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Characterization of a P_{2X}-purinoceptor in cultured neurones of the rat dorsal root ganglia

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1 The electrophysiological actions of the P_2-purinoceptor agonists, adenosine 5'-triphosphate (ATP), 2-methylthioATP (2-meSATP) and α,β-methyleneATP (α,β-meATP) and of uridine 5'-triphosphate (UTP) were studied under concentration and voltage-clamp conditions in dissociated neurones of 1-6 day old rat dorsal root ganglia.

2 ATP (10 nM-100 μM) applied rapidly via a U-tube perfusion system (equilibration time < 10 ms) activated concentration-dependent inward currents with a latency to onset of a few ms, an EC_{50} of 719 nM and a Hill slope of 1.47.

3 2-meSATP (10 nM-100 μM) and α,β-meATP (100 nM-100 μM) also evoked transient inward currents. The EC_{50} and Hill slopes were 450 nM and 1.58 for 2-meSATP and 1.95 μM and 1.53 for α,β-meATP respectively. There was no significant difference between the maximum currents evoked by the three agonists.

4 As the concentration of ATP increased so the rate of rise and decay of the currents also increased. At 100 and 300 nM ATP the decay of the current was best fitted by a single exponential, but at 1 μM and above two exponentials were required. Log-log plots of the rise time or time constants of decay versus concentration were linear. Currents evoked by 2-meSATP and α,β-meATP showed a similar concentration-dependence in their kinetics.

5 Inward currents evoked by ATP, 2-meSATP and α,β-meATP (300 nM) were abolished by the P_2-purinoceptor antagonist, suramin (100 μM).

6 UTP (10 μM) evoked similar transient inward currents, which were sensitive to suramin (100 μM). ATP (10 μM), applied 2 min beforehand, reduced the response to UTP (10 μM) by 80 $\pm$ 10%.

7 This study shows that ATP, 2-meSATP and α,β-meATP act via a suramin-sensitive P_{2X}-purinoceptor to evoke rapid, transient inward currents in dissociated neurones of rat dorsal root ganglia. The pyrimidine nucleotide, UTP, was also active. It is likely that the agonists were acting at the P_{2X3}-subtype to produce these effects.

Keywords: Rat dorsal root ganglia; sensory neurones; P_{2X}-purinoceptor; ATP; UTP

Introduction

The actions of adenosine 5'-triphosphate (ATP) as a fast neurotransmitter at peripheral neuro-effector junctions are now well characterized (Burnstock, 1990). More recently, a rapid neurotransmitter action of ATP was also determined at neuro-neuronal synapses in the brain (Edwards *et al.*, 1992) and in sympathetic neurones in culture (Evans *et al.*, 1992). When released ATP acts at the P_{2X}-subtype of P_2-purinoceptors, ligand-gated cation channels were originally defined in smooth muscle preparations by an agonist potency order of α,β-methyleneATP (α,β-meATP) > > 2-methylthioATP (2-meSATP) $\geq$ ATP (Burnstock & Kennedy, 1985; Kennedy, 1990). However, it is now clear that in many tissues ATP and 2-meSATP, but not α,β-meATP, are rapidly broken down by ecto-ATPase and if this breakdown is prevented, then 2-meSATP and ATP are in fact more potent than α,β-meATP (see Kennedy & Leff, 1995a).

The amino acid sequence of several subtypes of the P_{2X}-purinoceptor have now been published (Brake *et al.*, 1994; Valera *et al.*, 1994; Bo *et al.*, 1995; Chen *et al.*, 1995; Lewis *et al.*, 1995). These form a new family of ligand-gated ion channels, with a predicted structure of short, intracellular amino and carboxy domains, two transmembrane spanning regions and a large extracellular domain. ATP, 2-meSATP and α,β-meATP are all active at the P_{2X1}- and P_{2X3}-subtypes, with only small differences in potency. However, α,β-meATP is a weak agonist at the P_{2X2}- and P_{2X4}-purinoceptors. A further difference is that the P_{2X1}- and P_{2X3}-subtypes both show rapid desensitization in the continued presence of agonist, whereas desensitization of the P_{2X2}- and P_{2X4}-subtypes is much slower.

Whilst mRNA for the P_{2X1}-, P_{2X2}- and P_{2X4}-subtypes is distributed throughout the body, that for the P_{2X3}-subtype has only been found at high levels in sensory neurones (Chen *et al.*, 1995; Lewis *et al.*, 1995). Pretreatment with capsaicin substantially reduces the level of P_{2X3}-mRNA (Chen *et al.*, 1995), suggesting that P_{2X3}-purinoceptors are selectively expressed in C-fibre afferents, which are predominantly nociceptive. The aim of this study was to characterize the pharmacological and biophysical properties of the P_{2X}-purinoceptor(s) present in neurones of the rat dorsal root ganglia. These cells have previously been shown to respond to ATP with a rapidly developing inward current (Bean, 1990; Bouvier *et al.*, 1991), but it is not known which subtype(s) of P_{2X}-purinoceptor they functionally express. A preliminary account of these results has been published (Roberston *et al.*, 1995).

Methods

Single cell isolation

Neuronal cells of rat dorsal root ganglia were isolated by a modification of the method of Stansfeld & Mathie, (1993). Rat pups (1-6 days old) were killed by cervical dislocation fol-

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lowed by decapitation. The ganglia were dissected out and incubated for 20 min at 37°C in 3 ml of a modified Ca^{2+}- and Mg^{2+}-free Hanks balanced salt solution containing papain (20 units ml^{-1}). Papain was pre-activated by incubation with L-cysteine (4 mg ml^{-1}) for 10 min. The ganglia were then transferred to 3 ml of Ca^{2+}- and Mg^{2+}-free Hanks solution containing collagenase Type 1 (150 units ml^{-1}) and dispase (8 mg ml^{-1}) and incubated for a further 45 min. Mild trituration was performed with a 5 ml Gilson pipette every 15 min and a 200 μl Gilson pipette at 45 min; 3 ml of L-15 (Leibovitz) medium was then added to the enzyme solution and the cells centrifuged at 75 g for 5 min. The cells were re-suspended in 3 ml of L-15 medium, centrifuged at 75 g for 5 min and finally re-suspended in a modified L-15 medium containing 10% foetal calf serum, $NaHCO_3$ (26 mM), glucose (30 mM), nerve growth factor (10–100 ng ml^{-1}), glutamax (4 mM), penicillin (30 units ml^{-1}) and streptomycin (30 μg ml^{-1}). The cells were plated onto glass coverslips (Fisons) coated with poly-L-ornithine (18 μg ml^{-1}) and laminin (10 μg ml^{-1}) or with calf skin collagen (0.001% solution) and grown at 37°C in a 100% humidified 95% air/5% CO_2 atmosphere. Cells were used within 4 days.

Electrophysiological recordings

Cells were superfused (2 ml min^{-1}) at room temperature with a solution of the following composition (mM): NaCl 140, KCl 5, Na_2HPO_4 0.06, glucose 10, HEPES 10, $MgCl_2$ 1.2, $CaCl_2$ 2.5, titrated to pH 7.3 with NaOH and osmolarity 310 mOsm. Whole cell currents were recorded in cells of 20–40 μm diameter by the patch clamp technique with an Axopatch 1D amplifier (Axon Instruments, U.S.A.). The resistance of the pipettes was 2–5 MΩ when filled with a solution containing (mM): KCl 145, $MgCl_2$ 2, HEPES 10, EGTA 5, Na_2ATP 2, Na_2GTP 0.1, titrated to pH 7.03 with KOH and osmolarity 300 mOsm. Pipettes were coated with Sigmacoat to reduce capacitance artefacts. The liquid junction potential was subtracted using the d.c. offset on the amplifier. In most cells series resistance was not compensated for. In all experiments the membrane potential was clamped at −65 mV. Data were collected on an IBM compatible PC using WCP software (Dr John Dempster, Strathclyde University) with a National Instruments Lab PC plus interface at a sampling frequency of 1 kHz.

Application of drugs

In initial experiments, bath perfusion of ATP (30–60 s) evoked small inward currents which took at least 30 s to develop and showed marked desensitization. Therefore, throughout this study agonists were applied rapidly by use of a solenoid valve-controlled U-tube application system placed 200 μm from the cell (Fenwick *et al.*, 1982). The delay to onset of solution change (approximately 60 ms) and the equilibration time (< 10 ms), calculated as the time between 10–90% maximum change, were measured by monitoring the change in resistance of a patch electrode when deionized water was applied from the U-tube. The increase in resistance was maintained for the duration of the application pulse, indicating that the U-tube was providing an effective concentration-clamp.

Routinely, at the start of each recording a 500 ms pulse of the extracellular bathing solution was applied to a cell via the U-tube and any cells displaying a change in holding current (i.e. mechanical artefacts) were discarded. The first application of agonist was applied to the cell 5 min after whole cell mode was achieved, in order to standardize any time-dependent effects of cell dialysis. Only one cell per coverslip was tested for responses to the drugs.

Statistics

Data are expressed throughout as the mean ± s.e.mean and were compared by Student's unpaired t test or one way analysis of variance and Tukey's comparison as appropriate. $P < 0.05$ was considered to be statistically significant. Concentration-response curves were fitted to the data by logistic (Hill equation), non-linear regression analysis (Fig.P., Biosoft, Cambridge, U.K.).

Drugs and solutions

Ca^{2+}- and Mg^{2+}-free Hanks balanced salt solution, L-15 (Leibovitz) medium, collagenase Type 1, papain, L-cysteine, $NaHCO_3$ 7.5% solution, glucose 45% solution, foetal calf serum, nerve growth factor, penicillin, streptomycin, calf skin collagen, laminin and poly-L-ornithine were obtained from Sigma, dispase from Boehringer Mannheim and Glutamex from Gibco.

Stock solutions of ATP (sodium salt), α,β-meATP (lithium salt), uridine 5'-triphosphate (UTP) (sodium salt), (all Sigma), purified UTP (a gift from Drs P. Leff and K. McKechnie, Astra, Charnwood), 2-meSATP (Research Biochemicals Inc.) and suramin (Bayer, U.K.) were dissolved in deionized water and stored frozen. All drugs were then diluted in extracellular bathing solution.

Results

Responses to rapid application of ATP

Isolated neurones of the rat dorsal root ganglia had a resting membrane potential of −55.0 ± 0.7 mV and cell capacitance of 27.5 ± 1.2 pF (n = 140). When applied rapidly for 500 ms ATP (1 nM) had no effect on the holding current in 4/5 cells (see Figure 1) and produced a small (41 pA) inward current in the remaining cell. However, higher concentrations of ATP (10 nM–100 μM) evoked an inward current in 50/53 cells, with a delay to onset of several ms (Figure 1). The peak amplitude of the current was concentration-dependent and when the Hill equation was fitted to the data an EC_{50} of 719 nM (95% confidence limits = 503–981 nM) and a Hill slope of 1.47 were obtained. There was no indication of a higher Hill slope value at low concentrations, as reported by Bean (1990). Initial experiments showed that if 10 min was allowed between applications, then ATP at concentrations up to and including 1 μM could be repeatedly administered with little current rundown being apparent. However, at concentrations greater than 1 μM, substantial rundown was seen.

At each concentration of ATP there was a large cell-to-cell variation in the peak current amplitude, as reflected by the relatively large standard error bars in Figure 1b. For example, at 300 nM ATP the peak amplitude range was 58–1500 pA. This was not due to differences in cell size, as the currents showed a similar large degree of variation when cell size was taken into account, i.e. when calculated as pA pF^{-1}. Thus, at 300 nM ATP the peak amplitude range was 2.8–55.5 pA pF^{-1}. A similar variation in the amplitude of inward currents evoked by ATP has been reported previously in this (Bean, 1990) and other cell types (Evans & Kennedy, 1994) and presumably reflects variability in the number of receptors expressed in different cells.

At low concentrations, ATP-evoked currents developed and decayed slowly (Figures 1a, 2), but as the concentration of ATP increased so the current reached a peak and decayed more rapidly. A plot of log rise time versus log concentration was linear (Figure 2a). The decay of the current in the continued presence of ATP was best described by a single exponential in 5/6 cells at 100 nM and 15/20 cells at 300 nM ATP. In the remaining cells and in all cells at 1 μM ATP and above, decay was best fitted by two exponentials. Plots of log τ_{decay} versus log concentration for both components were linear (Figure 2b). This shows that the rise and decay times vary with the concentration of ATP in an exponential manner.

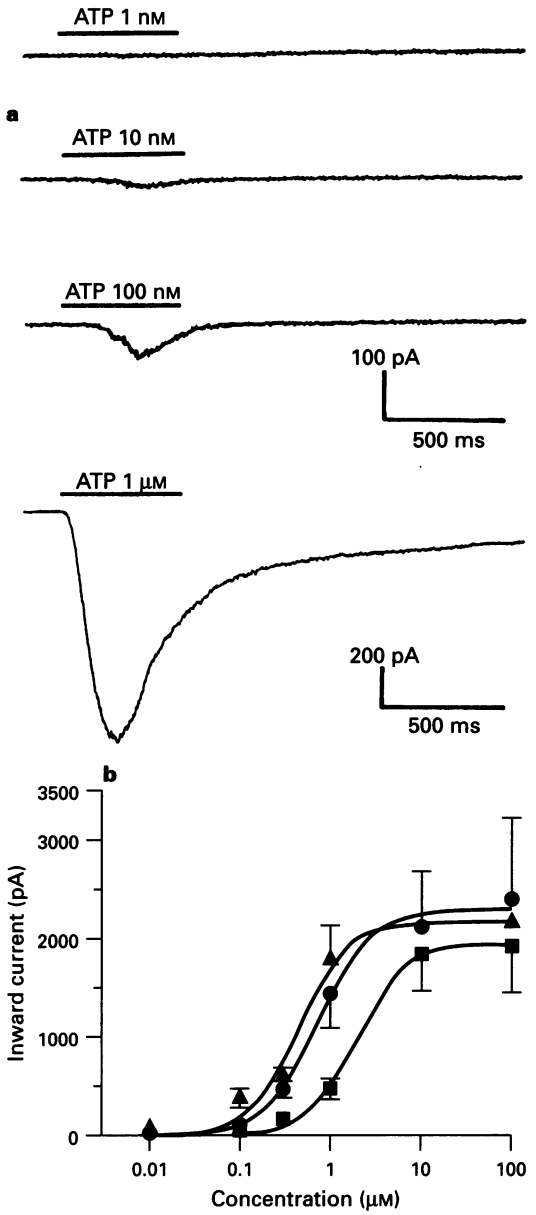

Figure 1 ATP evokes fast inward currents in acutely dissociated neurones of the rat dorsal root ganglia: (a) traces show inward currents evoked by ATP (1 nM – 1 μM) in the same cell, when applied rapidly for 500 ms, as indicated by the solid bars. Note the difference in scale for the current evoked by 1 μM ATP. ATP was applied at 10 min intervals to minimize current rundown. (b) Mean peak inward current amplitude (±s.e.mean) is plotted against log concentration of ATP (●), 2-meSATP (▲) and α,β-meATP (■) $n = 4-23$. Error bars for several points have been omitted for clarity where necessary.

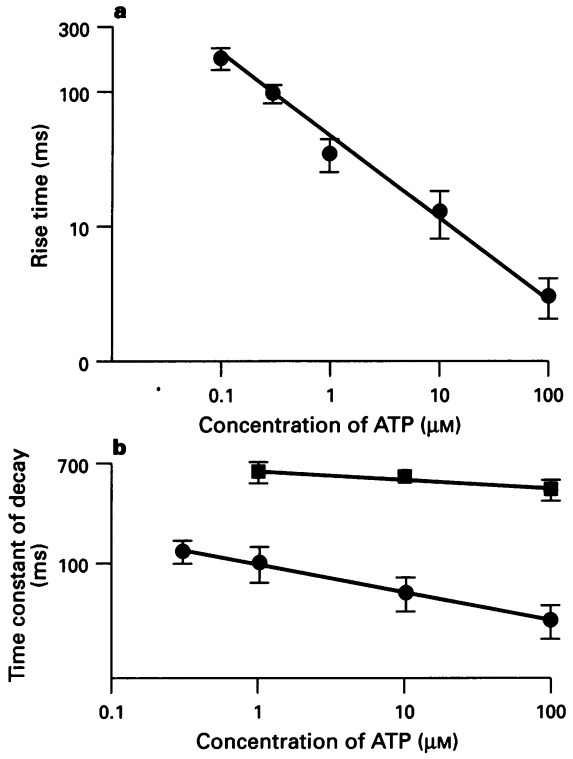

Figure 2 Inward currents develop and decay more rapidly as the concentration of ATP increases: (a) log mean (±s.e.mean) rise time is plotted against log concentration of ATP (100 nM – 100 μM) (slope = −0.58, $r = 0.996$). (b) Log mean (±s.e.mean) time constant of both components of decay are plotted against concentration of ATP (300 nM – 100 μM). Fast component - slope = −0.24, $r = 0.999$, slow component - slope = −0.079, $r = 0.977$. In some cells the computed time constant of the slower component was greater than 1 s, i.e. much greater than the period of ATP application and so these values have been omitted from the analysis.

Responses to rapid application of 2-meSATP and α,β-meATP

In order to characterize pharmacologically the receptor at which ATP was acting, the effects of the P_2-purinoceptor agonists 2-meSATP and α,β-meATP were also determined. 2-meSATP (10 nM – 100 μM) and α,β-meATP (100 nM – 100 μM) evoked inward currents in a concentration-dependent manner (Figures 1, 3). The EC_{50} and Hill slope values were 450 nM (95% confidence limits = 181 – 719 nM) and 1.58 for 2-meSATP and 1.95 μM (95% confidence limits = 1.60 – 3.3 μM) and 1.53 for α,β-meATP respectively. There was no significant difference between the maximum currents evoked by each agonist. Similar to those evoked by ATP, these currents developed and decayed more rapidly with increasing concentration (not shown). Thus, the agonist potency order in these cells

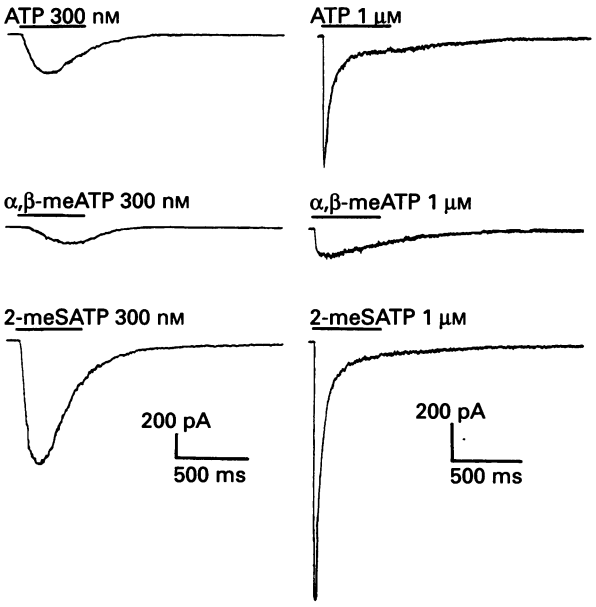

Figure 3 ATP, α,β-meATP and 2-meSATP evoke fast inward currents in acutely dissociated neurones of the rat dorsal root ganglia: the left hand traces show currents evoked in a single cell by 300 nM of each agonist when applied rapidly for 500 ms, as indicated by the solid bars. The right hand traces show currents evoked in another cell by 1 μM of each agonist. Note the difference in scale bars. Agonists were applied at 10 min intervals.

was 2-meSATP≈ATP>α,β-meATP, but the differences in potency were small.

Effects of suramin

The involvement of P_2-purinoceptors in the generation of the inward currents was studied by use of the P_2-purinoceptor antagonist, suramin. A control response was obtained to ATP, 2-meSATP or α,β-meATP (all 300 nM), applied via the U-tube, before cells were exposed to suramin (100 μM) for 5 min in the superfusate. The agonist, plus suramin, was then readministered via the U-tube.

Suramin abolished the inward currents evoked by ATP ($n=10$) (Figure 4), 2-meSATP ($n=11$) (not shown) and α,β-meATP ($n=4$) (not shown). Reversal of the inhibitory action of suramin developed slowly, even though its washout was rapid. After 10 min washout the amplitude of the currents tended to be approximately 50% of the control response. The records shown in Figure 4 are from the only cell out of 25 tested in which the recording was maintained long enough to see full recovery of the agonist response. Thus, ATP, 2-meSATP and α,β-meATP evoked inward currents through activation of P_2-purinoceptors which are sensitive to suramin.

Responses to rapid application of UTP

Finally, we determined the effects of UTP, which was shown recently to be a relatively weak agonist at the P_{2X3}-purinoceptor cloned from neurones of the rat dorsal root ganglion (Chen *et al.*, 1995). When applied rapidly for 500 ms, UTP (1 μM) had no effect on the holding current in 3/3 cells. However, UTP (10 μM) evoked an inward current in 8/10 cells with a mean peak amplitude of 526±104 pA (range=134–910 pA) (Figure 5). This was significantly smaller than the response to ATP (10 μM) ($P<0.05$), which was near maximal (2133±562 pA, $n=8$). The 2 cells which were unresponsive to UTP responded to ATP (10 μM) with an inward current. The decay of the UTP-evoked current could be described by a single exponential (600±91 ms).

UTP provided by Sigma was assayed by h.p.l.c. and found to be 86–95% pure, but it is unlikely that the activity of UTP was due to a contaminating nucleotide as ATP was not present and no other nucleotide was identified. Furthermore, when this UTP was purified it was still as effective at evoking the inward currents (not shown).

Suramin (100 μM) abolished the inward currents evoked by UTP in 3/4 cells (Figure 5) and decreased the response by 90% in the remaining cell. Although partial recovery from the inhibitory effect of suramin could be seen following its washout, the recordings were not maintained long enough to see full recovery of the agonist response in any of the four cells tested.

To investigate further the site of action of UTP, cross-desensitization studies with ATP were performed. First, a control response to UTP (10 μM) was obtained, followed 10 min later by application of ATP (10 μM); 3 min later UTP (10 μM) was reapplied to test for desensitization. In the 4 cells tested and the second response to UTP was reduced by 80±10% compared with the first (Figure 6). If 10 min was then allowed before a third application of UTP (10 μM), some recovery of the response was seen (Figure 6). However, the recordings were not maintained long enough to see full re-

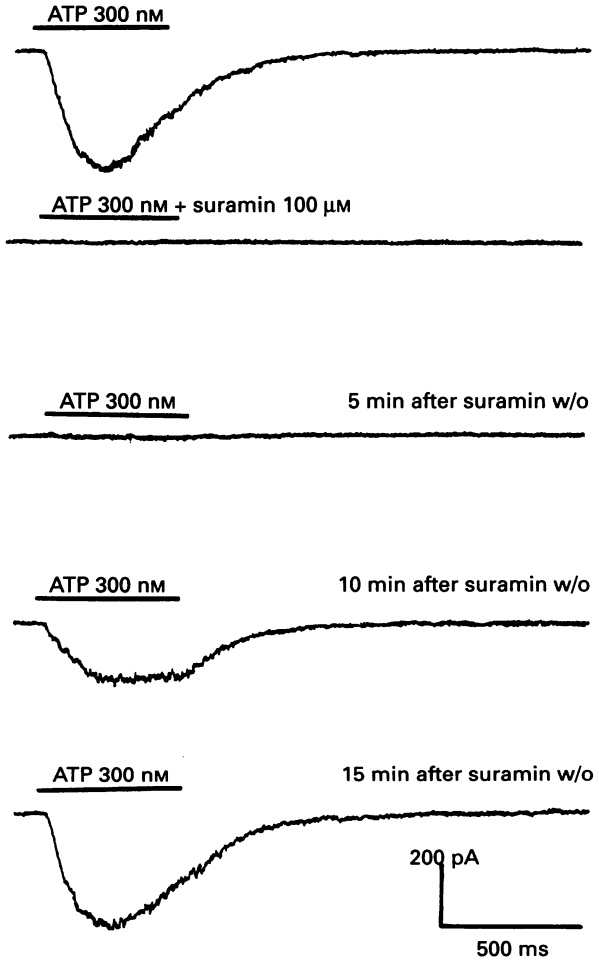

Figure 4 Suramin abolishes inward currents evoked by ATP in acutely dissociated neurones of the rat dorsal root ganglia: traces show inward currents evoked in a single cell by ATP (300 nM) when applied rapidly for 500 ms, as indicated by the solid bars, in the absence of suramin, after 5 min bath superfusion with suramin (100 μM) and at 5 min intervals following washout of suramin.

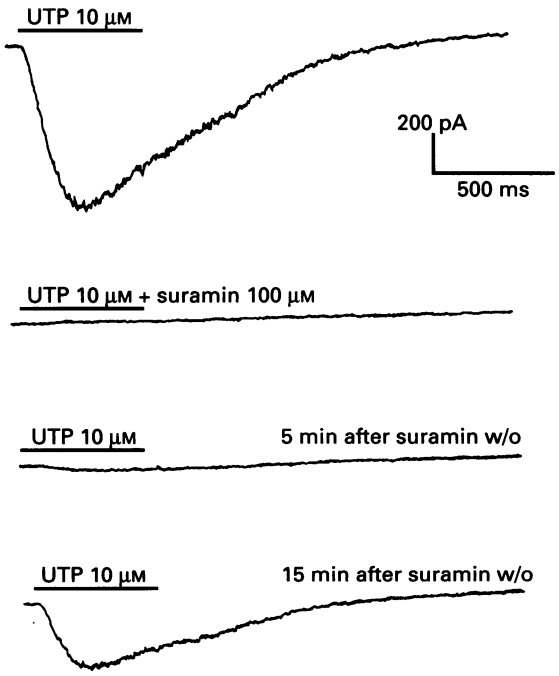

Figure 5 UTP evokes inward currents in acutely dissociated neurones of the rat dorsal root ganglia: traces show inward currents evoked by UTP (10 μM) in a single cell when applied rapidly for 500 ms, as indicated by the solid bars. UTP was first applied in the absence of suramin, then after 5 min bath superfusion with suramin (100 μM) and 5 min and 15 min following the washout of suramin.

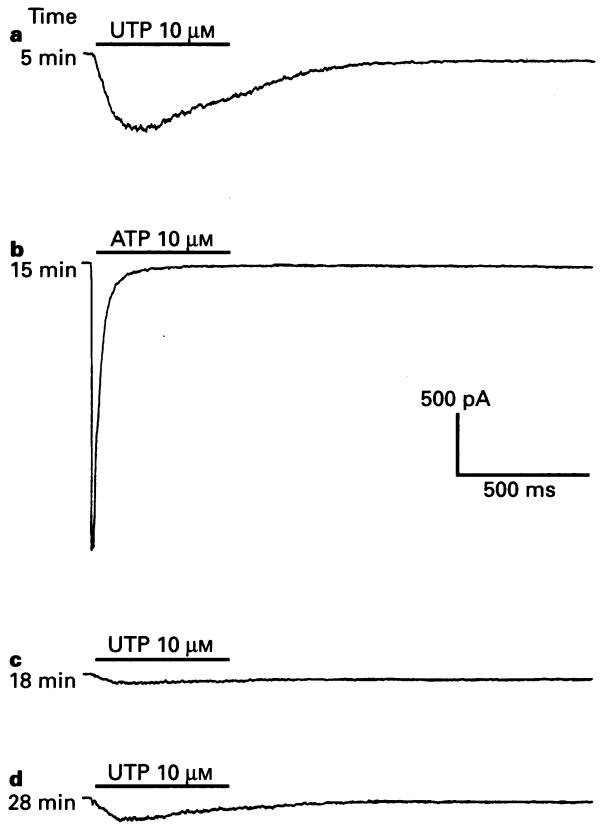

Figure 6 ATP causes desensitization of UTP-evoked inward currents in acutely dissociated neurones of the rat dorsal root ganglia: traces show inward currents evoked by UTP and ATP (both 10 μM) in a single cell when applied rapidly for 500 ms, as indicated by the solid bars. UTP was applied first, 5 min after the whole cell mode had been achieved (a), followed 10 min later by ATP (b). UTP was then reapplied a further 3 min (c) and 13 min (d) later.

covery of the agonist response in any of the four cells tested. Thus, ATP appears to cause cross-desensitization of the response to UTP.

Discussion

This study shows that P_2-purinoceptor agonists activate a suramin-sensitive, inward current in dissociated sensory neurones of the neonatal rat dorsal root ganglia. The delay to onset of the current was only a few ms, consistent with activation of a P_{2X}-purinoceptor, a ligand-gated ion channel. 2-meSATP and ATP were active at submicromolar concentrations, with EC_{50} values of 450 nM and 719 nM respectively, while α,β-meATP was only slightly less potent. This is similar to the potency of these agonists in a number of single cell systems (see Kennedy & Leff, 1995a), but differs from the original definition of the P_{2X}-purinoceptor in smooth muscle tissues, where α,β-meATP > > 2-meSATP ≥ ATP (Burnstock & Kennedy, 1985; Kennedy, 1990). However, it is now clear that in many tissues ATP and 2-meSATP, but not α,β-meATP, are rapidly broken down by ecto-ATPase and that if breakdown is prevented, then 2-meSATP and ATP are in fact more potent than α,β-meATP at the P_{2X}-purinoceptor (Kennedy & Leff, 1995a), as was seen here. This also suggests that under our recording conditions ATP and 2-meSATP were subject to minimal breakdown.

The multiple subtypes of P_{2X}-purinoceptor which have been cloned and characterized show two broad patterns of agonist activity. The absolute and relative potentcies of ATP, 2-meSATP and α,β-meATP seen in the present study are consistent with both the P_{2X1}- and P_{2X3}-subtypes (Brake *et al.*, 1994; Chen *et al.*, 1995; Lewis *et al.*, 1995). However, as α,β-meATP was a potent agonist the involvement of P_{2X2}- and P_{2X4}-purinoceptors can be discounted since α,β-meATP is a weak agonist at these sites (Valera *et al.*, 1994; Bo *et al.*, 1995).

UTP also evoked a rapidly developing, transient, suramin-sensitive inward current in these cells, but was at least 100 fold less potent than ATP. It is likely that ATP and UTP were acting at the same receptor, as ATP caused cross-desensitization of the response to UTP, though we cannot discount the possibility that they acted via different receptors and that the desensitization was heterologous. For example Ca^{2+} influx via the NMDA channel can cause desensitization of the AMPA receptor in rat central neurones (Mulkey *et al.*, 1994; Kroyzis *et al.*, 1995) and P_{2X}-purinoceptors are known to be Ca^{2+}-permeable (Valera *et al.*, 1994; Lewis *et al.*, 1995).

The excitatory effect of UTP was surprising as it has not generally been found to act at P_{2X}-purinoceptors. Here, UTP was active at 10 μM, similar to the cloned P_{2X3}-purinoceptor (Chen *et al.*, 1995), but 50 μM UTP had no effect at the cloned P_{2X2}-purinoceptor (Brake *et al.*, 1994) and 300 μM UTP was ineffective at the cloned P_{2X4}-purinoceptor (Bo *et al.*, 1995). Likewise, UTP was ineffective at 200 μM at the native receptor in dissociated smooth muscle cells of the rat vas deferens (Friel, 1988) and at 100 μM at the P_{2X1}-purinoceptor cloned from this tissue (Valera *et al.*, 1994) and the human urinary bladder (Evans *et al.*, 1995). However, it shoud be noted that we have previously found UTP to activate a suramin-sensitive inward current, similar to that evoked by ATP, in single smooth muscle cells of the rat tail artery and this was probably due to activation of the P_{2X1}-purinoceptor (McLaren *et al.*, 1995). The reason for this difference is not known.

The currents activated by ATP, 2-meSATP, α,β-meATP and UTP in these experiments all showed rapid desensitization in the continued presence of agonist. The time constant of the fast component of decay decreased from 200 to 20 ms with increasing agonist concentration, which is similar to the properties of the functionally expressed cloned P_{2X3}-purinoceptor (Chen *et al.*, 1995; Lewis *et al.*, 1995), but different from the P_{2X1}-purinoceptor studied in single cells of the rat vas deferens, where the time-constant of decay was concentration-independent (Khakh *et al.*, 1995b). This suggests that we were studying the P_{2X}-purinoceptor in dissociated neurones of the neonatal rat dorsal root ganglia. Consistent with this conclusion is the report that while mRNA for all P_{2X}-purinoceptor subtypes can be detected in sensory neurones, the levels of the P_{2X3}-subtype are much higher than the others (Chen *et al.*, 1995). If this is mirrored at the level of protein expression, then the current carried by P_{2X3}-purinceptors is likely to be the major component of current activated by ATP.

The rapid desensitization of the currents seen in this study is very different from previous reports on neurones of the rat dorsal root ganglia (Bean, 1990; Bouvier *et al.*, 1991) and nodose ganglia (Krishtal *et al.*, 1983; Li *et al.*, 1993; Khakh *et al.*, 1995a), where the currents decayed over at least several seconds. It has been suggested that these slow currents may be due to the formation of a novel channel phenotype following heteropolymerization of P_{2X2}- and P_{2X3}-subunits (Lewis *et al.*, 1995). If so, then such heteropolymerization does not appear to have occurred in the neurones used in the present study and consequently the current was carried by P_{2X3}-purinoceptors alone.

P_{2X3}-purinoceptors have a very restricted distribution, being selectively expressed at high levels in nociceptive C-fibre afferents. This has lead to the proposal that ATP may play a role in nociception since if P_{2X3}-subunits are present in the cell bodies of nociceptive neurones, then they may also be expressed in the terminals and so be a target for ATP in the extracellular fluid (Chen *et al.*, 1995; Kennedy & Leff, 1995b; Lewis *et al.*, 1995). ATP, released perhaps from damaged or stressed cells, could activate the P_{2X3}-purinoceptors, initiating depolarization, action potentials and so a nociceptive signal. In support of this proposal, P_{2X}-purinoceptors have been shown to be present on capsaicin-sensitive sensory nerve terminals in

rat tail (Rea & Wallis, 1992; Trezise *et al.*, 1996) and canine lung (Pelleg & Hurt, 1996). This is consistent with the ability of ATP to evoke pain in a human blister-base model (Bleehen & Keele, 1977) and with studies which show that P_{2X}-purinoceptor antagonists are analgesic in animals (Ho *et al.*, 1992; Driessen *et al.*, 1994). Clearly if ATP, and more specifically P_{2X3}-purinoceptors, are involved in nociception, then the development of a selective P_{2X3}-antagonist could prove useful in pain relief. The highly selective localization of P_{2X3}-purinoceptors could confer a degree of specificity, leading to fewer side-effects.

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