



Zinc Changes AMPA Receptor Properties: Results of Binding Studies and Patch Clamp Recordings

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Summary—The influence of zinc ions on α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors was investigated using binding studies with [3 H]AMPA to rat cortical membranes and patch clamp recordings from cultured superior colliculus neurones. In Tris-HCl buffer, zinc (1–10 mM) significantly increased the specific binding of [3 H]AMPA whereas this increase was negligible in the presence of CaCl₂ (2.5 mM) and KSCN (100 mM). This effect was associated with a dramatic increase in B_{\max} but a decrease in both agonist and antagonist affinity. Association and dissociation experiments showed that equilibrium [3 H]AMPA binding is reached with faster kinetics in the presence of zinc. At low concentrations (0.3 mM) zinc also concentration-dependently potentiated both peak and plateau components of whole cell current responses to AMPA (100 μ M). This effect was accompanied by a reduction of the degree, and slowing of the rate, of AMPA receptor desensitisation. In contrast, higher concentrations of zinc (1–3.0 mM) inhibited AMPA responses to some degree, but slowed desensitisation further. This ability of zinc to change AMPA receptor properties may be relevant to neurotoxicity associated with AMPA receptor activation. Copyright © 1996 Elsevier Science Ltd.

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In the last few years a number of agents including nootropics, benzothiadiazines, AMPAkinases and 2,3-benzodiazepines have been shown to modulate α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor activity leading to the suggestion that the AMPA receptor may possess positive and negative modulatory binding sites (for reviews see Lee and Benfield, 1994; Danysz *et al.*, 1995). Another modulatory effect on AMPA receptor properties may be produced by zinc (see Smart *et al.*, 1994). Zinc selectively attenuates *N*-methyl-D-aspartate (NMDA) receptor-mediated excitotoxicity but has been found to potentiate AMPA receptor-mediated neurotoxicity in cultured cortical neurones (Peters *et al.* 1987; Koh and Choi, 1988). A recent study considered the possibility that the increased AMPA receptor-mediated toxicity reflects AMPA potentiation of inherent zinc toxicity, perhaps mediated by neuronal depolarisation and zinc entry through voltage-

gated calcium channels (Weiss *et al.*, 1993). In contrast, electrophysiological data indicate that these neurotoxic effects of zinc may be due to a direct modulatory action at AMPA receptors. Investigations on mouse hippocampal neurones in cell cultures and *Xenopus* oocytes injected with rat mRNA are consistent with the assumption that zinc potentiates AMPA receptor-mediated currents at low concentrations (μ M range) while inhibiting these responses at higher concentrations (mM range) (Mayer *et al.*, 1989; Rassendren *et al.*, 1990; Reichling *et al.*, 1991). For several reasons such a modulatory effect of zinc on excitatory amino acid receptors may be of particular interest: zinc is widely distributed in the mammalian central nervous system and is present in nerve terminals and synaptic vesicles from where it is released during synaptic transmission (Assaf and Chung, 1984; Pérez-Claussel and Danscher, 1985). Previous studies indicate that this zinc is specifically associated with glutamatergic pathways (Ottersen and Storm-Mathisen, 1986; Cotman *et al.*, 1987) and particularly high concentrations (200–300 μ M) are present in mossy fibre terminals of the hippocampus (Frederickson *et al.*, 1983; Aniksztejn *et al.*, 1987).

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Previous patch clamp studies on the effects of zinc on AMPA receptor activation kinetics utilised non-selective ligands in primary culture. Moreover, no results from receptor binding studies are available. As such, it seems pertinent to compare the effects of zinc on [³H]AMPA receptor binding and AMPA-induced currents under concentration-clamp in cultured neurons.

METHODS

Receptor binding on rat brain homogenates

Male Sprague–Dawley rats (200–250 g) were decapitated and their forebrains were removed rapidly. Tissue preparation was performed according to Foster and Wong (1987). In brief, the brain tissue was homogenised in 20 vols of sucrose (0.32 M) using a glass-Teflon homogenizer. The homogenate was centrifuged at 1000 g for 10 min. The pellet was discarded and the supernatant centrifuged at 20,000 g for 20 min. The resulting pellet was re-suspended in 20 volumes of distilled water and centrifuged for 20 min at 8000 g. Then the supernatant and the buffy coat were centrifuged three times (48,000 g for 20 min) in the presence of Tris–HCl (5 mM) pH 7.4. All centrifugation steps were carried out at 4°C. After re-suspension in 5 vols of Tris–HCl (5 mM) pH 7.4 the membrane suspension was frozen rapidly at –80°C.

On the day of assay the membranes were thawed and washed four times by re-suspension in Tris–HCl (5 mM) pH 7.4 and centrifugation at 48,000 g for 20 min. The final pellet was re-suspended in different buffers containing: (1) Tris–HCl (10 mM); (2) Tris–HCl (10 mM) and CaCl₂ (2.5 mM) or (3) Tris–HCl (10 mM), CaCl₂ (2.5 mM) and potassium thiocyanate (KSCN 100 mM) respectively (final protein concentration was 0.15–0.20 mg/ml determined by the method according to Lowry *et al.*, 1951). The relatively low concentration of Tris–HCl (10 mM) was chosen to make the assay conditions more comparable to those used for other glutamate receptor binding assays (normally 5–10 mM). However, similar levels of binding were seen in the presence of Tris–HCl (30 mM)—a concentration typically used in [³H]AMPA receptor binding assays.

All displacement and stimulation experiments were carried out in triplicates with a radioligand concentration of [³H]AMPA of 5 nM (46.1 Ci/mmol, obtained from New England Nuclear) at a temperature of 4°C. Non-specific binding was determined with L-glutamate (1 mM). Receptor–ligand complexes were separated by using a Millipore filter system for vacuum filtration over glass fibre filters (Whatman GF/C). The membranes retained on the glass fibre filters were rinsed three times with 2.5 ml ice cold Tris–HCl (10 mM) buffer.

For stimulation experiments binding of [³H]AMPA was determined in the presence of different concentrations of ZnCl₂ (0.1 mM–10 mM) and in different buffers. In association and dissociation experiments bound ligand was determined over a range of incubation times (10 s to 60 min) in the presence of Tris–HCl (10 mM), CaCl₂

(2.5 mM) and KSCN (100 mM) or Tris–HCl (10 mM) and ZnCl₂ (10 mM) at 4°C.

In competition studies increasing concentrations of cold AMPA, L-glutamate, kainate or 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) were added under standard conditions [Tris–HCl (10 mM), CaCl₂ (2.5 mM) and KSCN (100 mM)] and in the presence of Tris–HCl (10 mM) buffer with ZnCl₂ (10 mM).

For saturation studies AMPA concentrations up to 20 nM were achieved by increasing the concentration of [³H]AMPA, while those above 20 nM were produced by adding unlabeled AMPA to a fixed [³H]AMPA concentration of 20 nM. Under standard conditions the assay was carried out in the presence of Tris–HCl (10 mM), CaCl₂ (2.5 mM) and KSCN (100 mM) with an incubation time of 45 min at 4°C. The saturation experiment was then repeated in the presence of Tris–HCl (10 mM) with ZnCl₂ (10 mM).

Binding assays were all carried out at two centres (Merz+Co. and the Royal Danish School of Pharmacy) with corresponding results.

Patch clamp technique

Patch clamp recordings were made from cultured rat superior collicular neurones (11–14 days *in vitro*; see Parsons *et al.*, 1994 for details) in the whole cell mode with the aid of an EPC-7 amplifier (List) at a membrane potential of –70 mV. Patch clamp electrodes were pulled with a horizontal puller (DMZ) and had an internal tip diameter between 1.0 and 1.4 μm and a tip resistance of 4–8 M. Cells were continuously superfused via one of eight channels of a custom made fast superfusion system with a common outflow. Test substances then were applied by rapidly switching channels—complete exchange of the superfused solution was achieved within 10 ms. The application of solutions and the synchronised online electronic acquisition of data were controlled by the IBM program PCLAMP.

The contents of the intracellular (electrode) solution were as follows (mM): CsCl (120), TEACl (20), EGTA (10), MgCl₂ (1), CaCl₂ (0.2), Glucose (10), ATP (2), cAMP (0.25). The extracellular solutions had the following basic composition (mM): NaCl (140), KCl (3), Glucose (10), HEPES (10), CaCl₂ (0.2), Sucrose (4.5), Glycine (2.5 × 10⁻³). In addition, neurones were pharmacologically isolated from one another by the inclusion of 0.3 μM tetrodotoxin to block voltage-activated sodium currents. AMPA (100 μM) and ZnCl₂ (0.3–3 mM) were added to this basic solution.

RESULTS

[³H]AMPA binding to rat cortical membranes was performed using three different buffer compositions. In the absence of ZnCl₂, specific binding of [³H]AMPA (final concentration 5 nM) reached values of 380 ± 93 dpm when assays were carried out with Tris–HCl/CaCl₂ buffer and 2294 ± 298 dpm with

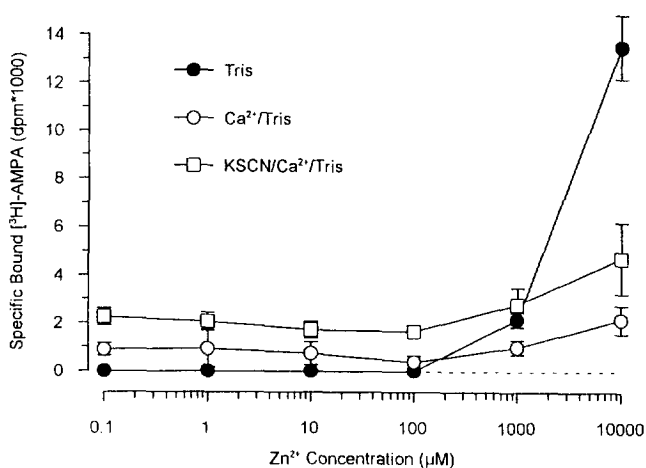


Fig. 1. Concentration-dependent influence of ZnCl_2 on specific bound $[^3\text{H}]\text{AMPA}$ (final concentration 5 nM) using different buffers. Data represent mean \pm SEM of three replicate experiments.

Tris-HCl/ CaCl_2 /KSCN buffer. No specific binding was obtained when membranes were re-suspended in 10–30 mM Tris-HCl buffer alone. However, some specific binding was seen with 10–20 nM of $[^3\text{H}]\text{AMPA}$ and with higher final protein concentrations (data not shown). Within a low concentration range of 0.1–100 μM ZnCl_2 had only negligible effects on specific $[^3\text{H}]\text{AMPA}$ binding in the presence of the different buffers (Tris-HCl, Tris-HCl/ CaCl_2 , Tris-HCl/ CaCl_2 /KSCN). However, in the presence of ZnCl_2 (1 mM) specific binding was increased to 1005 ± 302 dpm in Tris-HCl/ CaCl_2 buffer, 2795 ± 702 dpm in Tris-HCl/ CaCl_2 /KSCN buffer and 2241 ± 410 dpm in Tris-HCl buffer (Fig. 1). This effect was even more pronounced in the presence of ZnCl_2 (10 mM). Non-specific binding was similar under all conditions investigated (Fig. 2).

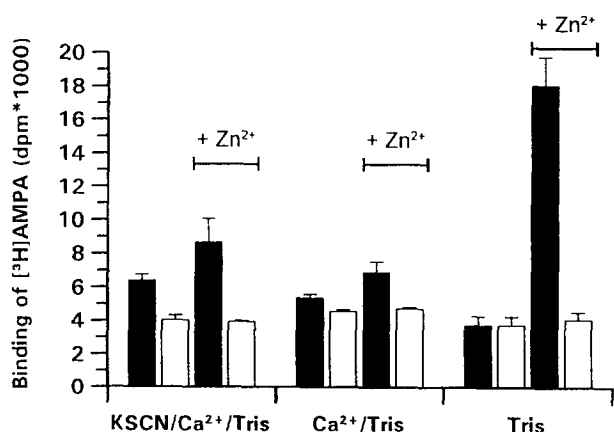


Fig. 2. Stimulation of $[^3\text{H}]\text{AMPA}$ (final concentration 5 nM) with 10 mM zinc. Bars depict mean \pm SEM of total binding (filled bars) and non-specific binding (open bars) of $[^3\text{H}]\text{AMPA}$ in the presence and absence of 10 mM zinc chloride using (a) 10 mM Tris-HCl/2.5 mM CaCl_2 /100 mM KSCN (b) 10 mM Tris-HCl/2.5 mM CaCl_2 or (c) 10 mM Tris-HCl as assay buffers.

Table 1. Scatchard analysis of the binding of $[^3\text{H}]\text{AMPA}$ under standard conditions (control) and in the presence of ZnCl_2 (10 mM)

	K_D [nM]	B_{max} [pmol/mg protein]
Control	High affinity: 19.1 ± 7.9 Low affinity: 375 ± 114	High affinity: 0.62 ± 0.24 Low affinity: 3.23 ± 0.69
+ ZnCl_2 (10 mM)	$23,100 \pm 960$	350 ± 204

Specific binding in Tris-HCl/ CaCl_2 /KSCN buffer reached equilibrium within 25 min with half-maximal specific binding being achieved at 4.5 min. Dissociation was completed within 30 min after addition of unlabelled glutamate (final concentration 1 mM). Using Tris-HCl buffer in the presence of ZnCl_2 (10 mM) equilibrium was reached almost instantaneously and dissociation was complete within 4–5 min. However, the time resolution of the assay used was not fast enough to assess exact rate constants in the presence of zinc.

Saturation experiments carried out under standard assay conditions resulted in the detection of high- and low-affinity binding sites with values comparable to those found by previous groups (Honoré and Drejer, 1988; Hall *et al.*, 1992). In contrast, Scatchard analysis in the presence of ZnCl_2 (10 mM) and Tris-HCl (10 mM) revealed only a single class of binding sites with lower affinity and a dramatic increase in B_{max} (Table 1). Hill coefficients ($n_H = 1.08 \pm 0.02$) were not different from unity and therefore did not indicate any cooperativity of $[^3\text{H}]\text{AMPA}$ binding or the existence of high- and low-affinity binding sites in the presence of zinc.

Displacement studies performed in the presence of zinc revealed a large increase of K_i values for the investigated standard compounds. Thus, ZnCl_2 (10 mM) decreased the affinity of AMPA, L-glutamate and kainate by approximately 220-, 210- and 30-fold, respectively (Table 2). The affinity of CNQX was also decreased more than 300-fold (Table 2). All results are given as mean \pm SEM for 3–4 determinations.

Whole cell current responses to AMPA (100 μM) rose rapidly to a peak level (906 ± 80 pA, 15–85% rise time 12.1 ± 0.3 ms, $n = 8$) and then desensitised rapidly to a lower plateau level (242 ± 35 pA, $\tau_{\text{desen}} = 25.3 \pm 1.6$ ms, Figs 3 and 4). ZnCl_2 (0.3 mM) slightly poten-

Table 2. Inhibition of $[^3\text{H}]\text{AMPA}$ (5 nM final concentration) by standard compounds on rat cortical membranes in the presence and absence of ZnCl_2 (10 mM). K_i values are given as mean \pm SEM of 3–4 separate determinations. Values were calculated using the equation $K_i = \text{IC}_{50}/(1 + ([L]/K_D))$ (Cheng and Prusoff, 1973)

Competitor	K_i [μM] Control	K_i [μM] + ZnCl_2 (10 mM)
AMPA	0.0450 ± 0.0065	10.05 ± 2.8^a
L-glutamate	0.728 ± 0.208	156.7 ± 38.4^a
kainate	10.05 ± 2.91	311.3 ± 171.6
CNQX	0.37 ± 0.04	$>100^a$

^a Significant different from control, $p < 0.05$ (Student's *t*-test)

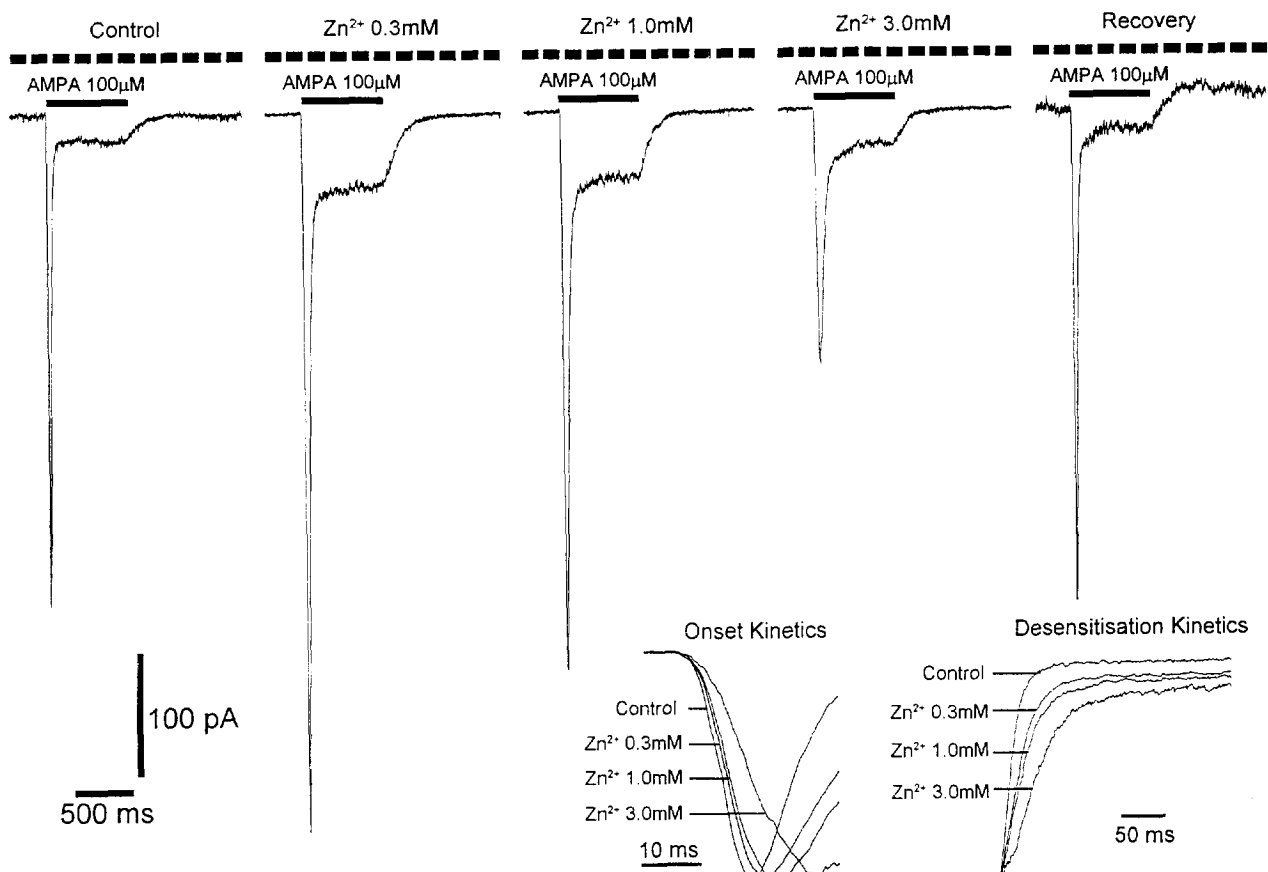


Fig. 3. Concentration-dependent effects of zinc on AMPA-induced current responses of a single cultured superior colliculus neurone. AMPA ($100 \mu\text{M}$) was applied for 700 ms every 15 s at a constant membrane potential of -70 mV . The left and right panels show control and recovery responses to AMPA. The middle three panels show AMPA responses in the continuous presence of ZnCl_2 (0.3–3.0 mM). Note the appearance of unstable leak currents following removal of ZnCl_2 (3.0 mM, recovery). The inserts show the effects of these same ZnCl_2 concentrations on AMPA response onset and desensitisation kinetics (different time scales).

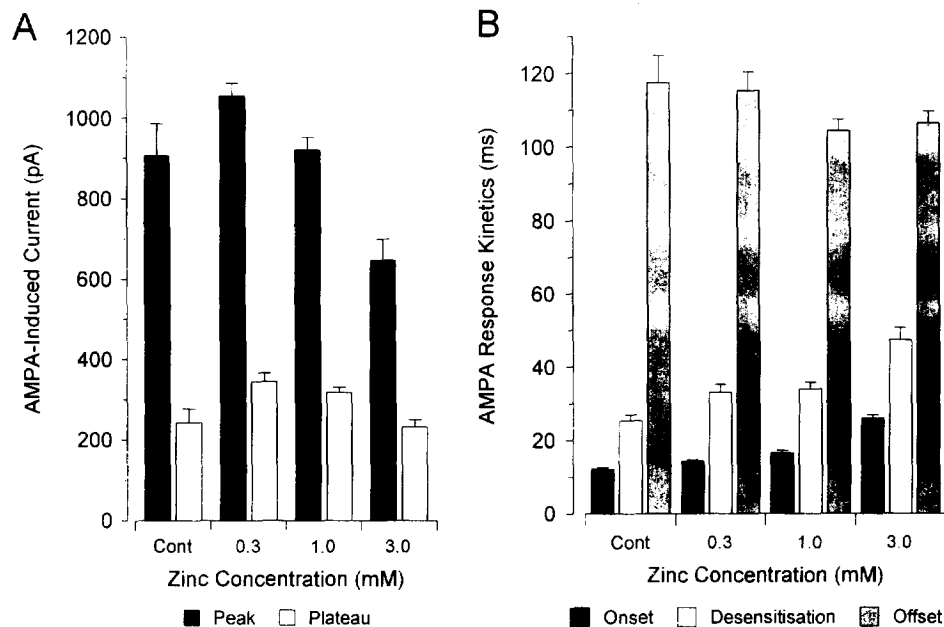


Fig. 4. Concentration-dependent effects of zinc on pooled AMPA-induced current responses. AMPA ($100 \mu\text{M}$) was applied as in Fig. 2. (A) Mean peak and plateau (steady-state) currents have been plotted against zinc concentration. (B) Onset, desensitisation and offset kinetics have been plotted against zinc concentration. Error bars represent SEM ($n = 8$).

tiated peak AMPA current responses but had a greater effect in reducing the degree, and slowing the rate, of desensitisation (peak $116.5 \pm 3.4\%$ of control, plateau $142.4 \pm 8.8\%$ of control, $\tau_{\text{desen}} = 33.0 \pm 2.2$ ms, $n = 8$). In the presence of ZnCl_2 (1.0 mM) the effects on peak currents were no longer apparent, whereas the effects on desensitisation were of similar magnitude (peak $101.8 \pm 3.5\%$ of control, plateau $131.2 \pm 5.6\%$ of control, $\tau_{\text{desen}} = 34.0 \pm 1.8$ ms, $n = 8$). In the presence of ZnCl_2 (3.0 mM) peak current responses were reduced and the potentiation of plateau currents was no longer apparent (peak $71.5 \pm 5.7\%$ of control, plateau $96.0 \pm 7.4\%$ of control, $n = 8$). In contrast, the slowing in the rate of desensitisation was most pronounced at this concentration ($\tau_{\text{desen}} = 47.3 \pm 3.3$ ms). Thus, zinc seems to have two opposing effects on AMPA receptors (1) a reduction in the rate and degree of desensitisation at lower concentrations and (2) an antagonistic effect at higher concentrations which masks the former effect to some degree. The antagonistic effects of zinc may be related to a decrease in agonist affinity as the onset rate of AMPA-induced currents was slowed in a concentration-dependent manner (15–85% rise times ZnCl_2 : 0.3 mM = 14.3 ± 0.5 ms, 1.0 mM = 16.5 ± 0.7 ms, 3.0 mM = 25.9 ± 1.0 ms). However, the effects on offset kinetics were not so apparent (Fig. 4). It is therefore possible that the effects on onset kinetics are due to chelation of AMPA by higher concentrations of zinc. An interesting observation was the fact that leak currents became consistently unstable for several minutes after returning from ZnCl_2 (3.0 mM) to normal extracellular solutions (see Fig. 3, recovery).

DISCUSSION

The data from the present study clearly indicate a change in binding properties of [^3H]AMPA and a slowing of rapid desensitisation of whole cell current responses to AMPA by zinc. The increase of [^3H]AMPA binding in the presence of ZnCl_2 was most pronounced in the absence of KSCN and CaCl_2 . Experiments with ZnCl_2 (10 mM) carried out in buffer containing Tris-HCl (10 mM) and CaCl_2 (2.5 nM) showed only a marginal enhancement of specific [^3H]AMPA binding. This could be taken as evidence that the effect of zinc is not due to a change in the ionic strength of the buffer although the possibility that Ca^{2+} screens the Zn^{2+} binding site can not be excluded. [^3H]AMPA binding assays are usually carried out in the presence of Tris-HCl, CaCl_2 and KSCN in order to increase specific binding (e.g. Honoré and Drejer, 1988) whereas binding assays for NMDA receptor recognition sites (e.g. PCP/MK-801 or strychnine-insensitive glycine binding sites) are usually carried out in the presence of lower concentration Tris-buffers without added CaCl_2 or KSCN. This may be one explanation for the fact that no AMPA binding studies supporting the potentiation of AMPA toxicity by zinc have been reported to date.

The very low affinities determined in saturation and displacement experiments may indicate that the AMPA binding site in the presence of zinc possesses a different pharmacological profile. In the presence of ZnCl_2 there is a loss of high-affinity AMPA binding sites which may be due to a conformational change of the receptor complex. The number of binding sites increased profoundly (around 100-fold) compared to standard assay conditions. It could therefore be supposed that the AMPA binding site in the presence of zinc is the result of an unmasking of non-specific AMPA binding sites or revealing binding to other receptors e.g. high affinity kainate receptors. However, this did not seem to be the case as non-specific binding was not influenced by zinc and specific binding was displaced by standard AMPA receptor ligands in the same rank order of potencies as seen under standard conditions. In addition, this stimulatory effect of zinc on [^3H]AMPA binding is suggested to be specific for this binding site as only inhibitory effects of ZnCl_2 (1 mM) have been reported for both [^3H]glutamate and [^3H]MK-801 binding under comparable conditions (Monahan and Michel, 1987; Reynolds and Miller, 1988). Finally, it could be argued that the AMPA binding site revealed in the presence of zinc is, in fact, more pharmacologically relevant as the profoundly reduced affinities of AMPA and glutamate are actually more in line with those determined in electrophysiological experiments (Kiskin *et al.*, 1986; Trussell and Fischbach, 1989; Patneau and Mayer 1991; Patneau *et al.*, 1992; Hall *et al.*, 1993; Hennegriff *et al.*, 1994; Parsons *et al.*, 1994). On the other hand, zinc has also been reported to enhance kainate binding and neurotoxicity in the hippocampus *in vivo* (Shiraishi *et al.*, 1993; Nave and Conner, 1993). Clearly, a more thorough study on the rank order of potency of several other AMPA/kainate receptor ligands would be necessary to exclude the possibility that the site labelled represents AMPA binding to other receptors.

Previous electrophysiological investigations on cultured hippocampal neurones and *Xenopus* oocytes injected with rat brain mRNA have revealed that zinc has both stimulatory and inhibitory effects on AMPA receptor-mediated currents (Mayer *et al.*, 1989; Rassen-dren *et al.*, 1990). In agreement with the present study, zinc exhibited potentiating effects at AMPA receptors at concentrations up to 300 μM whereas inhibition was seen at higher concentrations. Smart *et al.* (1994) suggested that this dual effect of zinc on AMPA receptors may be mediated via separate mechanisms. Since the present receptor binding studies did not reveal any inhibitory effect on [^3H]AMPA binding, irrespective of the conditions used, the findings reported in this study may provide additional support for this assumption.

It is well accepted that desensitisation of AMPA receptors is accompanied by an increase in agonist affinity (Kiskin *et al.*, 1986; Trussell and Fischbach, 1989; Patneau and Mayer 1991; Patneau *et al.*, 1992; Hall *et al.*, 1993; Hennegriff 1994; Parsons *et al.*, 1994). This in turn, can be taken to imply that high affinity

[³H]AMPA binding represents selective recognition of inactive—i.e. desensitised—high affinity AMPA receptors. Indeed, thiocyanate has also been shown to enhance AMPA receptor desensitisation in electrophysiological studies (Arai *et al.*, 1995). Moreover, some positive modulators of AMPA receptors such as cyclothiazide, which markedly decrease AMPA receptor desensitisation (Livsey *et al.*, 1993; Partin *et al.*, 1993; Patneau *et al.*, 1993; Yamada and Tang, 1993) also decrease high affinity [³H]AMPA binding (Hall *et al.*, 1992, 1993; Hennegriff *et al.*, 1994). As such, one possible explanation for the effects observed in the present study, is that zinc also decreases AMPA receptor desensitisation and high affinity [³H]AMPA binding in a similar manner to cyclothiazide. This effect is, however, masked by the additional antagonistic effects of higher concentrations of zinc on AMPA-induced currents and the appearance of low affinity [³H]AMPA binding sites. Moreover, it should be noted that other positive modulators of AMPA receptors such as certain nootropic drugs and BDP have been claimed to decrease AMPA receptor desensitisation but also to increase high affinity [³H]AMPA binding (Copani *et al.*, 1992; Arai *et al.*, 1994; Lee and Benfield, 1994). Clearly, further studies are necessary to determine whether the effect of zinc on AMPA receptor desensitisation are related to the observed changes in the relative proportions of high and low affinity AMPA recognition sites or due to changes in the coupling of the AMPA recognition site to other domains on the receptor protein.

The potentiation of AMPA binding and plateau AMPA-induced currents by zinc could have important consequences under pathological conditions such as ischaemia. Thus, the presence of high concentrations of zinc in the dentate gyrus might be expected to potentiate the toxic effects of elevated concentrations of glutamate in the hippocampus (for review see Danysz *et al.*, 1995). This idea is supported by the fact that the main effect of lower concentrations of zinc is a reduction of AMPA receptor desensitisation, a process which may normally represent an endogenous mechanism to prevent prolonged pathological activation of AMPA receptors whilst permitting fast AMPA receptor mediated neurotransmission (May and Robinson 1993; Rock and Campbell, 1993; Moudy *et al.*, 1994; see Danysz *et al.*, 1995 for review).

In conclusion, the mechanism by which zinc increases [³H]AMPA binding and decreases AMPA receptor desensitisation remains unclear and indirect effects such as chelation of AMPA or an influence on membrane properties can not be excluded. Moreover, it should be born in mind that most binding assays, including those of the present study, are normally carried out under extremely unphysiological conditions. It is therefore highly speculative to make any conclusive assumptions from receptor binding studies concerning their biological relevance. However, since *in vivo* endogenous zinc concentrations have been reported to reach values of

about 200–300 μ M (Frederickson *et al.*, 1989; Ebadi *et al.*, 1990; Tønder *et al.*, 1990) it is conceivable that the reported observations may be of relevance under excitotoxic conditions.

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REFERENCES

- Aniksztejn L., Charton G. and Ben-Ari Y. (1987) Selective release of endogenous zinc from the hippocampal mossy fibers *in situ*. *Brain Res.* **404**: 58–64.
- Arai A., Kessler M., Ambro-Singerson J., Xiao P., Rogers G. and Lynch G. (1994) A centrally active drug that modulates AMPA receptor gated currents. *Soc. Neurosci. Abs.* **20**: 343–346.
- Arai A., Silberg J., Kessler M. and Lynch G. (1995) Effect of thiocyanate on AMPA receptor mediated responses in excised patches and hippocampal slices. *Neuroscience* **66**: 815–827.
- Assaf S. Y. and Chung S. H. (1984) Release of endogenous zinc from brain tissue during activity. *Nature* **308**: 734–736.
- Cheng Y. C. and Prusoff W. H. (1973) Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **22**: 3099–3108.
- Copani A., Genazzani A. A., Aleppo G., Casabona G., Canonico P. L., Scapagnini U. and Nicoletti F. (1992) Nootropic drugs positively modulate α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-sensitive glutamate receptors in neuronal cultures. *J. Neurochem.* **58**: 1199–1204.
- Cotman C. W., Monaghan D. T., Ottersen O. P. and Storm-Mathisen J. (1987) Anatomical organization of excitatory amino acid receptors and their pathways. *Trends Neuroscience* **7**: 273–280.
- Danysz W., Parsons C. G., Bresink I. and Quack G. (1995) Glutamate in CNS disorders. A revived target for drug development? *Drug News Perspect.* **8**: 261–277.
- Ebadi M., Murrin L. C. and Pfeiffer R. F. (1990) Hippocampal zinc thionein and pyridoxal phosphate modulate synaptic functions. *Ann. N.Y. Acad. Sci.* **585**: 189–201.
- Foster A. C. and Wong E. H. F. (1987) The novel anticonvulsant MK-801 binds to the activated state of the *N*-methyl-D-aspartate receptor in rat brain. *Brit. J. Pharmacol.* **91**: 403–409.
- Frederickson C. J., Klitenick M. A., Manton W. I. and Kirkpatrick J. B. (1983) Cytoarchitectonic distribution of zinc in the hippocampus of man and the rat. *Brain Res.* **273**: 335–339.
- Frederickson C. J., Hernandez M. D. and McGinty J. F. (1989) Translocation of zinc may contribute to seizure-induced death of neurons. *Brain Res.* **480**: 317–321.
- Hall R. A., Kessler M. and Lynch G. (1992) Evidence that high-affinity and low-affinity DL- α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) binding sites reflect membrane-dependent states of a single receptor. *J. Neurochem.* **59**: 1997–2004.
- Hall R. A., Kessler M., Quan A., Ambrosingerson J. and Lynch G. (1993) Cyclothiazide decreases [³H]AMPA binding to rat brain membranes—evidence that AMPA receptor desensitization increases agonist affinity. *Brain Res.* **628**: 345–348.

- Hennegriff M., Hall R. A., Bahr B. A., Neve R. L. and Lynch G. (1994) Effects of cyclothiazide on [³H]AMPA binding to AMPA receptor subunits stably expressed in HEK 293 cells. *Soc. Neurosci. Abs.* **20**: 484(Abs.).
- Honoré T. and Drejer J. (1988) Chaotropic ions affect the conformation of quisqualate receptors in rat cortical membranes. *J. Neurochem.* **51**: 457–461.
- Kislin N. I., Krishtal O. A. and Tsyndrenko A. Y. (1986) Excitatory amino acid receptors in hippocampal neurons: kainate fails to desensitize them. *Neurosci. Lett.* **63**: 225–230.
- Koh J. and Choi D. W. (1988) Zinc alters excitatory amino acid neurotoxicity on cortical neurones. *J. Neurosci.* **8**: 2164–2171.
- Lee C. R. and Benfield P. (1994) Aniracetam—an overview of its pharmacodynamic and pharmacokinetic properties, and a review of its therapeutic potential in senile cognitive disorders. *Drugs and Aging* **4**: 257–273.
- Livsey C. T., Costa E. and Vicini S. (1993) Glutamate-activated currents in outside-out patches from spiny versus aspiny hilar neurons of rat hippocampal slices. *J. Neurosci.* **13**: 5324–5333.
- Lowry O. H., Rosebrough N. J., Farr A. L. and Randell R. J. (1951) Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193**: 265–275.
- May P. C. and Robison P. M. (1993) Cyclothiazide treatment unmasks AMPA excitotoxicity in rat primary hippocampal cultures. *J. Neurochem.* **60**: 1171–1174.
- Mayer M. L., Vyklicky L. Jr. and Westbrook G. L. (1989) Modulation of excitatory amino acid receptors by group IIB metal cations in cultured mouse hippocampal neurones. *J. Physiol.* **415**: 329–350.
- Monahan J. B. and Michel J. (1987) Identification and characterization of *N*-methyl-D-aspartate-specific L-[³H]glutamate recognition site in synaptic plasma membranes. *J. Neurochem.* **48**: 1699–1708.
- Moudy A. M., Yamada K. A. and Rothman S. M. (1994) Rapid desensitization determines the pharmacology of glutamate neurotoxicity. *Neuropharmacology* **33**: 953–962.
- Nave J. M. and Connor J. D. (1993) Influence of ZnCl₂ pretreatment on behavioral and histological responses to kainic acid in rats. *Brain Res.* **604**: 298–303.
- Ottersen O. P. and Storm-Mathisen J. (1986) Excitatory amino acid pathways in the brain. In: *Excitatory Amino Acids and Epilepsy* (Schwarcz R. and Ben-Ari Y., Eds.), pp. 263–284. Plenum Press, NY.
- Parsons C. G., Gruner R. and Rozental J. (1994) Comparative patch clamp studies on the kinetics and selectivity of glutamate receptor antagonism by 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline (NBQX) and 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5h-2,3-benzodiazepine (GYKI 52466). *Neuropharmacology* **33**: 589–604.
- Partin K. M., Patneau D. K., Winters C. A., Mayer M. L. and Buonanno A. (1993) Selective modulation of desensitization at AMPA versus kainate receptors by cyclothiazide and concavalin-A. *Neuron* **11**: 1069–1082.
- Patneau D. K. and Mayer M. L. (1991) Kinetic analysis of interactions between kainate and AMPA: evidence for activation of a single receptor in mouse hippocampal neurons. *Neuron* **6**: 785–795.
- Patneau D. K., Mayer M. L., Jane D. E. and Watkins J. C. (1992) Activation and desensitization of AMPA/kainate receptors by novel derivatives of willardine. *J. Neurosci.* **12**: 595–606.
- Patneau D. K., Vyklicky L. and Mayer M. L. (1993) Hippocampal neurons exhibit cyclothiazide-sensitive rapidly desensitizing responses to kainate. *J. Neurosci.* **13**: 3496–3509.
- Perez-Claussel J. and Danscher G. (1985) Intravesicular localization of zinc in rat telencephalic boutons. A histochemical study. *Brain Res.* **337**: 91–98.
- Peters S., Koh J. and Choi D. W. (1987) Zinc selectively blocks the action of *N*-methyl-D-aspartate on cortical neurones. *Science* **236**: 589–593.
- Rassendren F. A., Lory P., Pin J. P. and Nargeot J. (1990) Zinc has opposite effects on NMDA and non-NMDA receptors expressed in *Xenopus* oocytes. *Neuron* **4**: 733–740.
- Reichling D. B. and MacDermot A. B. (1991) Lanthanum actions on excitatory amino acid-gated currents and voltage-gated calcium currents in rat dorsal horn neurons. *J. Physiol.* **441**: 199–218.
- Reynolds I. J. and Miller R. J. (1988) [³H]MK-801 binding to the NMDA receptor/ionophore complex is regulated by divalent cations: evidence for multiple regulatory sites. *Eur. J. Pharmacol.* **151**: 103–112.
- Rock D. M. and Campbell G. W. (1993) Cyclothiazide and GYKI-52466 affect AMPA-induced neuronal damage of cultured cortical neurons by actions at distinct sites. *Soc. Neurosci. Abs.* **19**: 294.
- Shiraishi K., Nakazawa S. and Ito H. (1993) Zinc enhances kainate neurotoxicity in the rat brain. *Neurol. Res.* **15**: 113–116.
- Smart T. G., Xie X. M. and Krishek, B. J. (1994) Modulation of inhibitory and excitatory amino acid receptor ion channels by zinc. *Prog. Neurobiol.* **42**: 393–441.
- Tønder N., Johansen F. F., Frederickson C. J., Zimmer J. and Diemer N. H. (1990) Possible role of zinc in the selective degeneration of dentate hilar neurones after cerebral ischemia in the adult rat. *Neurosci. Lett.* **109**: 247–252.
- Trussell L. D. and Fischbach G. D. (1989) Glutamate receptor desensitization and its role in synaptic transmission. *Neuron* **3**: 209–218.
- Weiss J. H., Hartley D. M., Koh J. Y. and Choi D. W. (1993) AMPA receptor activation potentiates zinc neurotoxicity. *Neuron* **10**: 43–49.
- Yamada K. A. and Tang C. M. (1993) Benzothiadiazides inhibit rapid glutamate receptor desensitization and enhance glutamatergic synaptic currents. *J. Neurosci.* **13**: 3904–3915.