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The novel pyridoxal-5′-phosphate derivative PPNDS potently antagonizes activation of $P2X_1$ receptors

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Abstract

Pyridoxal-5′-phosphate-6-(2′-naphthylazo-6′-nitro-4′,8′-disulfonate) (PPNDS) potently antagonized $P2X_1$ receptor-mediated responses in rat vas deferens ($pK_B = 7.43$) and *Xenopus laevis* oocytes ($pIC_{50} = 7.84$). It showed lower (up to 20,000-fold) inhibitory potency on ecto-nucleotidase in *Xenopus* oocytes and on $P2Y_1$ receptors in guinea-pig ileum ($pA_2 = 6.13$). PPNDS did not interact with α_{1A}-adrenoceptors, adenosine A_1 and A_{2B}, histamine H_1 and muscarinic M_3 receptors. Thus, PPNDS is a novel, specific P2 receptor antagonist and represents the pyridoxal-5′-phosphate derivative with the highest potency at $P2X_1$ receptors.

Keywords: P2 receptor antagonist; PPNDS (pyridoxal-5′-phosphate-6-(2′-naphthylazo-6′-nitro-4′,8′-disulfonate)); PPADS (pyridoxal-5′-phosphate-6-phenylazo-2′,4′-disulfonate)

One of the current challenges in the P2 receptor field is to relate the cloned ionotropic P2X (seven subunits known) and metabotropic P2Y (at least five subtypes known) receptors to the diverse native responses (Ralevic and Burnstock, 1998). Progress in this field has been impeded because of the lack of useful ligands, especially subtype-selective antagonists and radioligands (Ralevic and Burnstock, 1998; Lambrecht et al., 1999). We have provided an important advance by introducing pyridoxal-5′-phosphate-6-phenylazo-2′,4′-disulfonate (PPADS; Lambrecht, 1996; Lambrecht et al., 1992). Unfortunately, PPADS is a non-selective (but non-universal) P2 receptor antagonist that blocks recombinant $P2X_1$ and $P2Y_1$ receptors with similar potency (Ralevic and Burnstock, 1998). In order to increase the potency of PPADS at the $P2X_1$ receptor and, hence, to alter its antagonistic properties in favour of the $P2X_1$ subtype, we have recently synthesized a series of 6-naphthylazo analogues. One of these analogues, pyridoxal-5′-phosphate-6-(2′-naphthylazo-6′-nitro-4′,8′-disulfonate) (PPNDS), was the subject of a detailed pharmacological investigation. The parent compound PPADS was used as reference drug.

Experimental methods used were those detailed in the literature (for references, see Lambrecht et al., 1999; see also Table 1). The results (Table 1) are presented as means ± S.E.M. from 3–12 observations.

PPADS and PPNDS had no effect on the basal tone of the preparations used, and their inhibitory effect at P2 receptors was reversed on repeated wash out, albeit slowly (up to 120 min; data not shown).

In rat vas deferens, PPADS and PPNDS (60- to 120-min exposure) inhibited α,β-methylene ATP-induced isometric contractions, mediated by $P2X_1$ receptors, in a concentration-dependent and apparently pseudoirreversible manner. The resulting pK_B estimates indicate that PPNDS is seven-fold more potent at native $P2X_1$ receptors than the

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Table 1
Functional potencies of P2 receptor antagonists obtained in tissues or cells that are endowed with P2X or P2Y receptor subtypes
IC_{50} values ($pIC_{50} = -\log IC_{50}$) are the molar concentrations of antagonists necessary to inhibit the response to a single dose of agonist by 50%. pA_2 values were determined by Schild analysis, and pK_B values were derived using a double-reciprocal plot of equieffective agonist concentrations in the absence and presence of the antagonists (Kenakin, 1993). The results are presented as means ± S.E.M. from 3–12 observations. Concentrations (μM; −log interval = 0.5) of antagonists are given in parentheses.

Antagonists	$P2X_1$ (ratVD)[a]	$rP2X_1$ (XLO)[b]	$P2Y_1$ (GPI)[c]	
	pK_B	pIC_{50}	pK_B	pA_2
PPADS	6.59 ± 0.14[d]	7.06 ± 0.03	6.20 ± 0.05[e]	–
	(1–30)	(0.01–1)	(1–10)	–
PPNDS	7.43 ± 0.04[d]	7.84 ± 0.05	–	6.13 ± 0.03[f]
	(0.3–10)	(0.001–0.3)	–	(3–30)

[a] Inhibition of contractions to single doses of α,β-methylene ATP (0.1–300 μM; tension generated in control experiments to 30 μM α,β-methylene ATP = 2004 ± 95 mg) in prostatic segments of rat vas deferens (ratVD; $P2X_1$ receptors).
[b] Inhibition of inward currents to a fixed concentration of ATP (1 μM; $EC_{50} = 0.96 \pm 0.12$ μM) in *Xenopus laevis* oocytes (XLO) expressing homomeric cloned rat $P2X_1$ receptors. Two-electrode voltage clamp recordings were carried out at a holding potential of −60 mV.
[c] Inhibition of contractions to ADPβS (0.3–300 μM; $EC_{50} = 4.86 \pm 0.25$ μM; maximum tension generated in control experiments = 1373 ± 56 mg) in the presence of atropine (0.3 μM) in longitudinal smooth muscle of guinea-pig ileum (GPI; $P2Y_1$ receptors).
[d] The antagonist pK_B values were derived from the effects of 10 μM PPADS and 1 μM PPNDS.
[e] The antagonist pK_B value was derived from the effects of 3 μM PPADS.
[f] Schild analysis yielded a slope of the regression line (0.93 ± 0.08) that was not significantly ($P > 0.05$) different from unity.

parent compound PPADS. Increasing concentrations of PPADS and PPNDS (5-min pre-incubation) reduced and finally abolished the inward current evoked by ATP in follicle cell free *Xenopus* oocytes expressing the rat $P2X_1$ receptor. Again, PPNDS was considerably more potent (six-fold) than PPADS.

In the longitudinal smooth muscle of guinea-pig ileum, PPNDS (60-min exposure) caused a pure competitive antagonism of isometric responses to adenosine 5′-*O*-(2-thiodiphosphate) (ADPβS), whereas PPADS (90-min exposure) acted as a pseudoirreversible antagonist. The resulting potency estimates for the two antagonists at the ileal $P2Y_1$ receptors were not significantly different ($P > 0.05$).

Generation of inorganic phosphate (P_i) by ecto-nucleotidases in folliculated *Xenopus* oocytes, using ATP (100 μM; 2.49 ± 0.04 nmol P_i/30 min per cell, produced in control incubations) as substrate, was inhibited by PPNDS, leaving a residual ecto-nucleotidase activity of 30.0 ± 5.5% at the high concentration of 300 μM. A similar low inhibitory potency has been reported for PPADS (Ziganshin et al., 1996).

PPNDS (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) in guinea-pig taenia coli (data not shown). Thus, the antagonism of PPNDS appeared to be specific for P2 receptors. A similar high P2 receptor specificity has been reported for the parent compound PPADS (Lambrecht, 1996; Lambrecht et al., 1992).

The data presented here illustrate that PPNDS is a novel, specific and high-affinity $P2X_1$ receptor antagonist. In contrast to PPADS, PPNDS also is remarkably selective for $P2X_1$ vs. $P2Y_1$ receptors (up to 52-fold). These properties of PPNDS make it unique among the pyridoxal-5′-phosphate derivatives reported to date (Jacobson et al., 1998; Kim et al., 1998). It might fill the long-standing need for a $P2X_1$-selective radioligand. However, further experiments are needed to clarify the inhibitory properties of PPNDS at all known P2 receptor subtypes.

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