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Role of zinc in blockade of excitotoxic action of quinolinic acid by picolinic acid

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Summary. This study examined whether picolinic acid (PIC) inhibits quinolinic acid (QUIN) – induced excitotoxicity through zinc chelation. Injection of QUIN into the nucleus basalis magnocellularis significantly depleted cortical choline acetyltransferase activity 7 days post injection and PIC inhibited this response. Zinc augmented the QUIN- but not NMDA-induced response. When PIC was co-administered with zinc, PIC failed to attenuate the QUIN-induced response. The inhibition of QUIN – induced cholinergic toxicity by PIC may involve chelation of zinc.

Keywords: Picolinic acid – Quinolinic acid – Zinc – Excitotoxicity

Introduction

Metabolism of tryptophan via the kynurenine pathway yields quinolinic acid (QUIN) and other products which have the ability to activate or block central excitatory amino acid (EAA) receptors (Schwarcz et al., 1984). QUIN, a pyridine dicarboxylic acid, is an NMDA receptor agonist which produces neuronal excitation and excitotoxic cell death after focal injections into specific brain areas. In contrast, kynurenic acid acts as a non-selective EAA receptor antagonist which blocks the excitatory (Stone and Connick, 1985) and excitotoxic (Jhamandas et al., 1994) action of QUIN and other NMDA or non-NMDA receptor agonists. Picolinic acid (PIC), a pyridine monocarboxylate, itself lacks excitatory or excitotoxic effects but acts as a low potency antagonist against the excitotoxic action of QUIN on brain cholinergic and dopaminergic neurons (Cockhill et al., 1992; Beninger et al., 1994). The mechanism by which PIC exerts this action is unknown. Since PIC chelates zinc (Suzuki et al., 1957), a divalent cation which modulates the ionic responses mediated by EAA receptors (Smart et al., 1994), this property may contribute to its antagonism of QUIN-induced excitotoxicity. To examine this possibility the present study investigated the interaction of QUIN and PIC with zinc in an *in vivo* model of central cholinergic toxicity.

Materials and methods

Male Sprague-Dawley rats (275–300 g) were anaesthetized with 4% halothane and placed in a stereotaxic frame. A stainless steel cannula was placed in the right nucleus basalis magnocellularis (nbm) using the co-ordinates: 2.6 mm lateral, 0.8 mm posterior to bregma, and ventral to the skull surface with incisor bar set at –3.3 mm (see Cockhill et al., 1992) An injection of 1ul saline or solution of the test agent was infused through the cannula over 2.5 minutes and the cannula retained for an additional 3 minutes. Seven days post recovery, the animal was sacrificed, the brain removed and the frontoparietal cortex was dissected from injected and uninjected sides for assay of choline acetyltransferase (ChAT) by the method of Fonnum (1975). The protein content was measured using the method of Lowry et al. (1951). Enzyme activity values for injected and un-injected hemispheres in the same animal were compared to determine changes produced by different treatments. The data were expressed as mean ± SEM and compared using a one-way analysis of variance followed by Newman-Keuls test. Differences in mean values were considered significant at $p < 0.05$.

Results

An unilateral injection of QUIN (60 nmol) into the rat nbm produced approximately 30% decrease in cortical ChAT activity 7 days post-injection when compared with a similar injection of saline (Fig. 1A). Co-injection of PIC with QUIN in different molar ratios produced a dose-related attenuation of QUIN action on cortical ChAT activity (Fig. 1A). The highest dose of PIC which itself did not influence cortical ChAT activity (Fig. 2), abolished the QUIN effect. In contrast, when QUIN (60 nmol) was co-injected with different doses of zinc, the cation significantly enhanced the effect of QUIN on cortical ChAT activity (Fig. 1A). Zinc by itself did not significantly influence cortical ChAT activity.

Figure 1B shows comparative action of zinc on the effects of 30 nmol dose of QUIN or NMDA on cortical ChAT activity. Injection of QUIN into the nbm produced a decrease in cortical ChAT comparable to that produced by the saline injection (Fig. 1B). However, when co-injected with zinc this dose of QUIN produced a significant depletion of cortical ChAT activity. Administration of 30 nmol NMDA into the nbm produced approximately 25% decrease in cortical ChAT activity. When NMDA was delivered with zinc there was no enhancement of its action on cortical ChAT activity. Thus, zinc augmented the effect of QUIN but not of NMDA.

In subsequent experiments the potential of zinc to influence the inhibitory effect of PIC on QUIN-induced depletion of cortical ChAT activity was examined. In these experiments, QUIN (60 nmol) was delivered into the nbm with both PIC and zinc. Injection of QUIN alone reduced cortical ChAT 7 days post injection and PIC significantly attenuated this response (Fig. 2). However, when PIC was co-administered with the higher of the two zinc doses studied, it failed to antagonize the action of QUIN on cortical ChAT activity. In the presence of both PIC and zinc, the magnitude of the QUIN effect was not significantly different from that seen in the absence of these two agents. At the doses tested, zinc alone did not significantly affect cortical ChAT activity. Thus, in these experiments the inhibitory action of PIC against QUIN could not be observed in the presence of zinc.

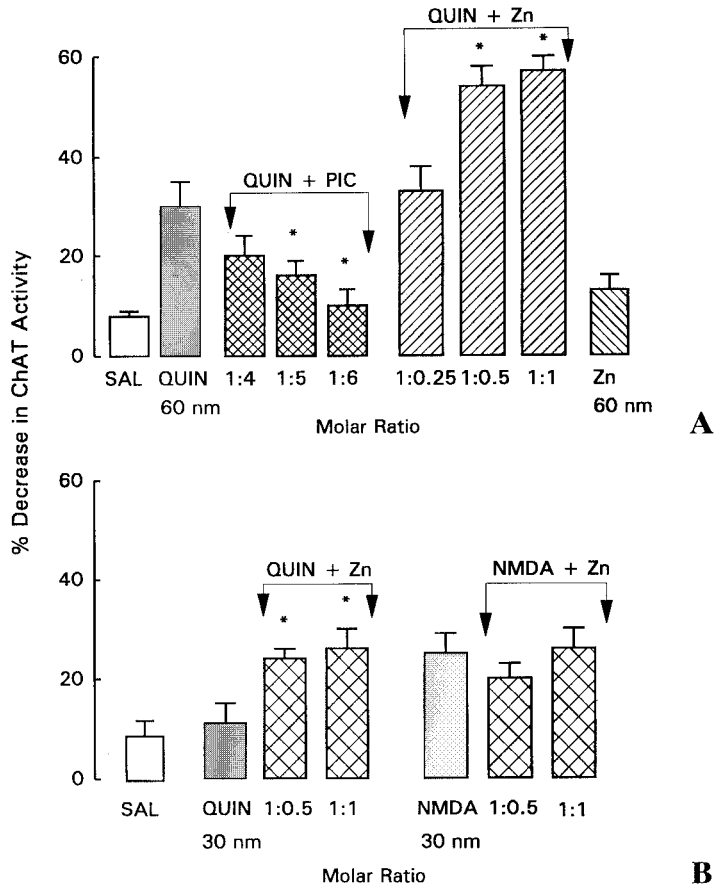

Fig. 1. **A** Changes in cortical ChAT activity 7 days after unilateral injection of different molar ratios of quinolinic acid (*QUIN*) and picolinic acid (*PIC*) or QUIN and zinc into the nucleus basalis magnocellularis. Each value is the mean ± SEM from four to six experiments. * Significantly different ($p < 0.05$) from QUIN alone. **B** Changes in cortical ChAT activity 7 days after unilateral injection of different molar ratios of quinolinic acid (*QUIN*) and zinc or NMDA and zinc into the nucleus basalis magnocellularis. Each value is the mean ± SEM from four experiments. * Significantly different ($p < 0.05$) from QUIN alone

Discussion

The reduction of cortical ChAT activity, a biochemical marker of cholinergic neurons, produced by focal QUIN injection reflects loss of cholinergic neurons projecting from the nbm to the fronto-parietal cortex. This study shows that PIC, a zinc chelator, inhibits QUIN-induced cholinergic toxicity. If the PIC effect is related to zinc chelation then this cation would be expected to augment QUIN-induced toxicity. Indeed, zinc augmented the neurotoxic response to the focal injection of QUIN. This facilitatory effect of zinc showed selectivity for QUIN since a similar response to the injection of NMDA was not affected. QUIN and NMDA apparently produce cholinergic excitotoxicity by activating different types of NMDA receptors (Pawley et

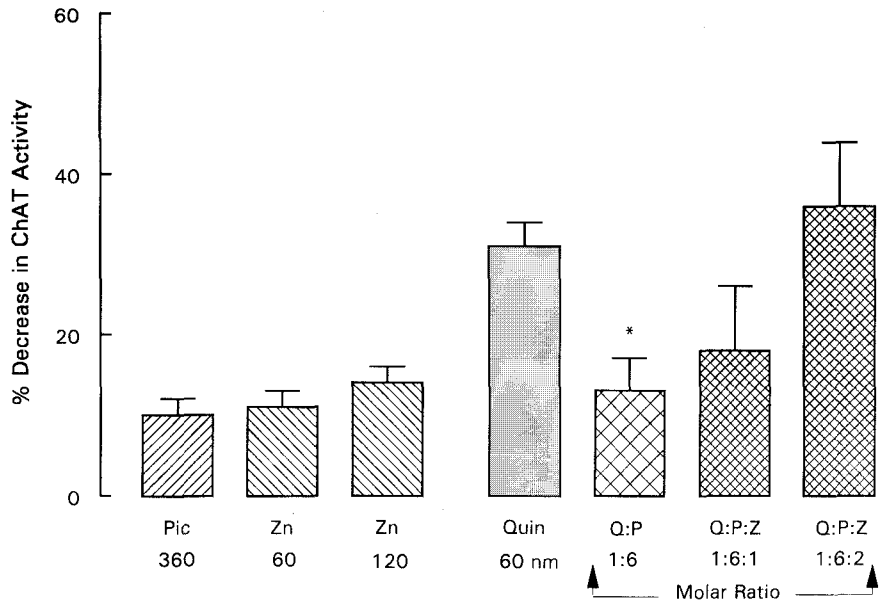

Fig. 2. Effect of picolinic acid (*PIC*) with and without zinc on the depletion of cortical ChAT activity produced by unilateral injection of quinolinic acid (*QUIN*) into the nucleus basalis magnocellularis. Each value is mean ± SEM from four to six experiments. * Significantly different ($p < 0.05$) from the effect of quin (60 nmol)

al., 1996). Thus, ability of zinc to selectively influence QUIN's effect suggests that this cation exerts its action primarily at the QUIN-sensitive NMDA receptor subtype. The finding that zinc facilitates the effect of an NMDA receptor agonist may appear to be at variance with the general view that zinc inhibits NMDA receptor function. However, molecular studies on NMDA receptors have shown that submicromolar concentrations of zinc can potentiate the ionic function of certain splice variants of the NMDA receptors (Hollman et al., 1993).

The inhibitory effect of PIC on QUIN-induced depletion of ChAT activity was not observed in the presence of zinc. This observation suggests that *endogenous* zinc is essential for the neurotoxic action of QUIN on the nbm cholinergic neurons and PIC most likely inhibits this action by chelating *endogenous* zinc. However, when PIC is administered with exogenous zinc it loses it ability to chelate endogenous zinc and thus fails to inhibit the QUIN effect. The involvement of zinc in PIC's action is further supported by the observation that known zinc chelators such as dipicolinic acid (Cockhill et al., 1992) and diethyldithiocarbamate (Lee et al., 1996) can inhibit QUIN toxicity in the cholinergic model.

Acknowledgements

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