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Vasoconstrictor responses via P2X-receptors are selectively antagonized by NF023 in rabbit isolated aorta and saphenous artery

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1 The effects of NF023, the symmetrical 3′-urea of 8-(benzamido)naphthalene-1,3,5-trisulphonic acid), and its parent compound suramin were investigated on vasoconstrictor responses to α,β-methylene ATP in rabbit isolated saphenous artery and vasodilator responses to ATP in noradrenaline-precontracted rabbit isolated thoracic aorta.

2 In rabbit isolated saphenous artery, α,β-methylene ATP-induced vasoconstrictor responses via P2X-receptors were concentration-dependently and competitively antagonised by NF023 (30–300 μM; $pA_2 = 5.69 \pm 0.04$). Suramin (100–1000 μM) also competitively blocked vasoconstrictor responses to α,β-methylene ATP, albeit with lower potency ($pA_2 = 4.79 \pm 0.05$). In contrast, NF023 (100 μM) did not significantly affect contractile responses to noradrenaline or histamine in the saphenous artery.

3 In noradrenaline-precontracted rabbit isolated thoracic aorta preparations, ATP (3–3000 μM) concentration-dependently induced relaxations via endothelium-dependent or smooth muscle P2Y-receptor subtypes. NF023 (30–300 μM) failed to block relaxant responses to ATP at endothelium-dependent P2Y-receptors, whereas suramin (100–1000 μM) did antagonise endothelium-dependent vasodilator responses to ATP. Neither NF023 (100 μM) nor suramin (300 μM) influenced vasorelaxant responses to ATP via endothelium-independent P2Y-receptors.

4 In conclusion, this study outlines the selectivity of NF023 as an effective P2X-receptor antagonist in rabbit isolated blood vessels without affecting endothelium-dependent or endothelium-independent P2Y-receptor subtypes, adrenoceptors or histamine receptors.

Keywords: NF023; suramin; rabbit isolated saphenous artery; rabbit isolated thoracic aorta; P2X-receptors; P2Y-receptors; ATP; α,β-methylene ATP

Introduction

The effects of purine nucleosides and nucleotides on vascular preparations were shown first in the 1920s (Drury & Szent-Györgyi, 1929). Adenosine 5′-triphosphate (ATP) was assumed to have only vasodilator actions, or to cause vasodilatation after a transient vasoconstriction, as seen *in vivo* after systemic administration (Haddy & Scott, 1968). Following the discovery of the endothelium-derived relaxing factor (EDRF; Furchgott & Zawadski, 1980), ATP was shown to produce vasodilator responses via P2-receptors localised on endothelial cells (De Mey & Vanhoutte, 1981). ATP-induced relaxation was abolished after removal of the endothelial cells and constriction was seen instead, for example in the rat isolated femoral artery (Kennedy *et al*., 1985).

Subclassification of P2-receptors into P2X and P2Y subtypes was proposed mainly based on the rank order of potencies of purine agonists (Burnstock & Kennedy, 1985). P2X-receptors largely mediate contraction of smooth muscle while P2Y-receptors largely mediate vasodilatation via endothelial cells and release of NO, although in some vessels P2Y-receptors are also present on vascular smooth muscle cells (Kennedy & Burnstock, 1985). Endothelial P2Y-receptors were later shown to coexist with P2U-receptors (where ATP and UTP are equipotent) in several vascular tissues such as the rat and hamster mesenteric arterial beds (Ralevic & Burnstock, 1991; 1996b). Recently, the P2Y-receptors localised on endothelial cells and smooth muscle, mediating vasodilatation, have been distinguished as separate members of a P2Y-receptor family of G-protein linked ATP receptors (Abracchio & Burnstock, 1994).

The functional classification of P2-receptor subtypes continues to be hindered by the shortage of selective and competitive antagonists. The P2-agonist α,β-methylene ATP (α,β-mATP) is a selective P2X-receptor desensitising agent (Burnstock & Kennedy, 1985), and the photoaffinity label, arylazidoaminopropionyl ATP ($ANAPP_3$; Hogaboom *et al*., 1980), irreversibly antagonised only P2X-receptor-mediated responses.

Reactive blue 2 has been claimed to be a P2Y-selective antagonist (Burnstock & Warland, 1987a), but it also effectively antagonises P2X-receptor-mediated smooth muscle contractile responses and neuronal depolarisation (Humphrey *et al*., 1995). Pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS; Lambrecht *et al*., 1992) has so far been characterized as a P2-receptor antagonist being able to discriminate between native and recombinant P2X- and P2Y-receptor subtypes (Windscheif *et al*., 1994; 1995; Ziganshin *et al*., 1994; Ziyal *et al*., 1994; Brown *et al*., 1995; Humphrey *et al*., 1995; Collo *et al*., 1996; Ralevic & Burnstock, 1996a). Suramin, although being a P2-receptor antagonist, does not differentiate between P2-receptor subtypes (Hoyle *et al*., 1990; Humphrey *et al*., 1995). Additionally, several other compounds, including trypan blue, Evans blue or 4,4′-diisothiocyanatostilbene-2,2′-disulphonate (DIDS) act as P2-receptor antagonists (Bültmann & Starke, 1994; Bültmann *et al*., 1994; Humphrey *et al*., 1995). DIDS preferentially antagonised the $P2X_1$-receptor in the human

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bladder rather than the $P2X_2$-receptor in the rat PC12 (Humphrey *et al*., 1995).

NF023, the symmetrical 3′-urea of 8-(benzamido)naphthalene-1,3,5-trisulphonic acid, has recently been suggested to be a selective and competitive antagonist of P2X-receptors in the rabbit vas deferens (Ziyal *et al*., 1994; Lambrecht *et al*., 1996). It has also been shown *in vivo* that α,β-mATP-mediated vasoconstriction in pithed rats is antagonised by NF023 and its parent compound suramin (Urbanek *et al*., 1990).

In the present study we further characterized the antagonistic properties of NF023 at P2X-receptors which mediate contractions in the rabbit isolated saphenous artery (Burnstock & Warland, 1987b), and at the endothelium-dependent and endothelium-independent P2Y-receptor subtypes which mediate relaxations in the rabbit isolated thoracic aorta (Abracchio & Burnstock, 1994).

Methods

General procedure

Male New Zealand white rabbits (2–3 kg) were killed by an overdose of sodium pentobarbitone (Sagatal) injected into the marginal ear vein and exsanguination. All vessels were mounted horizontally under isometric tension in 5 ml organ baths by inserting two tungsten wires into the lumen (Bevan & Osher, 1972). The initial tension of 1000 mg was applied to the vessels before allowing them to equilibrate for at least 60 min. The tissues were bathed in Krebs solution of the following composition (mM): NaCl 133, KCl 4.7, $NaHCO_3$ 16.4, $MgSO_4$ 0.6, NaH_{2PO_4} 0.8, glucose 7.7 and $CaCl_2$ 2.52. The solutions were aerated with 95% $O_{2/5\%}$ CO_2 and maintained at 37°C. In experiments with the isolated thoracic aorta, cocaine (1 μM) was included in the Krebs solution in order to block the neuronal uptake of noradrenaline (NA). The Krebs solution was changed every 15 min by an overflow method throughout the experiment. Contractions were recorded by Grass FT03C force-displacement transducer and displayed on a Grass 79D ink-writing oscillograph.

Single concentrations of noradrenaline (NA; 1 μM; thoracic aorta) and α,β-mATP (1 μM; saphenous artery) were applied repeatedly at intervals of 30 min until a reproducible increase in tone was achieved.

Saphenous artery

Four ring segments, 4 mm in length, were removed from the proximal end of each saphenous artery, cleaned of the connective tissue and mounted as described above.

Preliminary studies Several experiments were necessary to design a new protocol to be used in the following studies.

To evaluate cumulative concentration-response curves to α,β-mATP in the saphenous artery, single addition and cumulative applications of the agonist were compared. Single addition concentration-response curves to α,β-mATP (0.03–30 μM) were determined with 30 min between consecutive concentrations. The tissues were allowed to recover for 60 min. Then two consecutive cumulative concentration-response curves to α,β-mATP (0.03–30 μM) were generated in the same tissue with 60 min between the cumulative curves.

Concentration-response relationships for α,β-mATP (0.03–30 μM) were determined before and after incubation with suramin (100–1000 μM) or NF023 (30–300 μM) for at least 60 min (Leff *et al*., 1990). Up to two concentrations of one antagonist were tested in one tissue. In parallel, two preparations from each animal were treated in the same manner but without an antagonist being added and were considered as time-matched controls.

In order to estimate the specificity of NF023, its effects were tested on contractile responses to cumulatively-added NA (0.03–30 μM) or histamine (1–1000 μM). Time-matched controls to either of the agonists were constructed as described above.

Aorta

The thoracic aorta was removed and cleaned of adherent tissue, with special care being taken to preserve the endothelium. The peparations were precontracted with 0.1 μM NA (approximate EC_{30} value) and relaxant responses to ATP (3–3000 μM) were determined cumulatively. Suramin (100–1000 μM) or NF023 (30–300 μM) were added in accordance with incubation times previously described for suramin by Leff *et al*. (1990). Additionally, the endothelium of some preparations was removed mechanically by gently rubbing with a silk thread. In order to examine whether the removal of the endothelium was successful, the preparation were cut longitudinally and treated with a silver staining technique, at the end of the experiments (Nakatsu *et al*., 1988). Similarly, suramin (300 μM) or NF023 (100 μM) was added in order to examine their effect on relaxant responses to ATP (3–1000 μM). Time-matched controls were generated as described above.

Drugs used

(−)-Noradrenaline bitartrate, adenosine 5′-triphosphate sodium salt, α,β-methylene ATP lithium salt and histamine were all obtained from Sigma Chemical Co. (U.K.) Suramin sodium salt (Germanine) was obtained from Bayer AG (Germany). NF023 was synthesized in the Department of Pharmaceutical Chemistry, Bonn, Germany (Nickel *et al*., 1986). NA was dissolved in 0.05% sodium bisulphite in saline. The other drug solutions were made up in saline.

Data analysis

Contractile and vasodilator responses to exogenous agonists were recorded as changes in tension (mg). In precontracted thoracic aorta preparations, vasodilator responses were expressed as % decrease of NA-induced tone. In experiments with denuded aorta preparations, responses were expressed as percentage of relaxation caused by 1 mM ATP. For evaluation of concentration-effect relationships, logistic curves were fitted to the weighted mean contractions or relaxations by use of equation no. 25 of Waud (Waud, 1976) and non-linear regression analysis. The calculation resulted in the maximal response, the negative log molar values inducing 50% of the maximal response ($-\log EC_{50} = pD_2$) and the slope parameter. When more than one antagonist concentration was used, pA_2 values were determined by means of Schild-analysis (Arunlakshana & Schild, 1959). Antagonist potencies calulated from single concentrations were estimated by the equation: apparent $pK_B = \log$ (dose-ratio − 1) − log (antagonist concentration). Statistical significance was evaluated by paired Student's t test as appropriate ($P < 0.05$ was accepted as significant). All data are shown as mean ± s.e.mean.

Results

Saphenous artery

Preliminary results Neither the potency of, nor the maximal response to α,β-mATP obtained from two consecutive cumulative concentration-response curves ($pD_2 = 6.41 \pm 0.09$, maximum = 2941.2 ± 524.9 mg, $n = 6$ for the first and $pD_2 = 6.31 \pm 0.08$, maximum = 2521.4 ± 477.8 mg, $n = 6$ for the second concentration-response curve, respectively) differed significantly from those derived from single additions ($pD_2 = 6.32 \pm 0.06$, maximum = 3236.4 ± 615.9; $n = 6$) constructed in the same tissue (Figure 1). Therefore cumulative concentration-response curves (Figure 2) were used throughout the rest of the study.

NF033 (30–300 μM) antagonised the vasoconstrictor responses to α,β-mATP (0.03–300 μM) in a concentration-dependent manner ($pA_2 = 5.69 \pm 0.04$; $n = 4$; Figure 3a). This antagonism was readily reversible after washing (data not shown). In time-matched control experiments, the second cumulative concentration-response curve for α,β-mATP in the absence of NF023 ($pD_2 = 6.23 \pm 0.10$; $n = 4$) was not significantly different from the first ($pD_2 = 6.23 \pm 0.12$; $n = 4$). Similarly, suramin blocked ($pA_2 = 4.79 \pm 0.05$; $n = 4$) the vasoconstrictor responses to α,β-mATP (0.1–3000 μM; Figure

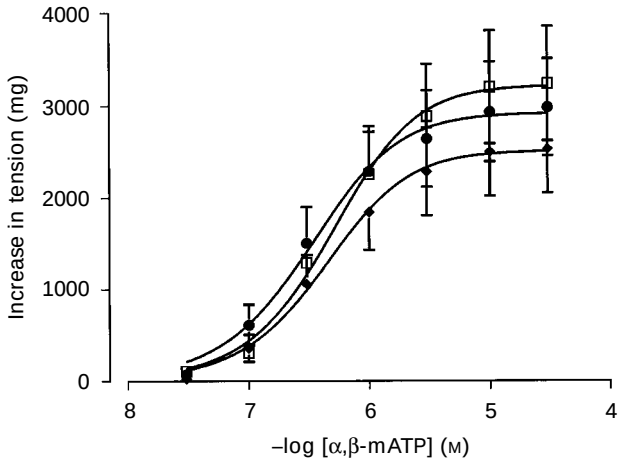

Figure 1 Concentration-response curves for the contractile responses to α,β-mATP derived from single addition (□, $n = 6$) and two consecutive cumulative applications of the agonist in the rabbit isolated saphenous artery (●, ◆, $n = 6$). Time interval between each concentration-response curve was 60 min. Data shown are means and vertical lines show s.e.mean. Error bars falling within the area covered by a symbol are not shown.

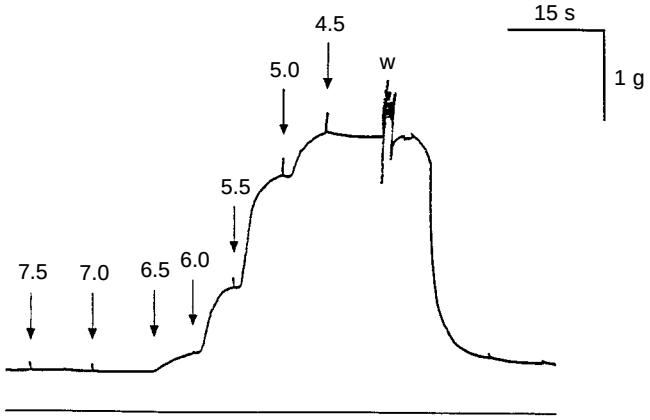

Figure 2 The original trace of concentration-dependent vasoconstrictor responses to α,β-mATP in the rabbit isolated saphenous artery. The application of exogenous α,β-mATP is indicated by the arrows and expressed as −log M. W denotes washing of the tissue.

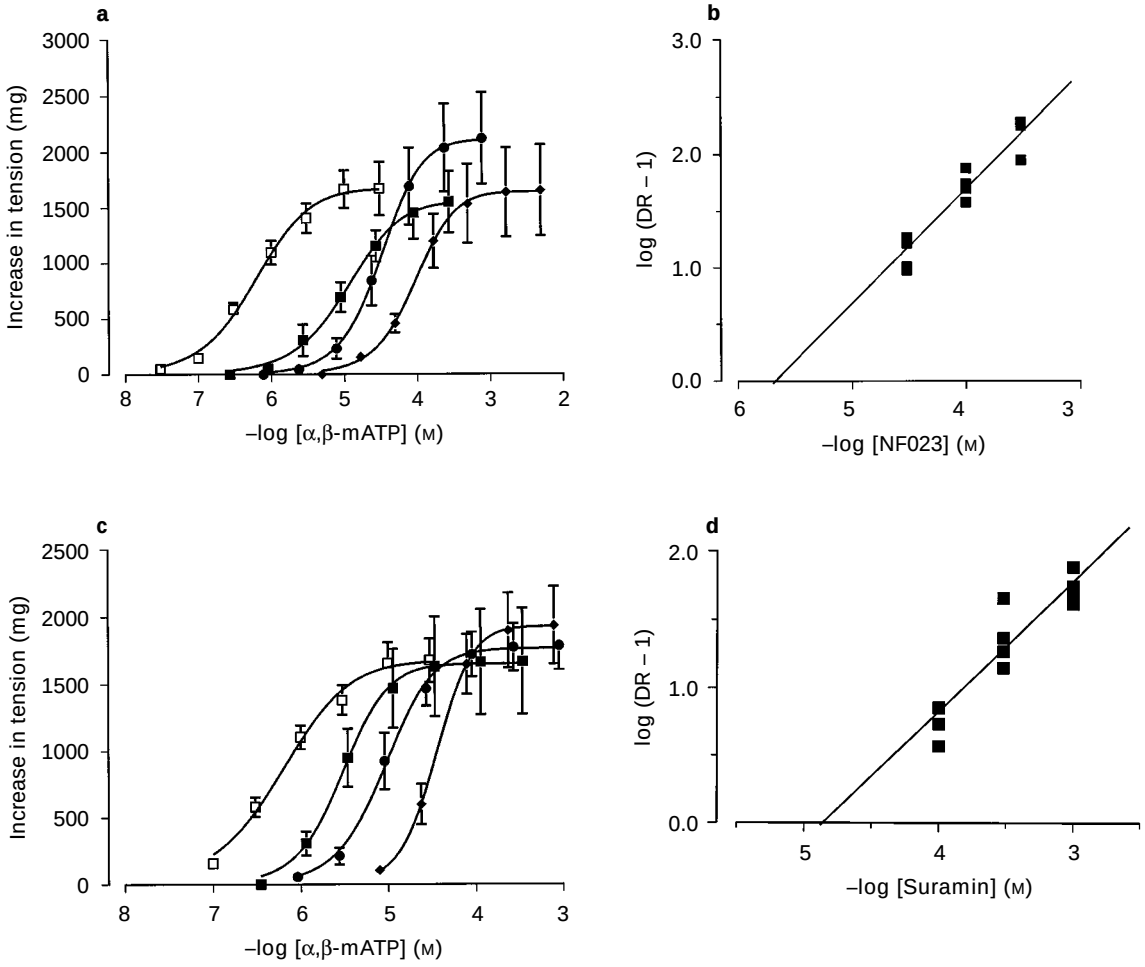

Figure 3 Cumulative concentration-response curves for the vasoconstrictor responses to α,β-mATP in the rabbit isolated saphenous artery (a) in the absence (□, $n = 12$) and in the presence of NF023 30 μM (■, $n = 4$), 100 μM (●, $n = 4$) and 300 μM (◆, $n = 4$) and (c) in the absence (□, $n = 9$) and in the presence of suramin 100 μM (■, $n = 4$), 300 μM (●, $n = 4$) and 1000 μM (◆, $n = 4$). For illustration purposes only the second curve data in the paired design where each tissue had its own control are shown. (b, d) The Schild-plots were derived from the associated paired dose-ratio (DR) data. Data shown are means and vertical lines indicate s.e.mean. Error bars falling within the area covered by a symbol are not shown.

3c). Time-matched control experiments for α,β-mATP (0.03–30 μM) in the absence of suramin revealed no significant difference between the first ($pD_2 = 6.11 \pm 0.09$; $n = 5$) and the second ($pD_2 = 6.26 \pm 0.11$; $n = 5$) cumulative curves. The slope of the Schild-plot for both NF023 (1.07 ± 0.06; Figure 3b) and suramin (0.98 ± 0.16; Figure 3d) did not significantly differ from unity. Neither of the antagonists had any significant effect on either the maximum contractile responses to α,β-mATP or on the slope parameters of the agonist concentration-response curves in this tissue.

Concentration-dependent contractions to NA (0.03–30μM; $pD_2 = 6.13 \pm 0.08$; $n = 9$) were unaffected by NF023 (100 μM; $pD_2 = 6.08 \pm 0.08$; $n = 9$; Figure 4a). Similarly, there was no significant change in the maximal vasoconstrictor response in the absence (4400.5 ± 333.8 mg; $n = 9$) and presence of NF023 (100 μM; 3960.1 ± 406.6 mg; $n = 9$). The response of NA in time-matched control preparations also remained unchanged (pD_2 values being 6.36 ± 0.09; $n = 4$ and 6.32 ± 0.07; $n = 4$ for the first and the second concentration-response curve, respectively).

The rabbit isolated saphenous artery was contracted by histamine (1–1000 μM) in a concentration-dependent manner. No significant difference could be seen in vasoconstrictor responses to histamine in the absence ($pD_2 = 4.97 \pm 0.04$; $n = 8$) and presence of NF023 (100 μM; $pD_2 = 4.73 \pm 0.03$; $n = 8$; Figure 4b). Also, the maximal response to histamine was not affected by NF023 (100 μM; 2679.2 ± 470.5 mg and 2479.2 ± 310.9 mg; $n = 8$ in the absence and in the presence of NF023, respectively). Time-matched controls revealed no significant difference in the effect of histamine between the first and second concentration-response curve ($pD_2 = 4.96 \pm 0.19$; $n = 5$ and $pD_2 = 4.72 \pm 0.24$; $n = 5$, respectively).

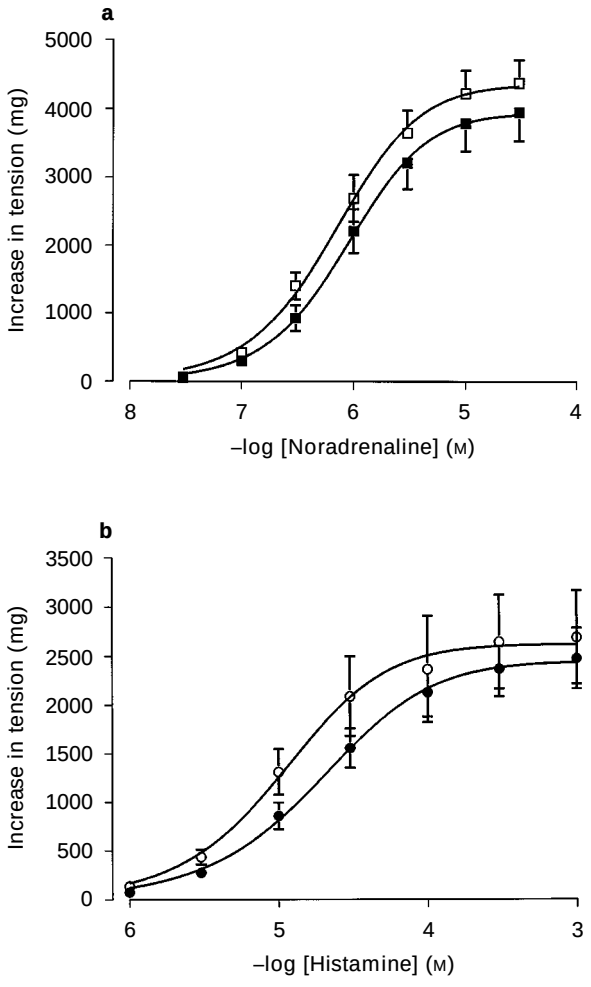

Figure 4 Cumulative concentration-response curves for the contractions (a) to noradrenaline in the absence (□, $n = 9$) and presence of 100 μM NF023 (■, $n = 9$) and (b) to histamine in the absence (○, $n = 8$) and presence of 100 μM NF023 (●, $n = 8$) in the rabbit isolated saphenous artery. Data shown are means and vertical lines indicate s.e.mean. Error bars falling within the area covered by a symbol are not shown.

Aorta

In the rabbit isolated thoracic aorta with intact endothelium precontracted with 0.1 μM NA to give a tone of approximately 30% of the maximal contraction, ATP evoked a fast and transient relaxation (Figure 5). NA itself caused concentration-dependent contractions in this tissue (0.01–300 μM; $pD_2 = 6.63 \pm 0.08$; $n = 9$). NF023 (30–300 μM) failed to affect the vasodilator responses to ATP (30–3000 μM; Figure 6a). Time-matched controls demonstrated that relaxant responses to ATP remained unchanged between the first ($pD_2 = 3.94 \pm 0.19$; $n = 5$) and second cumulative concentration-response curves ($pD_2 = 4.16 \pm 0.16$; $n = 5$). In contrast, suramin (100–1000 μM) significantly antagonised the relaxant responses to ATP (3–3000 μM; Figure 6b). Apparent pK_B values for the given concentrations were 4.43 ± 0.19 (100 μM; $n = 5$), 3.85 ± 0.09 (300 μM; $n = 9$) and 3.17 ± 0.13 (1000 μM; $n = 9$). Time-matched control experiments showed no difference between consecutive cumulative concentration-response curves to ATP (30–3000 μM; $pD_2 = 4.19 \pm 0.14$ and 4.28 ± 0.11; $n = 13$ for the first and for the second curve, respectively) in the absence of suramin.

In endothelium-denuded aortic preparations, a slow and sustained relaxation to ATP (3–1000 μM) occurred with a maximal response being significantly smaller ($51.6 \pm 2.2\%$, $n = 16$) than that in aorta preparations with intact endothelium. Control vasodilator responses to ATP ($pD_2 = 4.11 \pm 0.04$; $n = 12$) were not significantly different from those in the presence of suramin (300 μM; $pD_2 = 4.08 \pm 0.08$; $n = 6$) or NF023 (100 μM; $pD_2 = 4.15 \pm 0.13$; $n = 6$; Figure 7). In time-matched controls the vasodilator responses to ATP remained the same in the first ($pD_2 = 4.06 \pm 0.11$; $n = 4$) and the second ($pD_2 = 4.06 \pm 0.06$; $n = 4$) concentration-response curve.

Discussion

The results of this study indicate that in rabbit vascular preparations NF023 selectively inhibits P2X-receptor-mediated contractile responses without affecting relaxant responses mediated via endothelium-dependent and endothelium-independent P2Y-receptor subtypes.

Saphenous artery

α,β-mATP evokes transient contractile responses via P2X-receptors previously shown to be susceptible to desensitisation (Burnstock & Kennedy, 1985); therefore it is difficult to determine reproducible contractile responses to α,β-mATP.

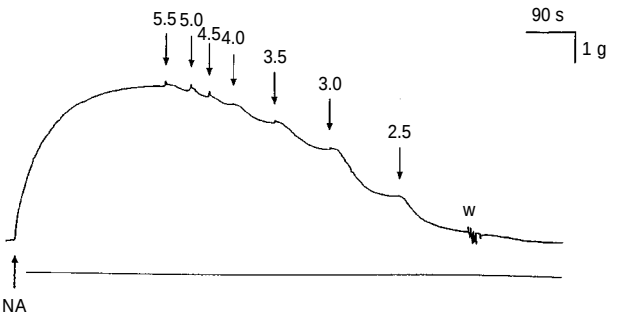

Figure 5 Single trace showing concentration-dependent vasodilatation responses to exogenous ATP in noradrenaline-preconstricted (0.1 μM) rabbit isolated thoracic aorta with intact endothelium. The application of the agonist is indicated by the arrows and expressed as −log M.W denotes washing of the tissue.

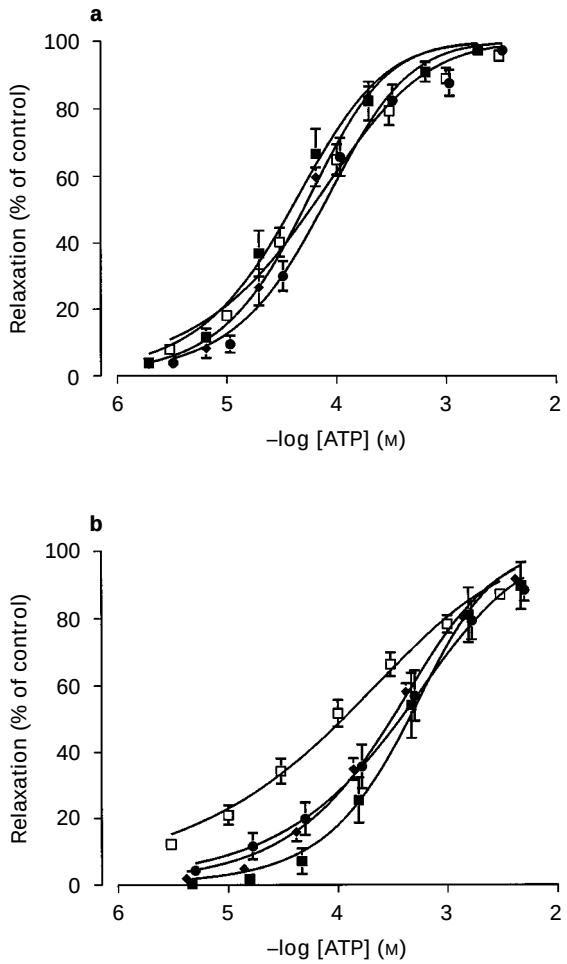

Figure 6 Relaxant responses to cumulatively applied ATP (a) in the absence (□, $n=9$) and presence of NF023 30 μM (■, $n=6$), 100 μM (●, $n=6$) and 300 μM (◆, $n=5$) and (b) in the absence (□, $n=22$) and presence of suramin 100 μM (■, $n=5$), 300 μM (●, $n=9$) and 1000 μM (◆, $n=9$) in noradrenaline (0.1 μM)-preconstricted rabbit isolated thoracic aorta preparations, with intact endothelium. For illustration purposes only the second curve data in the paired design where each tissue had its own control are shown. Responses are expressed as % decrease of noradrenaline-induced tone. Data shown are means and vertical lines indicate s.e.mean. Error bars falling within the area covered by a symbol are not shown.

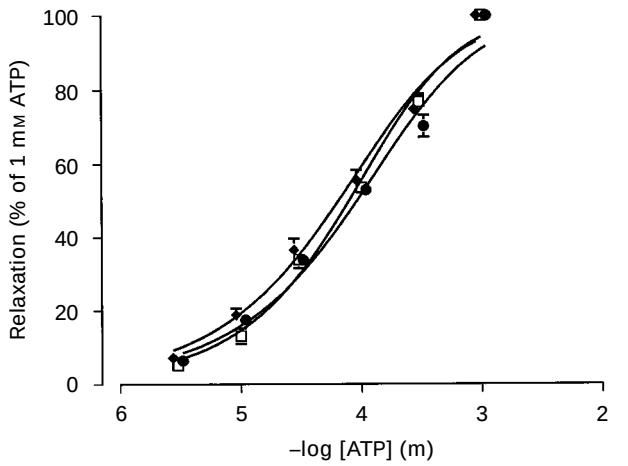

Figure 7 Cumulative concentration-response curves for relaxant responses to ATP in the absence (□, $n=12$) and presence of NF023 100 μM (◆, $n=6$) or suramin 300 μM (●, $n=6$) in noradrenaline (01. μM)-precontracted and endothelium-denuded rabbit isolated thoracic aorta preparations. For illustration purposes only the second curve data in the paired design where each tissue had its own control are shown. Responses are expressed as % of relaxation caused by 1 mM ATP. Data shown are means and vertical lines indicate s.e.mean. Error bars falling within the area covered by a symbol are not shown.

There have been several attempts to obtain cumulative concentration-response curves for nucleotide agonists in vascular preparations (Juul *et al*., 1993). With a new protocol in which cumulative applications of α,β-mATP were used, we tried to avoid the problem of a possible desensitisation of P2X-receptors in the rabbit isolated saphenous artery. Neither potency nor the achieved maximal response to α,β-,mATP changed significantly under the conditions chosen for the cumulative application of the agonist, indicating that no tachyphylaxis was induced in comparison to the single addition concentration curve within the time needed (approximately 60s) to obtain a cumulative concentration-response curve. Thus, the possibility of using cumulative application of nucleotide agonists should also be considered in other tissues.

In this study the suramin analogue, NF023, was 8 times more potent than its parent compound suramin in competitively antagonising the contractile responses to α,β-mATP mediated via P2X-responses on smooth muscle cells. Both antagonists produced rightward displacements of the α,β-mATP concentration-response curves in a concentration-dependent manner without significantly affecting either the maximal response or the slope parameter. Additionally, the slopes of the Schild-plots were not significantly different from unity. These results are consistent with our earlier findings describing the P2X-selective and competitive antagonism by NF023 in the rabbit vas deferens (Ziyal *et al*., 1994). In addition, the affinity estimate found for suramin in this study ($pA_2=4.79$) was identical with that determined in other vascular preparations such as the rabbit ear artery (Leff *et al*., 1990). In contrast, NF023 failed to block vasoconstrictor responses to NA or to histamine, underlining its P2-specificity.

Compared to NF023, other P2-receptor antagonists appear to have several disadvantages. PPADS has been shown to be selective for several native (Lambrecht *et al*., 1992; Ziganshin *et al*., 1994; Windscheif *et al*., 1994; 1995; Ziyal *et al*., 1994) and recombinant (Collo *et al*., 1996; Schachter *et al*., 1996) P2X-and P2Y-receptor subtypes. Furthermore, in one study PPADS showed pseudoirreversible antagonistic behaviour at P2X-receptors (Lambrecht *et al*., 1992). Additionally, it has low affinity for P2T- and P2U-receptors (Brown *et al*., 1995; Ralevic & Burnstock, 1996a). DIDS irreversibly antagonised P2Z-receptors in rat parotid acinar cells (Soltoff *et al*., 1993) and P2X-receptors in rat vas deferens (Bültmann & Starke, 1994). Evans blue and trypan blue, both of which are aromatic polysulphonic acids of relatively high molecular mass, have also been shown to possess antagonistic properties at P2X-receptors (Bültmann & Starke, 1993; Bültmann *et al*., 1994). Evans blue, differing from trypan blue in the position of the two sulphonic acid residues at the terminal naphthalene rings, reversibly blocked P2X-receptors in rat vas deferens without being competitive (Bültmann & Starke, 1993) and enhanced the maximum response to α,β-mATP. Likewise, trypan blue increased contractile responses to α,β-mATP in rat vas deferens (Bültmann *et al*., 1994). Suramin, the parent compound of NF023, has been shown to be competitive, but is not able to discriminate between P2-receptor subtypes (Hoyle *et al*., 1990). It has also been found that suramin enhances the maximum responses to α,β-mATP suggesting that it may abolish relaxant effects of high α,β-mATP-concentrations (Mallard *et al*., 1992).

Aorta

The receptors in the rabbit isolated thoracic aorta, mediating vasorelaxant responses, were found to be heterogeneous (Ziganshin *et al*., 1994). The heterogeneity of the P2-receptor population in this tissue is also consistent with the fact that the

P2Y-agonist, 2-MeSATP, produced a lower maximum response than ATP (author's unpublished observations), similar to that described in several other vascular preparations including the precontracted bovine aortic collateral artery (Wilkinson *et al*., 1994). However, NF023 (100 μM) did not affect the vasorelaxant responses to ATP via either subtype, whether endothelium-dependent or endothelium-independent. The P2-receptor on endothelial cells has been cloned and identified as a P2Y subtype (Webb *et al*., 1993; Henderson *et al*., 1995). However, the P2Y-receptor subtype on the vascular smooth muscle is pharmacologically different from the endothelium-dependent P2Y subtype (Burnstock *et al*., 1994) and has recently been sequenced (Chang *et al*., 1995).

In contrast, suramin (100 – 1000 μM) significantly antagonised the vasodilator responses to ATP via endothelium-dependent P^{2Y}-receptors. The suramin-induced rightward shift of the vasodilator response to ATP was not concentration-dependent and an approximately 4 fold decrease in the apparent antagonistic affinity could be seen as the suramin concentration became greater. As suramin has been shown to inhibit the ecto-nucleotidases as well as being a P2 antagonist its functional behaviour in this tissue may be explained by 'self-cancellation' as previously described by Crack *et al*. (1994). The surface-located nucleotidases are likely to play a crucial part in the rapid hydrolysis of the extracellular ATP and so are of major pharmacological interest. The enzyme inhibitory and P2-receptor antagonistic properties may not oppose each other when a less susceptible agonist to ectonucleotidase hydrolysis like α,β-mATP is used. Thus, the development of a specific ecto-nucleotidase inhibitor such as FP 67156, previously been shown to exhibit some 50 fold selective inhibitory activity for the ecto-nucleotidases (Crack *et al*., 1995), would be the next important step to facilitate receptor subtype characterization.

In conclusion, the suramin analogue NF023 competitively and selectively antagonises vascular P2X-receptors, having no effect on adrenoceptors, histamine receptors or on endothelium-independent P2Y-receptor subtypes. Thus, NF023 has advantages over other known P2-antagonists and the investigation of the P2-receptor activity of further suramin congeners may be of major pharmacological interest, in the near future.

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