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# $P_2$-purinoceptor antagonists: III. Blockade of $P_2$-purinoceptor subtypes and ecto-nucleotidases by compounds related to suramin

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**Abstract** Effects of suramin and five analogs or fragments of suramin were studied on contractions of the rat vas deferens elicited by $\alpha,\beta$-methylene ATP ($\alpha,\beta$-MeATP; mediated by $P_{2X}$-purinoceptors), relaxations of the carbachol-precontracted guinea-pig taenia coli elicited by adenosine 5′-O-(2-thiodiphosphate) (ADP$\beta$S; mediated by $P_{2Y}$-purinoceptors), and the degradation of ATP by rat vas deferens tissue. One compound, NF023, differed from suramin by removal of two *p*-methylbenzamido groups, whereas another, BSt101, differed from NF023 by additional removal of the three sulphonate residues from one of the terminal naphthalene rings.

The compounds all shifted the concentration-response curve of $\alpha,\beta$-MeATP in the rat vas deferens to the right and simultaneously increased the maximum of the curve. Where three concentrations were tested, the Arunlakshana-Schild regression was linear, and the slope did not differ from 1. The apparent $K_d$ values were between 1 and 3672 μM. In the guinea-pig taenia coli, the compounds shifted the concentration-response curve of ADP$\beta$S to the right in a parallel manner, but in the one case where three concentrations were tested, the slope of the Arunlakshana-Schild regression was lower than 1. Apparent $K_d$ values were between 10 and 786 μM. The removal of ATP from the medium by vas deferens tissue was decreased only by suramin, NF023 and BSt101, with $IC_{25\%}$ values between 170 and 590 μM.

The results indicate that $P_{2X}$-purinoceptor affinity, $P_{2Y}$-purinoceptor affinity and the ecto-nucleotidase effect all increase with the size of the molecule. BSt101 resembled NF023 in potency at all three sites, indicating that the possession of a second naphthalene-trisulphonate group is not a prerequisite for relatively high affinity. NF023 is interesting because it is $P_{2X}$- versus $P_{2Y}$-selective and, in addition, the compound with the highest $P_{2X}$- versus ecto-nucleotidase-selectivity presently available.

**Key words** Rat vas deferens · Guinea-pig taenia coli · $P_2$-purinoceptor antagonists · $P_{2X}$-purinoceptor · $P_{2Y}$-purinoceptor · Ecto-nucleotidase · Suramin · Suramin derivatives

## Introduction

In the first two studies of the present series we examined $P_{2X}$-purinoceptor, $P_{2Y}$-purinoceptor and ecto-nucleotidase effects of derivatives of 4,4′-diisothiocyanato-2,2′-disulphonate (DIDS; Bültmann et al. 1996b) and of Evans blue and trypan blue (Wittenburg et al. 1996b). One observation was that, by and large, activity increased with molecular size. The large molecules, however, were also symmetric, containing two of the putative receptor binding moieties. Thus, the results could also be taken to indicate that, instead of or in addition to an increase in size, the possession of two binding molecular structures increased biological activity (Wittenburg et al. 1996b). The aim of this last study was to re-examine the role played by size, the existence of two binding structures, or both, in a number of suramin derivatives. The approach again was to test smaller fragments, and modifications of smaller fragments, of the parent compound (see Bültmann et al. 1996b).

Suramin is the most widely used $P_2$ antagonist ever since its introduction by Dunn and Blakeley (1988). Of its smaller derivatives (Fig. 1), NF023 has been examined at the $P_{2X}$-purinoceptors mediating an increase in blood pres-

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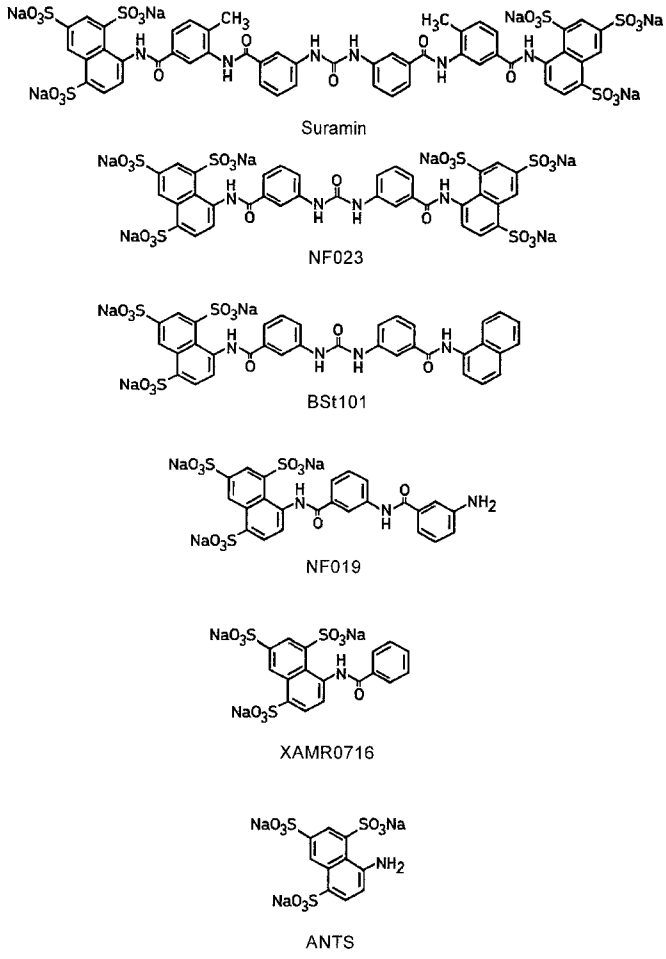


**Fig. 1** Structures of suramin and congeners

sure in pithed rats (Urbanek et al. 1990), at the $P_{2Y}$-purinoceptors of turkey erythrocytes (van Rhee et al. 1994), and in various other smooth muscle preparations (Ziyal et al. 1994, 1996; Lambrecht et al. 1996) and has been shown to be $P_{2X}$- versus $P_{2Y}$- and $P_{2U}$-selective (Ziyal et al. 1994, 1996). BSt101 is a new, non-symmetrical derivative of NF023 lacking the three sulphonic acid residues at one of the naphthalene rings. NF019 has previously been tested in pithed rats and turkey erythrocytes (Urbanek et al. 1990; van Rhee et al. 1994), whereas XAMR0716 and ANTS have been tested in turkey erythrocytes only (van Rhee et al. 1994).

As in the preceding work (Bültmann et al. 1996b; Wittenburg et al. 1996b), effects of the putative antagonists were assessed on contractions of the rat vas deferens elicited by $\alpha,\beta$-methylene ATP ($\alpha,\beta$-MeATP; mediated by $P_{2X}$-purinoceptors), relaxations of the carbachol-precontracted guinea-pig taenia coli elicited by adenosine 5′-O-(2-thiodiphosphate) (ADP$\beta$S; mediated by $P_{2Y}$-purinoceptors), and the ecto-nucleotidase-catalyzed degradation of ATP by rat vas deferens tissue. Some results have been presented in abstract form (Wittenburg et al. 1996a).

## Methods

*General.* Methods were those of the first paper of this series (Bültmann et al. 1996b). Briefly, contractions of prostatic portions of the rat vas deferens were elicited by either $\alpha,\beta$-MeATP or high $K^+$ (addition of 40 mM, final $K^+$ concentration therefore 45.7 mM). Relaxations of strips of the guinea-pig taenia coli, precontracted with carbachol (50–90 nM), were elicited by either ADP$\beta$S or noradrenaline. Agents causing contraction (vas deferens) or relaxation (taenia coli) were washed out after responses had peaked. Removal of ATP was measured in 0.6 ml medium containing 10 µM of ATP and single pieces (8 mg on average) of rat vas deferens.

Logistic curves were fitted to contraction or relaxation values of agonist ($\alpha,\beta$-MeATP, ADP$\beta$S, noradrenaline) concentration-response experiments using equation No. 25 of Waud (1976) and non-linear regression. The calculation yielded the maximal agonist effect and the $EC_{50}$, i.e. the concentration producing 50% of the maximum of that curve. Curves were fitted to weighted mean values except for the calculation of antagonist $K_d$ values. In the latter case, curves were fitted to values obtained in each of the two concentration-response curves of each single experiment, and the shift of the second concentration-response curve with respect to the first curve was read at the level of the $EC_{50}$. The shift obtained in each antagonist experiment was corrected for the mean shift occurring in solvent controls. The apparent antagonist $K_d$ value was then calculated using equation no. 4 of Furchgott (1972) or (in the case of three antagonist concentrations and if the slope of the regression line did not differ from 1) regression according to Arunlakshana and Schild (1959).

*Statistics.* Data are expressed as either the arithmetic mean ± SEM or, in the case of $EC_{50}$ values and maximal effects, the SE as defined by Waud (1976). Means were tested for a significant difference by the Mann-Whitney test, with Bonferroni correction if applicable. Fitted curves were tested for a significant difference according to p. 371 of Motulsky and Ransnas (1987). $P<0.05$ was taken as the limit of statistical significance.

*Materials.* 8,8′-[Carbonylbis(imino-3,1-phenylenecarbonylimino)]-bis-(1,3,5-naphthalenetrisulphonate) hexasodium (NF023), 8-[3-aminophenylenecarbonylimino-(3,1-phenylene carbonylimino)]-1,3,5-naphthalenetrisulphonate trisodium (NF019) and 8-benzamido-1,3,5-naphthalenetrisulphonate trisodium (XAMR0716) were synthezised as previously described (Nickel et al. 1986; van Rhee et al. 1994). N-[3-(N-(1,3,5-Trisulpho-8-naphthyl)carbamoyl)phenyl]-N′-[3-(N-(1-naphthyl)-carbamoyl)phenyl]-urea trisodium salt (BSt101) was synthesized from $\alpha$-naphthylamine, 3-nitrobenzoylchloride, chloroformic acid phenyl ester (Aldrich, Steinheim, Germany) and 8-amino-1,3,5-naphthalenetrisulphonate trisodium (ANTS; Bayer, Leverkusen, Germany). The identity and purity of the products was confirmed by TLC or HPLC and $^1$H-NMR spectroscopy. Other drugs used were suramin (Bayer), adenosine 5′-O-(2-thiodiphosphate) trilithium (ADP$\beta$S), carbachol chloride, $\alpha,\beta$-methylene ATP dilithium ($\alpha,\beta$-MeATP) and (–)-noradrenaline bi-(+)-tartrate (Sigma, Deisenhofen, Germany). KCl for high $K^+$ was dissolved in medium. BSt101 was dissolved in dimethyl sulphoxide (DMSO; final concentration below 0.1%). All other drugs were dissolved in distilled water. Solutions of drugs were added to the organ bath in aliquots not exceeding 100 µl.

## Results

### Contraction of rat vas deferens

Two concentration-response curves of $\alpha,\beta$-MeATP were determined per vas deferens. In the first curve, increasing concentrations of $\alpha,\beta$-MeATP elicited increasing contraction with an $EC_{50}$ of 4.5±1.1 µM and a maximum of 25.3±1.8 mN ($n$ = 63). A second concentration-response curve, after addition of solvent (water or DMSO), was

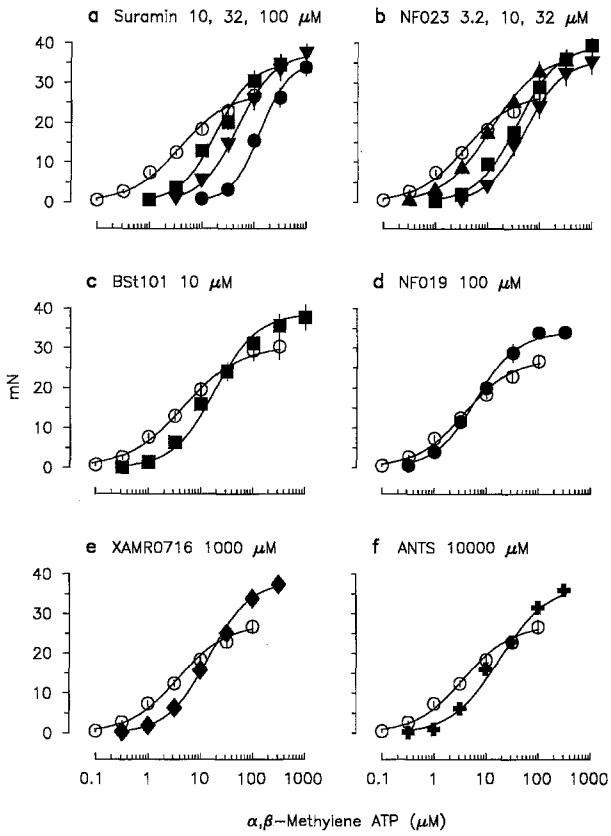


**Fig. 2** Effect of compounds related to suramin on the concentration-response curve of *α,β*-MeATP in rat vas deferens. Increasing concentrations of *α,β*-MeATP were added every 15 min and washed out immediately after the contraction had peaked. Two concentration-response curves were determined in each tissue. Solvent (○) or the antagonists (3.2 μM ▲; 10 μM ■; 32 μM ▼; 100 μM ●; 1000 μM ◆; 10000 μM ✚) were added to the medium after completion of the first curve and the second curve was determined 120 min later. *Abscissae*, concentration of *α,β*-MeATP. *Ordinates* show contraction (mN) in second curves. Means ± SEM from 4 to 7 experiments

close to the first ($EC_{50}$ 3.6±0.6 μM, maximum 28.7± 1.3 mN, $n$ = 10; empty circles in Fig. 2).

Suramin, NF023, BSt101, NF019, XAMR0716 and ANTS all shifted the concentration-response curve of *α,β*-MeATP to the right and increased the maximum contraction (Fig. 2). Where three concentrations were tested (suramin and NF023), plots according to Arunlakshana and Schild (1959) were linear and the slopes did not differ significantly from unity. Apparent $K_d$ values are summarized in Table 1.

Responses to high $K^+$ were studied using a different protocol. High $K^+$ (40 mM) was added twice, interval 60 min. It elicited rapid, transient contractions, amounting to 14.2±1.1 mN upon the first addition ($n$ = 33). Solvent (water or DMSO) or antagonists were administered immediately after the first response to high $K^+$. In solvent controls, $K^+$-evoked contractions remained constant (second response 100±5% of first; $n$ = 9). Suramin (100 μM), NF023 (32 μM), BSt101 (10 μM), NF019 (100 μM), XAMR0716 (1000 μM) and ANTS (10000 μM) did not change the response to high $K^+$ ($n$ = 3–5; not shown).

None of the antagonists altered the resting tension of the vas deferens.

## Relaxation of guinea-pig taenia coli

Two concentration-response curves of ADP*β*S or noradrenaline were determined in each preparation. When added during the plateau of the contraction elicited by carbachol (50–90 nM; force of contraction 98 mN on average), increasing concentrations of ADP*β*S caused increasing relaxation, $EC_{50}$ 98±1 nM and maximal relaxation by 100±2% ($n$ = 39; first concentration-relaxation curves). A second concentration-response curve, after addition of solvent (water or DMSO), was slightly to the left of the first ($EC_{50}$ 81±1 nM, maximal relaxation 101±1%; $n$ = 7; empty circles in Fig. 3).

NF019 (100 μM) did not alter the concentration-relaxation curve of ADP*β*S (Fig. 3d). Increasing concentrations of NF023 shifted the curve progressively to the right without changing the maximum (Fig. 3b). The regression ac-

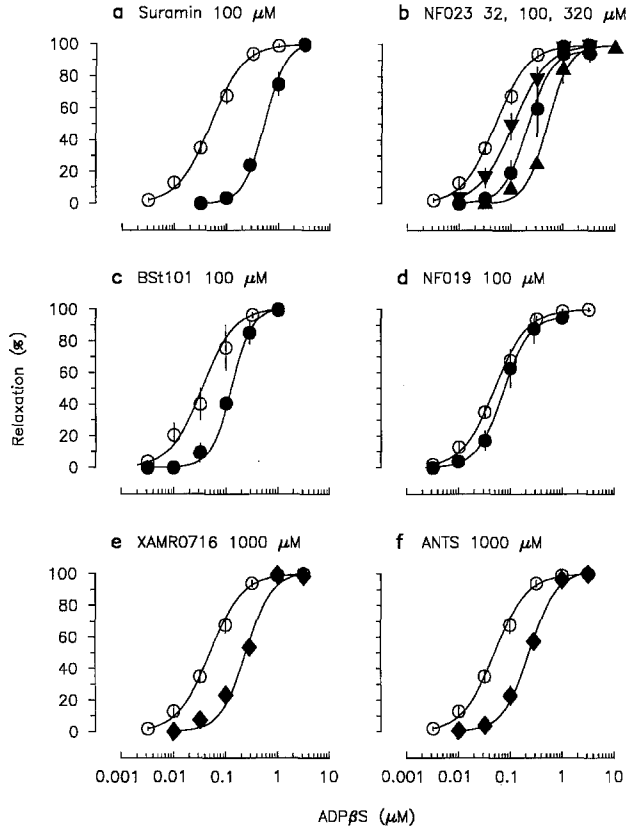


**Fig. 3** Effect of compounds related to suramin on the concentration-response curve of ADP*β*S in the carbachol-precontracted guinea-pig taenia coli. Increasing concentrations of ADP*β*S were added with each successive carbachol dose, i.e. every 15 min. Two concentration-response curves were determined in each tissue. Solvent (○) or the antagonists (32 μM ▼; 100 μM ●; 320 μM ▲; 1000 μM ◆) were added to the medium after completion of the first curve and the second curve was determined 120 min later. *Abscissae*, concentration of ADP*β*S. *Ordinates* show relation in second concentration-response curves as a percentage of the respective response to carbachol. Means ± SEM from 4 to 8 experiments

**Table 1** Apparent antagonist $K_d$ values of compounds related to suramin at $P_{2X}$-purinoceptors (rat vas deferens) and $P_{2Y}$-purinoceptors (guinea-pig taenia coli) and $IC_{25\%}$ values for inhibition of ATP breakdown (rat vas deferens)

| Compound | Apparent $K_d$ (μM) in rat vas deferens against $\alpha,\beta$-MeATP | | Apparent $K_d$ (μM) in guinea-pig taenia coli against ADP$\beta$S | | $K_i$ (μM) at ADP$\beta^{35}$S binding sites in turkey erythrocytes[a] | $IC_{25\%}$ (μM) for inhibition of ATP breakdown by rat vas deferens | |
|---|---|---|---|---|---|---|---|
| Suramin | 3.9[b,c] | (10, 32, 100) | 10.1[d] | (100) | 7.3 | 170 | (10, 100, 1000) |
| NF023 | 1.0[b] | (3.2, 10, 32) | 22.1, 28.4, 33.9 | (32, 100, 320) | 77 | 590 | (10, 100, 1000) |
| BSt101 | 4.8 | (10) | 30.8 | (100) | n.d. | 450 | (100, 1000) |
| NF019 | 93.9 | (100) | >100 | (100) | 58 | >1000 | (100, 1000) |
| XAMR0716 | 458 | (1000) | 511 | (1000) | >1000 | >10000 | (1000, 10000) |
| XAMR0721[e] | 515 | (1000) | 786 | (1000) | 19 | n.d. | |
| ANTS | 3672 | (10000) | 404 | (1000) | >1000 | >10000 | (10000) |

Apparent $K_d$ values in rat vas deferens and guinea-pig taenia coli are from experiments of Figs. 2 and 3, respectively. $IC_{25\%}$ values for ATP breakdown were interpolated from Fig. 4. Antagonist concentrations in parentheses. Where three $K_d$ values are given, they were obtained with the three concentrations indicated

[a] From van Rhee et al. (1994); [b] from regression according to Arunlakshana and Schild (1959); [c] 2.6 μM in Bültmann et al. (1994); [d] 6.5–16.1 μM in Bültmann et al. (1996a); [e] from Bültmann et al. (1996a; except $K_i$ in turkey erythrocytes; footnote a); n.d., not determined

cording to Arunlakshana and Schild (1959) was linear with a slope of 0.58 ($P<0.05$ vs. 1). Suramin, BSt101, XAMR0716 and ANTS, at the single concentration tested, also shifted the curve to the right without changing the maximum (Fig. 3). The apparent $K_d$ values are in Table 1.

Concentration-relaxation curves for noradrenaline were determined using the same protocol. The $EC_{50}$ of noradrenaline, its maximal effect, and the lack of change from the first to the second noradrenaline concentration-response curve in solvent (water or DMSO) experiments ($n = 8$) were similar to results in the accompanying isothiocyanate study (where the interval between the two curves was 30 min; Bültmann et al. 1996b). None of the compounds, at the highest concentration tested against ADP$\beta$S (Table 1), changed the concentration-response curve of noradrenaline ($n = 3$–4; not shown).

None of the antagonists altered the spontaneous activity of the taenia or the contraction to carbachol.

### Removal of ATP from medium

There was a loss of 4±1% of added ATP (10 μM) from the medium during 30 min of incubation in the absence of tissue ($n = 8$). Pieces of rat vas deferens removed 89.3±1.5% of added ATP within 30 min ($n = 10$). Suramin, NF023, BSt101 and XAMR0716 diminished the removal of ATP (Fig. 4). The $IC_{25\%}$ value of XAMR0716 exceeded the highest concentration tested (Table 1). DMSO, the solvent used for BSt101 ($n = 6$), as well as NF019 (100 and 1000 μM; Fig. 4d) and ANTS (10000 μM; Fig. 4f) had no effect. At the highest concentration tested, none of the antagonists altered the spontaneous loss of ATP ($n = 4$–6 each).

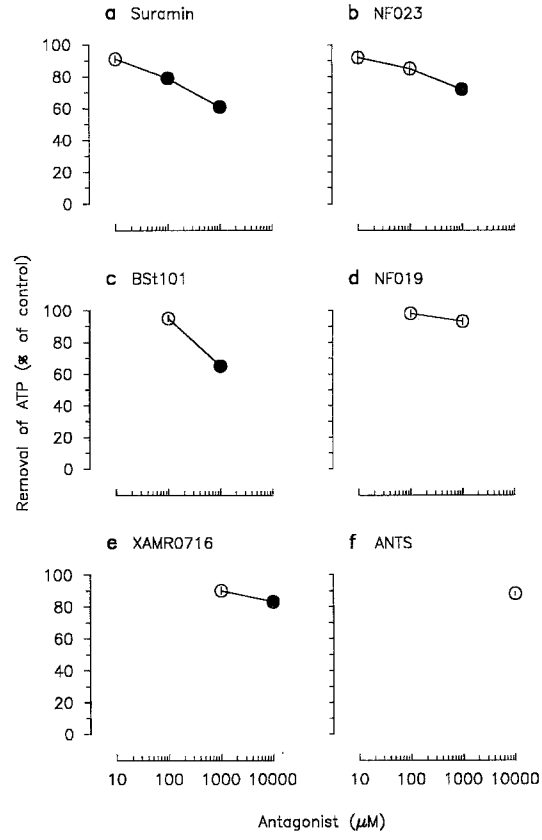


**Fig. 4** Effect of compounds related to suramin on the removal of ATP from the medium by rat vas deferens tissue. ATP, initial concentration 10 μM, was incubated with pieces of vas deferens for 30 min. *Abscissae*, antagonist concentration. *Ordinates* show removal of ATP in the presence of antagonists, expressed as a percentage of the average removal in solvent controls. *Filled symbols* indicate a significant difference from solvent controls ($P<0.05$). Means ± SEM from 4 to 6 experiments

## Discussion

Suramin, NF023, BSt101, NF019, XAMR0716 and ANTS antagonized $P_2$-purinoceptor-mediated responses in rat vas deferens and guinea-pig taenia coli (exception: NF019 in the taenia). Contraction of the vas deferens elicited by high $K^+$, contraction of the taenia coli elicited by carbachol, and relaxation of the taenia coli caused by noradrenaline remained unaltered, so the antagonism appeared to be specific for $P_2$-purinoceptors.

## $P_{2X}$-purinoceptor

Contraction of the rat vas deferens elicited by $\alpha,\beta$-MeATP is mediated by $P_{2X}$-purinoceptors (Bültmann and Starke 1994; Khakh et al. 1994, 1995a). Suramin and derivatives reduced these contractions in a surmountable manner (cf. Mallard et al. 1992; Bültmann et al. 1994 and Khakh et al. 1994 for suramin), and in the case of suramin and NF023 the slope of a plot according to Arunlakshana and Schild (1959) did not differ from unity. However, the antagonism was not purely competitive because all compounds enhanced the maximum of the agonist concentration-response curve (Fig. 2). Suramin also increases the maximum of the $\alpha,\beta$-MeATP concentration-response curves in other tissues containing $P_{2X}$-purinoceptors (Hoyle et al. 1990; von Kügelgen et al. 1990; Blakeley et al. 1991; Trezise et al. 1994), and Evans blue, trypan blue, reactive blue 2, cibacron blue 3GA and congo red, like suramin and congeners, increase it in the rat vas deferens (Bültmann and Starke 1993; Bültmann et al. 1994; Khakh et al. 1994; Wittenburg et al. 1996b; R. Bültmann, unpublished observation). The increase, hence, may be a common property of reversibly acting $P_2$-purinoceptor antagonists. The mechanism responsible for the increase of the maximum is not known (see Bültmann et al. 1994; Trezise et al. 1994; Wittenburg et al. 1996b).

Three structure-activity rules become manifest (see Table 1, which also contains values for XAMR0721, the 3,5-dinitro-derivative of XAMR0716, from Bültmann et al. 1996a). First, since even ANTS, the smallest compound, acted as a (weak) antagonist, the amino-naphthalene-trisulphonate moiety seems to be responsible for $P_{2X}$-purinoceptor binding, presumably also in the larger molecules: a conclusion similar to that drawn for the amino-hydroxy-naphthalene-disulphonate structure in the Evans and trypan blue series (Wittenburg et al. 1996b). Second, an increase in molecular size enhances $P_{2X}$ affinity until a maximum is reached in NF023; the further increase to suramin rather tends to reduce affinity. An order of potency NF023 $\geqslant$ suramin $\gg$ NF019 was also obtained for the blockade of the pressor response to $\alpha,\beta$-MeATP in pithed rats, another presumably $P_{2X}$-purinoceptor-mediated effect (Urbanek et al. 1990). Third, the finding that BSt101, which lacks the three sulphonic acid residues at one of its naphthalenes, was only slightly less potent than NF023 indicates that symmetry, or the possession of two binding (ANTS) structures per molecule, is not a prerequisite for high $P_{2X}$ affinity. Suramin and its congeners do not seem to bind to two sites of the $P_{2X}$-purinoceptor. The increase in affinity connected with the step from NF019 to NF023 is due to the increase in size rather than the additional binding moiety per antagonist molecule.

NF023 was the most potent antagonist at the $P_{2X}$-purinoceptor of rat vas deferens (Table 1). Comparison with other chemical classes shows that its apparent $K_d$ of 1.0 μM is similar to DIDS (2.5 μM), pyridoxalphosphate-6-azophenyl-2′,5′-disulphonic acid (iso-PPADS; 0.3 μM), cibacron blue 3GA (1.6 μM), reactive red 2 (0.4 μM), and Evans blue (2.5 μM) and its demethylated derivative NH01 (0.8 μM; see preceding paper: Wittenburg et al. 1996b).

## $P_{2Y}$-purinoceptor

Relaxation of the guinea-pig taenia coli caused by ADP$\beta$S is mediated by $P_{2Y}$-purinoceptors (Dudeck et al. 1995; Bültmann et al. 1996a). Suramin, NF023, BSt101, XAMR0716 and ANTS antagonized this relaxation in a surmountable manner (cf. Den Hertog et al. 1989, Hoyle et al. 1990 and Bültmann et al. 1996a for suramin and Ziyal et al. 1994 and Lambrecht et al. 1996 for NF023). Again, however, the antagonism was not purely competitive, at least for NF023 of which three concentrations were tested: the slope of the Arunlashana-Schild regression was lower than unity (cf. for suramin and several other $P_2$-purinoceptor antagonists Bültmann et al. 1996a; Lambrecht et al. 1996; Wittenburg et al. 1996b).

The three structure-activity rules derived above for the $P_{2X}$-purinoceptor also hold true for the $P_{2Y}$-purinoceptor (see Table 1): ANTS being the structure mainly responsible for binding, affinity increasing with size (although due to limited supply of substance we could not determine the $K_d$ of NF019), no detectable role of the possession of a second ANTS (compare BSt101 with NF023). On the whole, the range of affinities was smaller than at the $P_{2X}$-receptor, and NF023 was slightly less, not more, potent than suramin.

Twenty substances related to suramin were examined by van Rhee et al. (1994) for their effect on the binding of ADP$\beta^{35}$S to the $P_{2Y}$-purinoceptor of turkey erythrocytes. Pertinent results have been incorporated in Table 1. As pointed out previously, the $K_d$ of XAMR0721 in the taenia coli differs greatly from the $K_i$ at the erythrocyte $P_{2Y}$-receptor (Bültmann et al. 1996a). Combination with the present results permits the calculation of $K_d$ ratios. For example, the $K_d$ ratio XAMR0716/XAMR0721 is 0.65 at the receptor of the taenia but >53 at the erythrocyte receptor. The comparison bears out the view that the $P_{2Y}$-purinoceptors in turkey erythrocytes and the guinea-pig taenia coli differ (Burnstock et al. 1994; Bültmann et al. 1996a).

## Ecto-nucleotidases

Like suramin (Kurz et al. 1994; Khakh et al. 1995b), the compounds NF023, BSt101, NF019 and XAMR0716 reduced the removal of ATP from the medium by rat vas deferens tissue, or in other words inhibited ecto-nucleotidases (cf. Beukers et al. 1995 for NF023). The inhibitory potency was small. Like the $P_{2X}$ and $P_{2Y}$ activity, it increased with the size of the molecules and did not depend on symmetry or the possession of two amino-napthalene-trisulphonic acid residues (compare BSt101 with NF023).

## Selectivity

The difference between the guinea-pig taenia coli $P_{2Y}$-receptor and the turkey erythrocyte $P_{2Y}$-receptor (Table 1) strikingly shows that the selectivities found in the present study must not be extended to other $P_{2X}$- and $P_{2Y}$-purinoceptors (see Bültmann et al. 1996b). Suramin has little subtype selectivity (Table 1), as noted previously (see Cusack 1993). NF023 and to a lesser extent BSt101, on the other hand, are $P_{2X}$- versus $P_{2Y}$-selective (ratio "apparent $K_d$ at $P_{2Y}$/apparent $K_d$ at $P_{2X}$" > 5; cf. for NF023 Ziyal et al. 1994, 1996 and Lambrecht et al. 1996). Due to the overall low ecto-nucleotidase activity, no less than five of the six compounds are $P_{2X}$- versus ecto-nucleotidase-selective (ratio "$IC_{25\%}$ for ATP breakdown/apparent $K_d$ at $P_{2X}$" > 10). For the same reason, suramin, NF023, BSt101 and XAMR0716 are $P_{2Y}$- versus ecto-nucleotidase-selective (ratio "$IC_{25\%}$ for ATP breakdown/apparent $K_d$ at $P_{2Y}$" > 10). No $P_{2Y}$- versus $P_{2X}$-selective and no ecto-nucleotidase-selective drug was identified.

NF023 seems to be an interesting compound, being $P_{2X}$-versus $P_{2Y}$-selective, lacking a number of non-purinoceptor effects and, above all, being highly $P_{2X}$- versus ecto-nucleotidase-selective, with a ratio "$IC_{25\%}$ for ATP breakdown/apparent $K_d$ at $P_{2X}$" no less than 590.

## Conclusion

The aim of this study and the preceding two (Bültmann et al. 1996b; Wittenburg et al. 1996b) was to find $P_2$-purinoceptor antagonists and structure-activity relationships in three series of compounds: derivatives of DIDS, of Evans blue and trypan blue, and of suramin. What has been learnt?

A general experience, and not a new one (see Bültmann et al. 1996a), has been that $P_2$ antagonists tend to be non-selective and to act with kinetics not purely competitive. An example is the enhancement, by relatively low concentrations of Evans blue and most of its derivatives, of contractions of the rat vas deferens elicited by high $K^+$. Another example is the blockade of ecto-nucleotidases by all but two (NF019 and ANTS; present study) of the potential antagonists tested. A pervading feature has been the blockade of both the $P_{2X}$-receptor and the $P_{2Y}$-receptor by most antagonists, although selective compounds were obtained (see below). A non-competitive component was prominent in the $P_{2X}$ effect of the isothiocyanates. Deviations from competitive antagonism also included the increase of the maximum of the concentration-response curve of $\alpha,\beta$-MeATP in rat vas deferens by Evans blue and trypan blue and most of their derivatives as well as by suramin and its derivatives. Apparently simple competitive kinetics were found for the $P_{2Y}$-purinoceptor blockade by all four isothiocyanates (m-IBS, p-IBS, IB-2,4-dS, IB-2,5-dS) and for one trypan blue derivative (NH07) examined by Arunlakshana-Schild analysis.

Three general structure-activity conclusions can be drawn. First, in all three series affinity to the $P_{2X}$-purinoceptor, the $P_{2Y}$-purinoceptor as well as the ecto-nucleotidases increased with the size of the molecule. Second, in large symmetric molecules such as Evans blue and suramin, only one of the terminal naphthalene-sulphonate structures seems to be involved in purinoceptor and ecto-nucleotidase binding as shown directly for NF023 versus BSt101 (Table 1). Third, the position of the sulphonate residues (studied only for isothiocyanates and Evans blue and trypan blue analogues) may or may not influence activity: no influence at all was detectable in the isothiocyanate series; in the Evans and trypan blue series, the position of the sulphonate residues did not influence $P_{2X}$ but did influence $P_{2Y}$ and ecto-nucleotidase activity, a phenomenon leading to selectivities (see Wittenburg et al. 1996b) that might be improved in future new compounds.

Among the compounds tested, the isothiocyanate $\beta$-INS, the Evans blue analogue NH01 and the suramin derivative NF023 appear to be of particular interest. All three display considerable $P_{2X}$-selectivity. $\beta$-INS largely lacks non-purinoceptor effects at $P_{2X}$-blocking concentrations and possibly may be used to label the $P_{2X}$-receptor (Bültmann et al. 1996b). NH01 is the most selective $P_{2X}$ antagonist presently known. The ratio "apparent $K_d$ at $P_{2Y}$/apparent $K_d$ at $P_{2X}$" of >125 (Wittenburg et al. 1996b) is higher than the ratios for Evans blue (>40; Wittenburg et al. 1996b), pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS; 56; Lambrecht et al. 1996) and NF023 (about 30; Lambrecht et al. 1996; present study). $K^+$-evoked contractions of the rat vas deferens were enhanced only at high concentrations, and NH01 also was moderately $P_{2X}$- versus ecto-nucleotiase-selective, with a ratio "$IC_{25\%}$ for ATP breakdown/apparent $K_d$ at $P_{2X}$" of 23. Finally, NF023 is less $P_{2X}$- versus $P_{2Y}$-selective than NH01 but much more $P_{2X}$- versus ecto-nucleotidase-selective. With its ratio "$IC_{25\%}$ for ATP breakdown/apparent $K_d$ at $P_{2X}$" of 590, mentioned above, it is the most $P_{2X}$- versus ecto-nucleotidase-selective agent examined in the three studies. The limitation of these affinity and selectivity statements to the receptors tested should once again be recalled.

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