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Zn^{2+} modulation of ATP-responses at recombinant $P2X_2$ receptors and its dependence on extracellular pH

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1 Using recombinant $P2X_2$ receptors expressed in *Xenopus* oocytes, the modulatory effects of zinc (Zn^{2+}) on ATP-responses were studied under voltage-clamp conditions and at different levels of extracellular pH.

2 Zn^{2+} (0.3–300 μM) added to the bathing medium potentiated ATP-activated membrane currents, increasing ATP-responses by up to 20 fold. This potentiating effect was reversed on washout. Zn^{2+}-potentiation was reduced in an exponential manner (decaying 1/e in 42 s) as the interval was lengthened between adding Zn^{2+} then ATP to the superfusate.

3 The potentiating effect of Zn^{2+} was progressively diminished by acidic shifts in extracellular pH (pH_e) which, of itself, also potentiated ATP-responses at $P2X_2$ receptors. The maximal potentiating effects of Zn^{2+} and H^+ were not additive.

4 Neither Zn^{2+} nor H^+ potentiation of ATP-responses was abolished by diethylpyrocarbonate (DEPC, 0.3–3 mM), which irreversibly denatures histidyl residues. Nine histidyl residues are present in the extracellular loop of $P2X_2$ receptors.

5 Zn^{2+} also enhanced the blocking activity of the P2 receptor antagonist suramin at $P2X_2$ receptors. Therefore, Zn^{2+} also mimics H^+ in increasing suramin-activity at $P2X_2$ receptors.

6 In summary, Zn^{2+} and H^+ potentiate agonist and antagonist activity at $P2X_2$ receptors but their effects are not wholly alike for receptor agonism. There, the potentiating effects of Zn^{2+} are time-dependent and gradually convert to inhibition while those of H^+ are time-independent, persistent and more potent, suggesting that either these modulators interact in a different way with a single allosteric site or with different allosteric sites.

Keywords: Zinc; extracellular pH; ATP; P2X receptor; oocyte

Introduction

Adenosine 5′-triphosphate (ATP) acts as a fast neurotransmitter for a family of ligand-gated ion channels, the P2X receptors, of which seven subtypes ($P2X_{1-7}$) have been cloned from various tissues, including central and peipheral neurones, cardiac and smooth muscle, secretory epithelia and immune cells (for reviews, see: Burnstock, 1996; Buell *et al*., 1996; Burnstock & King, 1996; North, 1996). $P2X_{1-7}$ subunits share a common topology of intracellular N- and C-termini, two transmembrane spanning regions and a large extracellular loop (with 10 conserved cysteine residues) which is presumed to form the ATP binding pocket (North, 1996). The stoichiometry of cloned P2X subtypes and native P2X receptors is unknown (Buell *et al*., 1996).

Only one member of this ATP receptor/ion-channel family, the $P2X_2$ receptor, is strongly affected by extracellular Zn^{2+} which potentiates ATP-responses at recombinant $P2X_2$ receptors (Brake *et al*., 1994; Nakazawa & Ohno, 1996; 1997). Zn^{2+} also potentiates ATP-activated currents and ATP-evoked dopamine release from rat phaeochromocytoma PC12 cells (Koizumi *et al*., 1995) as well as potentiating ATP-activated currents in rat sensory (nodose) and sympathetic (SCG) neurones (Cloues *et al*., 1993; Li *et al*., 1993; 1996; Cloues, 1995). Since transcripts for the $P2X_2$ subunit were isolated from rat PC12 cells (Brake *et al*., 1994) and are also present in rat sensory neurones (Lewis *et al*., 1995) and rat sympathetic (SCG) neurones (Collo *et al*., 1996), a strong potentiating effect by Zn^{2+} may implicate the inclusion of $P2X_2$ subunits in these native P2X receptors.

Agonist activity at the $P2X_2$ receptor is also strongly affected by extracellular pH (pH_e), with acidification increasing and alkalinization decreasing agonist potency at this ATP-gated ion channel (King *et al*., 1996c; 1997; Wildman *et al*., 1997). A similar modulatory effect of pH_e was also found for ATP-responses at native P2X receptors in rat sensory neurones (Li *et al*., 1996). In the present study, we have investigated the modulatory effects of Zn^{2+} on ATP activity at the $P2X_2$ receptor and explored the relationship between pH_e and Zn^{2+} potentiation to see if Zn^{2+} and H^+ ions either act separately or by a common mechanism at $P2X_2$ receptors.

Methods

Oocyte preparation

Xenopus laevis were anaesthetized with Tricaine (0.1% w/v) and killed by decapitation. Ovarian lobes were excised and stored (at 4°C) in a Barth's solution (pH 7.45) 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 and gentamycin sulphate 50 $\mu g\,l^{-1}$. Mature oocytes (stages V and VI) were defolliculated by a two-step process involving collagenase treatment (Type 1A, 2 mg ml^{-1} in a Ca^{2+}-free Ringer, for 2–3 h) then stripping away the follicle cell layer with fine forceps. Defolliculation removes the native P1 and P2 receptors on the follicle cell layer that envelops oocytes (King *et al*., 1996a; b). Defolliculated oocytes were injected cytosolically with rat

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$P2X_2$ cRNA (40 nl, 0.1–1 μg ml^{-1}), then incubated at 18°C in Barth's solution for 48 h to allow full receptor expression, and stored at 4°C in Barth's solution for up to 12 days until used in electrophysiological experiments.

Electrophysiology

ATP-activated currents were measured from cRNA-injected oocytes held under voltage-clamp with a twin-electrode amplifier (Axoclamp 2B, with HS-2A head-stages). The voltage-recording (1–2 MΩ tip resistance) and current-recording (5 MΩ tip resistance) microelectrodes were filled with 0.6 M K_2SO_4 and 3.0 M KCl, respectively. Oocytes were placed in an electrophysiological chamber (0.5 ml vol) and superfused (5 ml min^{-1}, at 18°C) with a Ringer solution containing (mM): NaCl 110, KCl 2.5, HEPES 5, $CaCl_2$ 1.8; adjusted to pH 7.5. Where necessary, the pH of the bathing Ringer solution (pH_e) was adjusted with either 1.0 N HCl or 1.0 N NaOH, to achieve the desired acidic or alkaline shift. Electrophysiological data were filtered initially at 3 kHz, captured at a rate of 20 Hz on a computer with the MP100WSW interface (Biopac Systems, Inc.) and analysed by a commercial software package (Acqknowledge III, Biopac).

ATP-containing solutions

ATP was added to the Ringer solution (at the final concentrations given in the text) and superfused over dual-impaled oocytes by a gravity-fed continuous flow system, which allowed the rapid addition and washout of the agonist. ATP was applied for 120 s or until currents reached a plateau, then washed off with normal Ringer solution for a period of 5 min (or for times indicated in the text). The $P2X_2$ receptor is a slowly-desensitizing receptor and recovers rapidly (within 5 min) from agonist activation (Brake *et al*., 1994). For ATP concentration-response (C/R) curves, data were normalized to the maximum current (I_{max}) evoked by ATP (100 μM) at pH 7.5. The concentration that evoked 50% of the maximum response (EC_{50}) was taken from Hill plots constructed with the transform log (I/I_{max}-I), where I is the current evoked by each concentration of agonist. The Hill coefficient (n_H) was taken from the slope of Hill plots. Concentration-response curves for ATP shown in Results were fitted to the Hill equation by use of commercial software (Prism v1.03, GraphPad).

Zinc-containing solutions

The modulatory activity of Zn^{2+} at the $P2X_2$ receptor was tested in two ways. In most experiments, Zn^{2+} was added to ATP solutions (at the final concentrations given in the text) and both applied simultaneously to oocytes. In other experiments, Zn^{2+} was added to the Ringer solution and oocytes were pre-incubated for the times mentioned in the text before ATP was applied. In some experiments, the transition metal was added to the superfusate and $P2X_2$ receptors were superfused for 60 min before ATP concentration-response curves were constructed. In such experiments, Zn^{2+} was not removed from the superfusate during agonist washout. Data on Zn^{2+} potentiation of ATP-responses were normalized to control responses to 100 μM ATP (a supramaximal stimulus at pH 7.5). EC_{50} and n_H values for Zn^{2+} concentration-response curves were calculated from Hill plots, with the maximum effect of Zn^{2+} at each level of pH_e in calculations. Diethylpyrocarbonate (DEPC) was used in some experiments to denature histidyl residues (Miles, 1977) on the extracellular loop of $P2X_2$ receptors. Oocytes were pre-incuated for 10 min in Ringer solution containing DEPC (0.3 mM–3 mM) then washed with normal Ringer before ATP activity was retested in the presence of Zn^{2+} and under acidic conditions.

Statistics

Data are presented as mean ± s.e.mean of 4 sets of data from different oocyte batches. Significant differences were determined by unpaired Student's t test, by use of commercial software (Instat v2.05A, GraphPad).

Chemicals

Common salts and reagents were AnalaR grade (Aldrich Chemicals, U.K.). ATP disodium salt, diethylpyrocarbonate (DEPC) and zinc chloride were purchased from Sigma Chemical Co. (Dorset, U.K.). Suramin was a gift from Bayer plc (U.K.).

Results

Zn^{2+} potentiation of I_{ATP}

When Zn^{2+} (0.3–300 μM) and ATP (3 μM, $EC_{5\%}$) were added simultaneously to the superfusate (T = 0 min), ATP-responses at $P2X_2$ receptors were potentiated 2 fold at 0.3 μM Zn^{2+} and 15 to 20 fold at 30 μM, after which higher concentrations (50–300 μM) failed to enhance ATP activity further (Figure 1a). At

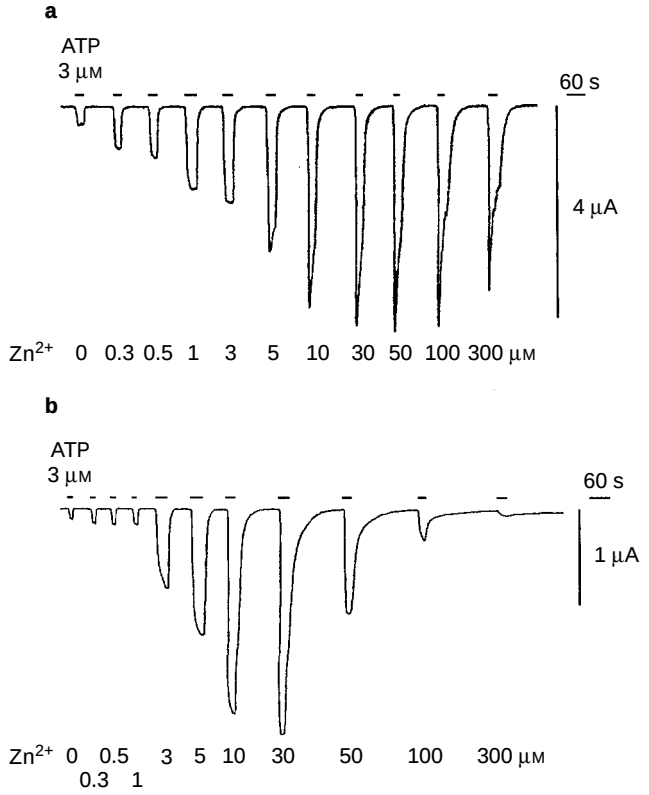

Figure 1 Zn^{2+} potentiation of I_{ATP} at $P2X_2$ receptors. Whole-cell membrane currents in *Xenopus* oocytes expressing $P2X_2$ receptors and activated by ATP (3 μM, $EC_{5\%}$ at pH 7.5) were potentiated in a concentration-dependent and time-dependent manner by Zn^{2+} (0.3–300 μM) added to the superfusate. In (a), Zn^{2+} and ATP were applied at the same time to oocytes while in (b), Zn^{2+} was applied continuously for 5 min before the application of ATP. Holding potential (V_h) was −80 mV; data in (a) and (b) from two separate oocytes.

high concentrations (300 μM and greater), the potentiating effect of Zn^{2+} appeared to wane slightly (see Figures 1a, 3a). The EC_{50} value for Zn^{2+} potentiation was 9.9 ± 1.9 μM ($n=4$) at pH 7.5 and this effect reversed on washout. When Zn^{2+} (0.3–300 μM) was applied 5 min before ATP (T = 5 min), ATP-responses were unaffected by 1 μM Zn^{2+} and potentiated 10 to 20 fold at 30 μM, after which higher concentrations (50–300 μM) progressively inhibited ATP activity (Figure 1b). In this case, the apparent EC_{50} value for Zn^{2+} potentiation was 6.1 ± 1.2 μM ($n=4$) at pH 7.5 (not significantly different from T = 0 data). Both the potentiation and inhibitory effects of Zn^{2+} were reversed on washout. The inhibitory effect was dependent on the length of Zn^{2+} pre-incubation but not Zn^{2+} concentration. For 300 μM Zn^{2+} (where the inhibitory effect was most profound), ATP-responses were initially potentiated when Zn^{2+} was applied simultaneously with ATP (Figure 2a). However, the degree of potentiation was progressively reduced when the pre-incubation period for Zn^{2+} was lengthened from 30 s to 300 s (Figure 2a). Averaged data for this run-down could be fitted to an exponential function, the time constant (decay by 1/e) for which was 42 ± 3 s ($n=4$) (Figure 2b).

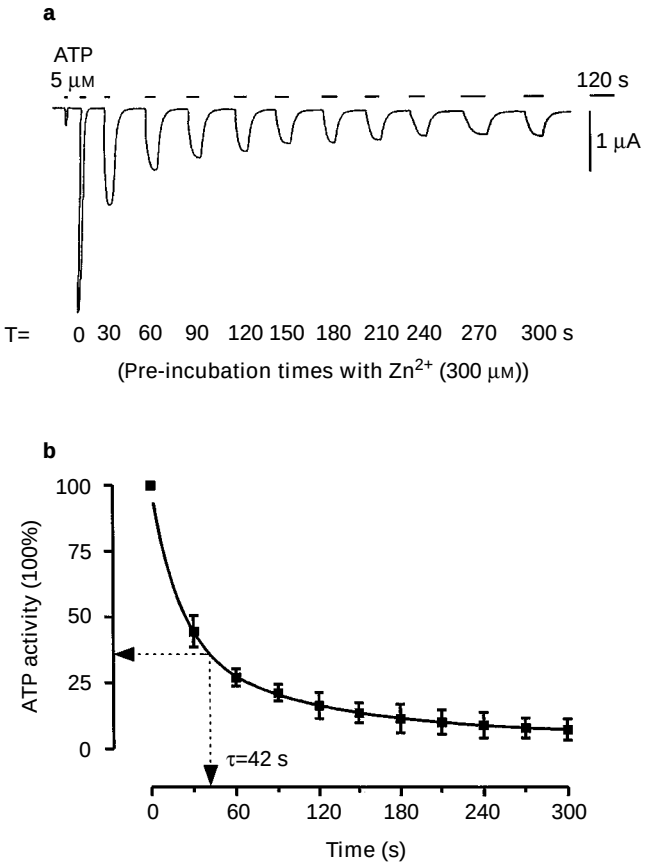

Figure 2 Run-down of Zn^{2+} potentiation of I_{ATP} at $P2X_2$ receptors. Whole-cell membrane currents to ATP and potentiated by Zn^{2+} (300 μM) showed run-down which was dependent on the duration of pre-incubation with the transition metal before the addition of the agonist. In (a), ATP (5 μM, at pH 7.5) produced a submaximal response ($V_h = -80$ mV) which was potentiated by Zn^{2+} (300 μM) added simultaneously with the agonist (T = 0). The amplitude of the ATP-response progressively decreased as the pre-incubation time with Zn^{2+} (300 μM) was increased from 30 s to 300 s. In (b), the relationship between the amplitude of I_{ATP} potentiated by Zn^{2+} (300 μM) and the duration of Zn^{2+} pre-incubation. The time constant (τ) for run-down by 1/e was 42 ± 3 s ($n=4$). The curve was fitted for exponential decay by use of commercial software (Prism v1.03, GraphPad).

Effect of pH on Zn^{2+} potentiation of I_{ATP}

The potentiating effect of Zn^{2+} on ATP-responses was dependent on extracellular pH, regardless of the length of Zn^{2+} pre-incubation. Where Zn^{2+} was applied simultaneously with ATP, the maximum potentiating effect of Zn^{2+} on ATP-response was reduced by 60% at pH 6.5 and abolished at pH 5.5 (Figure 3a). The potentiating effect of Zn^{2+} was not significantly different at pH 8.0 compared to its effect at pH 7.5 (Figure 3a). The EC_{50} values for the Zn^{2+} potentiation of I_{ATP} were ($n=4$): pH 8.0, 11.4 ± 1.7 μM; pH 7.5, 9.9 ± 1.9 μM; pH

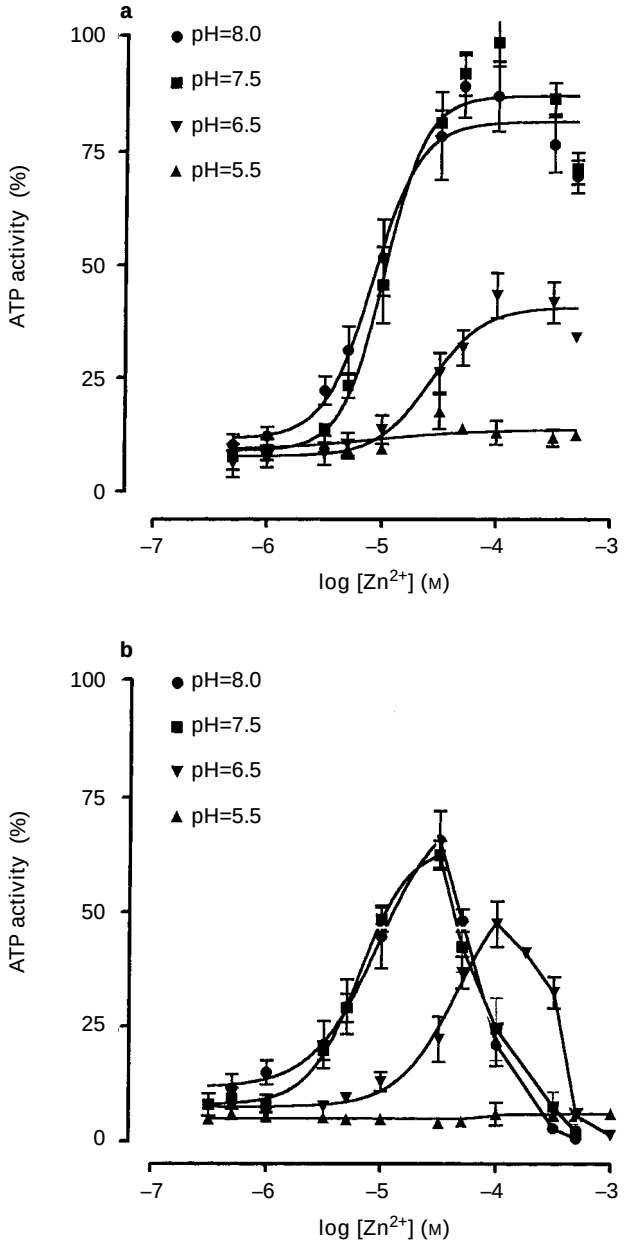

Figure 3 pH-dependence of Zn^{2+} potentiation of I_{ATP}. Concentration-response curves for the potentiating effect of Zn^{2+} (0.3–300 μM) on whole-cell membrane currents to ATP were studied at different levels of extracellular pH. In (a), Zn^{2+} and ATP were applied at the same time (T = 0 min) to oocytes while in (b), Zn^{2+} was applied continuously for 5 min (T = 5 min) before the application of ATP. Control ATP-responses were evoked by the $EC_{5\%}$ (i.e. control ATP-responses were amplitude matched for each pH level). Zn^{2+}-potentiated and control ATP-responses were normalized to the amplitude of responses to 100 μM ATP, which at pH 5.5–8.0 is a supramaximal stimulus.

6.5, 24.6±1.6 μM; pH 5.5, inactive. A similar pH-dependency was observed where $P2X_2$ receptors were pre-incubated with Zn^{2+} for 5 min (Figure 3b). Under these conditions, the apparent EC_{50} values for the Zn^{2+} potentiation of I_{ATP} were ($n=4$): pH 8.0, 7.4±1.1 μM; pH 7.5, 6.1±1.2 μM; pH 6.5, 35.3±4.7 μM; pH 5.5, inactive.

Effect of Zn^{2+} on concentration-dependence of I_{ATP}

When applied simultaneously with ATP (0.1–300 μM), 10 μM and 30 μM Zn^{2+} displaced the ATP concentration-response (C/R) curve leftwards, without changing the maximum response or the slope of the curve, while 100 μM and 300 μM Zn^{2+} did not significantly displace the ATP C/R curve further to the left (Figure 4a). Thus, the potentiating effect of Zn^{2+} reached a maximum at approximately 30 μM. EC_{50} values for ATP, at the following Zn^{2+} concentrations, were ($n=4$): 0 μM, 16.2±1.4 μM; 10 μM, 6.5±0.8 μM; 30 μM, 3.1±0.33 μM; 100 μM, 2.4±0.1 μM; 300 μM, 3.3±0.3 μM.

Prolonged pre-incubation with Zn^{2+} had an inhibitory effect on ATP activity and also reduced ATP potency. Where $P2X_2$ receptors were pre-incubated with 100 μM Zn^{2+} for 60 min (T=60 min), the ATP C/R curve was displaced to the right of the control curve and maximum ATP activity was reduced by about 50% (Figure 4b). This inhibition was reversed on washout. However, without pre-incubation 100 μM Zn^{2+} applied simultaneously with ATP enhanced agonist potency and displaced the ATP-curve to the left of the control curve (Figure 4b). EC_{50} values for ATP, at the following Zn^{2+} concentrations and incubation times, were ($n=4$): 0 μM, 16.2±1.4 μM; 100 μM (T=0), 2.4±0.1 μM; 100 μM (T=60), 50.9±8.0 μM. The inhibitory effect of prolonged Zn^{2+} pre-incubation on ATP was prevented by lowering pH to 5.5 (Figure 4c). EC_{50} values (at pH 5.5) for ATP, at the following Zn^{2+} concentrations and incubation times, were ($n=4$); 0 μM, 547±39 nM; 100 μM (T=0), 577±89 nM; 100 μM (T=60), 526±86 nM (none significantly different).

Zn^{2+} and pH interactions on concentration-dependence of I_{ATP}

Acidic shifts have been shown to increase ATP potency at $P2X_2$ receptors (King *et al*., 1996c; Wildman *et al*., 1997), with an apparent pK_a of 7.05±0.05 (King *et al*., 1997). To determine any interaction between Zn^{2+} and pH, the concentration-dependence of I_{ATP} was investigated at pH 7.5, 6.5 and 5.5 in the absence and presence of 100 μM Zn^{2+} (Figure 5a,b,c). With H^+ alone, ATP potency at $P2X_2$ receptors was increased at pH 6.5 and reached a maximum at pH 5.5. EC_{50} values for ATP were ($n=4$): pH 7.5, 16.2±1.4 μM; pH 6.5, 1.38±0.22 μM; pH 5.5, 547±39 nM. Thus, ATP potency was increased 12 fold at pH 6.5 and 30 fold at pH 5.5. Where Zn^{2+} (100 μM) was applied simultaneously with ATP, increases in ATP potency were progressively reduced as pH was lowered to 5.5. Thus, EC_{50} values for ATP (Zn^{2+} present) were ($n=4$): pH 7.5, 2.4±0.13 μM; pH 6.5, 363±43 nM; pH 5.5, 481±79 nM. Thus, Zn^{2+} increased the ATP potency at $P2X_2$ receptors by 7 fold at pH 7.5 and 4 fold at pH 6.5, but had no further effect at pH 5.5.

Effect of DEPC on Zn^{2+} and H^+ potentiation

The imidazole group on histidyl residues shows a strong avidity for both Zn^{2+} and H^+ ions and is irreversibly denatured by diethylpyrocarbonate (DEPC) to abolish Zn^{2+}

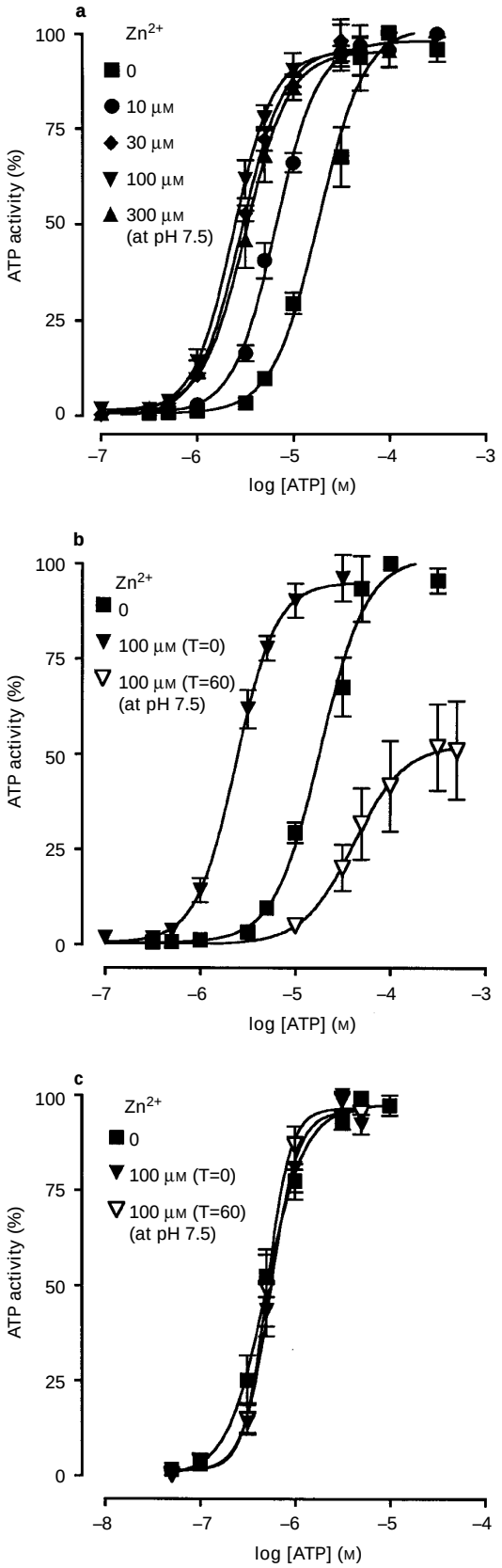

Figure 4 Effects of Zn^{2+} on concentration-dependence of I_{ATP}. In (a), concentration-dependence of whole-cell membrane currents to ATP (0.1–300 μM, at pH 7.5) in the presence of Zn^{2+} applied at the same time as the agonist. In (b) and (c), effect of Zn^{2+} (100 μM) applied at the same time as ATP (T=0) or continuously over a 60 min pre-incubation (T=60) on the concentration-dependence of ATP-responses at pH 7.5 (b) and pH 5.5 (c). Concentration-response curves to ATP in the absence of Zn^{2+} are also shown.

and H^+ binding (Miles, 1977). DEPC was applied briefly (10 min) in single doses (0.3, 0.5, 1 or 3 mM) to *Xenopus* oocytes ($n=3$, per dose) expressing the $P2X_2$ receptors to see if the potentiating effects of Zn^{2+} and H^+ depended on histidyl residues near the ATP binding site. At concentrations greater than 0.3 mM, DEPC reduced maximal ATP-responses by 90% and decreased ATP potency in an irreversible manner. In spite of this inhibitory action by DEPC, residual ATP-responses were potentiated by Zn^{2+} (100 μM) and pH (6.5). Histidyl residues did not appear to be crucial for the potentiating effect of Zn^{2+} and H^+ on I_{ATP}.

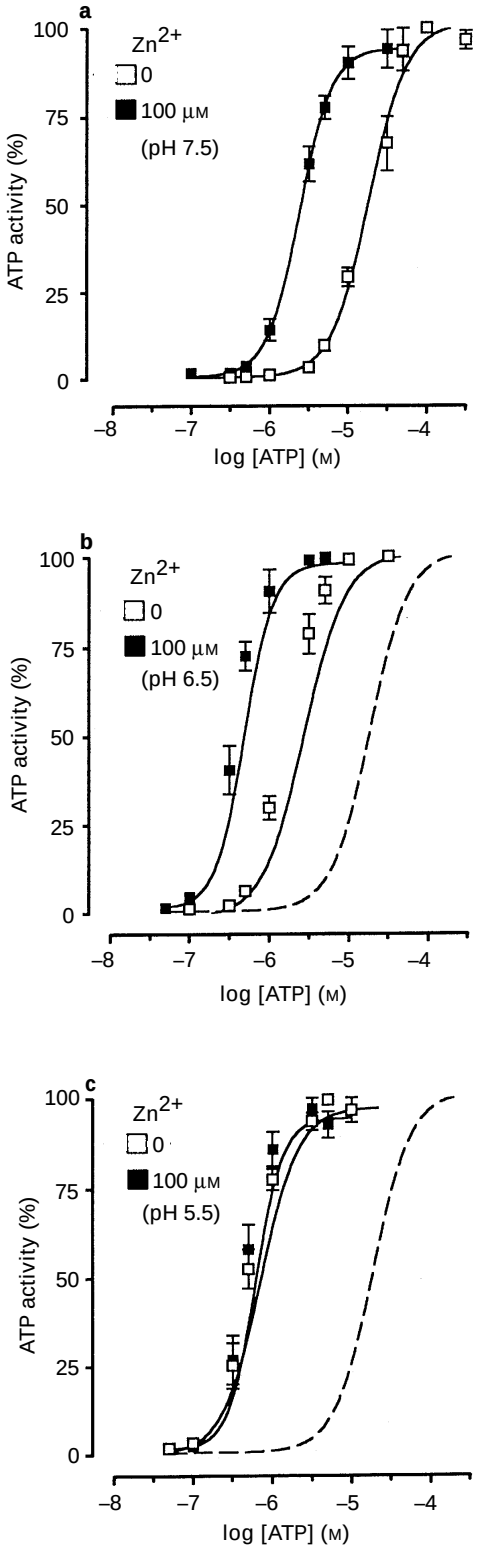

Figure 5 Interaction of Zn^{2+} and H^+ on concentration-dependence of I_{ATP}. Concentration-dependence of whole-cell membrane currents to ATP (0.03–300 μM) at pH 7.5 (a), pH 6.5 (b) and pH 5.5 (c), in the absence and presence of Zn^{2+} (100 μM) applied at the same time as the agonist. The ATP C/R curve was maximally displaced to the left by 100 μM Zn^{2+} at pH 7.5 (see (a); also Figure 4a). The control ATP C/R curve at pH 7.5 is shown as the dashed curve in (b) and (c).

Effect of Zn^{2+} on suramin blocking activity

The blocking activity of suramin at $P2X_2$ receptors is progressively increased by acidic shifts in extracellular pH (King *et al.*, 1997). Zn^{2+} (100 μM) mimicked this action of H^+ ions and enhanced blocking activity of suramin when Zn^{2+} was applied simultaneously with ATP (Figure 6). IC_{50} values for suramin at the following Zn^{2+} concentrations were ($n=4$): 0 μM, 1.74±0.17 μM; 100 μM, 225±23 nM.

Discussion

Extracellular Zn^{2+} and acidic solutions reversibly potentiate I_{ATP} at $P2X_2$ receptors, an effect caused by a leftwards displacement of the ATP concentration-response curve and increase in agonist potency. The potentiating action of Zn^{2+} and H^+ was saturable, reaching a maximum at 30 μM and pH 5.5 (i.e. $[H^+]=30$ μM), respectively. Zn^{2+} and H^+ also enhanced the blocking activity of suramin at $P2X_2$ receptors, thus showing that agonist and antagonist activity are both modulated and raising the possibility that Zn^{2+} and H^+ might act through a common mechanism. It is unlikely that these potentiating effects were due to an inhibitory action on ecto-ATPases, since the modulatory actions of Zn^{2+} and H^+ on ATP activity differ in time-course and the defolliculated oocyte

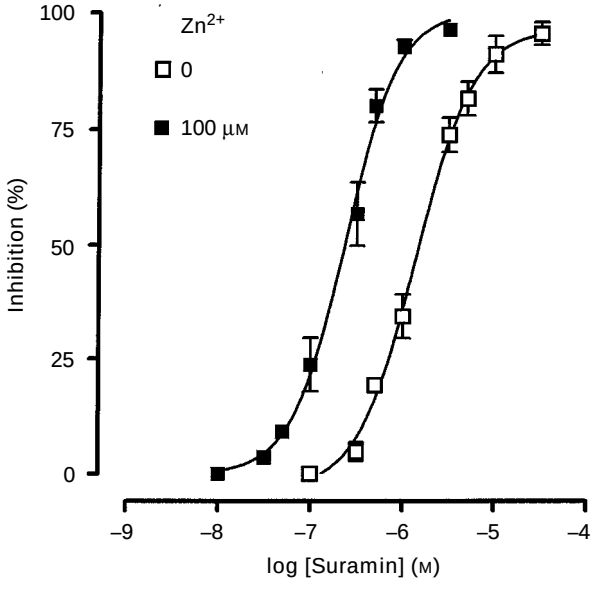

Figure 6 Zn^{2+} modulation of suramin antagonism of I_{ATP}. Concentration-dependence of suramin blockade of whole-cell membrane currents to ATP, in the absence and presence of Zn^{2+} (100 μM), applied at the same time as the agonist. Control ATP-responses were evoked with the EC_{30} (i.e. control ATP-responses were amplitude matched to account for the potentiating effect of Zn^{2+}) and the blocking activity of suramin tested. Experiments were carried out at pH 7.5.

is largely devoid of surface enzymes that degrade ATP (Ziganshin *et al*., 1995). Furthermore, the potentiating effects of Zn^{2+} and H^+ cannot be attributed to an interaction with ATP itself, since low pH levels and Zn^{2+} do not universally enhance ATP activity at all of the subtypes of cloned P2X receptors.

H^+ exerted a greater effect than Zn^{2+} on ATP-responses at $P2X_2$ receptors. For acid shifts, ATP potency was maximally increased 30 fold with the EC_{50} values of 547 ± 39 nM at pH 5.5 and 16.2 ± 1.4 μM at pH 7.5. However, for the transition metal ATP potency was maximally increased 7 fold with an EC_{50} value of 2.4 ± 0.1 μM in the presence of 100 μM Zn^{2+} compared to a control value of 16.2 ± 1.4 μM (at pH 7.5). The potentiating effects of H^+ persisted with time while Zn^{2+} acted in a time-dependent manner and potentiation showed run-down and, eventually, was replaced by an inhibitory action. The greater effectiveness and persistence of H^+ potentiation may help explain why the inhibitory actions of prolonged Zn^{2+} pre-incubation were reversed when pH_e was lowered to 5.5, a physiological antagonism of Zn^{2+} inhibition by a stronger and opposing H^+ potentiating effect. We also noticed that ATP potency decreases when the pH of the superfusate is raised from 7.5 to 8.0, while the extent of Zn^{2+} potentiation was no different at pH 7.5 and pH 8.0. This finding, together with the above differences in effectiveness of Zn^{2+} and H^+, raised a question mark over these modulators acting at the same allosteric site on the $P2X_2$ receptor.

Others have briefly commented on the potentiating effect of Zn^{2+} (Brake *et al*., 1994; Nakazawa & Ohno, 1996; 1997). Brake *et al*. (1994) tested 10 μM Zn^{2+} against I_{ATP} at $P2X_2$ receptors and found that this group IIb transition metal increased agonist potency by 4 fold. Here, we found a 3 fold increase in agonist potency under similar conditions. Nakazawa & Ohno (1996, 1997) also found that 100 μM Zn^{2+} was less effective than 10 μM at potentiating ATP-responses at $P2X_2$ receptors, confirming that the Zn^{2+} C/R curve is bell-shaped. However, this appears to be true only when $P2X_2$ receptors are pre-incubated with this transition metal.

The present results on H^+ potentiation of I_{ATP} agree with earlier findings on pH sensitivity of $P2X_2$ receptors (King *et al*., 1996c; 1997; Wildman *et al*., 1997), except for 2 minor differences. Previously, we found no significant difference in EC_{50} values for ATP at pH 6.5 and pH 5.5 (1.2 ± 0.1 μM and 1.1 ± 0.1 μM, respectively) (King *et al*., 1996c; 1997). However, a small difference in EC_{50} values was shown in the present study, i.e. 1.38 ± 0.22 μM (at pH 6.5) and 547 ± 39 nM (at pH 5.5) ($P < 0.05$). We explain this difference solely on a greater accuracy in determining EC_{50} values. We also found that an original estimate of suramin activity ($IC_{50} = 10.4 \pm 1.2$ μM) (King *et al*., 1997) did not agree with the present data ($IC_{50} = 1.74 \pm 0.17$ μM). The blocking activity of suramin is exceedingly sensitive to pH_e (King *et al*., 1997) and, therefore, small differences in setting pH_e have a profound impact on determinations of IC_{50} values. Also, we are now aware that the life-time and accuracy of pH-probes are often less than stated by their manufacturers.

Histidyl residues have been implicated in the effects of Zn^{2+} and H^+ on agonist activity/binding at adenosine and γ-aminobutyric acid (GABA) receptors (Smart, 1990; Allende *et al*., 1993; Wang *et al*., 1995; Krishek 1996). We used DEPC to denature histidyl residues on the extracellular loop of the $P2X_2$ receptor, although this reagent is not selective for histidine and will also act on arginyl, sulphydryl and tyrosyl residues when used at high concentrations (10 mM) and under very acidic conditions (pH 4) (Miles, 1977). DEPC did not block the potentiating effects of Zn^{2+} and H^+ at $P2X_2$ receptors and this outcome appeared to discount the involvement of histidyl residues as the allosteric sites near the ATP binding site. The precise locus of these sites on $P2X_2$ remains to be determined.

Cloues (1996) showed that the native P2X receptors on SCG neurones, PC12 cells and sensory neurones in rat all have similar biophysical and pharmacolaogical properties. The P2X receptors at these three sites are weakly stimulated by ADP (Rhoads *et al*., 1993; Khakh *et al*., 1995) and, interestingly, the $P2X_2$ receptor is also insensitive to ADP (King *et al*., 1997). ATP-responses at the above native P2X receptors are strongly affected by Zn^{2+} (Li *et al*., 1993; Koizumi *et al*., 1995) as are ATP-responses at the $P2X_2$ receptor. Acidic shifts potentiate and alkaline shifts inhibit ATP-responses at native P2X receptors of sensory neurones (Li *et al*., 1996) and, to date, only the $P2X_2$ receptors have been shown to react in the same way to pH changes (King *et al*., 1997; North, unpublished data). Thus, it appears that weak ADP activity, Zn^{2+} potentiation of ATP-responses and sensitivity to pH_e may represent key pharmacological features that could identify the presence of $P2X_2$ subunits in native P2X receptors. It is significant, therefore, that $P2X_2$ transcripts are contained in PC12 cells, sympathetic (SCG and coeliac) neurones and sensory (nodose and DRG) neurones (Brake *et al*., 1994; Collo *et al*., 1996).

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