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Profiling at recombinant homomeric and heteromeric rat P2X receptors identifies the suramin analogue NF449 as a highly potent $P2X_1$ receptor antagonist

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Abstract

P2X receptors are cation channels gated by extracellular ATP and related nucleotides. Because of the widespread distribution of P2X receptors and the high subtype diversity, potent and selective antagonists are needed to dissect their roles in intact tissues. Based on suramin as a lead compound, several derivates have been described that block recombinant P2X receptors with orders of magnitude higher potency than suramin. Here we characterized the suramin analogue 4,4′,4″,4‴-(carbonylbis(imino-5,1,3-benzenetriylbis(carbonylimino)))tetrakis-benzene-1,3-disulfonic acid (NF449) with respect to its potency to antagonize ATP or αβ-methyleneadenosine 5′-trisphosphate-induced inward currents of homomeric rat $P2X_1$–$P2X_4$ receptors or heteromeric $P2X_{1+5}$ and $P2X_{2+3}$ receptors, respectively. NF449 most potently blocked $P2X_1$ and $P2X_{1+5}$ receptors with IC_{50} values of 0.3 nM and 0.7 nM, respectively. Three to four orders of magnitude higher NF449 concentrations were required to block homomeric $P2X_3$ or heteromeric $P2X_{2+3}$ receptors (IC_{50} 1.8 and 0.3 μM, respectively). NF449 was least potent at homomeric $P2X_2$ receptors (IC_{50} 47 μM) and homomeric $P2X_4$ receptors ($IC_{50} > 300$ μM). Altogether, these results characterize NF449 as the so far most potent and selective antagonist of receptors incorporating the $P2X_1$ subunit such as the $P2X_1$ homomer and the $P2X_{1+5}$ heteromer.

Keywords: Suramin analogue; Recombinant P2X receptors; Xenopus oocytes; Two-electrode voltage-clamp electrophysiology; Antagonistic profile; Ligand-gated ion channel

1. Introduction

ATP and other endogenous purine and pyrimidine nucleotides induce neuronal and non-neuronal responses by acting on two types of cell surface receptors, the G protein-coupled metabotropic P2Y receptors and

Abbreviations: αβmeATP, α,β-methyleneadenosine 5′-trisphosphate lithium salt; rP2X, rat P2X; TNP-ATP, trinitrophenyl-ATP; IP_5I, diinosine pentaphosphate.

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the ligand-gated P2X receptor cation channels (North, 2002; Burnstock, 2004). P2X receptors are involved in a wide variety of essential cellular processes ranging from smooth muscle contraction, fast excitatory neurotransmission and nociception to interleukin-1 secretion from macrophages. Seven receptor subunits ($P2X_1$–$P2X_7$) have been identified in vertebrates, without considering the various isoforms originating from alternative splicing. Like other ligand-gated ion channels, P2X receptors are organized as oligomers. Both biochemical and electrophysiological studies indicate that a trimeric subunit organization is inherent to all functional homomeric and heteromeric P2X receptors (Nicke et al., 1998; Jiang et al., 2003; Aschrafi et al., 2004). In the *Xenopus* oocyte expression system, homomeric $P2X_1$, $P2X_2$, $P2X_3$, $P2X_4$, $P2X_5$, and $P2X_7$ receptors and heteromeric $P2X_{2+3}$ and $P2X_{1+5}$ receptors have been fully characterized with respect to kinetics and pharmacological profiles of agonists (North, 2002). $P2X_6$ subunits are unique in that they form homomeric channels only in a small subset of stably transfected HEK293 cells (Jones et al., 2004), but neither in transiently transfected HEK293 or COS cells nor in *Xenopus* oocytes (North, 2002).

The widespread and partially overlapping occurrence of P2X receptors and the high subtype diversity arising from homo- and heteropolymerization makes it difficult to determine which subtype mediates a particular response in native tissues. A panel of potent and subtype-selective antagonists is therefore needed to dissect the physiological and pathophysiological role of each subtype. Over the last several years, significant progress has been made in identifying potent and selective P2X receptor antagonists. These include trinitrophenyl-ATP (TNP-ATP), which potently antagonizes $P2X_1$, $P2X_3$, and $P2X_{2+3}$ receptors (Virginio et al., 1998), diinosine pentaphosphate (IP_5I), a $P2X_1$-selective antagonist (King et al., 1999), and the Abbott compound A-317491, a selective non-nucleotide antagonist of $P2X_3$ and $P2X_{2+3}$ receptors (Jarvis et al., 2002).

Another group of antagonists has its origin in suramin, which is a non-selective P2 receptor antagonist (Dunn and Blakeley, 1988; Ralevic and Burnstock, 1998; Lambrecht et al., 2002). Using suramin as a lead compound, derivatives such as NF023 (8,8′-(carbonylbis (imino-3,1-phenylene carbonylimino)(bis(1,3,5-naphthalenetrisulfonic acid)) and NF279 (8,8′-(carbonylbis (imino-4,1-phenylenecarbonylimino-4,1-phenylenecarbonylimino))bis(1,3,5-naphthalenetrisulfonic acid)) have been identified as potent $P2X_1$ receptor-selective blockers (Soto et al., 1999; Klapperstück et al., 2000; Rettinger et al., 2000). Most recently, we introduced the suramin derivative NF449 (4,4′,4″,4‴-(carbonylbis(imino-5,1,3-benzenetriylbis(carbonylimino)))tetrakis-benzene-1,3-disulfonic acid) as the most potent $P2X_1$ receptor antagonist yet described (Kassack et al., 2004), but its selectivity profile has so far only been characterized in intact tissues and at two recombinant P2X receptor subtypes, $P2X_1$ and $P2X_7$ (Braun et al., 2001; Hülsmann et al., 2003; Kassack et al., 2004). The objective of the present study was to evaluate the antagonistic potency of NF449 for the recombinant homomeric and heteromeric receptor subtypes $P2X_1$–$P2X_4$, $P2X_{1+5}$, and $P2X_{2+3}$. Our results establish NF449 as an extremely potent antagonist of receptors incorporating the $rP2X_1$ subunit such as the $rP2X_1$ homomer and the $rP2X_{1+5}$ heteromer.

2. Materials and methods

2.1. Materials

ATP was purchased from Roche Diagnostics (Mannheim, Germany); α,β-methyleneadenosine 5′-trisphosphate lithium salt (αβmeATP) was from Sigma (München, Germany). The suramin derivative NF449 (as octasodium salt) was synthesized and purified as previously described (Kassack et al., 2004). Molecular biology enzymes were obtained from New England Biolabs (Frankfurt, Germany), Epicentre (Madison, WI), Stratagene (La Jolla, CA), or Promega (Heidelberg, Germany). Desoxyoligonucleotides were purchased from Qiagen (Hilden, Germany). All other chemicals were purchased from either Sigma–Aldrich (Taufkirchen, Germany) or Merck (Darmstadt, Germany).

2.2. Plasmids encoding rat P2X receptors

Plasmids encoding the rat P2X subunits $rP2X_1$ (Valera et al., 1994), $rP2X_3$ (Chen et al., 1995), and $rP2X_5$ were available from previous studies (Garcia-Guzman et al., 1996; Nicke et al., 1998). cDNAs encoding $rP2X_2$ subunits (Brake et al., 1994) and $rP2X_4$ subunits (Bo et al., 1995) were amplified by RT-PCR using sequence-specific primers from NGF-treated PC12 cells and a Superscript rat brain cDNA library (Life Technologies), respectively, cloned into vector pNKS2 (Gloor et al., 1995), and fully sequenced. The encoded amino acid sequences are identical to GenBank sequences U14414 and X91200, respectively.

2.3. Electrophysiological current recordings of recombinant P2X receptors in Xenopus laevis oocytes

P2X receptor cRNAs were synthesized and injected at concentrations of 5 ng/μl ($rP2X_2$) or 0.5 μg/μl ($rP2X_1$, $rP2X_3$, $rP2X_4$, $rP2X_5$) in 50-nl volumes into collagenase defolliculated *Xenopus* oocytes prepared as described in detail previously (Schmalzing et al., 1991). For the expression of hetero-oligomeric $rP2X_{1+5}$ and $rP2X_{2+3}$