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Vasoconstrictor responses to the P_{2X}-purinoceptor agonist β, γ-methylene-L-ATP in human cutaneous and renal blood vessels

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1 Strips of human saphenous veins and of human renal arteries and veins were superfused with Krebs-Henseleit solution at 37°C. Constrictor responses were elicited by exogenous noradrenaline and the P_{2X}-purinoceptor-selective agonist, β,γ-methylene-L-ATP.

2 In human saphenous veins, β,γ-methylene-L-ATP (0.3–30 μM; EC_{50} 2.2 μM) induced marked constrictor responses. The maximal response to β,γ-methylene-L-ATP was similar to the maximal response to noradrenaline. The P_2-purinoceptor antagonist suramin (30 μM) shifted the concentration-response curve of β,γ-methylene-L-ATP to the right (apparent pK_B value 4.8); suramin (100 μM) markedly inhibited the responses to β,γ-methylene-L-ATP. The preferential P_{2X}-purinoceptor antagonist, pyridoxal-phosphate-6-azophenyl-2',4'-disulphonic acid (PPADS; 3 μM) slightly reduced the response to β,γ-methylene-L-ATP. At a ten times higher concentration (30 μM), PPADS almost abolished the responses to β,γ-methylene-L-ATP. PPADS (30 μM), in contrast, caused no significant change in the concentration-response curve of noradrenaline.

3 In extrarenal and intrarenal arteries, EC_{50} values and maximal responses to noradrenaline were similar when compared with responses to noradrenaline in saphenous veins. Noradrenaline also constricted extrarenal veins. However, in contrast to the results obtained on saphenous veins, β,γ-methylene-L-ATP caused almost no constrictor responses in extrarenal veins and arteries and only moderate responses in intrarenal arteries.

4 The results demonstrate marked differences in responsiveness of human blood vessels to the selective P_{2X}-purinoceptor agonist, β,γ-methylene-L-ATP, suggesting tissue differences in the occurrence or operation of P_{2X}-purinoceptors in human vascular tissues. Moreover, the results indicate that PPADS blocks P_{2X}-purinoceptors in human isolated blood vessels as previously demonstrated in animal blood vessels.

Keywords: P_{2X}-purinoceptors; β,γ-methylene-L-ATP; suramin; pyridoxal-phosphate-6-azophenyl-2',4'-disulphonic acid (PPADS); human saphenous vein; human renal artery; human renal vein

Introduction

P_{2X}-purinoceptors on blood vessels mediate vasoconstrictor responses to exogenous ATP and ATP analogues as well as to endogenous ATP released as a sympathetic cotransmitter (for review see Burnstock, 1990; Kennedy, 1990; Olsson & Pearson, 1990; Ralevic & Burnstock, 1991; von Kügelgen & Starke, 1991; Abbracchio & Burnstock, 1994). This extends to the rat kidney where ATP is involved as a sympathetic cotransmitter in nerve stimulation-induced vasoconstriction (Schwartz & Malik, 1989; Rump *et al.*, 1990; 1992; Bohmann *et al.*, 1995; for a controversial finding see Sehic *et al.*, 1994). Vasoconstriction-mediating P_{2X}-purinoceptors also operate on human subcutaneous, omental, skeletal muscle, pulmonary and pial arteries (Hardebo *et al.*, 1987; Pernow *et al.*, 1987; Liu *et al.*, 1989; Martin *et al.*, 1991) as well as on human mesenteric, saphenous and ovarian veins (Pernow *et al.*, 1987; Rump & von Kügelgen, 1994; Stones *et al.*, 1994). However, to our knowledge no information concerning P_{2X}-purinoceptor-mediated effects on blood vessels of the human kidney is available. Therefore, we analyzed the effects of the metabolically stable and P_{2X}-purinoceptor-selective agonist, β,γ-methylene-L-ATP (Hourani *et al.*, 1985; 1986; Leff *et al.*, 1990; see also von Kügelgen *et al.*, 1995) on human renal blood vessels and saphenous veins. In order to characterize the receptor mediating the responses to β,γ-methylene-L-ATP we also studied in saphenous veins the interaction with the non-subtype selective P_2-purinoceptor antagonist, suramin (Dunn & Blakely, 1988; von Kügelgen *et al.*, 1989; 1990; Hoyle *et al.*, 1990; Leff *et al.*, 1990) and the P_{2X}-preferential P_2-purinoceptor antagonist, pyridoxal-phosphate-6-azophenyl-2',4'-disulphonic acid (PPADS; Lambrecht *et al.*, 1992; Windscheif *et al.*, 1994; Ziganshin *et al.*, 1994).

Methods

This *in vitro* study was approved by the local ethics committee. Saphenous veins were obtained from patients (7 females, 60–83 years of age, mean age 68.3 ± 3.2; 24 males, 53–77 years of age, mean age 62.5 ± 1.3) undergoing open heart surgery for coronary bypass grafting. Renal blood vessels were obtained from patients (4 females, 63–75 years of age, mean age 70.7 ± 2.7; 4 males, 41–75 years of age, mean age 56.8 ± 7.1) undergoing renal surgery because of hypernephroma or epithelial carcinoma of the urinary tract. Portions of about 3–4 cm of the veins or 1.5–2 cm of the arteries were dissected free from surrounding connective tissue and cut into helical strips. This procedure removed the endothelium of the vessels; in preliminary experiments, arterial and venous strips precontracted with noradrenaline (10 μM) did not relax upon addition of acetylcholine (1 μM) or ATP (100 μM).

The strips were mounted in a jacketed chamber maintained at 37°C and superfused with Krebs-Henseleit solution at a

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constant rate of 6 ml min^{-1}. The superfusion fluid was gassed continuously with carbogen (95% O_2/5% CO_2) and passed through a heat exchanger (37°C) before it reached the vessels. The strips were connected to a force-displacement transducer (Biegestab K30, Hugo Sachs Electronics, March-Hugstetten, Germany) for measuring isometric tension with a multi-pen recorder (Rikadenki, Freiburg i. Br., Germany). Initial tension was set at 19.6 mN; during the first 60 (veins) or 90 min (arteries) of superfusion, the tissues relaxed to approximately 7 mN. After this equilibration period, vasoconstrictor responses were elicited by addition of noradrenaline or β,γ-methylene-L-ATP to the superfusion solution at increasing concentrations in a cumulative manner (see also Leff *et al.*, 1990). For interaction experiments with purinoceptor antagonists or their solvent (water), a second concentration-response curve for an agonist was performed on the same preparation after an interval of 60 min. Antagonists or their solvent were added to the medium immediately after the first concentration-response curve.

Vasoconstrictor responses were measured as the maximum increase in tension (mN) above resting tension. Responses in the second concentration-response curve (saphenous veins) were expressed as a percentage of the maximal response obtained in the first concentration-response curve. Where relevant, the sigmoid-shaped function No. 25 of Waud (1976) was fitted to averaged agonist concentration-response data. The function yielded the maximal constrictor response and the EC_{50} (concentration that caused 50% of the maximal response). For estimation of the affinity constant of suramin at the P_{2X}-purinoceptor in saphenous veins, an apparent pK_B($-\log K_B$) value of suramin was calculated from the shift of the concentration-response curve read at the level of 50% of the maximal response according to equation No. 4 of Furchgott (1972). Since only one antagonist concentration was used for calculation and a competitive character of the antagonism was not verified, the value is an *apparent* pK_B value (cf. von Kügelgen *et al.*, 1995).

Further experimental details are given in the Results section.

Materials

The following drugs were used: suramin hexasodium salt (Bayer, Wuppertal, Germany), β,γ-methylene-L-adenosine-5'-triphosphate tetrasodium salt (β,γ-methylene-L-ATP) (Biotrend, Research Biochemicals, Köln, Germany), pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid tetrasodium salt (PPADS) (Cookson Chemicals, Southampton, England), (−)-noradrenaline HCl, (±)-propranolol HCl (Sigma, Deisenhofen, Germany). Solutions of drugs were prepared with distilled water. The Krebs-Henseleit solution had the following composition (mM): NaCl 118; KCl 4.7; $CaCl_2$ 2.5, $MgSO_4$ 0.45; $NaHCO_3$ 25, KH_2PO_4 1.03, glucose 11.1, disodium EDTA 0.07; ascorbic acid 0.07, (experiments with veins) propranolol 0.005.

Statistics

Means ± s.e.mean are given throughout. Differences between means were tested for significance by the Mann-Whitney test. $P < 0.05$ or lower was taken as the criterion of statistical significance. For multiple comparisons with the same control, P levels were adjusted according to Bonferroni. n is the number of preparations.

Results

Human saphenous veins, extrarenal veins and arteries as well as intrarenal arteries were cut into helical strips and superfused with Krebs-Henseleit solution. Vasoconstrictor responses were elicited by exogenous noradrenaline or β,γ-methylene-L-ATP.

Saphenous veins

Noradrenaline as well as β,γ-methylene-L-ATP caused marked constrictor responses in saphenous veins. Concentration-response curves are shown in Figure 1a and representative tension tracings of one experiment with β,γ-methylene-L-ATP in Figure 2a. The maximal constrictor responses to noradrenaline and β,γ-methylene-L-ATP were similar (fitted maximal response to noradrenaline 26.9 mN; maximal response to β,γ-methylene-L-ATP 23.1 mN; see Methods). The respective EC_{50} values amounted to 0.1 μM for noradrenaline and 2.2 μM for β,γ-methylene-L-ATP.

In order to identify the receptor mediating the vasoconstrictor responses to β,γ-methylene-L-ATP, the interaction with P_2-purinoceptor antagonists was tested. For this purpose a second concentration-response curve was performed on each preparation after an interval of 60 min. Solvent or P_2-purinoceptor antagonists were added to the superfusion solution immediately after the first concentration-response curve. The responses to β,γ-methylene-L-ATP obtained in the second concentration-response curve in the presence of solvent did not differ from the respective responses obtained in the first concentration-response curve: e.g., the response to β,γ-methylene-L-ATP (10 μM) in the second concentration-response curve in the presence of solvent was 97.1 ± 5.3% ($n = 12$) of the response to β,γ-methylene-L-ATP (10 μM) in the first concentration-response curve. This excludes a significant, long lasting desensitization of the receptors mediating the responses to β,γ-methylene-L-ATP under the conditions of our experiments. Suramin (30 μM) shifted the concentration-response curve of

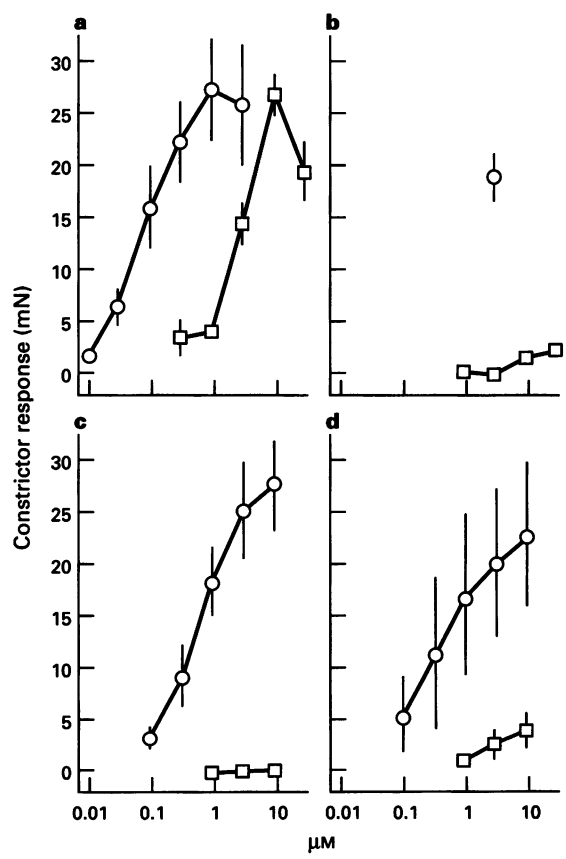

Figure 1 Vasoconstrictor responses of human blood vessels to noradrenaline (○) and β,γ-methylene-L-ATP (□). Saphenous veins (**a**), extrarenal veins (**b**), extrarenal arteries (**c**) and intrarenal arteries (**d**) were superfused with Krebs-Henseleit solution at 37°C. Vasoconstrictor responses were elicited by addition of noradrenaline or β,γ-methylene-L-ATP to the superfusion solution at increasing concentrations in a cumulative manner (exception: in extrarenal veins noradrenaline was given at a single concentration). Means ± s.e.mean of 6–41 experiments.

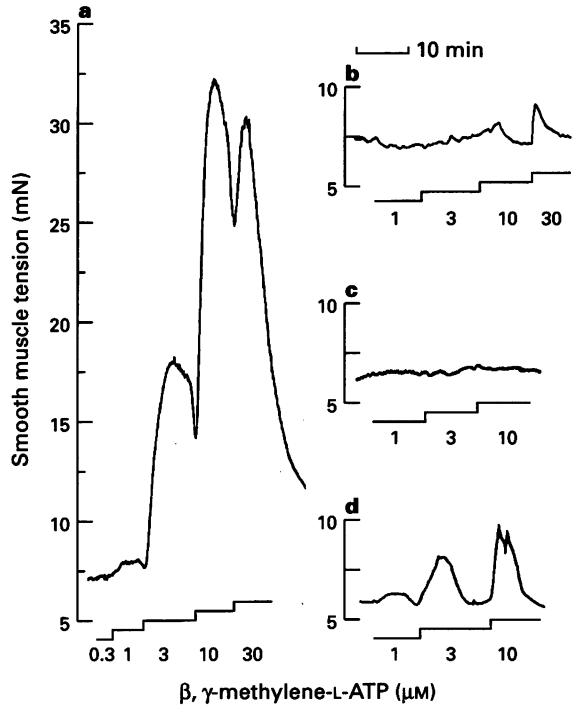

Figure 2 Effect of β,γ-methylene-L-ATP on human blood vessels. Human saphenous veins (**a**), extrarenal veins (**b**), extrarenal arteries (**c**) or intrarenal arteries (**d**) were superfused with Krebs-Henseleit solution at 37°C. β,γ-Methylene-L-ATP was added as indicated. Figure shows representative tracings from one of 41 (**a**), 6 (**b**), 8 (**c**) and 8 (**d**) experiments.

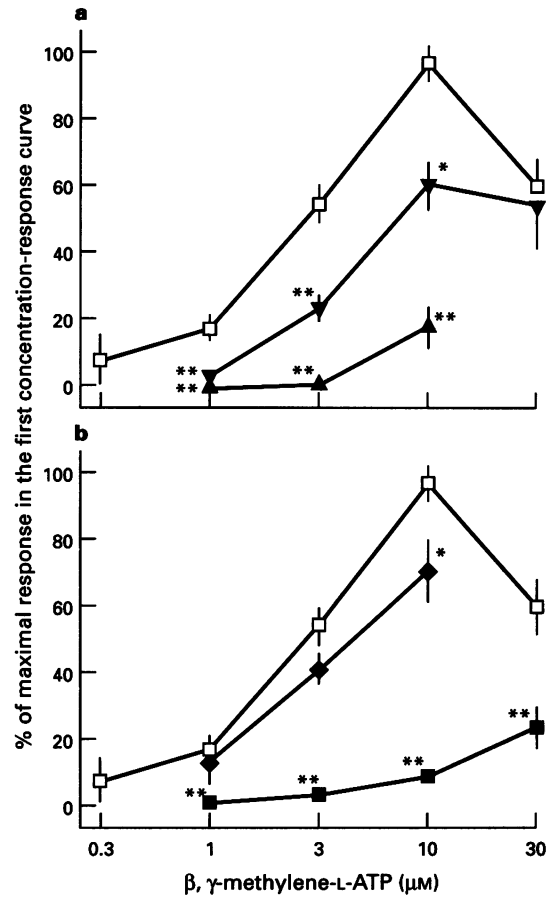

Figure 3 Interaction of suramin (**a**) and pyridoxal-phosphate-6-azophenyl-2',4'-disulphonic acid (PPADS; **b**) with β,γ-methylene-L-ATP in human saphenous veins. Two concentration-response curves of β,γ-methylene-L-ATP, separated by an interval of 60 min, were performed on a single preparation. Immediately after the first concentration-response curve, solvent (□), suramin (**a**; ▼, 30 μM; ▲, 100 μM) or PPADS (**b**; ◆, 3 μM; ■, 30 μM) was added to the superfusion solution for the remainder of the experiment. The figure shows constrictor responses obtained in the second concentration-response curve expressed as percentage of the maximal response obtained in the respective first concentration-response curve. Means ± s.e.mean of 5–12 experiments. Significant differences from corresponding value in the presence of solvent $*P<0.05$ and $**P<0.01$.

β,γ-methylene-L-ATP to the right (▼ in Figure 3a). From the shift at the level of 50% of the maximal response, an apparent pK_B value of suramin against β,γ-methylene-L-ATP of 4.8 was calculated (equation No. 4 of Furchgott, 1972). At a concentration of 100 μM, suramin very markedly inhibited responses to β,γ-methylene-L-ATP (▲ in Figure 3a). The P_{2X}-preferential antagonist PPADS used at a concentration of 3 μM slightly reduced the responses to β,γ-methylene-L-ATP (◆ in Figure 3b). At a ten times higher concentration (30 μM) PPADS very markedly inhibited the responses to β,γ-methylene-L-ATP with an obvious reduction in the maximal response (■ in Figure 3b). In contrast, PPADS (30 μM) caused no significant change in the concentration-response curve of noradrenaline (Figure 4).

Renal blood vessels

Responses to noradrenaline and β,γ-methylene-L-ATP in saphenous veins were compared to responses to these agonists in renal blood vessels. Noradrenaline caused similar maximal constrictor responses in extrarenal and intrarenal arteries (fitted maximal responses 29.2 mN and 23.6 mN, respectively; Figure 1c and d) when compared to responses in saphenous veins (Figure 1a; see also above). The respective EC_{50} values were 0.6 μM (extrarenal arteries) and 0.3 μM (intrarenal arteries). In extrarenal veins, noradrenaline (3 μM) also elicited a marked constrictor response amounting to 18.6 mN (Figure 1b). In contrast, β,γ-methylene-L-ATP elicited almost no responses in extrarenal veins (Figures 1b and 2b) and extrarenal arteries (Figures 1c and 2c) and only small responses in intrarenal arteries (Figures 1d and 2d). β,γ-Methylene-L-ATP also failed to cause a contraction of extrarenal arteries when it was added at one single high concentration (10 μM; not shown). In intrarenal arteries, no clear maximal responses to β,γ-methylene-L-ATP were obtained (Figure 1d).

Discussion

ATP has been shown to play an important role as a co-transmitter of noradrenaline, causing constrictor responses to sympathetic nerve stimulation in blood vessels; P_{2X}-purinoceptors mediate these effector responses (see Introduction). The aim of the present study was to investigate P_2-purinoceptor-mediated constrictor responses in human renal blood vessels as compared to responses in human saphenous veins.

In human saphenous veins, β,γ-methylene-L-ATP elicited marked constrictor responses as previously demonstrated in this tissue for the ATP analogue α,β-methylene-ATP (Rump & von Kügelgen, 1994). The interaction with the P_2-purinoceptor antagonists, suramin and PPADS indicate that the constrictor responses to β,γ-methylene-L-ATP are mediated by the P_{2X}-subtype of P_2-purinoceptor. Suramin has recently been shown to inhibit the constrictor responses to α,β-methylene-ATP in human saphenous veins without changing the responses to exogenous noradrenaline (Rump & von Kügelgen, 1994). Suramin also inhibited responses to β,γ-methylene-L-ATP with an apparent pK_B value (4.8) similar to the affinity constant of suramin at the P_{2X}-purinoceptor in animal tissues (4.7–5.2;

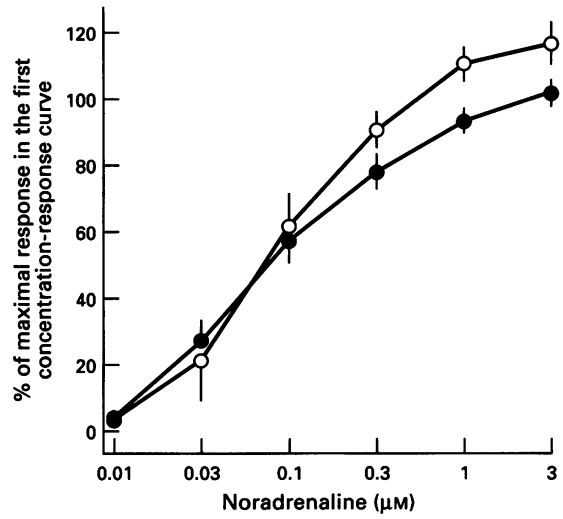

Figure 4 Interaction of pyridoxal-phosphate-6-azophenyl-2',4'-disulphonic acid (PPADS) with noradrenaline in human saphenous veins. Two concentration-response curves of noradrenaline, separated by an interval of 60 min, were obtained on a single preparation. Immediately after the first concentration-response curve, solvent (○) or PPADS (●, 30 μM) was added to the superfusion solution for the remainder of the experiment. The figure shows constrictor responses obtained in the second concentration-response curve expressed as percentage of the maximal response obtained in the respective first concentration-response curve. Means ± s.e.mean of 4 or 5 experiments.

Hoyle *et al.*, 1990; Leff *et al.*, 1990; von Kügelgen *et al.*, 1990). Moreover, the preferential P_{2X}-purinoceptor antagonist, PPADS (30 μM) markedly reduced the responses of human saphenous veins to β,γ-methylene-L-ATP while causing no change in the response to noradrenaline. PPADS is known to cause a selective, but non-competitive interaction with the P_{2X}-purinoceptor in rat and rabbit vascular tissues (Windscheif *et al.*, 1994; Ziganshin *et al.*, 1994). The reduction of the maximal response to β,γ-methylene-L-ATP by PPADS (30 μM) in human saphenous veins also indicates a non-competitive character of the interaction of PPADS with the P_{2X}-purinoceptor in human tissues.

In human renal blood vessels, β,γ-methylene-L-ATP caused almost no or only small constrictor responses despite the fact that the responses to noradrenaline were quite similar in all human blood vessels tested. This suggests tissue differences in the occurrence or operation of P_{2X}-purinoceptors in human vascular tissues. In line with regional differences in constrictor responsiveness of blood vessel in the kidney to activation of P_{2X}-purinoceptors (for rat kidney see Inscho *et al.*, 1994) β,γ-methylene-L-ATP caused some responses in intrarenal arteries but almost no responses in extrarenal arteries as well as veins.

In conclusion, this study demonstrates that the P_{2X}-purinoceptor agonist β,γ-methylene-L-ATP induces marked constrictor responses in the human saphenous vein while causing almost no or only moderate responses in renal blood vessels. This tissue difference in responsiveness to a P_{2X}-purinoceptor agonist may be important for the clinical use of related drugs. Thus, P_{2X}-purinoceptor agonists may increase renal blod flow (see also Harvey, 1964) due to an enhanced systemic blood pressure in the absence of an altered renal vascular resistance. Moreover, the results indicate that PPADS blocks vasoconstriction-mediating P_{2X}-purinoceptors in human blood vessels as previously demonstrated in rat and rabbit vascular tissues (Windscheif *et al.*, 1994; Ziganshin *et al.*, 1994).

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