Br. J. Pharmacol. (1994), **111**, 923–929

Selective antagonism by PPADS at P_{2X}-purinoceptors in rabbit isolated blood vessels

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1 Pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS), a P_2-purinoceptor antagonist, was investigated for its ability to antagonize: (1) P_{2X}-purinoceptor-mediated contractions of the rabbit central ear artery and saphenous artery evoked by either α,β-methylene ATP (α,β-MeATP) or electrical field stimulation (EFS); (2) P_{2Y}-purinoceptor-mediated relaxations of the rabbit mesenteric artery; (3) endothelium-dependent and endothelium-independent, P_{2Y}-purinoceptor-mediated relaxations of the rabbit aorta.

2 α,β-MeATP (0.1–100 μM) caused concentration-dependent contractions of the rabbit ear and saphenous arteries. The negative log[α,β-MeATP] that produced a contraction equivalent to the EC_{25} for noradrenaline (ear artery) or histamine (saphenous artery) in the absence of PPADS was 6.60 ± 0.18 (9) and 6.18 ± 0.17 (9) in the ear artery and saphenous artery, respectively. These effects of exogenous α,β-MeATP were concentration-dependently inhibited by PPADS (1–30 μM). In the ear artery, the negative log[α,β-MeATP] producing a contractile response equivalent to the EC_{25} of noradrenaline, in the presence of PPADS at 1, 3 and 10 μM was 6.16 ± 0.18 (8), 5.90 ± 0.18 (8) and 4.72 ± 0.36 (8), respectively ($P<0.01$). In the saphenous artery, the negative log[α,β-MeATP] values equivalent to the EC_{25} for histamine in the presence of PPADS at concentrations of 1, 3, 10 and 30 μM were 5.90 ± 0.19 (8), 5.73 ± 0.16 (8), 4.99 ± 0.14 (8) and 4.51 ± 0.13 (8), respectively ($P<0.01$).

3 PPADS at a concentration of 1 μM had no effect on contractions of the ear artery evoked by EFS (4–64 Hz; 1 μM phentolamine present). At higher concentrations (3–30 μM) it caused concentration-dependent inhibition of neurogenic contractions. In the saphenous artery, PPADS (1–30 μM) concentration-dependently inhibited contractions evoked by EFS at frequencies of 4, 8 and 16 Hz. Contractions evoked by EFS at frequencies of 32 and 64 Hz were significantly inhibited by PPADS only at concentrations of 10 and 30 μM.

4 PPADS (30 μM) had no effect on relaxations to 2-methylthio ATP (3 nM–3 μM) in rabbit mesenteric artery and to ATP (1 μM–1 mM) in rabbit aorta (with endothelium intact or removed). In addition, PPADS (30 μM) had no significant influence on the contractile potency of noradrenaline and histamine in rabbit ear and saphenous artery, respectively.

5 In conclusion, these results support the evidence that PPADS is a selective antagonist of P_{2X}-purinoceptor-mediated responses.

Keywords: PPADS; P_{2X}-purinoceptors; P_{2Y}-purinoceptors; rabbit blood vessels; α,β-methylene ATP; electrical field stimulation; 2-methylthio ATP

Introduction

The pharmacology of purinoceptors has advanced rapidly during the last 10 years (Hoyle & Burnstock, 1991; Abbracchio *et al.*, 1993), and there are now some adenosine receptor (P_1) agonists and antagonists that are selective for different subtypes of the P_1-purinoceptor (Williams, 1991; Mast, 1992). However, so far there is a lack of selective and reversible P_2-purinoceptor antagonists. Suramin has fairly specific antagonistic activity at P_2-purinoceptors but does not discriminate between P_{2X}- and P_{2Y}-subtypes (Hoyle *et al.*, 1990). Reactive blue 2 is only useful within a narrow range of concentrations and for limited time of exposure, above or after which it has non-specific effects (Burnstock & Warland, 1987b). Arylazidoaminopropionyl ATP ($ANAPP_3$), a photoaffinity ATP analogue, is a selective antagonist at P_{2X}-purinoceptors after photolysis, but its action is irreversible (Hogaboom *et al.*, 1980). α,β-Methylene ATP (α,β-MeATP) desensitization of P_{2X}-purinoceptors (Kasakov & Burnstock, 1983), although often used successfully (see Hoyle, 1992), does not provide competitive antagonism.

Recently we found that pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS) selectively inhibits P_{2X}-purinoceptor-mediated contractions in guinea-pig ileum resistance vessels (Bungardt *et al.*, 1992) and in the rabbit vas deferens (Lambrecht *et al.*, 1992). With this in mind, the present study was undertaken to investigate the selectivity of PPADS in inhibiting the P_{2X}-purinoceptor-mediated response in several rabbit isolated blood vessels.

P_2-purinoceptors in blood vessels are located both on endothelial cells and on smooth muscle cells (see Pearson & Gordon, 1989; Burnstock, 1990a). Generally, P_{2X}-purinoceptors found in vascular smooth muscles mediate contractile responses, while P_{2Y}-purinoceptors, present either on endothelial cells or on smooth muscle cells, mediate vasodilatation (Burnstock, 1988; Ralevic & Burnstock, 1991a). Recently it has been reported that in some tissues, for example rat mesenteric arterial bed (Ralevic & Burnstock, 1991b) and rabbit aorta smooth muscle (Chinelatto *et al.*, 1992) other, non-classical, types of P_{2Y}-purinoceptor exist at which UTP as well as ATP are effective in producing endothelium-independent vasodilatation.

In the present study we have tested the activity of PPADS in the rabbit central ear artery and saphenous artery for inhibition of P_{2X}-purinoceptor-mediated contractions (Ken-

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nedy & Burnstock, 1985; Warland & Burnstock, 1987; Burnstock & Warland, 1987a; O'Connor et al., 1990; MacDonald et al., 1992); in the rabbit mesenteric artery for antagonism of endothelium-independent relaxations mediated via P_{2Y}-purinoceptors in smooth muscle (Mathieson & Burnstock, 1985; Burnstock & Warland, 1987b); in the rabbit aorta for inhibition of either endothelium-dependent or endothelium-independent P_{2Y}-purinoceptor-mediated relaxation (Chinellato et al., 1992).

Methods

General procedure

Male New Zealand White rabbits (2–3 kg) were killed by an overdose of sodium pentobarbitone (Sagatal), which was injected via the marginal ear vein, and exsanguination. All vessels were suspended horizontally in 5 ml organ baths for isometric recording of mechanical activity (Bevan & Osher, 1972). An initial load of 750–1000 mg was applied to the vessels which were then allowed to equilibrate for at least 60 min. The Krebs solution had the following composition (mM): NaCl 133, KCl 4.7, $NaHCO_3$ 16.4, $MgSO_4$ 0.6, NaH_2PO_4 0.8, $CaCl_2$ 2.5 and glucose 7.7, and was gassed with 95% O_2/5% CO_2 (pH 7.3–7.4) and maintained at 37 ± 1°C. In experiments with electrical field stimulation (EFS), phentolamine (1 μM) was included in the Krebs solution in order to block the adrenergic component of contraction. Contractions were recorded by either a Dynamometer UF1 or Grass FT0C3 force-displacement transducer and were displayed on a Grass 79D ink-writing oscillograph.

Single concentrations of agonists (noradrenaline at 1 μM for the ear artery and aorta, noradrenaline at 10 μM for the mesenteric artery and histamine at 10 μM for the saphenous artery) were added repeatedly at intervals of 20 min, until reproducible increases in tone were obtained. Then these agonists were added cumulatively to determine the maximal contractile response of the blood vessels.

Central ear artery and saphenous artery

The proximal regions of the central ear artery and saphenous artery were removed and ring segments 4 mm in length were prepared and used as described above.

α,β-MeATP (0.1–100 μM) was added directly to the organ bath and washed out after a maximum contraction had been reached. In order to avoid desensitization, intervals of at least 30 min, with a wash every 10 min, were allowed between consecutive applications of α,β-MeATP. Concentration-response relationships for α,β-MeATP were constructed before and after incubation with PPADS (1–30 μM, at least 30 min).

EFS was applied via two platinum wire electrodes placed parallel to, and on each side of, the vessel, and was provided by a Grass S9 stimulator. Stimuli were applied at a given frequency (4–64 Hz) with a pulse width of 0.1 ms and supramaximal voltage for a period of 1 s. These parameters provide stimulation of intramural nerves without direct stimulation of muscle (Burnstock & Warland, 1987a; Stewart-Lee et al., 1991). Frequency-response relationships were determined before and after incubation with PPADS (1–30 μM) for at least 20 min.

When PPADS was tested against contractile responses induced by noradrenaline in the ear artery, the cumulative concentration-response relationships for noradrenaline (0.1–100 μM) were determined in parallel on two preparations from each animal. Then one preparation was incubated with PPADS at a concentration of 30 μM for at least 20 min, and the concentration-response relationships for noradrenaline on both preparations were determined again. Thus, the preparation without PPADS was considered as a time-matched control. The same procedure was used for histamine (1 μM–1 mM) in the saphenous artery.

Mesenteric artery

Side branches of the superior mesenteric artery were dissected and two ring segments 5 mm long were prepared. The initial maximum response to noradrenaline was established, the preparations were washed several times with fresh Krebs solution and were allowed to rest for at least 20 min. Then the segments were contracted by the approximate EC_{50} of noradrenaline (20 or 30 μM) and relaxant cumulative concentration-response relationships for 2-methylthio ATP (2-MeSATP, 3 nM–3 μM) were determined. One preparation was incubated with 30 μM PPADS for at least 20 min and a second concentration-response relationship for 2-MeSATP was constructed on each preparation. The parallel preparation, in which two concentration-response relationships for 2-MeSATP were determined without incubation with PPADS, was considered as a time-matched control.

Aorta

The thoracic aorta was removed, and after dissecting away connective tissue, two ring preparations 4 mm in length were prepared. The endothelium of one of the preparations was removed by gently rubbing with a silk thread passed through the lumen. After the maximum response to noradrenaline had been established, both preparations were precontracted by the approximate EC_{50} of noradrenaline (0.3 or 1 μM) and relaxant concentration-response relationships for ATP (1 μM –1 mM) were determined cumulatively. The concentration-response relationships were determined on the same preparations again after incubation with PPADS at 30 μM (at least 20 min). In the time-control preparations, the first and second relationships for ATP were determined with an interval of at least 20 min. At the end of the experiments, the preparations were opened longitudinally, and treated with a silver staining technique (Nakatsu et al., 1988) to examine the presence of endothelial cells under a microscope. In rubbed preparations the endothelium had been successfully removed, and in unrubbed preparations it was intact.

Drugs used

Rogitine (phentolamine sulphate) was obtained from Ciba. α,β-Methylene ATP lithium salt, adenosine 5'-triphosphate disodium salt (ATP), (−)-arterenol (noradrenaline) bitartrate and histamine dihydrochloride were obtained from Sigma Chemical Co. Ltd. 2-Methylthio ATP tetrasodium salt was obtained from Research Biochemicals Inc. (U.S.A.). Sagatal was supplied by May and Baker. PPADS was synthesized in one of our laboratories.

Solutions of noradrenaline were prepared in ascorbic acid (100 μM); other drug solutions were made up in distilled water.

Analysis of results

In the experiments with α,β-MeATP, responses were expressed as a percentage of the maximum contraction evoked by noradrenaline (ear artery) or histamine (saphenous artery). Because it was not practicable to construct complete concentration-response relationships for α,β-MeATP in the presence of PPADS, the negative log[α,β-MeATP] that produced a contraction equivalent to that produced by the EC_{25} for noradrenaline or histamine was calculated in controls and compared with the negative log[α,β-MeATP] that caused, in the presence of PPADS, contractions equivalent to the EC_{25} for noradrenaline or histamine.

In the experiments with EFS, responses were expressed as a percentage of the contraction evoked by stimulation at a frequency of 32 Hz (maximal contraction obtained) in the absence of PPADS.

When concentration-response curves for noradrenaline in the ear artery were constructed, responses were calculated as

a percentage of the maximal noradrenaline contraction. The mean response of the preparations from each animal was calculated at each concentration of noradrenaline applied. These mean responses for each animal were then subjected to a probit transformation (Bliss, 1935; Finney, 1971) which converts the sigmoidal log concentration curve to a straight line. Linear regression of the probit values against log concentration was then carried out in order to interpolate the log-concentrations yielding 1, 5, 10, 20, 35, 50, 65, 80, 90, 95 and 99% responses (Hoyle & Greenberg, 1988). The means and standard errors of the log concentrations for each of these percentage points were calculated and used to plot the summed concentration-response curve. The pD_2 values (negative logarithm of EC_{50}) in the absence and presence of PPADS and in time-control preparations were calculated from the concentration-response relationships and were then used in Student's paired t tests. The concentration-response histamine relationships for histamine were constructed in the saphenous artery in the same way.

The relaxant responses of 2-MeSATP on precontracted mesenteric arteries were expressed as a percentage of the maximal relaxation induced by 2-MeSATP, and were then used for probit analysis as described above.

In the aorta, the relaxant responses to ATP were expressed relative to the contraction evoked by the EC_{50} of noradrenaline. In preparations with intact endothelium, the $-$ log[ATP] that caused a 50% relaxation of the noradrenaline-induced contraction was calculated. In the cases when 50% relaxation was not reached in some endothelium-denuded preparations, the $-$ log[ATP] that caused 25% relaxation was calculated.

Means were compared by Student's paired and unpaired t tests. A probability of less than or equal to 0.05 was considered as significant. Data are presented as mean ± s.e.mean (n).

Results

In the rabbit isolated central ear artery, α,β-MeATP (0.1–100 μM) caused concentration-dependent contractions with a maximum response of 57.7 ± 4.3% (9) relative to the maximum contraction evoked by noradrenaline (Figure 1a). The negative log concentration of α,β-MeATP that caused a contraction equivalent to the EC_{25} for noradrenaline, was 6.60 ± 0.18 (9). PPADS at concentrations of 1–30 μM caused a concentration-dependent shift to the right of the concentration-response curve for α,β-MeATP (Figure 1a). The negative log[α,β-MeATP] producing a contractile response equivalent to the EC_{25} of noradrenaline, in the presence of PPADS at 1, 3 and 10 μM was 6.16 ± 0.18 (8), 5.90 ± 0.18 (8) and 4.72 ± 0.36 (8), respectively. All these values were significantly different from control ($P<0.01$, paired t tests). In the presence of PPADS at a concentration of 30 μM, the highest concentration of α,β-MeATP used (100 μM) did not produce an effect equivalent to the EC_{25} for noradrenaline.

In the saphenous artery, α,β-MeATP (0.1–100 μM) caused concentration-dependent contractions with a $-$ log[α,β-MeATP] that produced a contraction equivalent to the EC_{25} for histamine of 6.18 ± 0.17 (9) (Figure 1b). PPADS (1–30 μM) concentration-dependently shifted the concentration-response curve for α,β-MeATP to the right (Figure 1b). The negative log[α,β-MeATP] values equivalent to the EC_{25} for histamine in the presence of PPADS at concentrations of 1, 3, 10 and 30 μM were significantly less than control values being 5.90 ± 0.19 (8), 5.73 ± 0.16 (8), 4.99 ± 0.14 (8) and 4.51 ± 0.13 (8), respectively ($P<0.01$, paired t tests).

In the presence of phentolamine (1 μM), EFS of the intramural nerves caused frequency-dependent contraction of the rabbit ear artery, which although almost neglible at a frequency of 4 Hz, was maximal at a frequency of 32 Hz (Figure 2a). PPADS at a concentration of 1 μM did not significantly affect contractions of the ear artery evoked by EFS, but at 3–30 μM it caused significant inhibition. In the

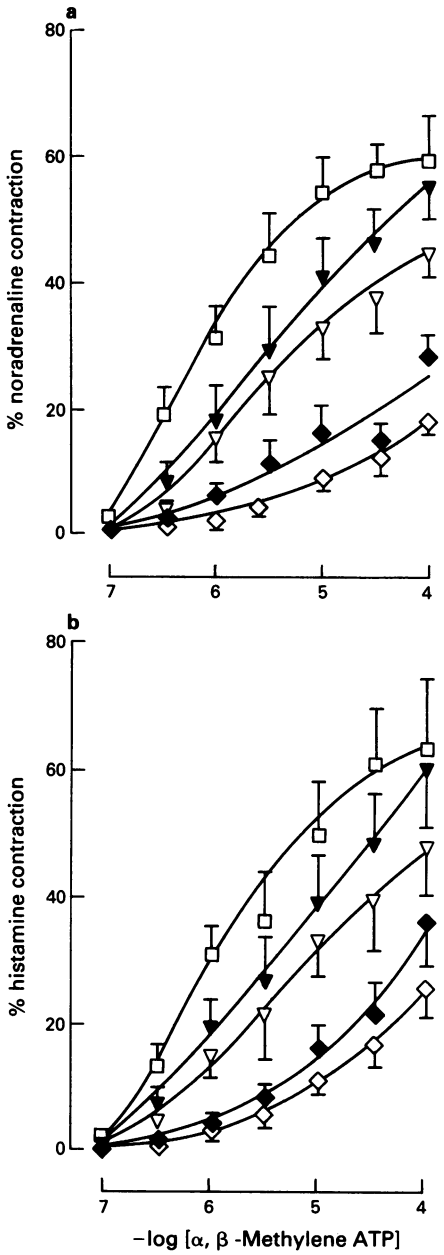

Figure 1 Concentration-response relationships for α,β-methylene ATP in the presence and absence of PPADS on rabbit isolated central ear artery (a) and saphenous artery (b). Responses (ordinate axis) are expressed as a percentage relative to the maximum contraction evoked by noradrenaline (ear artery) or histamine (saphenous artery). Concentration-response curves are shown for α,β-methylene ATP in the absence of PPADS (□, $n=8$ or 9) and presence of PPADS at concentrations of 1 μM (▼, $n=7$ or 8), 3 μM (▽, $n=7$ or 8), 10 μM (◆, $n=7$ or 8) and 30 μM (◇, $n=7$ or 8). The values shown represent the mean ± s.e.mean.

presence of 30 μM PPADS, the contractile response evoked by EFS at a frequency of 32 Hz was decreased by 80.0 ± 4.5% (8) relative to that in the absence of PPADS ($P<0.001$, paired t tests). The log EF_{50} (i.e. the frequency that causes a 50% contraction of the maximum response) of 1.00 ± 0.07 (8) in the absence of PPADS was significantly increased by PPADS at concentrations of 10 and 30 μM, being 1.22 ± 0.04 (7) and 1.31 ± 0.03 (6), respectively ($P<0.05$, paired t tests).

In the saphenous artery, EFS produced frequency-dependent contractions at all applied frequencies (4–64 Hz) being maximal at a frequency of 32 Hz (Figure 2b). PPADS (1–30

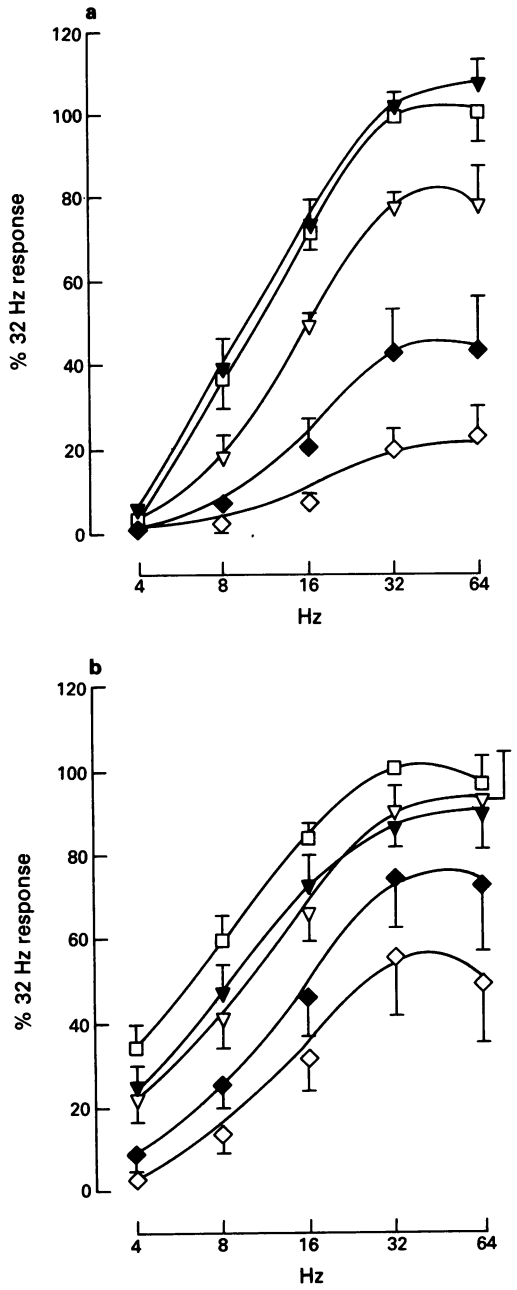

Figure 2 Frequency-response relationships for electrical field stimulation in rabbit isolated central ear artery (a) and saphenous artery (b). The Krebs solution contained 1 μM phentolamine. Responses (ordinate axis) are expressed as a percentage of contraction evoked by electrical field stimulation at a frequency of 32 Hz in controls. Frequency-response curves are shown for PPADS at zero (□, $n = 7$ or 8), 1 μM (▼, $n = 7$ or 8), 3 μM (▽, $n = 7$ or 8), 10 μM (◆, $n = 7$ or 8) and 30 μM (◇, $n = 7$ or 8). Values shown are mean ± s.e.mean. The abscissae are on a logarithmic scale.

μM) caused a concentration-dependent inhibition of neurogenic contractions of the saphenous artery at frequencies of 4, 8 and 16 Hz. Contractions evoked by EFS at frequencies of 32 and 64 Hz were significantly inhibited by PPADS only at concentrations of 10 and 30 μM. In the presence of PPADS at a concentration of 30 μM, contractions induced by EFS at 32 Hz were decreased by 44.7 ± 12.8% (9) relative to the control value. The log EF_{50} of 1.10 ± 0.03 (8) and 1.19 ± 0.03 (8) obtained in the presence of PPADS at concentrations of 10 and 30 μM, respectively, were significantly different from that of 0.89 ± 0.05 (8) obtained in the absence of PPADS ($P < 0.01$, paired t tests).

In mesenteric artery preparations in which tone has been raised with the EC_{50} of noradrenaline, 2-MeSATP at concentrations of 3–300 nM caused concentration-dependent sustained relaxations usually after an initial small transient contraction, but at concentrations of 1 μM and above it caused marked contractions without additional relaxation. In time-matched control preparations, the second concentration-response curve for 2-MeSATP was significantly shifted to the right with pD_2 values of 7.32 ± 0.17 (4) and 6.77 ± 0.13 (4) for the first and second curves, respectively ($P < 0.001$, paired t tests). In preparations incubated with 30 μM PPADS, there was also a significant rightward shift of the concentration-response curve with the pD_2 value being 7.42 ± 0.16 (4) in the absence and 6.73 ± 0.09 (4) in the presence of PPADS ($P < 0.01$, paired t tests). However, the shift of the second concentration-response curves for 2-MeSATP in preparations without and with incubation with PPADS were not significantly different being 0.55 ± 0.04 (4) and 0.69 ± 0.10 (4) log units, respectively ($P > 0.05$, unpaired t tests).

In preparations of the rabbit aorta with intact endothelium precontracted by the EC_{50} of noradrenaline, ATP (1 μM–1 mM) caused a concentration-dependent fast transient relaxation with a $-\log[ATP]$ that caused 50% relaxation of 4.13 ± 0.07 (6). In the presence of PPADS at a concentration of 30 μM, there was no significant change in this value, which was 3.89 ± 0.15 (6) (Figure 3a). Further, no significant difference was found when the slopes of the concentration-response curves for ATP were calculated, being 44.3 ± 3.4 (6) and 37.0 ± 3.4 (6)% log unit^{-1} in the presence and absence of PPADS, respectively ($P > 0.005$, paired t tests).

ATP (10 μM–1 mM) caused a slow, sustained relaxation of the precontracted endothelium-denuded preparations of the aorta, with a significantly smaller maximum response than in the endothelium-intact preparations at all concentrations of ATP. The $-\log[ATP]$, that caused 25% relaxation, 3.84 ± 0.12 (6), obtained before incubation with PPADS, was not significantly different from that obtained after 20 min incubation with PPADS at a concentration of 30 μM which was 4.00 ± 0.09 (6) (Figure 3b). No significant difference was found between the slopes of the concentration-response curves for ATP before and after incubation with PPADS being 35.0 ± 2.9 (6) and 34.0 ± 1.5 (6)% log unit^{-1}, respectively.

In time-matched control preparations, either with or without endothelium, there were no significant differences between any points of the concentration-response curves, or the $-\log[ATP]$ values causing 50% or 25% relaxation, or the slopes of the curves.

Noradrenaline, added cumulatively (0.1–100 μM), caused concentration-dependent contraction of the rabbit ear artery. The pD_2 values calculated for the first and second concentration-response curves in the time-matched control preparations were not significantly different being 6.07 ± 0.13 (4) and 5.99 ± 0.16 (4), respectively. PPADS (30 μM) had no effect on the potency of noradrenaline (Figure 4a). The pD_2 values in the absence and in the presence of PPADS were 6.08 ± 0.07 (4) and 5.95 ± 0.11 (4), respectively. There was no significant difference between these values. In addition, PPADS did not significantly change the maximum response to noradrenaline, which was 3.68 ± 0.35 (4) g before and 3.59 ± 0.32 (4) g after incubation with PPADS.

Histamine, added cumulatively (1 μM–1 mM), concentration-dependently contracted the rabbit saphenous artery. In the time-control preparations, the first pD_2 value was significantly greater than the second one being 5.46 ± 0.22 (4) and 5.12 ± 0.26 (4), respectively ($P < 0.05$, paired t tests). However, there was no significant difference between the pD_2 values calculated in the absence (5.46 ± 0.19, $n = 4$) and presence (5.27 ± 0.22, $n = 4$) of PPADS at a concentration of 30 μM (Figure 4b). Further, there was no significant difference between the shift in the concentration-response curve due to time alone and that due to PPADS as well, these values being 0.19 ± 0.07 (4) and 0.34 ± 0.10 (4) log units, respectively ($P > 0.05$, unpaired t tests). No significant differ-

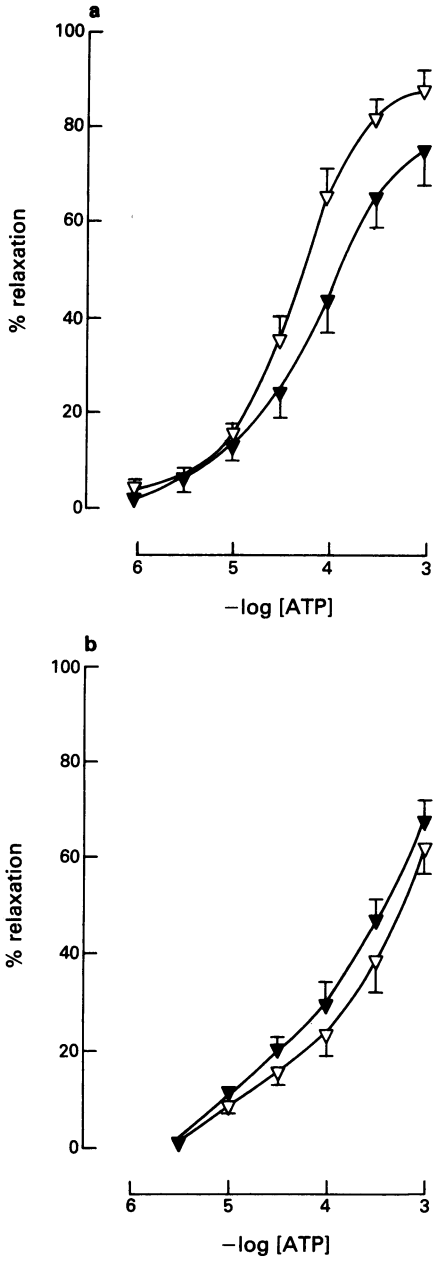

Figure 3 Concentration-response relationships for ATP in the rabbit aorta with (a) and without endothelium (b). Curves are shown in the absence (▽, $n = 4$) and presence of PPADS at 30 μM (▼, $n = 4$). Values shown are mean ± s.e.mean. $^*P < 0.05$.

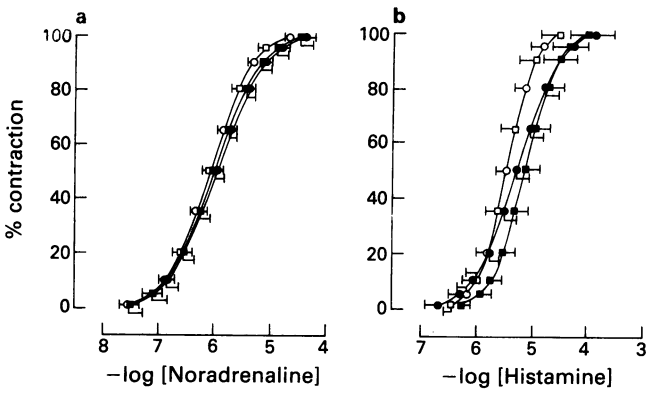

Figure 4 Rabbit isolated arteries: concentration-response relationships for noradrenaline in central ear artery (a) and for histamine in saphenous artery (b) calculated using probit analysis. Curves are shown for the first (□, $n = 4$) and the second (■, $n = 4$) relationships in time-control preparations, and in the absence (○, $n = 4$) and in the presence of PPADS at 30 μM (●, $n = 4$). Values shown are mean with s.e.mean.

ence was found when the maximum responses for histamine were calculated in the presence and absence of PPADS, being 3.08 ± 0.25 (4) g and 3.25 ± 0.29 (4) g, respectively.

Discussion

The results described in this study demonstrate that PPADS selectively antagonized responses mediated via P_{2X}-purinoceptors in the rabbit central ear artery and saphenous artery without significantly affecting responses mediated via P_{2Y}-purinoceptors in the rabbit mesenteric artery and aorta. Further, PPADS did not antagonize α-adrenoceptor or histamine receptor-mediated responses. These results are consistent with those of Bungardt *et al.* (1992) who showed that PPADS strongly inhibited contractions to exogenous α,β-MeATP (mediated via P_{2X}-purinoceptors) in guinea-pig ileum resistance vessels, without any action on the resting vessel diameter or the diameter after preconstriction with noradrenaline. In addition, our findings are also consistent with those of Lambrecht *et al.* (1992) who observed that PPADS caused selective inhibition of neurogenic contractions in the rabbit vas deferens, which also possesses postjunctional excitatory P_{2X}-purinoceptors (Kennedy & Burnstock, 1985).

Both the rabbit central ear and saphenous arteries possess P_{2X}-purinoceptors on the smooth muscle cells, which mediate the contractile actions of α,β-MeATP and purinergic nerve stimulation (Kennedy *et al.*, 1986; Burnstock & Warland, 1987a; Saville & Burnstock, 1988). In both preparations, PPADS caused a concentration-dependent inhibition of the contractile effects of α,β-MeATP. However, from our results it is difficult to decide whether or not the antagonism is competitive, since in both tissues the maximum effect of α,β-MeATP had not been reached in the presence of PPADS.

During the last few years sympathetic purinergic excitatory co-transmission in various blood vessels has been established (Burnstock, 1990b; Von Kügelgen & Starke, 1991; Hoyle 1992). Burnstock & Warland (1987a), using EFS, established the involvement of noradrenaline and ATP in sympathetic co-transmission in the rabbit saphenous artery, where there is a large prazosin-resistant purinergic component. Similar co-transmission has been demonstrated in the rabbit central ear artery (Kennedy *et al.*, 1986; Saville & Burnstock, 1988), however, in this preparation the prazosin-resistant component is much less dominant. In our experiments with EFS, the contractile responses were purinergic because the α-adrenoceptors had been blocked by phentolamine. At the highest PPADS concentration tested (30 μM), the inhibition of contractions evoked by EFS at frequencies of 32 and 64 Hz was about 2 times greater in the ear artery than in the saphenous artery. However, the changes of the EF_{50} due to PPADS in both the arteries were approximately the same, as were the shifts in concentration-response curves for α,β-MeATP in both tissues. Thus, it seems that the greater % inhibition due to PPADS in the ear artery is because the total amount of purinergic transmitter released by EFS in the ear artery is less than that in the saphenous artery at any given frequency, but the affinity of PPADS for P_{2X}-purinoceptors in the two arteries is the same. When PPADS was tested against the direct action of histamine in the saphenous artery there was no effect that did not occur with time in any case.

In order to determine the selectivity of PPADS for P_{2X}-purinoceptors, it was also tested on vessels that possess other subtypes of purinoceptors. P_{2Y}-purinoceptors in the rabbit

mesenteric artery are present, unlike many other vessels, not on endothelial cells, but on smooth muscle cells (Burnstock & Warland, 1987b). In the present study, we have shown that P_{2Y}-purinoceptor-mediated relaxations of the mesenteric artery are susceptible to desensitization, since there was a highly significant difference between the two pD_2 values for 2-MeSATP obtained in the time-control experiments. However, PPADS did not significantly affect this relationship, indicating an absence of inhibitory action on these P_{2Y}-purinoceptors.

The purinoceptors that mediate relaxation in the rabbit aorta appear to be different from those in the mesenteric artery. There are P_{2Y}-purinoceptors on endothelial cells, but another type of ATP-receptor, which is not a P_{2X}-purinoceptor, is present on the smooth muscle cells (O'Connor *et al.*, 1991; Chinellato *et al.*, 1992). At endothelial P_{2Y}-purinoceptors the rank order of potency of agonists is 2-MeSATP >> ATP = ADP >> UTP (O'Connor *et al.*, 1991), which is inherent for 'classical' P_{2Y}-purinoceptors as in the guinea-pig taenia coli (Burnstock & Kennedy, 1985). However, the order of potency of agonists at the aorta smooth muscle receptors is ATP = UTP > ADP > 2-MeSATP, which is not consistent with a classical P_{2Y}-purinoceptor (O'Connor *et al.*, 1991). Whatever the subtypes of P_2-purinoceptor, in testing the effects of PPADS in the aorta, the concentration-response relationship for ATP in preparations either with or without endothelium was not significantly altered by PPADS.

Thus, in the present study we have established that PPADS is a selective antagonist of vascular P_{2X}-purinoceptors having no effect at P_{2Y}-purinoceptors, α-adrenoceptors or histamine receptors. This is in line with previous observations (Lambrecht *et al.*, 1992; Bungardt *et al.*, 1992).

Note added in proof

Since this Manuscript was submitted and accepted for publication we have also shown that PPADS antagonizes P_{2X}-purinoceptors in the rabbit urinary bladder. (Ziganshin, A.U., Hoyle, C.H.V., Bo, X., Lambrecht, G., Mutschler, E., Bäumert, H.G. & Burnstock, G. (1993). PPADS selectively antagonizes P_{2X}-purinoceptor-mediated responses in the rabbit urinary bladder. *Br. J. Pharmacol.*, **110**, 1491–1495.)

The authors thank the Wellcome Trust (A.U.Z.), the Fonds der Chemischen Industrie (Germany) (G.L., E.M.), the Deutsch Forschungsgemeinschaft (H.G.B., SFB 169) and the British Heart Foundation for financial support. The authors would like to thank Dr W.R. Stones for assistance with the histochemistry and Dr A.L. Brizzolara for assistance with preparation of the vessels.

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(Received July 5, 1993
Revised October 30, 1993
Accepted November 18, 1993)