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NF279: a novel potent and selective antagonist of P2X receptor-mediated responses

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Abstract

8,8′-(Carbonylbis(imino-4,1-phenylenecarbonylimino-4,1-phenylenecarbonylimino))bis(1,3,5-naphthalenetrisulfonic acid) (NF279) antagonized P2X receptor-mediated contractions in rat vas deferens, evoked by α,β-methylene ATP (10 μM; $p\mathrm{IC}_{50} = 5.71$) without affecting responses mediated via α_{1A}-adrenoceptors, adenosine A_1 and A_{2B} receptors, histamine H_1, muscarinic M_3 and nicotinic receptors. The low inhibitory potency of NF279 on P2Y receptors in guinea-pig taenia coli ($p\mathrm{A}_2 = 4.10$) and at ecto-nucleotidases in folliculated *Xenopus laevis* oocytes ($\mathrm{IC}_{50} > 100$ μM) indicates that NF279 is a novel specific and selective P2X receptor antagonist.

Keywords: P2 receptor antagonist; Suramin; NF279 (8,8′-(Carbonylbis(imino-4,1-phenylenecarbonylimino-4,1-phenylenecarbonylimino))bis(1,3,5-naphthalenetrisulfonic acid))

P2 receptor subtypes for extracellular nucleotides comprise an ionotropic (P2X, seven subunits known) and a metabotropic (P2Y, at least four subtypes known) family (Lambrecht, 1996; Bhagwat and Williams, 1997). The characterization of native and recombinant P2 receptors continues to be hindered by the lack of specific and subtype-selective antagonists. However, a number of compounds including suramin and its truncated analogue 8,8′-(carbonylbis(imino-3,1-phenylenecarbonylimino)bis(1,3,5-naphthalenetrisulfonic acid) (NF023), do exhibit selective P2 receptor subtype-blocking properties, and if used circumspectly, are of use in this respect (Humphrey et al., 1995; Bültmann et al., 1996; Lambrecht, 1996; Bhagwat and Williams, 1997). The present study was designed to evaluate the functional antagonistic properties of the novel suramin-related compound 8,8′-(carbonylbis(imino-4,1-phenylenecarbonylimino-4,1-phenylenecarbonylimino)) bis(1,3,5-naphthalenetrisulfonic acid) (NF279) on P2X receptors in rat vas deferens, P2Y receptors in guinea-pig taenia coli and ATP breakdown by ecto-nucleotidases in folliculated *Xenopus laevis* oocytes.

Prostatic segments of vasa deferentia from rats were incubated in Krebs solution as were strips of guinea-pig taenia coli (Khakh et al., 1995; Bültmann et al., 1996). Mechanical activity was recorded isometrically, and for evaluation of concentration–response relationships, a logistic function was fitted to the data using non-linear regression analysis (Jenkinson et al., 1995; Bültmann et al., 1996). $\mathrm{p}A_2$ values were calculated according to the Schild equation (Jenkinson et al., 1995). Ecto-nucleotidases activity of *Xenopus* oocytes was studied by measuring spectrophotometrically the production of inorganic phosphate (P_i) from the breakdown of extracellular ATP (Ziganshin et al., 1995, Ziganshin et al., 1996). The results are presented as means $\pm$ S.E.M. from n observations.

The three antagonists employed in the present study had no effect on the basal tone of the preparations used, and their inhibitory effect at P2 receptors was reversed on repeated wash out (up to 90 min; data not shown).

In the vas deferens, suramin, NF023 and NF279 (60–120 min exposure) concentration-dependently inhibited the contractions to single doses of α,β-methylene ATP (10

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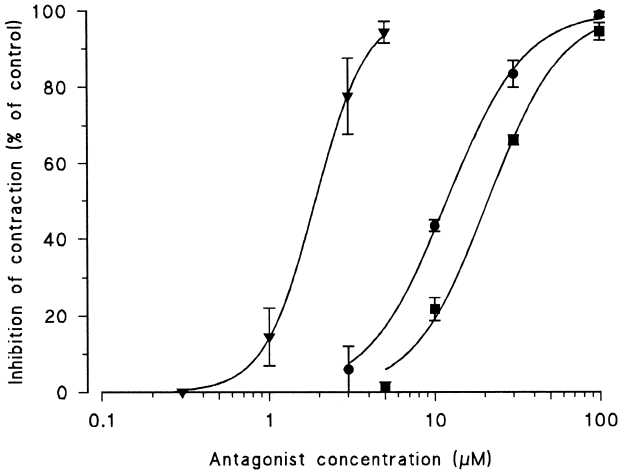

Fig. 1. Effect of NF279, NF023 and suramin on contractions of rat vas deferens elicited by single doses of α,β-methylene ATP (10 μM) mediated via P2X receptors. NF279 (▼, 0.3–5 μM), NF023 (●, 3–100 μM) or suramin (■, 5–100 μM) were incubated for 60–120 min. α,β-Methylene ATP was added at 30-min intervals. Each point represents the arithmetic mean derived from two to four experiments, vertical bars show S.E.M. where it exceeded the size of the symbol.

μM; tension generated in control experiments = 1499.7 ± 74.8 mg; $n = 15$) (Fig. 1). NF279 was considerably (up to 11-fold) more potent ($pIC_{50} = 5.71 \pm 0.01$) as an P2X receptor antagonist than either NF023 ($pIC_{50} = 4.92 \pm 0.02$) or suramin ($pIC_{50} = 4.67 \pm 0.03$; $n = 2–5$). NF279 (10 nM–3 μM) also was a highly potent antagonist ($pIC_{50} = 7.26 \pm 0.02$, $n = 5–9$) in studies with the cloned $P2X_1$ receptor expressed in *Xenopus* oocytes, inhibiting ATP (1 μM)-induced inward currents (two-electrode voltage-clamp recordings).

In the guinea-pig taenia coli precontracted by carbachol (0.1–0.3 μM), suramin (100 μM, $pA_2 = 4.47 \pm 0.05$), NF023 (300 μM, $pA_2 = 4.05 \pm 0.14$) and NF279 (100 μM, $pA_2 = 4.10 \pm 0.29$; $n = 3–4$) antagonized relaxant responses to adenosine 5′-O-(2-thiodiphosphate) ($EC_{50} = 0.63 \pm 0.09$ μM, $n = 12$) in a parallel and surmountable manner (60-min exposure), without affecting the carbachol-induced contractions. The resulting pA_2 values for NF023 and NF279 were very similar, these two compounds being approximately 3- to 4-fold less potent than suramin.

Generation of P_i by ecto-nucleotidases in oocytes, using ATP (100 μM; 4.54 ± 0.20 nmol P_i/30 min per cell, produced in control incubations; $n = 16$) as substrate, was inhibited with similar potency by suramin (300 μM, 41.2 ± 1.8%), NF023 (300 μM, 31.1 ± 2.2%) and NF279 (300 μM, 68.5 ± 2.6%; $n = 7–31$).

NF279 (100 μM) had no significant effects on either the potency or maximum response to noradrenaline (α_{1A}-adrenoceptors) in rat vas deferens, 2-chloro-N^6-cyclopentyladenosine (adenosine A_1 receptors), histamine (H_1 receptors), arecaidine propargyl ester (muscarinic M_3 receptors) in guinea-pig ileum and 2-chloroadenosine (adenosine A_{2B} receptors) as well as nicotine (neuronal nicotinic receptors) in guinea-pig taenia coli, respectively (data not shown). Thus, the antagonism of NF279 appears to be specific for P2 receptors.

In conclusion, NF279 is a novel and highly potent antagonist blocking P2X receptor-mediated responses and, to the best of our knowledge, the compound with the highest P2X- versus P2Y- and ecto-nucleotidases-selectivity presently available. If used at concentrations lower than 30 μM, effective blockade of P2X receptors by NF279 may be achieved, with little or no inhibition of P2Y receptors or ecto-nucleotidases. However, further experiments are needed to clarify its exact mechanism of actions at the known P2X and P2Y receptor subtypes.

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