presence of UTP (0.3 mM) (F). Effect of suramin (Sur) 0.1 and 0.3 mM on the contractile effect of UTP (0.3 mM) and ATP (1 mM) on basal tone (G). The magnitude of sustained contractions in response to ATP was determined by considering the tone increased by UTP as the basal level. Each point is the mean ± S.E.M. ($n \geq 6$). $*P < 0.05$ and $**P < 0.01$ for paired data.

(30 μM to 10 mM) in the presence of UTP (0.3 mM) (Fig. 7C, A). However, pertussis toxin significantly inhibited the contraction induced by UTP (0.3 mM) (Fig. 7C).

Pertussis toxin, 2 μg/ml for 3 h, reduced the contractile response to 30 mM sodium fluoride by 70.0% (compared to the contractions in response to 30 mM sodium fluoride in the absence of pertussis toxin) and the response to 1 μM of noradrenaline by 31.4% (compared to the contractions with 1 μM of noradrenaline).

3.7. Further pharmacological characterization of the receptor site mediating the sustained contractile effect

To characterize the receptor involved in the sustained contraction elicited by ATP in rat aortic strips with their tension increased by UTP, the effects of P_2 purinoceptor antagonists and of desensitization by α,β-methylene ATP were studied.

The P_2 purinoceptor antagonist, reactive blue 2 (10 and 30 μM), inhibited the contraction elicited by ATP in the presence of UTP (Fig. 7D). Suramin, another P_2 purinoceptor antagonist, did not affect the sustained contractions in response to ATP at 0.1 mM but reduced them at 0.3 mM (Fig. 7F). Reactive blue 2 (10 and 30 μM), did not inhibit the transient contractions in response to ATP (1 mM) during basal tone whereas suramin (0.1 and 0.3 mM) reduced these contractions. Both reactive blue 2 (10 and 30 μ M) and suramin (0.1 and 0.3 mM) inhibited UTP (0.3 mM)-induced contractions (Fig. 7E, G).

α,β-methylene ATP (1 to 30 μ M), like ATP, induced a biphasic contractile response in rat aortic strips in the presence of UTP. The sustained response to ATP, 2-methylthio ATP and α,β-methylene ATP given on top of a UTP contraction, was not affected by 1 h exposure of the preparations to α,β-methylene ATP (30 μ M), which is known to desensitize P_{2X} purinoceptors. However, as ex-

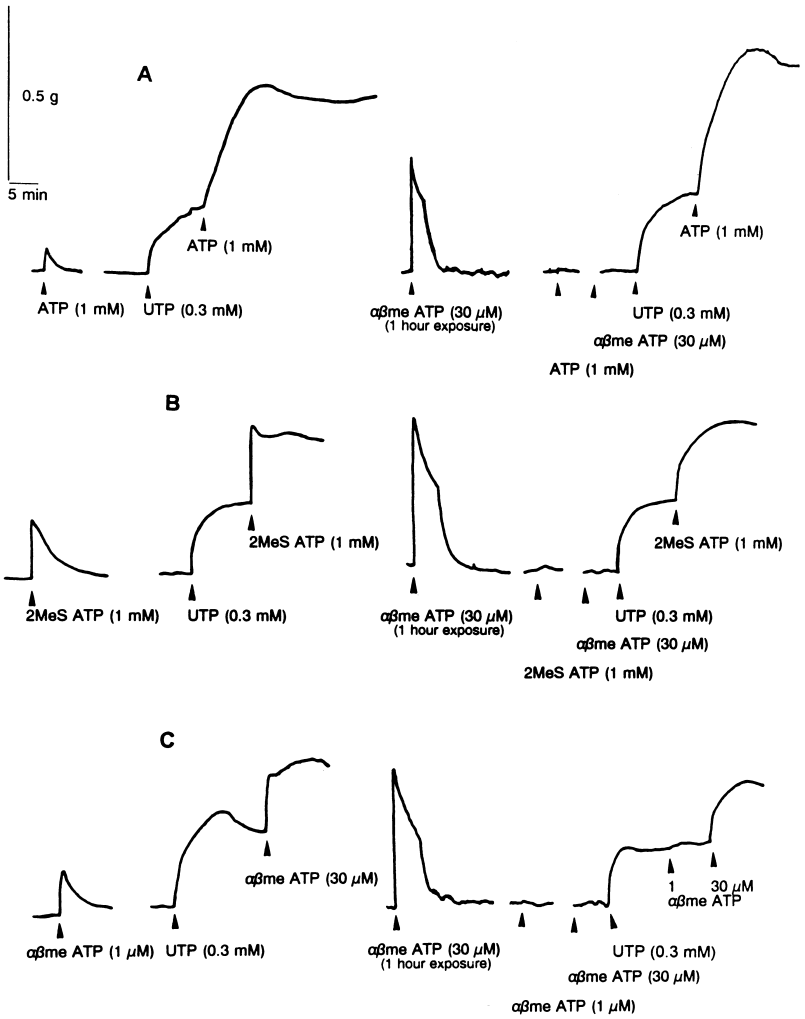

Fig. 8. Recordings of fast transient contractions in response to ATP (1 mM) (A), 2-methylthio ATP (2MeS ATP, 1 mM) (B) and α,β-methylene ATP (α,βme ATP, 1 μM) (C) during basal tone and the sustained contractions elicited, by the same concentrations, during UTP (0.3 mM) exposure in rat aortic strips. Effect of incubation with α,βme ATP (30 μM) for 1 h on the fast contraction elicited by ATP (1 mM) (A), 2MeS ATP (1 mM) (B) and α,βme ATP (1 and 30 μM) (C) during basal tone and on the sustained contractions in response to ATP (1 mM) (A), 2MeS ATP (1 mM) (B) and α,βme ATP (1 and 30 μM) (C) after UTP (0.3 mM) exposure. The recordings shown are representative of four similar experiments.

pected, α,β-methylene ATP exposure abolished the phasic contractions elicited by α,β-methylene ATP itself as well as those with ATP and 2-methylthio ATP during basal vascular tone. α,β-methylene ATP also abolished the phasic component of the contractions elicited by 2-methylthio ATP and α,β-methylene ATP given on top of UTP (Fig. 8). The desensitization induced by α,β-methylene ATP exposure of the receptor which induces the fast response was, therefore, not abolished by UTP, UDP or ATPγS.

4. Discussion

Our results showed that exposure to UTP modifies the size and the kinetics of the contraction elicited by ATP in rat aortic strips: a sustained, concentration-dependent contraction in response to ATP developed in the presence of UTP instead of the characteristic transient response observed during basal tone, which is related to P_{2X} purinoceptors (García-Velasco et al., 1995). The transient contractions during basal tone and the sustained contraction in the presence of UTP were observed with a similar concentration range of ATP.

4.1. The receptor mediating the ATP-induced sustained contraction in the presence of UTP

The characteristics of the response to ATP in the presence of UTP suggest that the response could be mediated through receptors. A P_2 purinoceptor may be involved, since adenosine is not an agonist in this response, as it induces relaxation, thus excluding via P_1 purinoceptors. The sustained contraction in response to ATP probably is also not produced via a UTP receptor as the responses to these agonists in isolated rat aorta are additive. Moreover, both nucleotide-induced contractions are coupled to different transduction pathways. Thus, the UTP-induced effect, as described for other preparations (Sánchez-Fernández et al., 1993; Lazarowski and Harden, 1994), is pertussis toxin-sensitive, but the effect of ATP in the presence of UTP is not. Therefore, the change in the time course of contractions elicited by ATP could be related to the existence of a purinoceptor different from the P_{2X} purinoceptor functionally characterized on vascular smooth muscle (Burnstock and Kennedy, 1985; Fredholm et al., 1994; Kennedy and Leff, 1995; Surprenant et al., 1995). Additive effects of ATP and UTP were also described for other vascular preparations (Von Kügelgen and Starke, 1990), although in those experiments the response to ATP was transient and not sustained as observed in rat aortic strips.

This ATP receptor is located on the vascular smooth muscle since the response was not modified by endothelium removal.

4.2. Nucleotides that enable ATP to induce sustained contraction

The sustained contraction elicited by ATP was associated with previous exposure to specific agonists. Among the compounds assayed, only UTP, UDP and ATPγS enabled ATP to induce a sustained contraction. In contrast, when the tone was raised by other agonists, such as noradrenaline or potassium chloride, ATP induced a concentration-dependent relaxation (García-Velasco et al., 1995). This indicates that a specific nucleotide, either UTP, UDP or ATPγS, is necessary to enable ATP to elicit these larger and prolonged contractions, and that the change is not related to an increase in the tone of the preparation or to an increase in the intracellular calcium as induced by noradrenaline and KCl.

The facilitatory effect of UTP was concentration-dependent, but not contraction-dependent, and the maximal effect was produced by 0.1 mM of UTP. This is suggested by the fact that even concentrations of UTP which did not induce contractions enabled ATP to elicit sustained contractions. Moreover, in the presence of concentrations of UTP (0.1 to 3 mM) that induced increases in the tone, the concentration-response curves to ATP did not differ. Similar to UTP, ATPγS ≥ 10 μM also induced the maximum facilitation.

It might be assumed that the observed modification of the response to ATP reflects a change in the association-dissociation rate at the P_{2X} purinoceptor and/or inhibition of ectonucleotidases. It is unlikely that the effect might be due to either changes in the association-dissociation rate (since the pharmacology of this postulated receptor is different from the P_2 purinoceptor eliciting contractions during basal tone), or to inhibition of ectonucleotidases. In the latter case the time course of the response should remain similar whereas the EC_{50} should change (Crack et al., 1995; Kennedy and Leff, 1995). In fact, however, differences in the time course and magnitude between ATP contractions induced during basal tone and during UTP exposure were observed. In addition, saturation of ectonucleotidases by the presence of ATP or other nucleotides to concentrations higher than that of UTP did not enable ATP to induce a sustained contraction. Furthermore, ATPγS, a non-hydrolyzable derivative, also enabled ATP to produce sustained contractions.

4.3. Nucleotides causing a sustained contraction in the presence of UTP

Besides the effect of ATP, the effect of ADP, AMP, dATP, 2-methylthio ATP, α,β-methylene ATP, GTP, GDP, GMP, CTP and ITP also was changed from transient to sustained in the presence of UTP. The characteristics of the response suggest that the sustained contractions could be mediated via common mechanisms. Accordingly, a different type or subtype of purinoceptor might mediate this nucleotide contraction.

Exposure to α,β-methylene ATP abolished not only the transient contraction induced by ATP, 2-methylthio ATP or α,β-methylene ATP during basal vascular tone (Kasakov and Burnstock, 1983; García-Velasco et al., 1995) but also the fast transient component of the ATP, 2-methylthio ATP and α,β-methylene ATP contraction during the tone increased by UTP. In contrast, in the same preparations, the sustained contractions in response to ATP, 2-methylthio ATP and α,β-methylene ATP in the presence of UTP or ATPγS were not modified after desensitization by α,β-methylene ATP. Consequently, the nucleotides causing a sustained contraction could act via a receptor unrelated to the P_{2X} purinoceptor (Surprenant et al., 1995).

4.4. Pharmacology of the receptor mediating the ATP-induced sustained contraction

A P_2 purinoceptor seems to mediate the ATP-induced sustained contractions, since these were inhibited by suramin, a non-selective antagonist of P_2 purinoceptors (Dunn and Blakeley, 1988; Evans and Kennedy, 1994). However, the pharmacology of the response is not consistent with that of the transient contractions during basal tone, as reactive blue 2, another P_2 purinoceptor antagonist (Burnstock and Warland, 1987; García-Velasco et al., 1995), did not affect ATP contractions from basal tone, but inhibited the ATP-induced sustained contractions in the presence of UTP.

According to our results, it seems clear that the properties of the receptor mediating the sustained response to ATP are not compatible with those of any purinoceptor described so far. Instead, our findings indicate the presence, in rat aorta, of a new, non-selective nucleotide receptor, activated by purines as well as pyrimidines and by ribose as well as deoxyribose nucleotides, and pharmacologically unrelated to P_2 purinoceptors known in vascular smooth muscle cells. In addition, the nucleotides, in order to bind to, or induce contractions via, this proposed receptor, require the presence of a specific nucleotide (UTP, UDP or ATPγS) which possibly induces an allosteric modification to facilitate the activation of this postulated receptor. The receptor could have physiological or pathophysiological significance for the interaction of several nucleotides which act synergistically to produce greater and longer-lasting effects.

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