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Trinitrophenyl-Substituted Nucleotides Are Potent Antagonists Selective for $P2X_1$, $P2X_3$, and Heteromeric $P2X_{2/3}$ Receptors

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ABSTRACT

There are currently seven P2X receptor subunits ($P2X_{1-7}$) defined by molecular cloning. The functional identification of these receptors has relied primarily on the potency of α,β-methylene-ATP relative to that of ATP and on the kinetics of receptor desensitization. In the present experiments we found that the 2′,3′-O-(2,4,6-trinitrophenyl)-substituted analogs of ATP are selective and potent antagonists at some but not all P2X receptors. The trinitrophenyl analogs of ATP, ADP, AMP, and GTP produced a reversible inhibition of ATP-evoked currents in human embryonic kidney 293 cells expressing $P2X_1$ receptors, $P2X_3$ receptors, or both $P2X_2$ and $P2X_3$ (heteromeric) receptors; IC_{50} values were close to 1 nM. These compounds were at least 1000-fold less effective in blocking currents in cells expressing $P2X_2$, $P2X_4$, or $P2X_7$ receptors ($P2X_5$ and $P2X_6$ not tested). GTP, 2,4,6-trinitrophenol, and the 2′,3′-trinitrophenyl analog of adenosine (0.1–10 μM) had no effect. Thus, we have identified a structural motif that confers antagonist action at P2X receptors that contain $P2X_1$ or $P2X_3$ subunits (the α,β-methylene-ATP-sensitive subclass).

There are seven P2X receptor subunits, which assemble into ATP-activated ion channels either as homomers or heteromers (reviewed by North, 1996; North and Barnard, 1997). At the molecular level, any pair of the subunits has 35–50% identical amino acids. At the functional level, several subgroups have been distinguished. For example, in one subgroup ($P2X_1$ and $P2X_3$ homomeric channels), $\alpha\beta$meATP and ATP are equally effective agonists, and the currents desensitize during agonist applications of more than several hundred milliseconds. None of the other homomeric channels is activated by $\alpha\beta$meATP, and the currents show much less desensitization. A distinct class of channel is formed by the coexpression of $P2X_2$ and $P2X_3$ subunits; this class is activated by $\alpha\beta$meATP and ATP but it shows little desensitization. A further distinguishing feature is the ability of PPADS to block the currents evoked by ATP; $P2X_4$, $P2X_6$, and $P2X_7$ receptors are relatively insensitive. Finally, $P2X_7$ homomeric channels are fundamentally different from all the others because repeated or prolonged agonist application results in cell permeabilization as measured by the uptake of fluorescent dyes and, eventually, cell lysis (North, 1996; Surprenant *et al.*, 1996; North and Barnard, 1997).

The assignment of functional roles for P2X receptors in intact tissues depends critically on the use of receptor antagonists. Indeed, the main evidence that ATP mediates synaptic transmission between neurons (Edwards *et al.*, 1992; Evans *et al.*, 1992) or from nerve to muscle (Sneddon and Westfall, 1984; Evans and Surprenant, 1992) has been the block of the postsynaptic responses by suramin and/or PPADS (Sneddon and Westfall, 1984; Dunn and Blakeley, 1988; Ziganshin *et al.*, 1994). However, the low affinity and limited specificity of these compounds restricts their usefulness and, as mentioned above, some P2X receptors are not blocked (Buell *et al.*, 1996). There is a clear need to identify more receptor antagonists.

Trinitrophenyl analogs of ATP have been widely used for the fluorescent labeling of ATP binding sites in proteins,

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ABBREVIATIONS: $\alpha\beta$meATP, α,β-methylene-ATP; HEK, human embryonic kidney; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; PPADS, pyridoxal 5-phosphate 6-azophenyl-2′,4′-disulphonic acid; TNP, trinitrophenyl; TNP-A, 2′,3′-*O*-(2,4,6-trinitrophenyl)-adenosine; TNP-ADP, 2′,3′-*O*-(2,4,6-trinitrophenyl)-ADP; TNP-AMP, 2′,3′-*O*-(2,4,6-trinitrophenyl)-AMP; TNP-ATP, 2′,3′-*O*-(2,4,6-trinitrophenyl)-ATP; TNP-GTP, 2′,3′-*O*-(2,4,6-trinitrophenyl)-GTP; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N′,N′,-tetraacetic acid.

including P2X receptors (Mockett *et al.*, 1994). We first examined their effects on cloned and expressed P2X receptors with such an application in mind. In the course of those experiments, it became clear that, for some P2X receptors, the analogs were able to block responses to ATP at nanomolar concentrations. Here we report the characterization of this observation.

Experimental Procedures

HEK 293 cells that stably or transiently express the following P2X receptors were used in these studies: human $P2X_1$, rat $P2X_2$, rat $P2X_3$, rat or human $P2X_4$, rat $P2X_2$ together with rat $P2X_3$ (heteromer), and rat $P2X_7$. Generation of stable P2X receptor-expressing cell lines and methods of transient lipofectin transfection have been described in detail previously (Evans *et al.*, 1995; Buell *et al.*, 1996; Evans *et al.*, 1996; Kawashima *et al.*, 1997). HEK cells stably transfected with the human $P2X_4$ receptor were generously provided by Professor W. Stuhmer, Max-Planck Institute (Gottingen, Germany). Cells were plated onto 12-mm glass coverslips and maintained in Dulbecco's modified Eagle's medium, Nutrient Mix F-12 (GIBCO-BRL, Bethesda, MD) supplemented with 10% heat-inactivated fetal calf serum (FAKOLA, Bern, Switzerland) and 2 mM L-glutamine at 37° in a humidified 5% CO_2 incubator.

Whole-cell recordings were made 12–48 hr after transient transfection (rat $P2X_1$, $P2X_3$, $P2X_4$) and 6–72 hr after passage of stable cell lines (human $P2X_1$, $P2X_3$, $P2X_4$, and rat $P2X_2$, $P2X_{2/3}$, and $P2X_7$). Currents were recorded with an EPC9 patch-clamp amplifier (HEKA Elektronik, Lambrecht/Pfalz, Germany), acquired (1–2 kHz) and analyzed with Pulse and PulseFit 8.02 (HEKA). Patch pipettes (4–7 M[/omega]) contained 140 mM NaCl, 10 mM HEPES, and 11 mM

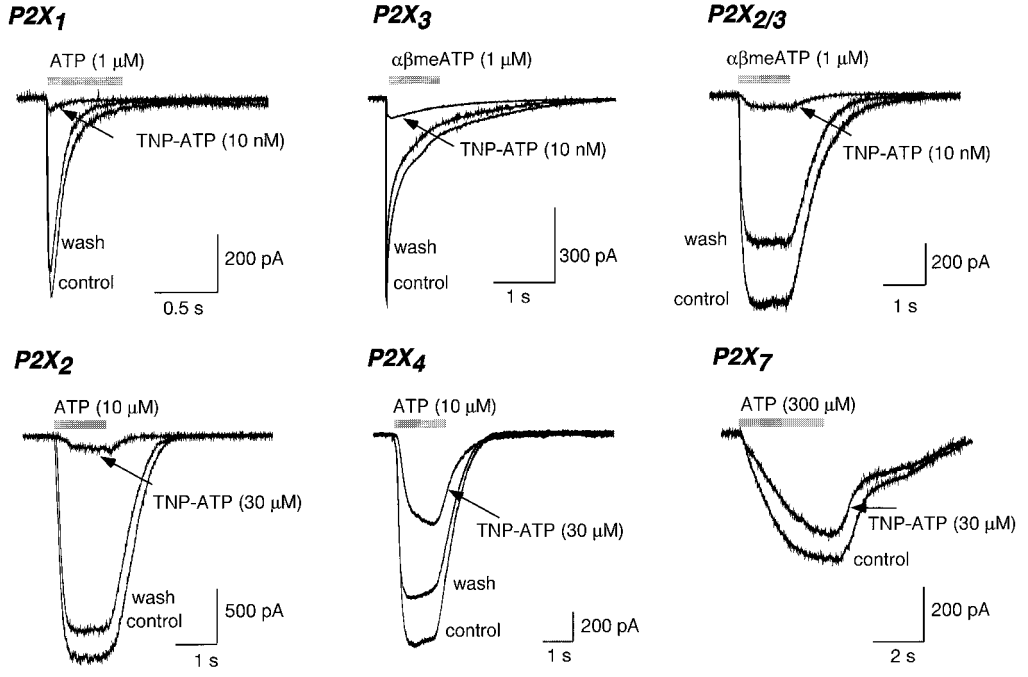

Fig. 1. TNP-ATP is a potent antagonist at $P2X_1$, $P2X_3$, and $P2X_{2/3}$ receptors. Each set of records consists of superimposed currents recorded from individual HEK 293 cells expressing the indicated receptor before, during, and after application of TNP-ATP at 10 nM ($P2X_1$, $P2X_3$, and $P2X_{2/3}$) or at 30 μM ($P2X_2$, $P2X_4$, and $P2X_7$). Currents shown in the presence of TNP-ATP are after 4-min application; currents shown after TNP-ATP are at 4-min wash except for $P2X_1$, in which case the washout was for 8 min.

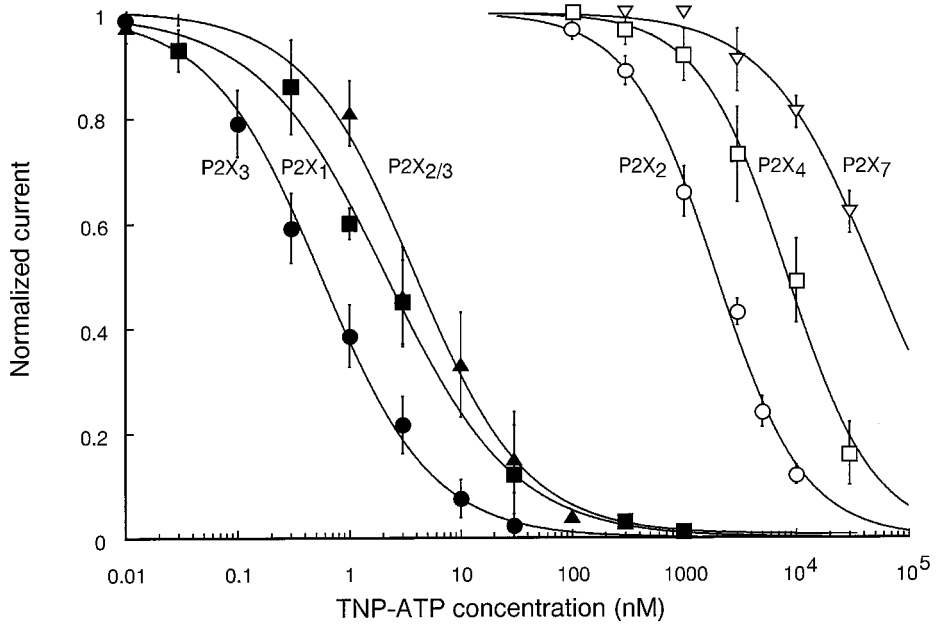

Fig. 2. TNP-ATP concentration-inhibition curves generated from all experiments as illustrated in Fig. 1. Results are plotted as normalized current, where current in absence of antagonist is equal to 1. *Points*, mean ± standard error of 4–8 experiments. *Lines*, least-square fits to a logistic equation (see Experimental Procedures).

EGTA. The external solution was 147 mM NaCl, 10 mM HEPES, 12 mM glucose, 2 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$. Osmolarity and pH values of both solutions were maintained at 300–315 mOsM/liter and 7.3, respectively. Unless otherwise stated, experiments were performed at a holding potential of −60 mV and at room temperature. Agonists were applied using a fast-flow U-tube delivery system (Fenwick *et al.*, 1982). Antagonists were added to both the bath superfusate and the fast-flow solution. ATP was the agonist in all experiments on $P2X_1$, $P2X_2$, $P2X_4$, and $P2X_7$ receptors. Both ATP and $\alpha\beta$meATP were used at the $P2X_3$ receptor and only $\alpha\beta$meATP was used at the heteromeric $P2X_{2/3}$ receptor (Kawashima *et al.*, 1997). Agonists were applied for 0.5–2-sec duration at 2 min intervals at all receptors except $P2X_1$ and $P2X_3$ where 4–5 min intervals were required to allow recovery from desensitization (Evans *et al.*, 1995).

Agonist concentration-response curves for each cell were fit by the least-squares method to $I = I_{max}[1 + (EC_{50}/[A])^{nH}]$ where I is the peak current evoked by agonist concentration $[A]$, I_{max} is the peak current evoked by a maximal agonist concentration, EC_{50} is the concentration giving half the maximal current, and n_H is the Hill coefficient. Antagonist concentration-inhibition curves were obtained in individual cells by using a fixed agonist concentration close to the EC_{50} (1 μM ATP at $P2X_1$, 10 μM ATP at $P2X_2$ and $P2X_4$, 300 μM ATP at $P2X_7$, 1 μM ATP or $\alpha\beta$meATP at $P2X_3$, and 5 μM $\alpha\beta$meATP at $P2X_{2/3}$), and progressively increasing the concentration of antagonist: IC_{50} values were calculated by least squares fitting to $I = I_0/[1 + (IC_{50}/[Ant])^{-nH}]$, where I and I_0 are peak currents in the presence and absence of antagonist at concentration $[Ant]$. All EC_{50} and IC_{50} values given in text and tables are the mean ± standard error from individual cells; however, the graphs in the figures were drawn by averaging results from all experiments and fitting a single concentration-response curve to the pooled data.

TNP-AMP sodium salt, TNP-ADP disodium salt, TNP-ATP trisodium salt, and TNP-GTP trisodium salt were obtained from Molecular Probes (Eugene, OR). GTP, ATP, and $\alpha\beta$meATP were from Sigma (St. Louis, MO) and 2,4,6-trinitrophenol (picric acid) was from Fluka (Buchs, Switzerland). TNP-A was prepared from adenosine and 2,4,6-trinitrobenzenesulfonate according to the procedure published by Azegami and Iwai (1975); the red precipitate of TNP-A that crystallized from the solution was purified by reprecipitation from acetone (1 ml) by adding 10 volumes of toluene.

Results

TNP-ATP strongly inhibited currents in cells expressing $P2X_1$, $P2X_3$, or $P2X_{2/3}$ receptors (IC_{50} about 1 nM), but was very much less effective in cells expressing $P2X_2$, $P2X_4$, or $P2X_7$ receptors ($IC_{50} > 1$ μM)(Figs. 1 and 2; Table 1). The inhibition was concentration-dependent and well fitted by the logistic function (see Experimental Procedures); the IC_{50} values are shown in Table 1, and the coefficient n_H was not significantly different from unity. The inhibition reversed within 4–15 min of TNP-ATP washout, although reversal

TABLE 1

Inhibition of currents evoked by ATP or $\alpha\beta$meATP in cells expressing P2X receptor

Values are mean ± standard error with numbers of experiments in parentheses.

	IC_{50}			
	TNP-ATP	TNP-ADP	TNP-AMP	TNP-GTP
	nM			
$P2X_1$	6 ± 3 (6)			
$P2X_3$	0.9 ± 0.2 (6)	1.3 ± 0.3 (4)	2.9 ± 1.5 (6)	0.4 ± 0.1 (4)
$P2X_{2/3}$	7 ± 3 (5)	5 ± 1 (3)	36 ± 10 (7)	1.2 ± 0.2 (4)
$P2X_2$	2,000 ± 200 (7)	1,200 ± 120 (7)	3,400 ± 900 (4)	4,400 ± 400 (7)
$P2X_4$	15,200 ± 410 (6)			
$P2X_7$	>30,000 (6)			

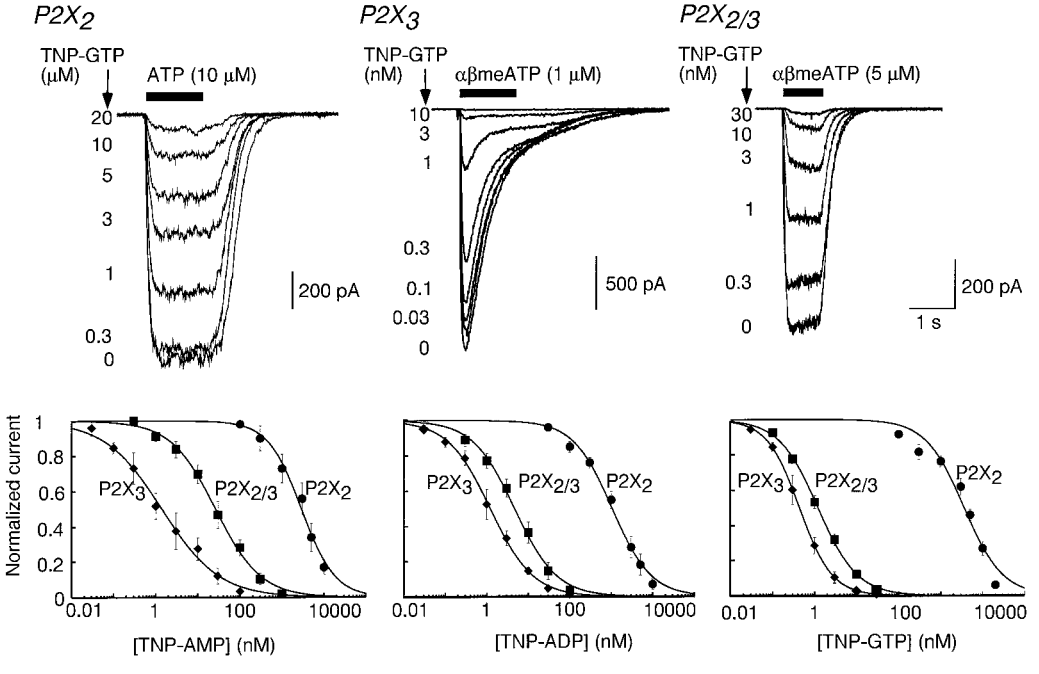

Fig. 3. TNP-AMP, TNP-ADP, and TNP-GTP are also potent antagonists at $P2X_3$ and $P2X_{2/3}$ receptors. *Sets of traces*, currents recorded from a single cell expressing $P2X_2$ (*left*), $P2X_3$ (*middle*), or $P2X_{2/3}$ (*right*) receptors in the absence and presence of increasing concentrations of TNP-GTP, as indicated. Graphs are concentration-inhibition curves obtained from all such experiments for TNP-AMP (*left*), TNP-ADP (*middle*), and TNP-GTP (*right*). *Points*, mean ± standard error of 4–8 experiments.

was sometimes incomplete for near maximal concentrations. The inhibition was the same at holding potentials of −60 mV and 40 mV (n = 4, 6, 5, and 3 for cells expressing $P2X_3$, $P2X_{2/3}$, $P2X_2$, and $P2X_4$ receptors, respectively). The $P2X_7$ receptor was particularly insensitive to blocking by TNP-ATP; at the highest concentration tested (30 μM), the inhibition was 39 ± 2% (n = 6). TNP-ATP (1 nM-30 μM) had no agonist action at any of the P2X receptors.

The effect of TNP-ATP was mimicked by TNP-ADP and TNP-AMP, as well as TNP-GTP (Fig. 3), although TNP-A had no effect (n = 4). These compounds were also highly effective at $P2X_1$, $P2X_3$, and $P2X_{2/3}$ receptors but much less so at $P2X_2$, $P2X_4$, and $P2X_7$ receptors. Complete antagonist-inhibition curves were generated for $P2X_2$, $P2X_3$, and $P2X_{2/3}$ receptors (Fig. 3), and IC_{50} values are provided in Table 1. The dose-inhibition curves at the heteromeric $P2X_{2/3}$ receptors were consistently to the right of those for the homomeric $P2X_3$ receptor (Fig. 3), although the difference in IC_{50} estimates was significant only in the case of TNP-ATP (Table 1). As for TNP-ATP, the other TNP-nucleotides (up to 30 μM) had no agonist action. TNP-A (0.1–1 μM), GTP (0.1–10 μM), and picric acid (10 μM) had no agonist or antagonist action at $P2X_3$, $P2X_{2/3}$, or $P2X_2$ receptors.

The nature of the inhibition was examined further in the case of the $P2X_3$ receptor by constructing full agonist concentration-response curves. With either ATP or αβmeATP as the agonist, TNP-ATP (3 and 10 nM, respectively) caused both a rightward shift and a depression of the maximal current, indicating insurmountable antagonism. For the two antagonist concentrations ([B])(3 and 10 nM, respectively), the curves were fit by an expression appropriate to noncompetitive antagonism [$I/I_{max} = [1 + (EC_{50}/[A])]^{-1} (1 + K_B/[B])^{-1}$], which provided estimates of K_B of about 2 nM. Similar results were obtained for TNP-ADP and TNP-GTP (data not shown).

Discussion

The results indicate that certain nucleotides with a ribose-substituted trinitrophenyl group are potent antagonists at those P2X receptors that can be activated by αβmeATP ($P2X_1$, $P2X_3$, and $P2X_{2/3}$). However, several observations are not readily reconciled with the notion that the TNP-nucleotides are binding to the site occupied by the agonists when they act to open the P2X receptor channel. First, the antagonism is noncompetitive (Fig. 4). Second, both guanine and adenine nucleotides are equally effective; this is in marked contrast to the lack of any agonist activity by GTP itself. Third, removal of one or even two phosphate groups from TNP-ATP had no significant effect on the antagonism; yet in terms of agonist action, ADP is more than 100-fold less potent than ATP at the $P2X_3$ receptor (Lewis *et al.*, 1995) and AMP (100 μM) has no effect at the $P2X_1$ (Evans *et al.*, 1995), $P2X_3$ (Chen *et al.*, 1995) or $P2X_{2/3}$ receptor (unpublished observations). Removal of the third phosphate, as in TNP-A, resulted in loss of antagonism. In brief, the antagonist binding site differs from the agonist binding site in that it does not discriminate between guanine or adenine bases, absolutely requires the 2′,3′-trinitrophenol, and will accept one, two, or three (but not zero) 5′-phosphates.

It is possible that the TNP-nucleotides directly block the conducting pathway of the channel, as found for the outwardly rectifying chloride channel (Paulmichi e*t al.*, 1992; Venglarik *et al.*, 1993). This seems unlikely both because ATP is negatively charged and the channel is cation-selective, and because the inhibition by TNP was not different for inward and outward currents. In any event the concentrations of extracellular ATP and TNP-ATP that block the outwardly rectifying chloride current are still some hundred-fold higher than those effective at $P2X_1$, $P2X_3$, and $P2X_{2/3}$ receptors. The most likely mechanism, therefore, is the binding of TNP-nucleotides to an allosteric site on the large extracellular region of the receptor. In this case, the $P2X_1$ and $P2X_3$ subunits might provide a common domain that interacts with the strongly electronegative trinitrophenyl moiety. It is interesting that the most potent antagonists in a series tested on the rat urinary bladder (which expresses $P2X_1$ receptors) also had large aromatic 3′ substitutions (Bo *et al.*, 1994; Burnstock *et al.*, 1994); these bound with affinities in the 10–100 nM range.

The weak antagonism of TNP-ATP at other receptors had previously been reported for cochlear hair cells isolated from guinea pig organ of Corti (Mockett *et al.*, 1994), which are known to express $P2X_2$ receptors (Housley *et al.*, 1995; Brandle *et al.*, 1997). In that case, 75 μM TNP-ATP almost completely blocked the current evoked by 10 μM ATP. TNP-ATP is not an effective antagonist in the rat parotid gland

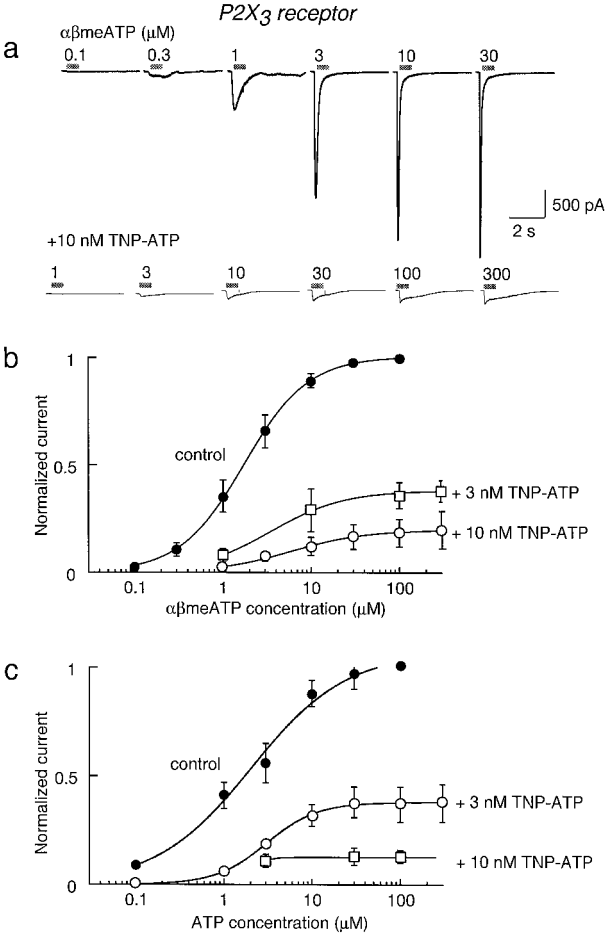

Fig. 4. TNP-ATP is a noncompetitive antagonist at the $P2X_3$ receptor. a, Currents recorded from a single cell expressing the $P2X_3$ receptor in the absence (*top*) and presence (*bottom*) of 10 nM TNP-ATP. b and c, Agonist concentration-response curves for αβmeATP (b) and ATP (c) in the absence and presence of 3 nM or 10 nM TNP-ATP. *Points*, mean ± standard error of 8–9 experiments for αβmeATP and 3–6 experiments for ATP.

(Soltoff *et al.*, 1993), which contains $P2X_4$ (Buell *et al.*, 1996) and $P2X_7$ receptors (Collo *et al.*, 1997). It will clearly be important to test the TNP analogs on P2X responses to ATP in other tissues. On the basis of the present work, one might expect blockade in the nanomolar concentration range to indicate that the underlying receptor contains $P2X_1$ or $P2X_3$ subunits. The results with heteromeric receptors, such as are expressed by some primary afferent neurons (Lewis *et al.*, 1995; Cook *et al.*, 1997), might be less straightforward. In the present work, we used $\alpha\beta$meATP as the agonist in experiments on the cells expressing the heteromeric $P2X_{2/3}$ receptor, on the assumption that it activates only heteromers and not any homomeric $P2X_2$ receptors that might also be present. The nerve-released transmitter would be ATP rather than $\alpha\beta$meATP, and a combined action of $P2X_2$ and $P2X_3$ subunits might result in an intermediate sensitivity to TNP-ATP. For the interpretation of such experiments it would also be useful to know whether these TNP analogs have blocking action at other receptors types, including the P2Y receptors.

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References

Azegami M and Iwai K (1975) Trinitrophenylation of nucleic acids and their constituents. *J Biochem* **78:**409–420.

Bo X, Fisher B, Maillard M, Jacobson KA, and Burnstock G (1994) Comparative studies on the affinities of ATP derivatives for P2X-purinoceptors in rat urinary bladder. *Br J Pharmacol* **112:**1151–1159.

Brandle U, Spielmanns P, Osteroth R, Sim J, Surprenant A, Buell G, Ruppersberg JP, Plinkert PK, Zenner H-P, and Glowatzki E (1997) Desensitization of the P2X(2) receptor controlled by alternative splicing. *FEBS Lett* **404:**294–298.

Buell G, Lewis C, Collo G, North RA, and Surprenant A (1996) An antagonist-insensitive P2X receptor expressed in epithelia and brain. *EMBO J* **15:**5–26.

Burnstock G, Fischer B, Hoyle CHV, Maillard M, Ziganshin AU, Brizzolara AL, Von Isakovics A, Boyer JL, Harden TK, and Jacobson KA (1994) Structure-activity relationships for derivatives of adenosine-5′-triphosphate as agonists at P2 purinoceptors: heterogeneity within P2X ad P2Y subtypes. *Drug Dev Res* **31:**206–219.

Chen C-C, Akopian AN, Silvotti L, Colquhoun D, Burnstock G, and Wood JN (1995) A P2X receptor expressed by a subset of sensory neurons. *Nature (Lond)* **377:**428–430.

Collo G, Neidhart S, Kawashima E, Kosco-Vilbois M, North RA, and Buell G (1997) Tissue distribution of the $P2X_7$ receptor. *Neuropharmacology* **36:**1277–1284.

Cook SP, Vulchanova L, Hargreaves KM, Elde RP, and McCleskey EW (1997) Distinct P2X receptors mediate ATP actions on nociceptive and non-nociceptive neurons. *Nature (Lond)* **387:**505–508.

Dunn PM and Blakeley AGH (1988) Suramin: a reversible P2-purinoceptor antagonist in the mouse vas deferens. *Br J Pharmacol* **93:**243–245.

Edwards FA, Gibb AJ, and Colquhoun D (1992) ATP receptor-mediated synaptic currents in the central nervous system. *Nature (Lond)* **359:**144–147.

Evans RJ, Derkach V, and Surprenant A (1992) ATP mediates fast synaptic transmission in mammalian neurons. *Nature (Lond)* **357:**503–505.

Evans RJ, Lewis C, Buell G, North RA, and Surprenant A (1995) Pharmacological characterization of heterologously expressed ATP-gated cation channels (P_{2X}-purinoceptors). *Mol Pharmacol* **48:**178–183.

Evans RJ, Lewis C, Virginio C, Lundstrom K, Buell G, Surprenant A, and North RA (1996) Ionic permeability of, and divalent cation effects on, two ATP-gated cation channels (P2X receptors) expressed in mammalian cells. *J Physiol (Lond)* **497:**413–422.

Evans RJ and Surprenant A (1992) Vasoconstriction of guinea-pig submucosal arterioles following sympathetic nerve stimulation is mediated by the release of ATP. *Br J Pharmacol* **106:**242–249.

Fenwick EM, Marty A, and Neher E (1982) A patch-clamp study of bovine chromaffin cells and of their sensitivity to acetylcholine. *J Physiol (Lond)* **331:**577–597.

Housley GD, Greenwood D, Bennett T, and Ryan AF (1995) Identification of a short form of the P2xR1-purinoceptor subunit produced by alternative splicing in the pituitary and cochlea. *Biochem Biophys Res Commun* **212:**501–508.

Kawashima E, Estoppey D, Fahmi D, Virginio C, Rees S, Surprenant A, and North RA (1997) A novel and efficient method for the stable expression of heteromeric ion channels in mammalian cells. *Receptors Channels,* in press.

Lewis C, Neidhart S, Holy C, North RA, Buell G, and Surprenant A (1995) Coexpression of $P2X_2$ and $P2X_3$ receptor subunits can account for ATP-gated currents in sensory neurons. *Nature (Lond)* **377:**432–434.

Mockett BG, Housley GD, and Thorne PR (1994) Fluorescent imaging of extracellular purinergic receptor sites and putative ecto-ATPase sites on isolated cochlear hair cells. *J Neurosci* **14:**6992–7007.

North RA (1996) Families of ion channels with two hydrophobic segments. *Curr Opin Cell Biol* **8:**474–483.

North RA and EA Barnard (1997) Nucleotide receptors. *Curr Opin Neurobiol* **7:**346–357.

Paulmichi M, Li Y, Wickman K, Ackerman M, Peralta E, and Clapham D (1992) New mammalian chloride channel identified by expression cloning. *Nature (Lond)* **356:**238–240.

Sneddon P and Westfall DP (1984) Pharmacological evidence that adenosine triphosphate and noradrenaline are co-transmitters in the guinea pig vas deferens. *J Physiol (Lond)* **347:**561–580.

Soltoff SP, McMillian MK, Talamo BR, and Cantlay LC (1993) Blockade of ATP binding site of P2 receptors in rat parotid acinar cells by isothiocyanate compounds. *Biochem Pharmacol* **45:**1936–1940.

Surprenant A, Rassendren F, Kawashima E, North RA, and Buell G (1996) The cytolytic P2Z receptor for extracellular ATP identified as a P2X receptor ($P2X_7$). *Science (Washington DC)* **272:**735–738.

Venglarik CJ, Singh AK, Wang R, and Bridges RJ (1993) Trinitrophenyl-ATP blocks colonic Cl^- channels in planar phospholipid bilayers. Evidence for two nucleotide binding sites. *J Gen Physiol* **101:**545–569.

Ziganshin AU, Hoyle CH, Bo X, Lambrecht G, Mutschler E, Baumert HG, and Burnstock G (1994) PPADS selectively antagonizes P2X-receptor-mediated responses in the rabbit urinary bladder. *Br J Pharmacol* **110:**1491–1495.

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