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Rat chromaffin cells lack P2X receptors while those of the guinea-pig express a P2X receptor with novel pharmacology

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1 Whole-cell patch-clamp recording was used to determine the functional expression and pharmacological properties of P2X receptors in chromaffin cells dissociated from adrenal medullae of rats and guinea-pigs.

2 In rat chromaffin cells maintained in culture for 1–7 days, ATP and UTP failed to evoke any detectable response.

3 Guinea-pig chromaffin cells responded to ATP (100 μM) with a rapidly activating inward current. The amplitude of the response to ATP increased over the period cells were maintained in culture and so did the number of cells giving a detectable response, with 69% of cells responding after $\geqslant$4 days of culture.

4 The response to ATP desensitized slowly, and had a reversal potential of 2.5 mV. The EC_{50} for ATP was 43 μM. The potency order for ATP analogues was 2-MeSATP > ATP > ADP. Adenosine, UTP and α,β-meATP were inactive.

5 Suramin (100 μM) and Cibacron blue (50 μM) inhibited the ATP (100 μM)-activated current by 51 and 47%, respectively. PPADS antagonized the response to ATP (100 μM) with an IC_{50} of 3.2 μM.

6 The ATP concentration-response curve shifted to the left at pH 6.8 (EC_{50}, 19 μM) and right at pH 8.0 (EC_{50}, 96 μM), without changing the maximal response. Zn^{2+} inhibited the response to ATP (100 μM) with an IC_{50} of 48 μM.

7 This study indicates that expression of ATP-gated cation channels in chromaffin cells is species dependent. The P2X receptors in guinea-pig chromaffin cells show many characteristics of the $P2X_2$ receptor subtype.

Keywords: Chromaffin; ATP; P2X; Suramin; PPADS; Cibacron blue; pH; Zn^{2+}

Abbreviations: ATP, adenosine 5′-triphosphate; DMEM, Dulbecco's modified Eagle's medium; DMPP, dimethylphenylpiperazinium iodide; EGTA, ethylene glycol-bis[β-aminoethylether]-N,N,N′,N′-tetraacetic acid; HBSS, Hank's balanced salt solution; α,β-meATP, α,β-methylene ATP; 2-MeSATP, 2-methylthio ATP; PPADS, pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid; TEAC1, tetraethylammonium chloride

Introduction

Adenosine 5′-triphosphate (ATP) is released from nerve terminals and acts as a neurotransmitter in the nervous system (see Burnstock, 1996). In addition, ATP has been suggested as an intercellular signalling molecule in a number of systems such as regulation of smooth muscle contraction and neuroendocrine secretion *via* ionotropic (P2X) and metabotropic (P2Y) receptors (see Abbracchio & Burnstock, 1994; Ralevic & Burnstock, 1998).

Chromaffin cells of the adrenal medulla are exposed to ATP from two distinct sources: splanchnic nerve terminals (Parker *et al*., 1990), where it is co-released with acetylcholine (Burnstock 1981; Vizi *et al*., 1997) and from chromaffin cells themselves. ATP is found to be co-stored with catecholamines in the granules of adrenal chromaffin cells at an ATP: catecholamines ratio of about 1 : 4 and co-released with catecholamines (Todorov *et al*., 1996). The occurrence of P2X receptors in rat adrenal gland has been suggested on the basis of Northern blot analysis and immunohistochemical studies (Bo *et al*., 1995; Vulchanova *et al*., 1996), indicating a possible role for ATP in the regulation of catecholamine secretion.

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Extracellular ATP stimulates catecholamine secretion from perfused guinea-pig and bovine adrenal glands, cultured chromaffin cells (Asano *et al*., 1995; Lin *et al*., 1995) and from rat phaeochromocytoma (PC12) cells (Inoue *et al*., 1989; Sela *et al*., 1991). This effect may be accomplished by increasing the influx of extracellular Ca^{2+} (Castro *et al*., 1995; Reichsman *et al*., 1995) *via* ATP-gated ion channels (Inoue *et al*., 1989; Nakazawa & Inoue, 1992). An ATP-activated inward current was observed in a subset of chromaffin cells from guinea-pig (Otsuguro *et al*., 1995). In contrast, ATP failed to produce any inward current in rat adrenal chromaffin cells (Hollins & Ikeda, 1997). ATP-activated membrane currents were reported in a subpopulation of bovine chromaffin cells (Diverse-Pierluissi *et al*., 1991), but were not observed in the studies by Currie & Fox (1996).

The relative proportion of adrenaline- and noradrenaline-containing cells varies in different species of animals with e.g. about 91% adrenaline-containing cells in the rat, 71% in cattle and 98% in the guinea-pig (Holzbauer & Sharman, 1972). Thus, dissociated chromaffin cells from both rats and guinea-pigs should provide a relatively homogeneous population of adrenaline-secreting cells. In the present study, we investigated the expression of ATP-activated currents in cultured chromaffin cells dissociated from rats and guinea-pigs and

characterized the pharmacological properties of the P2X receptors present on guinea-pig cells.

Methods

Chromaffin cell preparation

Chromaffin cells were obtained from the adrenal medullae of 17-day old male Sprague-Dawley rats and 17-day old male Duncan-Hartley guinea-pigs. Briefly, rats and guinea-pigs were killed by inhalation of a rising concentration of CO_2 and cervical dislocation. The adrenal glands were rapidly dissected out, cut transversely into 2–4 pieces and the darker cortex trimmed away from the medulla tissue and discarded. The adrenal medullae were minced and dissociated by incubation in 4 ml Ca^{2+}/Mg^{2+}-free Hank's balanced salt solution with 10 mM HEPES pH 7 buffer (HBSS) (Life Technologies, Paisley, U.K.) containing 1.5 mg ml^{-1} collagenase (Class-II, Worthington Biochemical Corporation, Reading, U.K.) and 6 mg ml^{-1} bovine serum albumin (Sigma, Poole, U.K.) at 37°C for 40 min. The adrenal tissue was subsequently incubated in 4 ml HBSS containing 1 mg ml^{-1} trypsin (Sigma) at 37°C for 15 min and then triturated with a fire polished glass pipette followed by centrifugation at 900 r.p.m. for 5 min. The dispersed cells were re-suspended in Dulbecco's modified Eagle's medium (DMEM) (Life Technologies) supplemented with 10% bovine serum, 2 mg ml^{-1} $NaHCO_3$, 5.5 mg ml^{-1} glucose, 200 IU ml^{-1} penicillin and 200 μg ml^{-1} streptomycin. Cells were plated in 35-mm culture dishes (Falcon) coated with collagen, or treated with 10 mg ml^{-1} laminin (Sigma) at room temperature for 1 h. Cultures were maintained at 37°C in a humidified atmosphere of 95% air and 5% CO_2 and used after 1–7 days.

Electrophysiology

Whole-cell voltage-clamp recording was carried out at room temperature using an Axopatch 200B amplifier (Axon Instruments, CA, U.S.A.). Patch pipettes were pulled from thin-walled glass capillary tubes with fine filament (GC 150 TF; Clark Electromedical Instruments, Reading, U.K.), using a two-stage puller (PP-830, Narishige, Tokyo, Japan), fire polished and filled with a solution containing (mM): citric acid 56, $MgCl_2$ 3, CsCl 10, NaCl 10, HEPES 40, EGTA 0.1, TEACl 10, pH 7.2 (adjusted with CsOH). A Cs^+-based pipette solution was used to prevent any complications arising from the activation of voltage- or Ca^{2+}-activated K^+ currents. These pipettes had a resistance of 2–5 MΩ when measured in the bath solution containing (mM): NaCl 154, KCl 4.7, $MgCl_2$ 1.2, $CaCl_2$ 2.5, glucose 5.6, HEPES 10, pH 7.4. Drugs were applied rapidly through a seven-barrel manifold composed of fused glass capillaries inserted into a common outlet tube with a tip diameter of ~200 μm (see Dunn *et al*., 1996). Solutions were delivered by gravity flow from independent reservoirs placed above the preparation. One barrel was used to apply drug free solution to enable rapid termination of drug application. Applications of agonists (10 s) were separated by intervals of 2 min, which was found to be sufficient to obtain reproducible responses to ATP. Furthermore, there was no indication of any interactions between DMPP and ATP responses when they were separated by a 2-min interval. The bath was continuously perfused with external solution at a flow rate of 0.5 ml min^{-1}. Suramin, Cibacron blue 3GA (Reactive blue 2), or pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS) were applied for 2 min prior to and during the ATP application.

Unless indicated otherwise, membrane potential was voltage clamped at −70 mV. Signals were filtered at 2 kHz and either stored on a digital tape recorder (Biologic DTR 1205) or recorded using a Gould TA240 chart recorder. Where examined, the pH of ATP-containing solutions was adjusted by addition of NaOH or HCl.

Drugs

ATP, related nucleotides and other chemicals were obtained from Sigma Chemical Co. (Poole, U.K.), except for 2-methylthio ATP (2-MeSATP), which was from RBI-SEMAT, (U.K.). Suramin was from Bayer AG (Leverkusen, Germany), and PPADS from Tocris Cookson (U.K.). Solutions of agonists and antagonists were prepared daily from a stock solution (10 or 100 mM, stored frozen) using extracellular bathing solution as the solvent.

Data analysis

The pooled data for concentration-effect curves were fitted with the Hill equation as defined by Prism v1.03, (GraphPad). The values presented are the fitted value ± s.e.mean. All responses to agonists were normalized to that evoked by ATP (100 μM) in the same cell (pH 7.4). All other data are expressed as the means ± s.e.mean. In this study, because agonist concentrations exceeding 300 μM were not used, the concentration-response curves did not always reach a maximum and, where necessary, the maximum has been estimated by the curve fitting routine. Statistical significance of results was assessed using Student's *t*-test, with a probability level of $P<0.05$ taken to be statistically significant; *n* refers to the number of cells tested.

Results

Chromaffin cells were identified using a combination of morphological and functional criteria. Recordings were only made from phase bright round cells having non-granular cytoplasm. Chromaffin cell plasma membranes are endowed with cholinergic nicotinic receptors. All cells tested were subjected to a standard brief test pulse of 10 μM dimethylphenylpiperazinium iodide (DMPP, an agonist at nACh receptors), and only those which responded with a pronounced inward current were studied further.

Response to ATP

In agreement with the observation of Hollins & Ikeda (1997), no detectable inward current was evoked by ATP (100 μM) in chromaffin cells dissociated from adrenal medullae of adult rats, despite a robust response to DMPP (10 μM) (Figure 1A). Although the responsiveness of guinea-pig chromaffin cells changed with time in culture (see below), rat cells cultured for 1–7 days failed to respond to ATP (100–300 μM). The presence of nerve growth factor in the culture medium, or the use of different media (DMEM or Leibovitz's L-15) failed to induce any ATP sensitivity.

In contrast, ATP (100 μM) did elicit an inward current in guinea-pig chromaffin cells (Figure 1B). The current activated rapidly, and on reaching its maximal amplitude, decreased slowly whilst in the continued presence of agonist. When compared with a current of similar amplitude produced by DMPP (10 μM), the half-decay time of the ATP-activated current (17.8 ± 3.2 s, $n=4$) was significantly longer ($P<0.05$)

than that of DMPP-evoked current (6.7±1.4 s, $n=3$). Current-voltage relations were determined from membrane potential ramps (−90 to +30 mV, 200 ms duration), before and during ATP (100 μM) application. An example of a subtracted current-voltage relationship is illustrated in Figure 1C. The ATP-evoked response had a mean zero current potential of 2.5±2.7 mV ($n=5$), and demonstrated profound inward rectification.

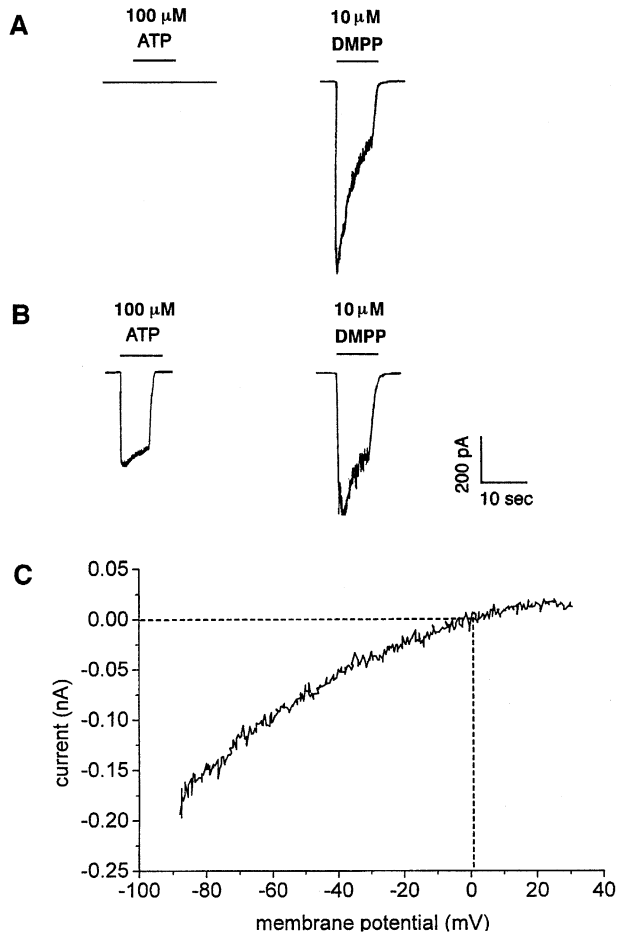

Figure 1 A comparison of inward currents evoked by extracellular application of ATP (100 μM) and DMPP (10 μM). Chromaffin cells dissociated from adrenal medulla of rat (A) and guinea-pig (B) were voltage clamped at a holding potential at −70 mV. Agonists were applied for 10 s (indicated by bar above tracing) and with a 2-min interval between successive responses. (C) Example of the current-voltage relationship for the ATP-activated current in a guinea-pig chromaffin cell. The mean zero current potential was 2.5±2.7 mV ($n=5$). Current-voltage relationships were obtained by subtracting the current evoked by a membrane potential ramp (−90 to +30, 200 ms) in the absence of agonist from that recorded in the presence of 100 μM ATP.

Effect of time in culture on the response to ATP

During intitial experiments on the ATP-activated currents, we found considerable variation in response from one experiment to another. Much of this variability was found to result from the time that the cells were maintained in culture as shown in Table 1. Data on the response to DMPP (10 μM) recorded from the same cells are shown for comparison. The response to ATP (100 μM) was observed only in a minor fraction (22%) of the cells cultured for 1–3 days and had a small amplitude 81.4±18.7 pA ($n=7$). However, 51 out of 57 cells cultured for 6–7 days gave responses to 100 μM ATP with a mean amplitude of 255.2±22.1 pA. With increasing time in culture, the amplitude of the peak current, which varied considerably from cell to cell, increased further. Detailed analysis of the frequency distributions of the ATP-activated current is shown in Figure 2. In contrast to the increasing percentage of responding cells and amplitude of response to ATP, the currents evoked by DMPP (10 μM) decreased during the time in culture (Table 1). The mean DMPP-evoked current produced by cells in culture for 6–7 days (128.6±11.6 pA; $n=57$) was 60% of that produced by cells cultured for 1–3 days (213.1±34.3 pA; $n=32$) ($P<0.01$).

The membrane capacitance remained relatively constant throughout the time the cells were kept in culture (Table 1). Similarly, the mean value of membrane capacitance (7.8±0.2 pF, $n=154$) obtained from the cells which produced responses to ATP (100 μM) was not significantly different from that (7.6±0.3 pF, $n=92$, $P>0.05$) for cells which produced no detectable response to same concentration ATP. The surface area of the chromaffin cells estimated from the cell capacitance (assuming specific membrane capacitance of 1 $\mu F/cm^2$) was $7.7\pm0.3\times10^{-6}$ cm^2 ($n=246$).

Agonist profile

Because the ATP response increased during the time in culture, all subsequent results were obtained from guinea-pig chromaffin cells cultured for 4–7 days. ATP induced a fast inward current in 69% (147/214) of these cells, with a mean amplitude of 196.1±22.9 pA ($n=147$) at a holding potential of −70 mV. Neither adenosine nor AMP produced any detectable response at concentration up to 1 mM ($n=4$). ADP did not evoke any current at 100 μM ($n=4$), but at 1 mM induced a response 34.0±2.6% ($n=7$) of that activated by 100 μM ATP. Membrane currents recorded from a guinea-pig chromaffin cell in response to increasing concentrations of ATP are shown in Figure 3A. Concentration-response relationships obtained for ATP and three analogues are shown in Figure 3B. Maximal currents were observed at a concentration of 300 μM for ATP and 2-MeSATP with EC_{50} values of 43±4 μM ($n=12$) and 34±4 μM ($n=6$) and Hill coefficients of 1.5±0.1 and 1.5±0.4, respectively. α,β-MeATP

Table 1 Effects of time in culture on responsiveness of cells to ATP

Days in culture	*Capacitance* (pF)	*DMPP* (pA)	*ATP* (pA)	*% Responding to ATP*
1–3	7.5±0.4 (32)	213.1±34.3 (32)	81.4±18.7 (7)	22
4	7.5±0.3 (82)	204.5±22.5 (82)	175.3±18.6 (43)**	52
5	8.0±0.3 (75)	146.6±11.2 (75)*	181.9±21.3 (53)**	71
6–7	7.9±0.5 (57)	128.6±11.6 (57)**	255.2±22.1 (51)**	89

All data are given as means±s.e.mean. Number of cells is given in parentheses. DMPP (10 μM) and ATP (100 μM) were applied in sequence separated by a 2 min interval to the same cells. For estimation of percentage of cells responding to ATP, a minimum detection threshold of 20 pA was used. Statistical significance (*$P<0.05$; **$P<0.01$) compared with the values at 1–3 days was determined by Student's *t*-test.

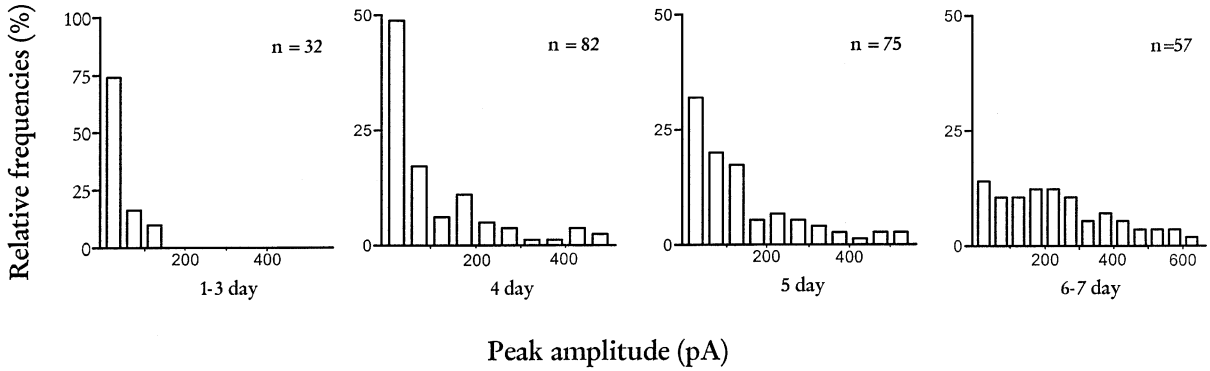

Figure 2 Relative frequency distribution for the amplitude of ATP (100 μM)-activated current in guinea-pig chromaffin cells cultured for varying periods. Relative frequencies were calculated as per cent of total responsive cells (n) within each distribution.

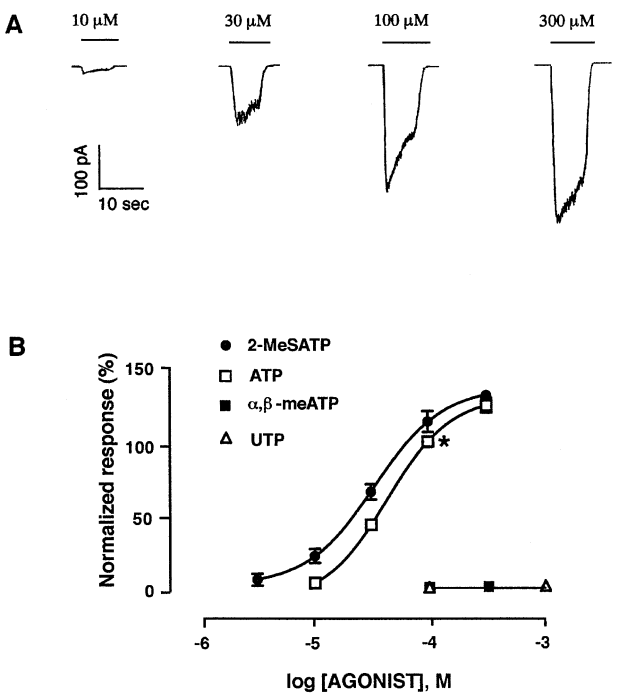

Figure 3 (A) Currents evoked by sequential application of ATP (10, 30, 100 and 300 μM) at a holding potential of -70 mV in a guinea-pig chromaffin cell. Agonists were applied at 2 min intervals. (B) Concentration-response curves for 2-MeSATP (●), ATP (□), α,β-meATP (■) and UTP (△). Peak amplitude of agonist-activated current was normalized to the response induced by 100 μM ATP (*) in the same cell. Each point represents the mean$\pm$s.e.mean from 6–12 cells.

(100–300 μM, $n=7$) and UTP (1 mM, $n=4$) failed to evoke any detectable response.

Effects of P2X receptor antagonists

Figure 4A shows the effects of suramin (100 μM), Cibacron blue (50 μM) , and PPADS (10 μM) on the currents activated by a submaximal concentration ATP (100 μM). On average, suramin (100 μM) reduced the response to 100 μM ATP by 51.7$\pm$6.0% ($n=6$). The inhibition by suramin reversed rapidly on washout, with the ATP-activated currents being 83.4$\pm$13.3% ($n=6$) of the control amplitude 4 min after returning to suramin free solution. Cibacron blue (10 μM) produced a small but not statistically significant increase of 9.5%$\pm$7.2% ($n=5$) in ATP-activated currents, but at 50 μM there was significant inhibition of 47.2$\pm$5.8% ($P<0.01$, $n=7$).

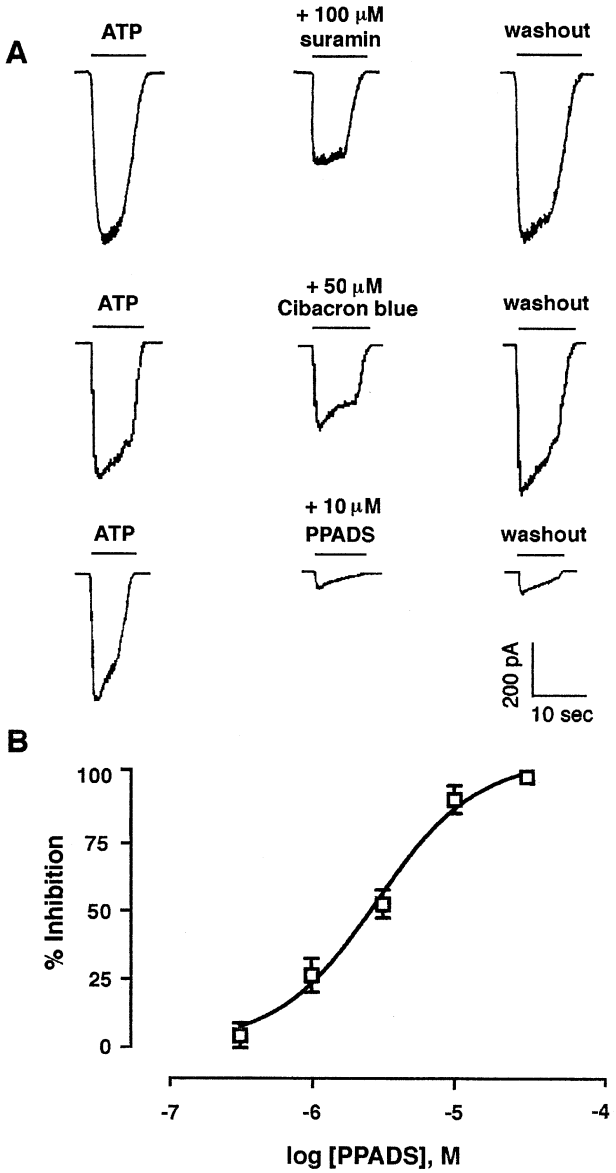

Figure 4 Blockade of ATP responses by P2 receptor antagonists. (A) Each row shows three consecutive currents evoked from the same chromaffin cell by 100 μM ATP at 2 min intervals before, in the presence of and 2 min after washing out the antagonist. Antagonists were present for 2 min before and during the second application of ATP. The cells were voltage-clamped at -70 mV. (B) Concentration-effect relationship for inhibition of ATP-activated currents by PPADS. Fitting the Hill equation to the data gave an IC_{50} value for PPADS of 3.2$\pm$0.5 μM. Each point represents the mean$\pm$s.e.mean from 4–6 cells.

PPADS (10 μM) attenuated the response to ATP (100 μM) by 90.0$\pm$4.1% (n=8). The recovery from this inhibition was slow and incomplete. In guinea-pig chromaffin cells, we found that PPADS (0.3–30 μM) reduced the amplitude of the ATP-activated current in a concentration-dependent manner (Figure 4B). The concentration of PPADS required to produce half-maximal inhibition of the inward current was 3.2$\pm$0.5 μM (n=8).

Effect of extracellular pH

No detectable current was observed when extracellular solutions at pH 6.8 or 8.0 were applied to guinea-pig chromaffin cells (n=5). The records in Figure 5A show currents activated when 100 μM ATP was applied to the same cell in solutions at pH 6.8, 7.4, and 8.0. Lowering pH to 6.8 produced a significant increase of the amplitude of the current activated by 30 μM to 207.4$\pm$16.5% ($P<0.01$, n=8) while that to 100 μM was not significantly changed being 113.1$\pm$15.0% ($P>0.05$, n=8) of the response evoked at pH 7.4. Elevating pH to 8.0 decreased the amplitude of the current activated by 30 and 100 μM ATP to 37.6$\pm$3.4% (n=8) and 66.1$\pm$5.3% (n=8) of that evoked at pH 7.4, respectively. Figure 5B shows concentration-response curves for the ATP-activated current at pH 6.8, and 8.0, compared with that obtained at pH 7.4. The EC_{50} values determined for these ATP concentration-response curves were 19$\pm$3 μM (n=8) at pH 6.8, and 96$\pm$15 μM (n=8) at pH 8.0. In contrast, neither the slopes nor maximal responses were significantly affected by the alteration of pH ($P>0.05$). The Hill coefficients of the ATP concentration-response curves were 1.4$\pm$0.2, 1.5$\pm$0.1 and 1.5$\pm$0.3 at pH 6.8, 7.4 and 8.0, respectively. The relative maximal responses (normalized to responses to 100 μM ATP at pH 7.4) were 134$\pm$13, 126$\pm$7 and 113$\pm$18% at pH 6.8, 7.4 and 8.0, respectively.

Inhibitory effect of Zn^{2+}

Micromolar concentrations of Zn^{2+} potentiate responses to ATP in rat autonomic neurons (Cloues, 1995; Zhong *et al.*, 1998). However we observed no potentiation, but a concentration dependent inhibition (Figure 6). At a concentration of 100 μM, co-application of Zn^{2+} with 100 μM ATP inhibited the response by 63.2$\pm$9.1% (n=6). This inhibition by Zn^{2+} was reversed following a 2 min washout. The concentration dependence of this effect is shown in Figure 6C. Fitting the Hill equation to the data yielded an IC_{50} of 48$\pm$7 μM (n=6).

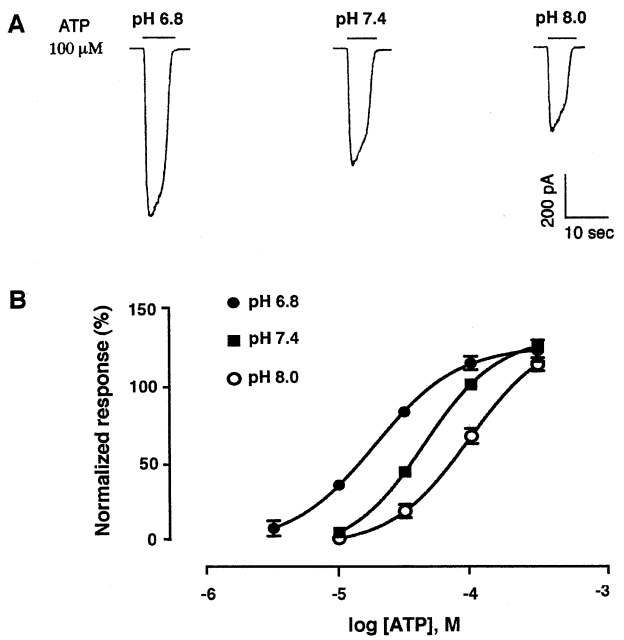

Figure 5 Effect of pH on ATP-activated currents. (A) Traces showing currents activated by successive applications of 100 μM ATP at pH 6.8, 7.4 and, 8.0. A 2-min interval separated each agonist application. (B) Concentration-response curves for ATP at pH 6.8 (●) and pH 8.0 (○). All responses were normalized with respect to that produced by 100 μM ATP at pH 7.4 in the same cell. The concentration-response curve obtained at pH 7.4 is reproduced from Figure 3 for comparison. Each curve was generated from a separate sample of cells. Changing the pH altered the EC_{50} for ATP from 43 μM at pH 7.4 to 19$\pm$3 μM (n=8), and 96$\pm$15 μM (n=8) at pH 6.8 and 8.0, respectively.

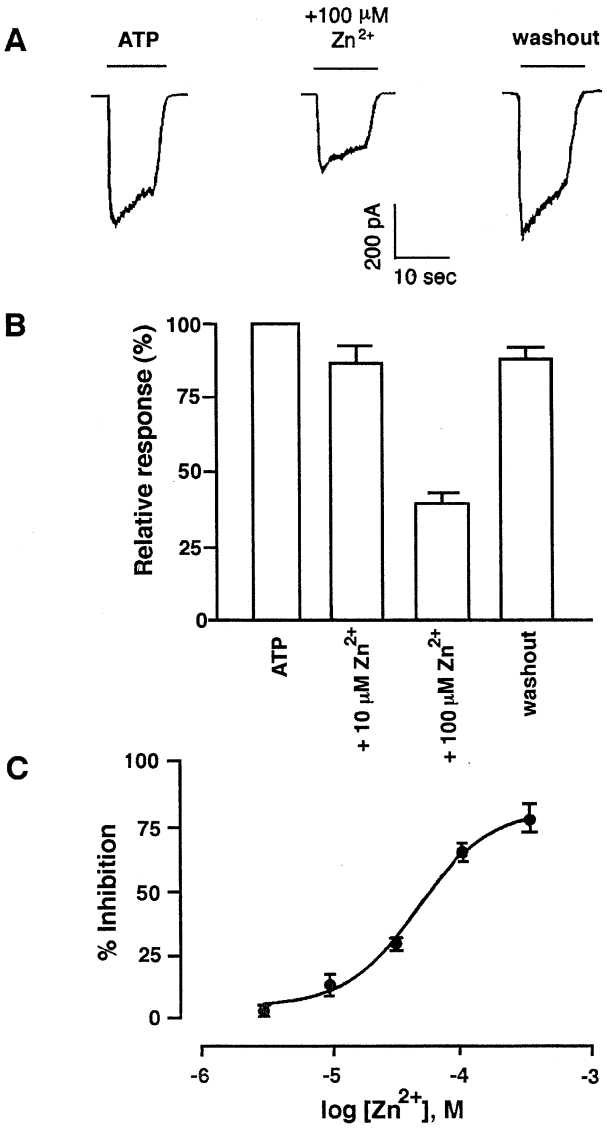

Figure 6 Effect of Zn^{2+} on ATP evoked responses. (A) Traces showing the inhibition by 100 μM Zn^{2+} of currents activated by ATP (100 μM). Zn^{2+} was applied simultaneously with ATP without any pre-equilibration. (B) Bar graph illustrating the inhibition of ATP-activated current by 10 and 100 μM Zn^{2+} and recovery after washout for 2 min (n=6). (C) Concentration-response relationship for inhibition of ATP (100 μM)-activated currents by Zn^{2+}. The curve is drawn according to the Hill equation with an IC_{50} value for Zn^{2+} of 48$\pm$7 μM and a maximum inhibition of 79$\pm$9%. Each point represents the mean$\pm$s.e.mean for six cells.

Discussion

Species dependence

P2X receptors are present on rat autonomic neurons (see Evans & Surprenant, 1996) and rat phaeochromocytoma (PC12) cells (Inoue *et al*., 1989). One might therefore expect to find them on adrenomedullary cells which are of the same embryological origin. We were consistently unable to detect any ATP-evoked currents in cultured chromaffin cells dissociated from adrenal medulla of adult rats. This observation is in agreement with the findings of Hollins & Ikeda (1997). In contrast, we were able to observe ATP-evoked responses in chromaffin cells dissociated from guinea-pig adrenal glands. On average, 69% guinea-pig chromaffin cells exhibited an inward current on application of ATP when they had been maintained in culture for 4–7 days. In a study on bovine chromaffin cells only a small fraction were responsive to ATP, showing an inward cationic current or calcium influx, when cultured for 7–14 days before use (Diverse-Pierluissi *et al*., 1991; Nuñez *et al*., 1995). These differences between chromaffin cells dissociated from adrenal medullae of rats, guinea-pigs or cattle are unlikely to result from the culture conditions because the chromaffin cells from rats or guinea-pigs used in this study were dissociated and cultured under the identical conditions. Thus, the expression of P2X receptors in chromaffin cells appears to be dependent on species.

Both $P2X_1$ and $P2X_2$ immunoreactivity has been described in chromaffin cells of the rat adrenal medulla and in PC12 cells (Vulchanova *et al*., 1996). Furthermore, RNA for $P2X_2$ and $P2X_4$ receptors has been found in the rat adrenal gland (Bo *et al*., 1995; Collo *et al*., 1996). However, in another study, neither $P2X_1$, $P2X_2$, nor $P2X_4$ receptors were detected on rat adrenal medullary cells using specific polyclonal antibodies (M. Afework, personal communication). Despite this conflicting biochemical evidence, while PC12 cells express functional P2X receptors, rat adrenomedullary cells do not, according to our present study.

Is the response to ATP an in vitro *phenomenon?*

The growing percentage of responding cells and increasing amplitude of the ATP-activated current during time in culture raises an important question: is the response to ATP physiologically significant or is it an *in vitro* phenomenon caused by the conditions of cell culture? A time-related increase of catecholamine secretion induced by extracellular ATP was observed with cultured bovine chromaffin cells (Lin *et al*., 1995). However, these authors were able to demonstrate ATP evoked catecholamine release from intact adrenal glands. Thus the increasing response to ATP with time in culture might indicate the replacement of receptors 'lost' during enzyme treatment rather than hyperexpression *per se*.

P2X receptor mediated agonist-activated current

The inward current on guinea-pig chromaffin cells appeared to be due to activation of P2X receptors for the following reasons: rapid activation and deactivation; reversal potential (close to 0 mV) expected for a non-selective cationic current; ADP is far less potent than ATP; neither UTP nor adenosine induced any obvious current.

What P2X subtype?

To date, seven P2X subunits have been cloned (see Ralevic & Burnstock, 1998). In addition, some exist as multiple spliced variants, and some can combine to form heteromultimeric receptors with unique properties (Lewis *et al*., 1995; Brändle *et al*., 1997; Parker *et al*., 1998). The ATP-gated cation channel in guinea-pig chromaffin cells shares a number of pharmacological properties with autonomic neurons, myenteric neurons and PC12 cells from which the rat $P2X_2$ receptor was originally cloned (Brake *et al*., 1994). For examples, α,β-meATP-insensitive, non-desensitising inward currents are the characteristics of responses in PC12 cells (Nakazawa *et al*., 1990), superior cervical neurons (Khakh *et al*., 1995), rat cardiac parasympathetic ganglia (Fieber & Adams, 1991), myenteric neurons of small intestine (Zhou & Galligan, 1996) and rat pelvic ganglion neurons (Zhong *et al*., 1998).

A distinct feature of the P2X receptor in guinea-pig chromaffin cells is the effect of Cibacron blue on ATP-activated currents. A low concentration (10 μM) had little effect, whereas at a high concentration (50 μM) it inhibited the ATP response. Although the lack of inhibitory effect of Cibacron blue at the low concentration might be due to its blockade of ecto-ATPases, this seems unlikely since the agonist was applied in a rapidly flowing solution. Furthermore, Cibacron blue has been shown to block ATP-activated currents in guinea-pig coeliac neurons (Silinsky & Gerzanich, 1993), rat parasympathetic cardiac neurons (Fieber & Adams, 1991), rat pelvic ganglion neurons (Zhong *et al*., 1998), and inhibits ATP-inward current and dopamine secretion in PC12 cells (Inoue *et al*., 1991). Interestingly, this antagonist appears to be less potent at guinea-pig receptors than it is on rat neurons and potentiation of responses to ATP was observed in guinea-pig myenteric neurons (Barajas-López *et al*., 1996). Cibacron blue potently inhibited ATP-responses at recombinant $rP2X_2$ receptors without any sign of potentiation at low concentrations (King *et al*., 1997).

Acid pH enhances the sensitivity of native ATP-gated ion channels in rat nodose ganglion neurons (Li *et al*., 1996), bullfrog dorsal root ganglion neurons (Li *et al*., 1997a), rat and guinea-pig autonomic neurons (Zhong *et al*., 1998; 1999). Of the recombinant $P2X_{1-4}$ receptors so far tested, only $P2X_2$ receptors are made more sensitive to ATP by lowering pH (King *et al*., 1996; Stoop *et al*., 1997), although the effects of pH remain to be determined on $P2X_5$ and $P2X_6$ receptors. The modulation of responses by pH observed in this study would be consistent with the receptor being of the $P2X_2$ subtype.

A striking pharmacological property of the ATP-activated current in guinea-pig chromaffin cell is its inhibition by Zn^{2+}. This is in marked contrast to the action of Zn^{2+} on rat autonomic (Cloues, 1995; Zhong *et al*., 1998) and sensory (Li *et al*., 1993) neurons, and recombinant $P2X_2$ receptors (Wildman *et al*., 1998), where it potentiates the effect of ATP. However, inhibition of P2X receptor-mediated response by Zn^{2+} has been observed in dorsal root ganglion neurons from bullfrog (Li *et al*., 1997b) and guinea-pig sympathetic neurons (Zhong *et al*., 1999). Of the recombinant P2X subunits tested, $P2X_1$ (S.S. Wildman, personal communication) and $P2X_7$ (Virginio *et al*., 1997) are inhibited by Zn^{2+}.

While some properties of the P2X receptor present on guinea-pig chromaffin cells: slow desensitization, insensitivity to α,β-meATP, potentiation by low pH and inhibition by suramin are consistent with those of the cloned $P2X_2$ receptors, other properties including inhibition by Zn^{2+} and low sensitivity to Cibacron blue are not. To date, three spliced variants of the guinea-pig $P2X_2$ receptor have been cloned

(Parker *et al*., 1998), although their functional properties and pharmacology have yet to be described. Thus, the pharmacological profile we have observed might be explained by differences between rat and guinea-pig receptors, the involvement of a novel spliced variant, or the presence of a novel heteromultimeric receptor.

In conclusion, our study has revealed three new observations regarding P2X receptors on chromaffin cells. Firstly, we found that the expression of functional P2X receptors is species-dependent. Secondly, cultured chromaffin cells gradually increased their response to ATP with time in culture. Most significantly, the pharmacological profile of the receptor present on guinea-pig chromaffin cells does not match that of any cloned receptor so far described.

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