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Modulatory activity of extracellular H^+ and Zn^{2+} on ATP-responses at $rP2X_1$ and $rP2X_3$ receptors

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1 The modulatory activity of extracellular H^+ and Zn^{2+} was examined on ATP-responses at rat $P2X_1$ ($rP2X_1$) and rat $P2X_3$ ($rP2X_3$) receptors expressed in *Xenopus* oocytes and studied under voltage-clamp conditions.

2 Superfused ATP (0.03–30 μM, at pH 7.5) evoked inward currents at $rP2X_1$ receptors (EC_{50} value, 300 ± 7 nM). ATP potency was reduced 2 fold at pH 6.5, and 6 fold at pH 5.5, without altering the maximum ATP effect. Alkaline conditions (pH 8.0) did not alter ATP activity.

3 Superfused ATP (0.01–300 μM, at pH 7.5) evoked inward currents at $rP2X_3$ receptors (EC_{50} value, 1.8 ± 0.3 μM). ATP activity was affected only at pH 5.5, reducing agonist potency 15 fold without altering the maximum ATP effect.

4 Extracellular Zn^{2+} inhibited ATP-responses at $rP2X_1$ receptors in a time-dependent manner, a 20 min pre-incubation being optimal (IC_{50} value, 1.0 ± 0.2 μM). However, the Zn^{2+} effect was pH-independent, suggesting Zn^{2+}- and H^+-inhibition of ATP-responses occur through independent processes.

5 Extracellular Zn^{2+} weakly potentiated ATP-responses at $rP2X_3$ receptors (EC_{50} value, 11 ± 1 μM). The Zn^{2+} effect was dependent on pre-incubation time and, with 20 min pre-incubation periods, Zn^{2+} potentiated then inhibited ATP-responses in a concentration-dependent, but pH-independent, manner.

6 In summary, ATP activity at $rP2X_1$ receptors was decreased by both extracellular H^+ and Zn^{2+} and their effects were additive. ATP activity at $rP2X_3$ receptors was less sensitive to H^+-inhibition and, in contrast, was potentiated by Zn^{2+} in a pH-independent manner. These differential effects may help distinguish $P2X_1$ and $P2X_3$ receptors in whole tissues.

Keywords: P2X receptor; ionotropic receptor; ion channel; ATP; extracellular pH; H^+; Zn^{2+}; oocyte

Abbreviations: ATP, adenosine 5′-triphosphate; α,β-meATP, α,β-methylene ATP; cRNA, capped ribonucleic acid; DRG, dorsal root ganglia; EC_{50}, concentration causing 50% of the maximum agonist response; IC_{50}, concentration inhibiting an agonist response by 50%; I_{ATP}, ATP-activated membrane current; 2-MeSATP, 2-methylthio-ATP; mRNA, messenger ribonucleic acid; n_H, Hill coefficient; pH_e, extracellular pH; PPADS, pyridoxal-α^5-phosphate-6-azophenyl-2′,4′-disulphonic acid; TNP-ATP, trinitrophenyl ATP; V_h, holding potential; Zn^{2+}, zinc ions

Introduction

Adenosine 5′-triphosphate (ATP) acts as a fast signalling molecule at P2X receptors in central, peripheral and enteric neurons and associated neuro-effector tissues (Burnstock, 1997). Seven P2X receptor subunits ($P2X_{1-7}$) have been identified thus far, of which $P2X_{1-6}$ subunit mRNA transcripts and receptor proteins are present in neurons (Humphrey *et al*., 1998; King, 1998). Homomeric $P2X_1$ and $P2X_3$ receptors examined in the present study are now classified (Group 1) P2X receptors, which are defined as α,β-meATP-sensitive, rapidly-inactivating, suramin-sensitive (Humphrey *et al*., 1998). However, there is limited scope to distinguish the operational profiles of $P2X_1$ and $P2X_3$ receptors. Previously, we have commented on the effects of H^+ ions (i.e., extracellular pH, pH_e) and Zn^{2+} ions on ATP-responses at homomeric $P2X_2$ (Group 2) and $P2X_4$ (Group 3) receptors, at which significant differences have been observed in their modulatory actions (King *et al*., 1996; 1997; Wildman *et al*., 1998; 1999b). We have extended our survey of H^+ and Zn^{2+} modulation to include Group 1 P2X receptors, in the hope of discriminating between $P2X_1$ and $P2X_3$ receptors as well as Group 1 from Groups 2 and 3 P2X receptors.

In an earlier study, the amplitude of ATP-responses at $P2X_1$ and $P2X_3$ receptors were shown to be reduced by lowering pH_e, although the H^+ effect on agonist potency and efficacy was not investigated (Stoop *et al*., 1997). An inhibitory effect by H^+ ions was also observed at $P2X_4$ receptors (Wildman *et al*., 1999b), which contrasted with a potentiating effect at $P2X_2$ receptors (King *et al*., 1996; 1997). Thus, the first objective of this study focused on changes in agonist potency and efficacy at $rP2X_1$ and $rP2X_3$ receptors under acidic and alkaline conditions, to see if such changes matched the H^+ effect at $P2X_4$ receptors. The second part of the present study focused on Zn^{2+} modulation of Group 1 P2X receptors, and the dependence of such modulatory effects on time and extracellular pH, in the knowledge that this transition metal also affected ATP-activity at $P2X_2$ and $P2X_4$ receptors in different ways in a time- and pH-dependent manner (see Wildman *et al*., 1998; 1999b). Finally, we have compared and contrasted the modulatory activity of H^+ and Zn^{2+} ions at $rP2X_1$, $rP2X_2$, $rP2X_3$ and $rP2X_4$ receptors to establish operational profiles distinct for each P2X subunit. Part of this study has been communicated to *The Physiological Society* (King *et al*., 1999).

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Methods

Oocyte preparation

Xenopus laevis frogs were anaesthetized in Tricaine (0.4% w v^{-1}), killed by decapitation, and ovarian lobes removed surgically. Oocytes (stages V and VI) were defolliculated by a 2-step process involving collagenase treatment (Type IA, 2 mg ml^{-1} in a Ca^{2+}-free Ringer's solution, for 2–3 h) and, thereafter, stripping away the follicular layer with fine forceps. Defolliculated oocytes were stored in Barth's solution (pH 7.5, at 4°C) 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; gentamycin sulphate, 50 $\mu g\,l^{-1}$. Defollicated oocytes were injected cytosolically with either $rP2X_1$ or $rP2X_3$ cRNA (40 nl, 1 $\mu g\,ml^{-1}$), incubated for 48 h at 18°C in Barth's solution then kept at 4°C for up to 12 days until used in electrophysiological experiments.

Electrophysiology

ATP-activated membrane currents (I_{ATP}) ($V_h = -60$ to -90 mV) were recorded from cRNA-injected oocytes using a twin-electrode voltage-clamp amplifier (Axoclamp 2B). The voltage-recording and current-recording microelectrodes (1–5 MΩ tip resistance) were filled with 3.0 M KCl. Oocytes were superfused with Ringer's solution (5 ml min^{-1}, at 18°C) containing (mM): NaCl 110, KCl 2.5, HEPES 5, $BaCl_2$ 1.8, adjusted to pH 7.5. Where stated, the pH of the bathing solution was adjusted using either 1.0 N HCl or 1.0 N NaOH to achieve the desired level. Electrophysiological data were stored on a computer using a MP100 WSW interface (Biopac Systems Inc.) and analysed using the software package Acqknowledge III (Biopac).

Solutions

All solutions were nominally Ca^{2+}-free to avoid the activation of an endogenous Ca^{2+}-dependent Cl^- current ($I_{Cl(Ca)}$) present in oocytes (Barish, 1983). ATP was prepared in a Ca^{2+}-free Ringer's solution (concentrations as stated in the text) and superfused by a gravity-feed continuous flow system which allowed rapid addition and washout. ATP was added for 120 s or until the current reached a peak, then washed out for a period of 20 min. Data were normalized to the maximum current (I_{max}) evoked by ATP at pH 7.5, including ATP-responses recorded at other pH levels. The concentration required to evoke 50% of the maximum agonist response (EC_{50}) was taken from Hill plots, constructed using the equation $\log (I/I_{max}\text{-}I)$, where I is the current evoked by each concentration of ATP.

The effects of extracellular zinc were investigated on agonist activity in two ways. First, C/R curves for the modulatory activity of Zn^{2+} ions were constructed using a submaximal concentration of ATP and data were normalized to the amplitude of control ATP-responses at pH 8.0, 7.5, 6.5 and 5.5. These experiments were carried out for different pre-incubation periods for Zn^{2+} (i.e., 0, 20 and 40 min) prior to the addition of ATP. Second, Zn^{2+} ions were added to ATP solutions and C/R curves for ATP were constructed and normalized to the maximal ATP effect under Zn^{2+}-free conditions at pH 7.5. In some experiments using $rP2X_3$, Zn^{2+} was added to the superfusate 20 min prior to the addition of ATP and C/R curves to ATP constructed.

Statistics

Data are presented as mean ± s.e.mean of four sets of data from different oocyte batches. Significant differences were determined by either unpaired Student's *t*-test or one-way analysis of variance (ANOVA) followed by Dunnett's test,

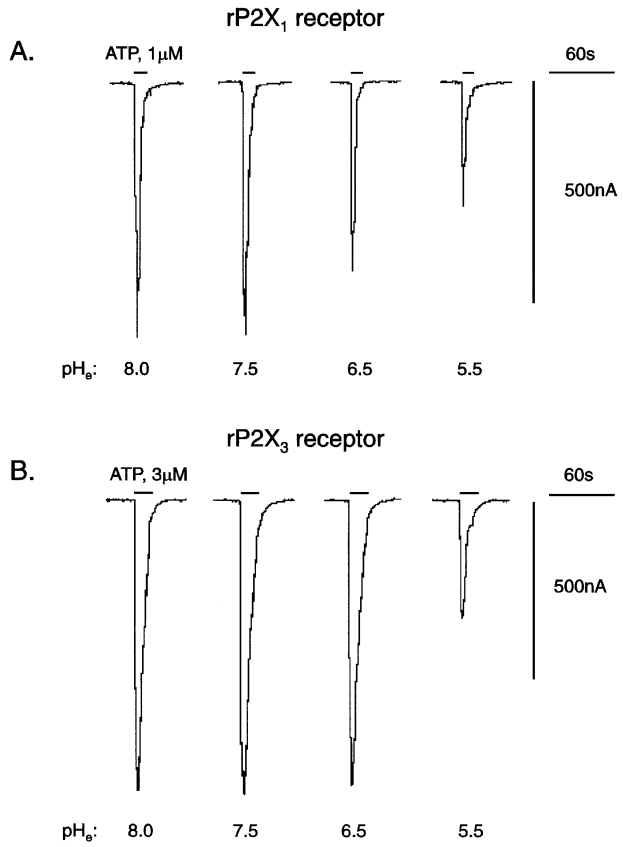

Figure 1 Extracellular pH modulates ATP activity at P2X (Group 1) receptors. (A) Whole-cell inward currents evoked by ATP (1 μM), at four levels of extracellular pH (8.0, 7.5, 6.5, 5.5), at $rP2X_1$ receptors expressed in *Xenopus* oocytes. All records from the same oocyte ($V_h = -60$ mV). (B) Whole-cell inward currents evoked by ATP (3 μM), at the same pH_e levels, at $rP2X_3$ receptors. All records from the same oocyte ($V_h = -90$ mV).

Table 1 ATP potency at Group 1 P2X receptors at different pH_e levels

P2X subunit	*pH_e levels*	*EC_{50} values*	*95% CI*	*Hill coefficient*
$rP2X_1$	8.0	305 ± 18 nM	193–354 nM	1.23 ± 0.21
	7.5	300 ± 7 nM	285–368 nM	1.47 ± 0.13
	6.5	601 ± 46 nM	413–811 nM	1.22 ± 0.23
	5.5	1698 ± 323 nM	870–2138 nM	1.04 ± 0.24
$rP2X_3$	8.0	1.61 ± 0.60 μM	0.79–2.19 μM	0.83 ± 0.16
	7.5	1.83 ± 0.28 μM	1.17–3.08 μM	0.75 ± 0.13
	6.5	1.30 ± 0.21 μM	0.54–1.28 μM	0.81 ± 0.12
	5.5	28.3 ± 18.3 μM	17.3–97.2 μM	0.56 ± 0.15

Determinations of EC_{50} values for ATP-activated inward currents were obtained from Hill plots of single sets of data, then expressed as mean ± s.e.mean of a population ($n = 4$). Confidence Intervals (CI, at the 95% level) for EC_{50} values were derived from pooled data (see Figure 2A,B) plotted using the Hill equation (Prism v2.0, GraphPad). Hill coefficients (n_H) were obtained from Hill plots of single sets of data then expressed as mean ± s.e.mean of a population ($n = 4$).

using commercially-available software (Instat v2.05a, GraphPad).

Drugs

All common salts and reagents were AnalaR grade (Aldrich Chemicals, U.K.). Adenosine 5′-triphosphate disodium salt (ATP) and zinc chloride were purchased from Sigma Chemical Co. (Poole, Dorset, U.K.). Drugs were dissolved in Ringer's solution with the pH adjusted to match individual experiments.

Results

Effects of extracellular pH on I_{ATP} at rP2X$_1$ and rP2X$_3$ receptor

Superfused ATP (0.03 – 30 μM) evoked rapidly-activating, rapidly-inactivating inward currents (I_{ATP}) in *Xenopus* oocytes expressing homomeric rP2X$_1$ receptors. The amplitude of the evoked I_{ATP} was dependent on extracellular pH (pH_e) and decreased as the superfusate was made more acidic (Figure 1A). This decrease was due to a fall in ATP potency, as judged by a change in the EC_{50} value (see Table 1) without a concomitant change in the maximum ATP effect (see Figure 2A). Thus, ATP potency was reduced 2 fold at pH 6.5 and 6 fold at pH 5.5, whereas alkaline conditions (pH 8.0) had no effect on agonist potency. The modulatory effects of H^+ at rP2X$_1$ receptors were reversed upon readjusting the superfusate to pH 7.5.

Superfused ATP (0.1 – 300 μM) also evoked rapidly-activating, rapidly-inactivating inward currents (I_{ATP}) from *Xenopus* oocytes expressing homomeric rP2X$_3$ receptors. The I_{ATP} of activated rP2X$_3$ receptors was insensitive to small changes to pH_e ($\pm$1 pH unit), although the amplitude of I_{ATP} decreased significantly ($P<0.05$) at pH 5.5 (Figure 1B). This decrease was due to a fall in ATP potency ($\sim$15 fold decrease) (see Table 1), without a concomitant change in the maximum ATP effect (Figure 2B). The modulatory effects of H^+ at rP2X$_3$ receptors were also reversed upon readjusting the superfusate to pH 7.5.

Modulatory effects Zn^{2+} ions of I_{ATP}

Extracellular Zn^{2+} (0.01 – 300 μM) inhibited I_{ATP} at rP2X$_1$ receptors, yet potentiated I_{ATP} at rP2X$_3$ receptors, in a reversible manner (Figure 3A, B). Zn^{2+}-inhibition of rP2X$_1$ receptors was not only dependent on concentration but also the pre-incubation time for Zn^{2+} (Figure 4A). Zn^{2+} was approximately 10 fold more potent, as judged by changes in IC_{50} values (Table 2), when superfused for 20 min or longer prior to ATP application. The ability of Zn^{2+} to inhibit I_{ATP} at rP2X$_1$ was not altered appreciably over the range of pH 8.0 – 5.5 (Figure 4B). Since agonist potency at rP2X$_1$ is already reduced as the extracellular solution was made more acidic,

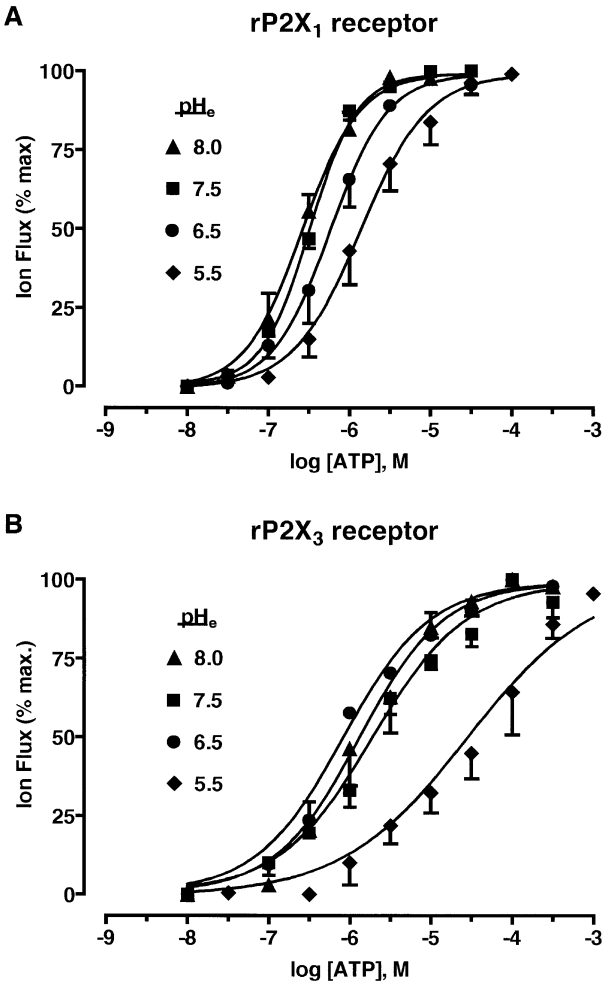

Figure 2 pH-dependent changes in ATP potency at P2X (Group 1) receptors. (A) Concentration/response (C/R) curves for whole-cell inward currents (I_{ATP}) evoked by ATP (10 nM – 100 μM), at four levels of pH_e (8.0, 7.5, 6.5, 5.5), at rP2X$_1$ receptors. The amplitude of I_{ATP} was normalized to the maximum ATP effect at pH 7.5. (B) C/R curves for I_{ATP} at rP2X$_3$ receptors under the same conditions. Data points are means $\pm$ s.e.mean, $n=4$.

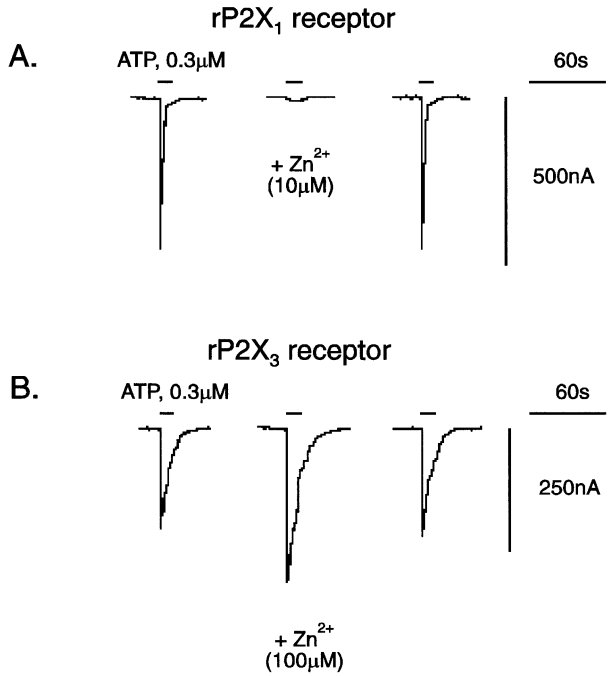

Figure 3 Zn^{2+} ions modulate ATP activity at P2X (Group 1) receptors. (A) Whole-cell inward currents evoked by ATP (0.3 μM, approx. EC_{50}) before, during and after washout of Zn^{2+} (10 μM; 20 min pre-incubation; at pH 7.5) at rP2X$_1$ receptors. All records from the same oocyte ($V_h=-60$ mV). (B) Whole-cell inward currents to ATP (0.3 μM, approx. EC_{20}) before, during and after washout of Zn^{2+} (100 μM; 0 min pre-incubation; at pH 7.5). All records from the same oocyte ($V_h=-60$ mV). Note that the Zn^{2+} effect at rP2X$_1$ and rP2X$_3$ receptors reversed on washout (20 min).

Zn^{2+} and H^+-inhibition appear to be additive and to occur by independent processes.

Zn^{2+}-potentiation of I_{ATP} at $rP2X_3$ receptors was dependent on both concentration and the pre-incubation time for Zn^{2+}.

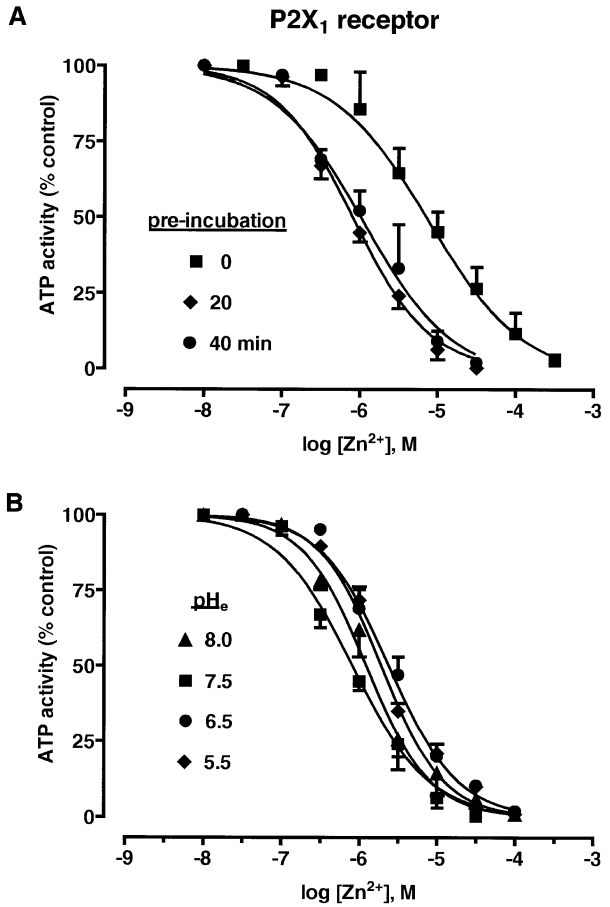

Figure 4 Zn^{2+}-inhibition of ATP-responses at $rP2X_1$ receptors. (A) Inhibition curves for Zn^{2+} modulation of whole-cell inward currents (I_{ATP}) evoked by ATP (0.3 μM, approx. EC_{50}), where Zn^{2+} was applied over three pre-incubation periods (0, 20 and 40 min), at pH 7.5. (B) Inhibition curves for Zn^{2+} against I_{ATP}, at four different levels of pH_e (8.0, 7.5, 6.5, 5.5). Each concentration of Zn^{2+} was applied 20 min prior to and during ATP superfusion. Data points are mean$\pm$s.e.mean, $n=4$.

Table 2 Inhibitory activity of Zn^{2+} ions at $rP2X_1$ receptors

Conditions	*IC_{50} values*	*95% CI*	*Hill coefficient*
Preincubation time			
0 min	9.34$\pm$0.64 μM	3.49–17.3 μM	−0.71$\pm$0.2
20 min	0.82$\pm$0.14 μM	0.58–1.16 μM	−0.88$\pm$0.13
40 min	1.1$\pm$0.14 μM	0.49–2.50 μM	−0.75$\pm$0.22
Extracellular pH			
8.0	1.24$\pm$0.23 μM	0.64–2.38 μM	−1.06$\pm$0.31
7.5	0.82$\pm$0.21 μM	0.58–1.16 μM	−0.88$\pm$0.13
6.5	2.40$\pm$0.69 μM	1.68–3.46 μM	−1.08$\pm$0.14
5.5	1.93$\pm$0.58 μM	1.49–2.59 μM	−0.98$\pm$0.15

Determinations of IC_{50} values for Zn^{2+}-inhibition of ATP-responses were obtained from Hill plots of single sets of data, then expressed as mean$\pm$s.e.mean of a population ($n=4$). Confidence Intervals (CI, at the 95% level) for IC_{50} values were derived from pooled data (see Figure 4A,B) plotted using the Hill equation (Prism v2.0, GraphPad). Hill coefficients (n_H) were obtained from Hill plots of single sets of data then expressed as mean$\pm$s.e.mean of a population ($n=4$).

When applied simultaneously with ATP, Zn^{2+} (0.1–300 μM) potentiated I_{ATP} by approximately 50% above control responses in a manner described by a sigmoid C/R curve (EC_{50}, 10.9$\pm$0.74 μM, $n=4$) (Figure 5A). However, the effects of Zn^{2+} were more complex when applied prior to ATP, with a 20 min pre-incubation period for Zn^{2+} proving optimal. Under these circumstances, Zn^{2+} showed a greater degree of potentiation, which was then followed by inhibition, in a manner best described by a bell-shaped C/R curve (Figure 5A). The ability of Zn^{2+} to potentiate I_{ATP} at $rP2X_3$ receptors (where Zn^{2+} was applied simultaneously with ATP) was not altered appreciably over the range pH 8.0–5.5 (Figure 5B).

Effect of Zn^{2+} on ATP potency and efficacy at Group 1 P2X receptors

ATP potency and efficacy at $rP2X_1$ and $rP2X_3$ receptors were reassessed in the presence of Zn^{2+} to help explain its modulatory effects. For $rP2X_1$, Zn^{2+} was applied for 20 min prior to ATP and at a concentration matching its IC_{50} value (1 μM). Under these conditions, ATP potency was reduced by 5 fold without changing the maximum ATP effect (Figure 6A).

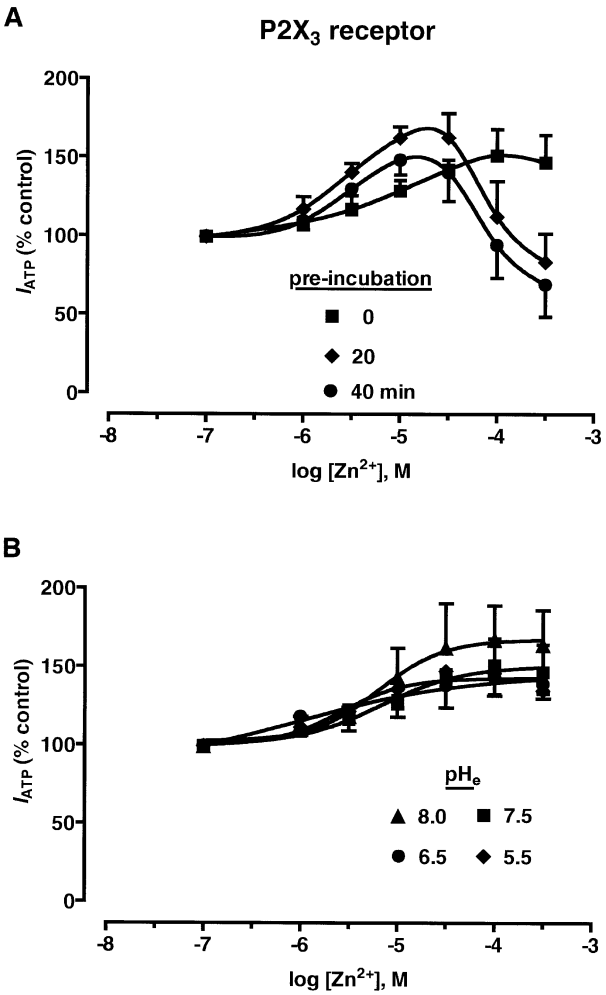

Figure 5 Zn^{2+}-modulation of ATP-responses at $rP2X_3$ receptors. (A) Concentration/response (C/R) curves for Zn^{2+} modulation of whole-cell inward currents (I_{ATP}) to ATP (0.3 μM, approx. EC_{20}), where Zn^{2+} was applied over three pre-incubation periods (0, 20, 40 min), at pH 7.5. (B) C/R curves for Zn^{2+} against I_{ATP}, at four different levels of pH_e (8.0, 7.5, 6.5, 5.5). Each concentration of Zn^{2+} was applied simultaneously with ATP. Data points are means $\pm$s.e.mean, $n=4$.

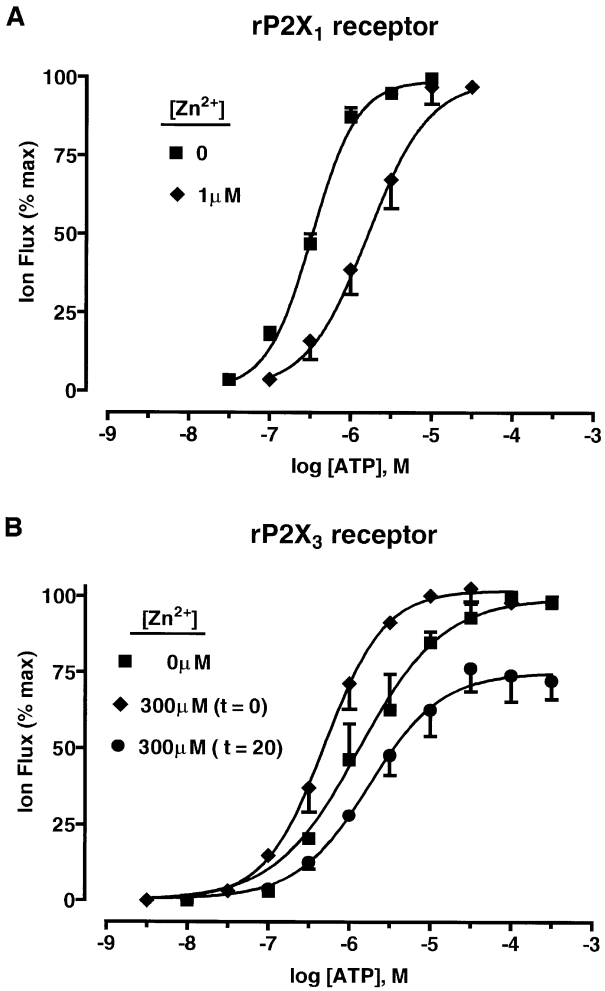

Figure 6 Zn^{2+} modulation of agonist potency and efficacy at P2X (Group 1) receptors. (A) concentration/response (C/R) curves for whole-cell inward currents to ATP (30 nM–30 μM) at $rP2X_1$ receptors, in the absence and present of Zn^{2+} (1 μM), at pH 7.5. Zn^{2+} was applied for 20 min prior to each concentration of ATP. (B) C/R curves for whole-cell inward currents to ATP (3 nM–300 μM) at $rP2X_3$ receptors, in the absence and presence of Zn^{2+} (300 μM) at pH 7.5. Zn^{2+} was applied either simultaneously with ATP ($t=0$) or 20 min prior to ATP ($t=20$). Data points are means±s.e.mean, $n=4$.

Table 3 Effect of Zn^{2+} ions on ATP potency at Group 1 P3X receptors

Conditions	*EC_{50} values*	*95% CI*	*Hill coefficient*
$rP2X_1$ receptor			
Zn^{2+}, 0 μM	325±23 nM	287–392 nM	1.56±0.18
Zn^{2+}, 1 μM	1646±198 nM	918–2951 nM	1.15±0.39
$rP2X_3$ receptor			
Zn^{2+}, 0 μM	1.83±0.28 μM	0.79–2.19 μM	0.75±0.13
Zn^{2+}, 300 μM ($t=0$)	0.50±0.19 μM	0.36–0.69 μM	1.18±0.21
Zn^{2+}, 300 μM ($t=20$)	1.75±0.37 μM	0.95–3.21 μM	0.97±0.26

Determinations of EC_{50} values for ATP-activated inward currents were obtained from Hill plots of single sets of data, then expressed as mean±s.e.mean of a population ($n=4$). Confidence Intervals (CI, at the 95% level) for EC_{50} values were derived from pooled data (see Figure 6A,B) plotted using the Hill equation (Prism v2.0, GraphPad). Hill coefficients (n_H) were obtained from Hill plots of single sets of data then expressed as mean±s.e.mean of a population ($n=4$).

EC_{50} values and Hill coefficients are given in Table 3. For $rP2X_3$, Zn^{2+} was applied either simultaneously with ATP (0 min) or prior to ATP (20 min) at a concentration (300 μM) which gave maximal potentiation and inhibition for the appropriate pre-incubation period. Zn^{2+}-potentiation (0 min pre-incubation) was due to a 3–4 fold increase in ATP potency, without any change in agonist efficacy (Figure 6B). Zn^{2+} inhibition (with 20 min pre-incubation) was due to a 30±5% ($n=3$) decrease in the maximum ATP effect (decreased agonist efficacy), without any change in agonist potency (Figure 6B). EC_{50} values and Hill coefficients are given in Table 3.

Discussion

H^+ modulation of P2X (Group 1) receptors

The two members of Group 1 (α,β-meATP-sensitive, fast-inactivating, suramin-sensitive) P2X receptors were affected in a similar manner by acidification of the extracellular solution. Agonist potency was decreased at both $rP2X_1$ and $rP2X_3$ receptors, without a concomitant change in agonist efficacy, and this effect was reversed by restoring $[H^+]_o$ to pH 7.5. Although the H^+ effect was broadly similar for these two recombinant P2X receptors, there were subtle differences in the way this was manifested. There was a gradual decrease in agonist potency at $rP2X_1$ receptors over the range of pH 7.5–5.5, and the H^+ effect was rather modest (a 6 fold increase in the EC_{50} value for ATP at pH 5.5). In contrast, agonist potency at $rP2X_3$ receptors was altered only at pH 5.5, at which point the H^+ effect was more substantial (a 15 fold increase in the EC_{50} value for ATP).

The H^+ effect at Group 1 P2X receptors is different from the modulatory activity of H^+ ions at $rP2X_2$ (Group 2) and $rP2X_4$ (Group 3) receptors. H^+ ions potentiate ATP-responses at $rP2X_2$ receptors, by increasing agonist potency without changing efficacy, showing maximum activity at levels as low as pH 5.5 (King *et al*., 1996; 1997, Stoop *et al*., 1997). However, it was also reported that highly acidic conditions (<pH 5.0) result in a progressive loss in agonist efficacy at $rP2X_2$ receptors (Stoop & Quayle, 1998). The H^+ effect at $rP2X_4$ is characterized by a progressive decrease in agonist potency over the range of pH 7.5–5.5, and a loss of agonist efficacy at pH 5.5 (Wildman *et al*., 1999b). To some extent, an H^+ effect has also been observed at $rP2X_7$ (Group 4) receptors at which acidic conditions (pH 7.5–5.5) progressively inhibited membrane current (through the P2Z ion-channel) and YO-PRO uptake (through the P2Z-activated pore) (Virginio *et al*., 1997). However, there is no information on changes in agonist potency and efficacy to explain the H^+-inhibition of ATP-responses at $rP2X_7$ receptors.

The H^+ effect is potentially important for P2X receptor signalling for two reasons. First, large pH shifts (2–3 pH units) occur with the localized metabolic acidosis associated with bone fracture (pH 4.7), ischaemia (pH 5.7), inflammation (pH 5.4), epileptic seizures and injuries related to CNS degenerative changes (DeSalles *et al*., 1987; Chesler, 1990; Ransom & Philbin, 1992; Steen *et al*., 1992). Second, transient acidic shifts also occur during CNS neurotransmission (Krishtal *et al*., 1987; Chesler, 1990; Rose & Dietmer, 1995; Yanovsky *et al*., 1995), a phenomenon due to the way transmitters are packaged in vesicles. Vacuolar H^+-ATPase (V-ATPase) establishes a H^+-gradient across the wall of synaptic vesicles, rendering the intravesicular space acidic (pH 5.5) (Cidon & Sihra, 1989). The resultant electrochemical

Table 4 H^+ and Zn^{2+} modulation of ATP-responses at $P2X_{1-4}$ receptors

	rP2X$_1$	*rP2X$_2$*	*rP2X$_3$*	*rP2X$_4$*
H^+ effect	ATP potency↓ ATP efficacy⇄ (time independent)	ATP potency↑ ATP efficacy⇄ (time independent)	ATP potency↓ ATP efficacy⇄ (time independent)	ATP potency↓ ATP efficacy↓ (time independent)
Zn^{2+} effect	ATP potency↓ ATP efficacy⇄ (time dependent)	ATP potency↑,↓ ATP efficacy⇄, ↓ (time dependent)	ATP potency↑, ↓ ATP efficacy⇄, ↓ (time dependent)	ATP potency↑ ATP efficacy↓ (time independent)
H^+ & Zn^{2+} combined	ATP potency↓ ATP efficacy⇄ (effects additive)	ATP potency↑ ATP efficacy⇄ (H^+ effect dominant)	ATP potency↑, ↓ ATP efficacy⇄, ↓ (Zn^{2+} effect dominant)	ATP potency↑ ATP efficacy↓ (effects additive)

The modulatory activity of H^+ and Zn^{2+} was assessed by changes in agonist potency and efficacy at each of the above homomeric $P2X_{1-4}$ receptors. Symbols: ↑, increase; ↓, decrease; ⇄, no change; ↑,↓, bi-directional changes, dependent on incubation time; ⇄,↓, no change initially, but a decrease with prolonged incubation.

gradient for H^+ ions assists in concentrating neurotransmitters inside storage vesicles (for a review, see: Moriyama *et al*., 1992). The acidic shift associated with neurotransmission may have a significant bearing on the sensitization of postjunctional P2X receptors activated by purinergic nerves, particularly during high frequency transmission where significant accretion of vesicular H^+ will occur at the synapse. Little is known, however, about the pH-dependence of purinergic transmission in smooth muscles where $P2X_1$ receptors are concentrated. Similarly, the effect of localized acidosis has not been investigated for $P2X_3$ receptor-mediated currents in sensory nerve endings.

Zn^{2+} modulation of P2X (Group 1) receptors

The Zn^{2+} effect, in contrast to the above H^+ effect, was dissimilar at $rP2X_1$ and $rP2X_3$ receptors–inhibition and potentiation, respectively. The inhibitory action of Zn^{2+} at $rP2X_1$ receptors was characterized by a fall in agonist potency without any change in agonist efficacy. The immediacy of Zn^{2+}-inhibition, where Zn^{2+} was applied simultaneously with ATP, indicated an extracellular locus for its site of action. However, the time-dependency and increased potency of Zn^{2+}-inhibition at $rP2X_1$ receptors also pointed to the involvement of a second intracellular site of action. Time-dependent Zn^{2+}-inhibition of ATP-responses was also seen at $rP2X_2$ receptors (Wildman *et al*., 1998) and, to some extent, at $rP2X_3$ receptors (present study).

The actions of Zn^{2+} at $rP2X_3$ receptors were complex, yet bore a similarity to its actions at $rP2X_2$ receptors (Wildman *et al*., 1998). When applied simultaneously with ATP, the main Zn^{2+} effect was potentiation of ATP-responses at $rP2X_3$ receptors. The same effect occurs at $rP2X_2$ receptors, although the magnitude of the Zn^{2+} effect is much greater. With Zn^{2+}-preincubation, the Zn^{2+} effect at $rP2X_3$ receptors was characterized by a concentration-dependent potentiation followed by inhibition; the same occurs at $rP2X_2$ receptors. Potentiation was due to an increase in agonist potency at $rP2X_3$ receptors, whereas inhibition was primarily due to a decrease in agonist efficacy (by 30%). Zn^{2+}-inhibition at $rP2X_2$ receptors is characterized by a 50% loss of agonist efficacy and a decrease in agonist potency (Wildman *et al*., 1998). The observed Zn^{2+}-inhibition (and Zn^{2+}-potentiation) at P2X receptors expressed in oocytes is not due to alterations in ecto-ATPase activity since the enzyme is absent in defolliculated cells (Ziganshin *et al*., 1995).

The Zn^{2+} effect at P2X (Group 1) receptors is important for two reasons. First, it now provides a simple means to distinguish $rP2X_1$ receptors from $rP2X_3$ receptors when, presently, there are no P2X subunit-selective agonists and antagonists. Second, Zn^{2+} ions are taken up and concentrated in synaptic vesicles of defined subsets of central neurons (for a review, see: Smart *et al*., 1994), although the accumulation of Zn^{2+} in peripheral nerves has not yet been studied. It is not known if vesicular Zn^{2+}, like vesicular H^+, acts as an agent for packaging of neurotransmitters and/or serves as a neuromodulator of receptor function. The Zn^{2+} content of some synaptic boutons is as high as 200–300 μM, and the vesicular concentration may be higher still (Smart *et al*., 1994). The plasma Zn^{2+} concentration in rat is 21 μM, although much (~99%) is bound to seral proteins (Walker & Kelleher, 1978). The impact of either vesicular-released or extracellular-fluid Zn^{2+} on purinergic signalling has not been assessed in tissues where $P2X_1$ and $P2X_3$ subunits are concentrated.

H^+ and Zn^{2+} modulation of recombinant rP2X$_{1-4}$ receptors

This present study of Group 1 P2X subunits represents a part of an extended survey of H^+ and Zn^{2+} modulation of P2X subunits known to be associated with neurotransmission. We have already commented at length on the modulatory activity of H^+ and Zn^{2+} at $rP2X_2$ and $rP2X_4$ receptors (King, 1998; King *et al*., 1996; 1997; Wildman *et al*., 1998; 1999b). The modulatory activity of H^+ and Zn^{2+} ions is important because of their presence in synaptic vesicles and likelihood they are co-released with ATP during purinergic transmission. The outcome of the present study, when taken with our past results, shows a spectrum of H^+ and Zn^{2+} effects at homomeric $P2X_{1-4}$ receptors. Table 4 summarizes the available information on these effects and establishes an operational profile for each P2X subunit. Such information on the H^+ and Zn^{2+} effects, together with distinct agonist profiles for such agents as the naturally-occurring diadenosine polyphosphates series (Brown *et al*., 1999: Wildman *et al*., 1999a), may be helpful in the future to identify the presence of P2X subunits in native P2X receptors at a time when P2X subunit-selective antagonists are not available.

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