British Journal of Pharmacology (2000) 130, 1378–1384 © 2000 Macmillan Publishers Ltd All rights reserved 0007–1188/00 $15.00

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Diinosine pentaphosphate: an antagonist which discriminates between recombinant P2X$_3$ and P2X$_{2/3}$ receptors and between two P2X receptors in rat sensory neurones

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1 We have compared the antagonist activity of trinitrophenyl-ATP (TNP-ATP) and diinosine pentaphosphate (Ip$_5$I) on recombinant P2X receptors expressed in *Xenopus* oocytes with their actions at native P2X receptors in sensory neurones from dorsal root and nodose ganglia.

2 Slowly-desensitizing responses to α,β-methylene ATP (α,β-meATP) recorded from oocytes expressing P2X$_{2/3}$ receptors were inhibited by TNP-ATP at sub-micromolar concentrations. However, Ip$_5$I at concentrations up to 30 μM was without effect.

3 Nodose ganglion neurones responded to α,β-meATP with slowly-desensitizing inward currents. These were inhibited by TNP-ATP (IC$_{50}$, 20 nM), but not by Ip$_5$I at concentrations up to 30 μM.

4 In DRG neurones that responded to ATP with a rapidly-desensitizing inward current, the response was inhibited by TNP-ATP with an IC$_{50}$ of 0.8 nM. These responses were also inhibited by Ip$_5$I with an IC$_{50}$ of 0.1 μM. Both antagonists are known to inhibit homomeric P2X$_3$ receptors.

5 Some DRG neurones responded to α,β-meATP with a biphasic inward current, consisting of transient and sustained components. While the transient current was abolished by 1 μM Ip$_5$I, the sustained component remained unaffected.

6 In conclusion, Ip$_5$I is a potent antagonist at homomeric P2X$_3$ receptors but not at heteromeric P2X$_{2/3}$ receptors, and therefore should be a useful tool for elucidating the subunit composition of native P2X receptors.

British Journal of Pharmacology (2000) **130,** 1378–1384

Keywords: P2X receptor; TNP-ATP; Ip$_5$I; DRG; nodose ganglion; *Xenopus* oocyte

Abbreviations: ATP, adenosine 5′-triphosphate; DRG, dorsal root ganglion; HBSS, Hanks' balanced salt solution; Ip$_5$I, diinosine pentaphosphate; α,β-meATP, α,β-methylene ATP; TNP-ATP, 2′,3′-O-(2,4,6-trinitrophenyl)-ATP

Introduction

ATP can excite sensory neurones from dorsal root and nodose ganglia as well as neurones in the dorsal horn of the spinal cord (Jahr & Jessel, 1983; Krishtal *et al*., 1983). Furthermore, when ATP is injected intradermally, it causes intense pain, thus suggesting a role for ATP in nociception and primary afferent neurotransmission (for review see Burnstock & Wood, 1996; Burnstock, 2000). Immunohistochemical and *in situ* hybridization studies indicate that of the seven P2X subunits cloned, P2X$_1$–P2X$_6$ are expressed in sensory ganglia (Chen *et al*., 1995; Collo *et al*., 1996; Vulchanova *et al*., 1997; Xiang *et al*., 1998). Functional studies *in vitro* so far suggest that it is the P2X$_2$ and P2X$_3$ subunits that are important for the activation of primary afferents by ATP (Lewis *et al*., 1995; Robertson *et al*., 1996; Cook *et al*., 1997). However, the lack of suitable pharmacological tools has so far prevented the characterization of P2X receptors on sensory neurones *in vivo*.

P2X receptors are oligomeric complexes of uncertain stoichiometry, but possibly composed of either three subunits (Nicke *et al*., 1998; Stoop *et al*., 1999) or four subunits (Kim *et al*., 1997; Ding & Sachs, 2000). In sensory neurones, the expression of P2X$_2$ and P2X$_3$ subunits can give rise to the formation of both homomeric P2X$_2$ and P2X$_3$ receptors as well as at least one population of heteromeric P2X$_{2/3}$ receptors (Lewis *et al*., 1995; Thomas *et al*., 1998; Grubb & Evans, 1999). The development of more selective agonists and antagonists will help to clarify the involvement of different subunits in the formation of native P2X receptors and the role of these different receptors in physiological and pathophysiological processes.

Diinosine pentaphosphate (Ip$_5$I) is a very potent antagonist at recombinant P2X$_1$ receptors (pA$_2$, 8.2) and is quite active at P2X$_3$ receptors with a pA$_2$ of 6.3 (King *et al*., 1999). In this study, we have investigated the antagonist activity of Ip$_5$I at the recombinant heteromeric P2X$_{2/3}$ receptor. We have then compared it with trinitrophenyl-ATP (TNP-ATP) as an antagonist at the rapidly-desensitizing P2X receptors present on dorsal root ganglion (DRG) neurones and the slowly-desensitizing α, β-meATP sensitive receptor present on nodose ganglion neurones. While the former are believed to be homomeric P2X$_3$ (Robertson *et al*., 1996; Cook *et al*., 1997), the latter are thought to be heteromeric P2X$_{2/3}$ receptors (Lewis *et al*., 1995).

Methods

Native receptors in sensory neurones

Dissociated sensory neurones were prepared following a protocol used previously for isolation of autonomic ganglion neurones (Zhong *et al*., 1998). Rat pups (0–2-days-old) were

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killed by cervical dislocation followed by decapitation. Dorsal root or nodose ganglia were removed and placed in Ca^{2+}/Mg^{2+} free Hanks' Balanced Salt Solution containing 10 mM HEPES (pH 7.3) buffer (HBSS). Ganglia were treated with collagenase 1.5 mg ml^{-1} (CLS II; Worthington Biochemical Corporation, Reading, U.K.) for 40 min, followed by trypsin 1 mg ml^{-1} (Sigma) for 15 min. The ganglia were then suspended in growth medium comprising of L-15 medium (Gibco) supplemented with 10% bovine serum albumin, 2 mg ml^{-1} $NaHCO_3$, 5.5 mg ml^{-1} glucose, 200 IU ml^{-1} penicillin and 200 μg ml^{-1} streptomycin. Following gentle trituration with a fire-polished Pasteur pipette single cells were

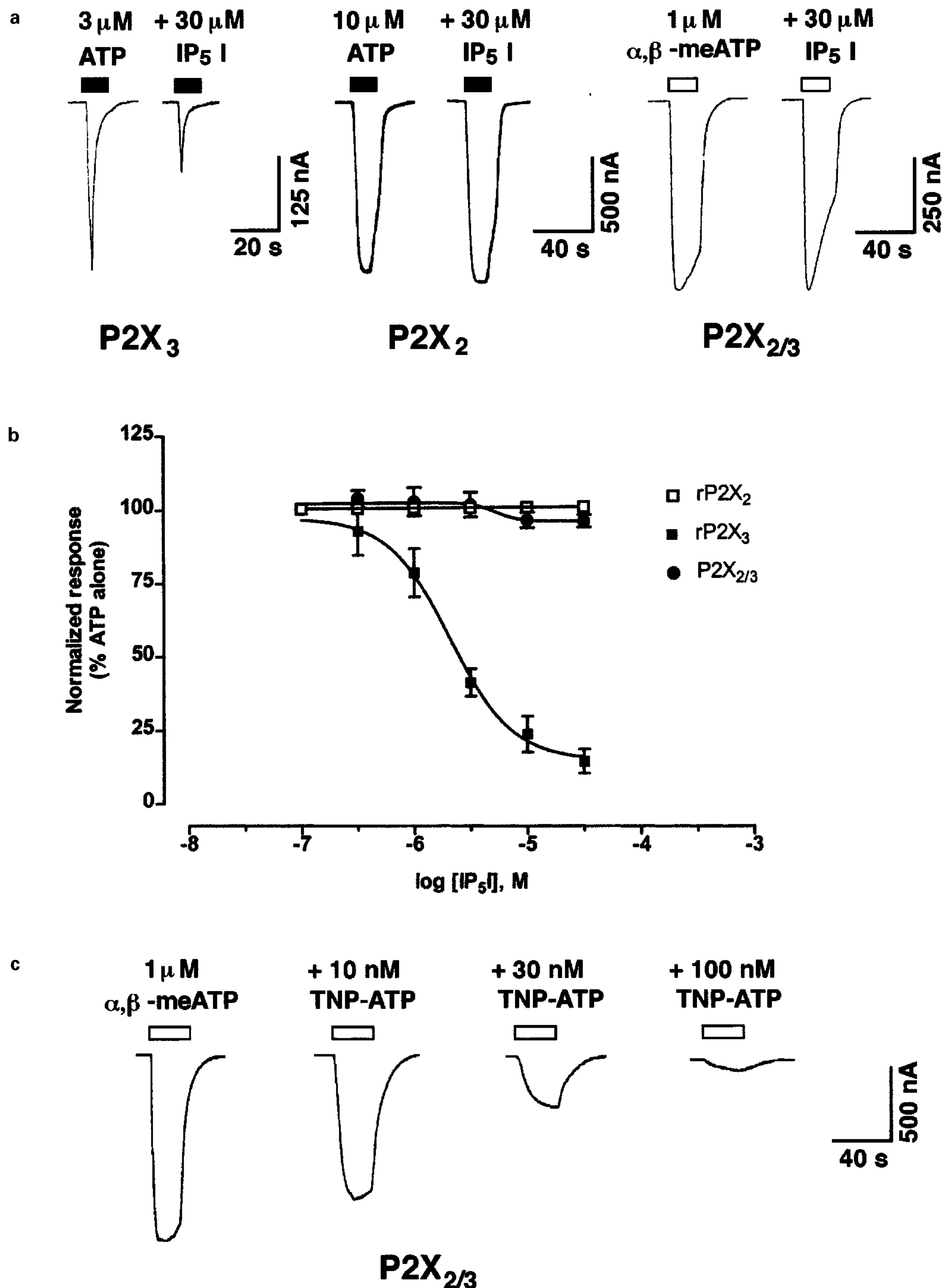

Figure 1 Comparison of the action of TNP-ATP and Ip_5I on recombinant $P2X_2$, $P2X_3$ and $P2X_{2/3}$ receptors. (a) Response of three different *Xenopus* oocytes expressing $P2X_3$, $P2X_2$ and $P2X_{2/3}$ receptors, to ATP or α,β-meATP alone, and in the presence of 30 μM diinosine pentaphosphate (Ip_5I). (b) Concentration-effect curves for the inhibition of $P2X_3$, $P2X_2$ and $P2X_{2/3}$ receptors by Ip_5I. ATP was used as the agonist at 3 μM and 10 μM for $P2X_3$ and $P2X_2$ receptors respectively, while 1 μM α,β-meATP was used in experiments on the heteromeric receptor. Points represent the mean$\pm$s.e.mean from five cells. Antagonists were given for 60 s before, and during the agonist application. In the presence of Ip_5I, the peak amplitude of agonist evoked responses at $P2X_2$ and $P2X_{2/3}$ receptors were not significantly different from control ($P>0.05$). (c) Responses to α,β-meATP recorded from an oocyte expressing $P2X_{2/3}$ receptors in the presence of increasing concentrations of TNP-ATP. Comparable results were obtained in a further three cells. Oocytes were voltage-clamped at a holding potential of -50 mV. Antagonists were present for 60 s before and during the agonist applications. In oocytes co-injected with $P2X_2$ and $P2X_3$ transcripts, the transient current due to activation of homomeric $P2X_3$ receptors was abolished by a condition application of α,β-meATP (10 μM, 20 s) given 60 s before the test response, leaving only the sustained response mediated by $P2X_{2/3}$ receptors.

harvested by centrifugation at 900 r.p.m. for 5 min. The resultant pellet was re-suspended in growth medium and plated onto 35 mm culture dishes that had been pre-treated with 10 μg ml^{-1} laminin (Sigma). Cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2, and used between 2 and 10 h after plating.

Whole cell voltage-clamp recording was carried out at room temperature using an Axopatch 200B amplifier (Axon Instruments, Foster City, CA, U.S.A.). Membrane potential was held at −70 mV. External solution contained (mM): NaCl 154, KCl 4.7, $MgCl_2$ 1.2, $CaCl_2$ 2.5, HEPES 10, Glucose 5.6, the pH was adjusted to 7.4 using NaOH. Recording electrodes (resistance 2–4 MΩ) were filled with internal solution that contained (mM): CsCl 120, HEPES 10, tripotassium citrate 10, the pH was adjusted to 7.2 using CsOH. Data were acquired using pCLAMP software (Axon Instruments). Signals were filtered at 2 kHz (−3 dB frequency, Bessel filter, 80 dB/decade).

Drugs were applied rapidly through a 4-barrel manifold comprising fused glass capillaries inserted into a common outlet tube (tip diameter of ~200 μm) which was placed about 200 μm from the cell (Dunn *et al*., 1996). Solutions were delivered by gravity flow from independent reservoirs with solution flow controlled by computer driven solenoid valves. One barrel was used to apply drug free solution to enable rapid termination of drug application. Solution exchange measured by the change in open tip current on switching from 150 mM NaCl to 150 mM KCl solution was complete in 20 ms; however, complete exchange of solution around an intact cell was considerably slower ($\leqslant$100 ms). Traces were acquired using Fetchex (pCLAMP software) and plotted using Origin (Microcal, Northampton, MA, U.S.A.).

Recombinant receptors expressed in Xenopus *oocytes*

Methods for preparation of defolliculated oocytes from *Xenopus laevis* have been described in detail previously (King *et al*., 1997b). Defolliculated oocytes were injected (40 nl) cytosolically with capped ribose nucleic acid (cRNA) encoding either rat $P2X_2$ or rat $P2X_3$ receptors. In coexpression experiments, oocytes were injected with a mixture of cRNAs prepared by mixing $rP2X_2$ cRNA (2 μg ml^{-1}) with $rP2X_3$ cRNA (1 mg ml^{-1}), yielding a concentration ratio of 1 : 500. RNA-injected oocytes were incubated at 18°C in Barth's solution (pH 7.5) containing (mM): NaCl 110, KCl 1, $NaHCO_3$ 2.4, Tris-HCl 7.5, $Ca(NO_3)_2$ 0.33, $CaCl_2$ 0.41, $MgSO_4$ 0.82, supplemented with gentamycin sulphate 50 μg l^{-1} for 48 h to allow full receptor expression and then stored at 4°C for up to 12 days.

Membrane currents were recorded under voltage-clamp conditions using a twin-electrode amplifier (Axoclamp 2B). The microelectrodes had a resistance of 1–2 MΩ when filled with 3 M KCl. Oocytes were perfused constantly (at 5 ml min^{-1}) with Ringer's solution containing (mM): NaCl 110, KCl 2.5, HEPES 5, $BaCl_2$ 1.8, pH 7.5. All recordings were made at room temperature (18°C) and at a holding potential of −50 mV unless stated otherwise. Electrophysiological data were recorded on a chart recorder (Gould recorder 2200s).

Solutions were delivered by gravity flow from independent reservoirs placed above the preparation. Applications of agonists were separated by a 20 min interval unless otherwise stated. The P2 receptor antagonists TNP-ATP (3–1000 nM) and Ip_5I (0.03–100 μM) were applied for 1 min prior to and during agonist application. For concentration-inhibition curves, data were normalized with respect to the response evoked in the absence of antagonist.

Data analysis

All data are expressed as the means ± s.e.mean. Statistical analysis (Student's *t*-test) was performed using Origin 5 (Microcal, Northampton, MA, U.S.A.); *P* values <0.05 were considered significant. Concentration-response data were fitted with the Hill equation: $Y = A/[1 + (K/X)^n]$, where: A is the maximum effect, K is the EC_{50}, and n is the Hill coefficient, using Origin 5. The combined data from the given number of cells were fitted, and the results are presented as values ± s.e., determined by the fitting routine.

Drugs and reagents

All common salts were AnalaR grade (BDH, Lutterworth, U.K.). Diinosine pentaphosphate (Ip_5I) was prepared by enzymatic degradation of diadenosine pentaphosphate (Ap_5A) (see King *et al*., 1999). Trinitrophenyl-ATP (Molecular Probes, Leiden, Netherlands). All other drugs were from Sigma Chemical Co (Poole, Dorset, U.K.).

Results

Recombinant receptors expressed on Xenopus *oocytes*

Oocytes injected with $P2X_3$ cRNA responded to ATP (3 μM) with a rapidly-desensitizing inward current (Figure 1a). Micromolar concentrations of Ip_5I produced a concentration dependent inhibition of this response (Figure 1a,b), which is in keeping with the previously published IC_{50} of 2.8 ± 0.7 μM (King *et al*., 1999). In contrast, oocytes injected with $P2X_2$ cRNA gave a slowly-desensitizing response to ATP that was not affected by Ip_5I at concentrations up to 30 μM (Figure 1a,b). In oocytes co-injected with $P2X_2$ and $P2X_3$ cRNA, it was necessary to use α,β-meATP as the agonist, to prevent activation of homomeric $P2X_2$ receptors. Nevertheless, α,β-

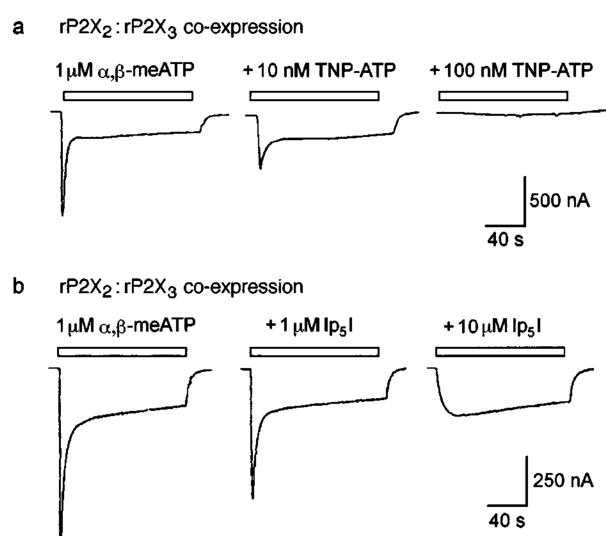

Figure 2 The effect of Ip_5I and TNP-ATP on composite responses at recombinant P2X receptors. (a) Response of an oocyte, co-injected with $rP2X_2$ and $rP2X_3$ transcripts and expressing both $P2X_3$ and $P2X_{2/3}$ receptors, to 1 μM α,β-meATP, alone and in the presence of 10 and 100 nM TNP-ATP. (b) Response of another oocyte to 1 μM α,β-meATP alone, and in the presence of 1 and 10 μM Ip_5I. Similar effects of TNP-ATP and Ip_5I were observed in three and five cells respectively. Oocytes were voltage-clamped at a holding potential of −50 mV. Antagonists were present for 60 s before and during the agonist application.

meATP evoked a biphasic response (see Figure 2) that we attribute to the activation of homomeric $P2X_3$ and heteromeric $P2X_{2/3}$ receptors ($n=8$). By using a pre-application of 10 μM α,β-meATP to produce selective desensitization of the $P2X_3$ receptors, the heteromeric receptor could be studied in isolation. Ip_5I at concentrations up to 30 μM produced no inhibition of the slowly-desensitizing response to 1 μM α,β-meATP (Figure 1a,b). In contrast, sub-micromolar concentrations of TNP-ATP (0.01–0.1 μM) produced a concentration dependent reduction in the response to α,β-meATP (Figure 1c), consistent with the previously published IC_{50} of 7 nM (Virginio *et al*., 1998). Similar results were obtained in all four oocytes tested. We next investigated the effect of these two antagonists on co-injected oocytes, without prior desensitization of the $P2X_3$ receptors. Under these conditions α,β-meATP evoked a biphasic response composed of transient and sustained inward currents. TNP-ATP produced a concentration dependent reduction in both the transient and sustained parts of the response (Figure 2a). In contrast, Ip_5I selectively

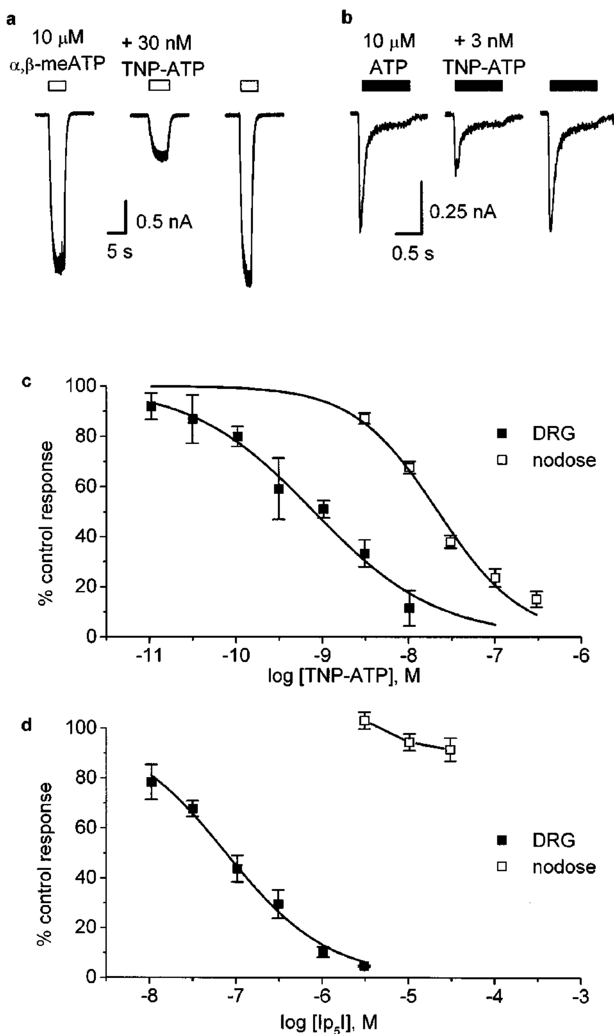

Figure 3 Inhibition of P2X receptors in sensory neurones by TNP-ATP. (a) Response of a nodose ganglion neurone to 10 μM α,β-meATP before, in the presence of, and following washout of 30 nM TNP-ATP. (b) Response of a dorsal root ganglion neurone to 10 μM ATP before, in the presence of and following washout of 3 nM TNP-ATP. Cells were voltage-clamped at a holding potential of -60 mV. Responses were recorded at 3.5 min intervals and antagonists were present for 3 min before, and during the second agonist application. (c) Concentration-effect curves for the inhibition of the response of DRG neurones and nodose ganglion neurones by TNP-ATP. The agonist used was 10 μM ATP and 10 μM α,β-meATP for DRG and nodose ganglion neurones respectively. Each point represents the mean$\pm$s.e.mean from 3–12 cells. Solid lines show least squares fit of the Hill equation to the data which yielded IC_{50} values of 0.77 ± 0.12 μM and 20.6 ± 2.8 nM and Hill coefficients of 0.6 and 1.3 for DRG and nodose ganglion neurones respectively. (d) Concentration-effect curves for the inhibition by Ip_5I of the response of DRG neurones and nodose ganglion neurones to 10 μM ATP and 10 μM α,β-meATP, respectively. Each point represents the mean$\pm$s.e.mean from 3–6 cells. The solid curve show least squares fit of the Hill equation to the data for DRG neurones which yielded an IC_{50} value of 0.07 ± 0.007 μM and a Hill coefficient of 0.73. Responses of nodose ganglion neurones in the presence of Ip_5I at concentrations up to 30 μM were not significantly different from control responses ($P>0.05$).

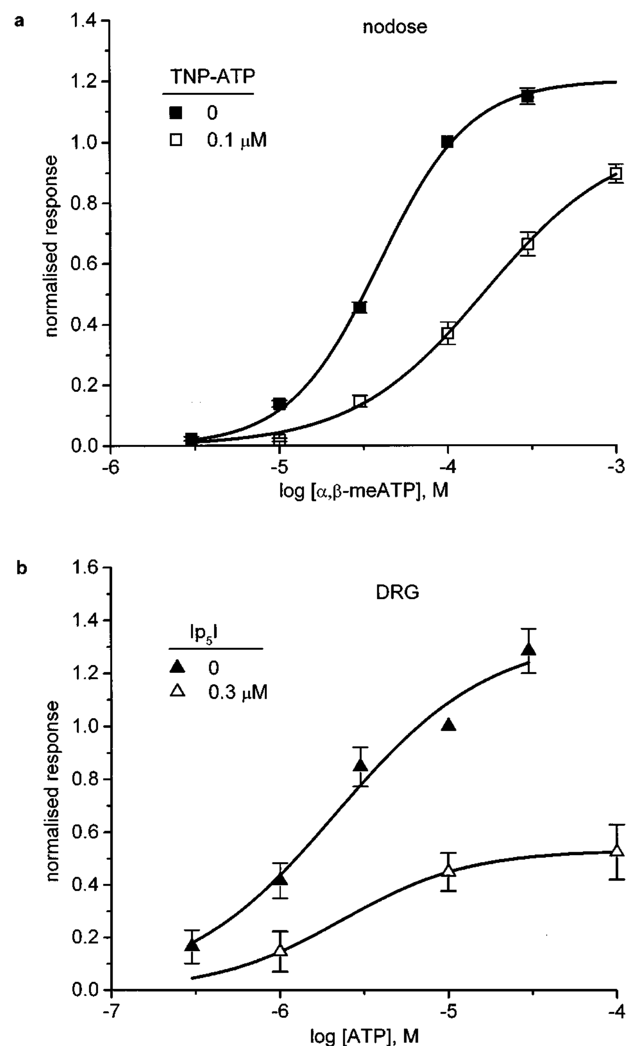

Figure 4 The nature of the antagonism produced by TNP-ATP and Ip_5I. (a) Concentration-response curves for α,β-meATP evoked inward currents recorded from nodose ganglion neurones in the absence or presence of 0.1 μM TNP-ATP. Cells were voltage-clamped at -60 mV. Responses were normalized with respect to that produced by 100 μM α,β-meATP in the absence of antagonist recorded from the same cell. Points represent the mean$\pm$s.e.mean from 3–6 cells. The curves show least squares fit of the Hill equation to the data, which gave EC_{50} values of 39.2 ± 2.5 μM and 167 ± 22 μM and Hill coefficients of 1.6 and 1.1 in the absence and presence of antagonist respectively. (b) Concentration-response curves for the rapidly-desensitizing inward currents produced by ATP in DRG neurones in the absence and presence of 0.3 μM Ip_5I. Points represent the mean$\pm$s.e.mean from 4–6 cells. Responses were normalized with respect to that produced by 10 μM ATP in the same cell, in the absence of antagonist.

abolished the transient component of the response while leaving the sustained current unaffected (Figure 2b).

Native receptors in nodose ganglion neurones

In agreement with the observations of Khakh and colleagues (1995), all neurones dissociated from neonatal rat nodose ganglia responded to 10 μM α,β-meATP with a rapidly-activating and slowly-desensitizing response (Figure 3a) attributed to the activation of heteromeric $P2X_{2/3}$ receptors. These neurones posses both homomeric $P2X_2$ and heteromeric $P2X_{2/3}$ receptors (Thomas *et al*., 1998), so α,β-meATP was used as the agonist to avoid activation of homomeric $P2X_2$ receptors. Transient responses to α,β-meATP are not evoked in these neurones, indicating the absence of homomeric $P2X_3$ receptors. Consequently, the desensitizing pre-pulse used in the oocyte experiments was not necessary. TNP-ATP produced a concentration dependent inhibition of this response with an IC_{50} of 20.5 ± 2.8 nM (Figure 3c). We investigated further the antagonism produced by TNP-ATP, by looking at its action on the α,β-meATP log-concentration-response curve. In the presence of 0.1 μM TNP-ATP, a concentration that we had found to reduce the response to 10 μM α,β-meATP by about 75%, there was a rightward shift in the α,β-meATP log-concentration-response curve, increasing the EC_{50} from 39–167 μM (Figure 4a). However, there was also a marked reduction in the slope of the curve, suggesting that TNP-ATP does not act as a simple competitive antagonist. In contrast, Ip_5I at concentrations up to 30 μM failed to produce any antagonism of the α,β-meATP response in nodose ganglion neurones (Figure 3d).

Native receptors in DRG neurones

DRG neurones can respond to ATP with either rapidly-desensitizing, sustained, or biphasic responses (Robertson *et al*., 1996; Burgard *et al*., 1999; Grubb & Evans, 1999). In our experiments, approximately 50% (105/209) of dissociated DRG neurones, responded to 10 μM ATP with only a fast activating and rapidly-desensitizing inward current. This response declined to less than 10% of the peak by the end of a 1 s agonist application and is believed to be mediated by homomeric $P2X_3$ receptors (Figure 3b). TNP-ATP produced a rapid and reversible inhibition of this response. The effect of TNP-ATP was concentration dependent with an IC_{50} of 0.77 ± 0.12 nM (Figure 3c). Ip_5I also produced a reversible, concentration dependent inhibition of the transient ATP response in DRG neurones, with an IC_{50} of 0.12 ± 0.03 μM (Figure 3d). We investigated the nature of the antagonism produced by Ip_5I, by examining its effect on the ATP log-concentration response curve. In the presence of 0.3 μM Ip_5I, a concentration predicted to give approximately 75% inhibition, the maximum response to ATP was greatly reduced, while the EC_{50} was almost unchanged (2.3 μM compared with 2.1 μM; Figure 4b).

A small percentage of DRG neurones responded to α,β-meATP with a clearly biphasic inward current, consisting of both transient and sustained components, similar to those observed in co-injected oocytes. In four such cells where the current at 1 s was 36 ± 6% of the peak current, 1 μM Ip_5I, abolished the transient component, while leaving the sustained current unaffected (Figure 5).

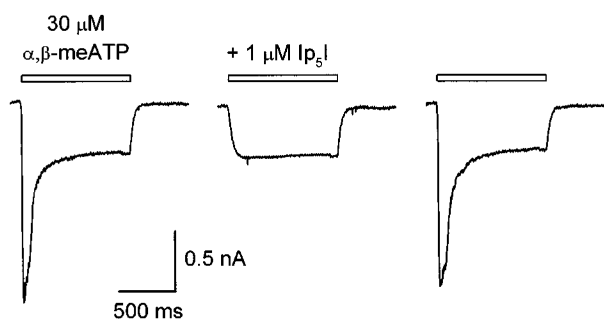

Figure 5 Effect of Ip_5I on mixed responses in dorsal root ganglion neurones. The traces show membrane currents evoked by 30 μM α,β-meATP in a DRG neurone voltage-clamped at -60 mV, alone and in the presence of 1 μM Ip_5I. While the transient component of the response was abolished, the sustained component was unaffected by the antagonist. Similar results were observed in a total of four neurones.

Discussion

Ip_5I is an antagonist at recombinant $P2X_1$ and $P2X_3$ receptors, but is ineffective at $P2X_2$ receptors (King *et al*., 1999). We have now extended these studies, and found that this compound is also ineffective as an antagonist at the heteromeric $P2X_{2/3}$ receptor. It thus shows at least 100 fold selectivity for $P2X_3$ over $P2X_{2/3}$ receptors. In contrast, although TNP-ATP is much more potent than Ip_5I as an antagonist at $P2X_3$ receptors, it is also very active at heteromeric $P2X_{2/3}$ receptors, with at best a 10 fold selectivity between these receptor sub-types (Virginio *et al*., 1998).

It has been suggested that the rapidly-desensitizing ATP responses in sensory neurones are due to activation of homomeric $P2X_3$ receptors (Robertson *et al*., 1996, Cook *et al*., 1997). In contrast, the slowly-desensitizing α,β-meATP sensitive receptor found in nodose ganglion cells results from the activation of heteromeric $P2X_{2/3}$ receptors (Lewis *et al*., 1995). In keeping with this view, we have not only confirmed that the rapidly-desensitizing receptor is blocked by nanomolar concentrations of TNP-ATP, but we have also demonstrated its sensitivity to Ip_5I. In contrast, the slowly-desensitizing response to α,β-meATP in nodose ganglion neurones is not affected by Ip_5I at concentrations up to 30 μM (the highest concentration tested). This is in keeping with our observation that the recombinant $P2X_{2/3}$ receptor is resistant to Ip_5I.

In dissociated neurones, responses mediated by homomeric $P2X_3$ receptors can readily be distinguished from those of heteromeric $P2X_{2/3}$ receptors by their time course. However in multicellular preparations and *in vivo*, this distinction may be less clear (see Kirkup *et al*., 1999; Rong *et al*., 2000). In such

Table 1 Comparison of the antagonst activity of diinosine pentaphosphate (Ip_5I) and trinitrophenyl-ATP (TNP-ATP) at recombinant and native P2X receptors

	IC_{50} (nM)	
Receptor	*Ip_5I*	*TNP-ATP*
$P2X_1$	3.1 ± 0.4^a	6 ± 3^b
$P2X_2$	ND	2000 ± 200^b
$P2X_3$	2800 ± 700	0.9 ± 0.2^b
$P2X_{2/3}$	ND	7 ± 3^b
Nodose neurone	ND	21 ± 3
DRG neurone (fast)	70 ± 7	0.8 ± 0.1

The IC_{50} values determined at recombinant P2X receptors expressed in *Xenopus* oocytes or HEK 293 cells are presented along with those determined at native receptors in sensory neurones. ND, no inhibition detected at concentrations up to 30 μM. [a]data from King *et al.*, (1999); [b]data from Virgino *et al.*, (1998).

situation, an antagonist like Ip_5I should be very useful in identifying the type of P2X receptor involved.

The nature of the antagonism produced by TNP-ATP and Ip_5I appear to be complex. Thus, at the recombinant $P2X_3$ receptor, TNP-ATP produces a non-surmountable antagonism (Virginio *et al*., 1998). Such behaviour may in part result from the very rapid desensitization of this receptor, which will prevent equilibrium being established between the receptor, agonist and antagonist. The antagonism at the $P2X_{2/3}$ receptor in nodose ganglion neurones produced by 0.1 μM TNP-ATP appeared to be surmountable, although there was a reduction in the slope of the log-concentration-response curve indicating a non-competitive interaction. At recombinant $P2X_1$ and $P2X_3$ receptors low concentrations of Ip_5I produce parallel shifts in the agonist log-concentration response curves, but the antagonism was found to be non-competitive (King *et al*, 1999). Our experiments investigating the antagonism of the native P2X receptor in DRG neurones by Ip_5I showed a non-surmountable, non-competitive antagonism. However, the very rapid desensitization of this response prevents true equilibrium being established and may again complicate interpretation of the results. The rapid desensitization of $P2X_3$ and $P2X_1$ receptors is determined by the intracellular domains (Werner *et al*., 1996; King *et al*., 1997a; Smith *et al*., 1999). It would therefore be interesting to see how TNP-ATP and Ip_5I interact with chimeric receptors possessing the extracellular loop of the $P2X_3$ receptor and the cytoplasmic and trans-membrane segments of the $P2X_2$ receptor. Such receptors should retain the pharmacology of the $P2X_3$ receptor, but with the slow desensitization of the $P2X_2$ receptor.

Our results on the action of TNP-ATP and Ip_5I at recombinant P2X receptors expressed in *Xenopus* oocytes, are in broad agreement with our results on the native receptors found in sensory neurones (Table 1). However, the results are not identical. In particular, Ip_5I produces a non-surmountable antagonism of the rapidly-desensitizing receptor in DRG neurones and it is considerably more potent than at the recombinant $P2X_3$ receptor. The reason for this is at present unclear, but receptors expressed in *Xenopus* oocytes do show some differences from those expressed in mammalian cells (Evans *et al*., 1995; Lewis *et al*., 1997). An alternative, though perhaps less likely explanation is that the rapidly-desensitizing receptor present on DRG neurones is not in fact a homomeric $P2X_3$ receptor, but incorporates some additional subunit(s). However in general, our results thus support the notion that the slowly-desensitizing α,β-meATP response in nodose ganglion neurones is mediated by heteromeric $P2X_{2/3}$ receptors, while the rapidly-desensitizing response seen in DRG neurones is mediated by homomeric $P2X_3$ receptors.

In conclusion, we have demonstrated that Ip_5I is an effective antagonist of the homomeric $P2X_3$ with an IC_{50} of 3 μM, but is inactive at the heteromeric $P2X_{2/3}$ receptor. The ability of Ip_5I to discriminate between the rapidly-desensitizing responses in DRG neurones and the slowly-desensitizing α,β-meATP response in nodose ganglion neurones supports the notion that these responses are mediated by $P2X_3$ and $P2X_{2/3}$ receptors respectively. Ip_5I should be a useful tool for further elucidating the subunit composition of native P2X receptors.

We are grateful to Roche Bioscience (Palo Alto, U.S.A.) for financial support, and to Dr J. Pintor and Dr J. Gaulix for preparation of Ip_5I. We thank Professor David Julius (UCSF, U.S.A.) and Professor John Wood (UCL, U.K.) for providing $rP2X_2$ and $rP2X_3$ plasmids. We acknowledge the assistance of Dr Andrea Townsend-Nicholson (UCL, U.K.) in preparing cRNAs and the editorial assistance of Mr Roy Jordan.

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(Received January 17, 2000
Revised March 30, 2000
Accepted March 30, 2000)