British Journal of Pharmacology (2000) 130, 851–856
www.nature.com/bjp

Interaction between copper and zinc at $GABA_A$ receptors in acutely isolated cerebellar Purkinje cells of the rat

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1 Nanomolar concentrations of Cu^{2+} induce a slowly reversible block of $GABA_A$ receptor-mediated currents which can be removed by chelating substances.

2 The possible interaction of Cu^{2+} with the Zn^{2+} binding site on the $GABA_A$ receptor complex was studied in acutely isolated Purkinje cells using whole-cell recording and a fast drug application system.

3 When Zn^{2+} was applied together with 2 μM GABA, the Zn^{2+}-induced block of GABA-mediated currents was not additive to the Cu^{2+}-induced block. In the presence of 0.1 μM Cu^{2+} in the bath solution the degree of inhibition of GABA-mediated responses by Zn^{2+} was strongly attenuated.

4 Preapplication of 100 μM Zn^{2+} during 10 s, terminated 1 s before exposure to 2 μM GABA did not affect the GABA current in Cu^{2+}-free solution, but relieved its block by 0.1 μM Cu^{2+}. This effect of Zn^{2+} was concentration-dependent with an EC_{50} of 72 μM.

5 When the Cu^{2+}-induced block was removed by histidine, preapplication of Zn^{2+} did not increase the GABA current, indicating that the relief of Cu^{2+} block by Zn^{2+} is the result of its ability to actively remove Cu^{2+} from the GABA receptor complex.

6 It is proposed that the inhibitory effects of Zn^{2+} and Cu^{2+} on GABA-induced currents result from an action of these metal ions at distinct, but conformationally linked sites on the $GABA_A$ receptor protein. Under physiological conditions Zn^{2+} would liberate Cu^{2+} from the $GABA_A$ receptor, thus facilitating Cu^{2+} turnover and its binding by other endogenous chelating molecules. *British Journal of Pharmacology* (2000) **130,** 851–856

Keywords: Cu^{2+}; Zn^{2+}; GABA; cerebellum; histidine; patch-clamp; Wilson disease

Introduction

Cu^{2+} and Zn^{2+} are essential nutrients with close physiological interactions. High dietary levels of Zn^{2+} depress, Zn^{2+}-deficiency increases Cu^{2+} absorption (Cousins, 1985). Both Zn^{2+} and Cu^{2+} are cofactors in the metalloenzyme Cu^{2+}, Zn^{2+}-superoxide dismutase. Zn^{2+} competes with Cu^{2+} for the binding site, it decreases the ability of Cu^{2+} to transfer electrons in certain conditions. Both metals are present in remarkably high levels in the brain, largely bound to proteins; the exact levels of the free ions in the extracellular space are not known. They occur in presynaptic terminals and are released with synaptic activity (Assaf & Chung, 1984; Frederickson, 1989; Hartter & Barnea, 1988; Kardos *et al*., 1989). Zn^{2+} ions can modulate a number of ligand- and voltage-gated ion channels (Harrison & Gibbons, 1994; Xie & Smart, 1991). Both Zn^{2+} and Cu^{2+} block $GABA_A$ receptor-mediated currents. The mechanisms of Zn^{2+} and Cu^{2+} modulation of GABA-mediated responses have been analysed separately in a number of preparations (Celentano *et al*., 1991; Draguhn *et al*., 1990; Kilic *et al*., 1993; Ma & Narahashi, 1993; Smart & Constanti, 1990; Smart *et al*., 1991; Trombley & Shepherd, 1996; White & Gurley, 1995; Yakushiji *et al*., 1987). Cu^{2+} and Zn^{2+} had a very similar action on GABA-mediated responses when studied in dorsal root ganglion cells. In this preparation Cu^{2+} antagonized the blocking action of Zn^{2+}, suggesting that Cu^{2+} and Zn^{2+} may share a common site on the $GABA_A$ receptor.

We have recently found copper blocking GABA-induced currents on Purkinje cells with very high affinity (Sharonova *et al*., 1998). The block appeared at Cu^{2+} concentrations of about 10 nM, half-maximal inhibition (IC_{50}) at 35 nM. It developed within about 1 min but washout took several minutes. We have now analysed the interaction between these two trace metals and suggest closely related but not identical sites of action at the $GABA_A$ receptor.

Methods

Preparation of Purkinje cells

Neurons were isolated from cerebella of 2–3 week-old male Wistar rats (Versuchstieranstalt Heinrich-Heine-Universität, Düsseldorf). Saggital slices were cut by vibratome perpendicularly to the cerebellar cortex surface and were incubated at room temperature for 1–8 h on a mesh near the bottom of a 150 ml beaker. The incubation solution was continuously bubbled with carbogene (5% CO_2 + 95% O_2). The solution had a following composition (in mM): NaCl 125; KCl 5; $CaCl_2$ 1.5; $MgCl_2$ 1.5; NaH_2PO_4 1.28; $NaHCO_3$ 25; glucose 10; phenol red 0.01%. One at a time, slices were transferred to the recording chamber and neurons were dissociated using a vibrating tip of a fused glass pipette (Vorobjev, 1991). The solution for dissociation and recording had the following composition (in mM): NaCl 150; KCl 5; $CaCl_2$ 2.7; $MgCl_2$ 2.0; HEPES 10, pH adjusted to 7.4 with NaOH. In most experiments 100 nM Cu_2SO_4 was present in the solution.

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Whole-cell recording

Voltage-clamp recording was obtained using the whole-cell configuration of the patch-clamp technique (Hamill *et al*., 1981). Glass recording patch pipettes were prepared from filament-containing borosilicate tubes using a two-stage puller. The electrodes, having resistances of 4–5 MΩ, were filled with recording solution of the following composition (in mM): KCl 140; $CaCl_2$ 0.5, $MgCl_2$ 4; HEPES 10; EGTA 5; ATP-Na 3 (pH adjusted to 7.3 with KOH). When high concentrations of GABA (10–50 μM) were used, recordings were performed with an intracellular solution containing low Cl^- concentration resulting in a reduced amplitude of agonist induced current, in order to minimize the error due to the voltage drop through the uncompensated series resistance. 125 mM KCl in the intracellular solution were replaced by potassium methanesulphonate. Recordings were carried out at room temperature (22–26°C) using an EPC-9 patch-clamp amplifier. Currents were filtered at 2 kHz, sampled at 1 kHz, and stored on a computer disk. Data were collected with commercially available software (TIDA for Windows). The holding potential was maintained at −70 mV.

Drug application

A fast perfusion technique was used to apply GABA and drugs (Vorobjev *et al*., 1996). Isolated Purkinje cells were first patch clamped and then lifted into the outflow of the control bath solution. The substances were applied through two different glass capillaries, 0.1 mm in diameter, and, for continuous perfusion, they were added to the bath solution. The delivery ports of the capillaries were positioned within 0.4 mm from the cell under study. For exposure the application system was moved so as to place the cell in the solution stream leaving one of the application capillaries. One capillary was used to apply GABA or GABA together with substances (coapplication). Another capillary was used for perfusion of the cell with substances applied without GABA. The flow through each tube was gravity-driven. For activation of $GABA_A$ channels, in most experiments 2 μM GABA was applied for periods of 1 s, at 30 s intervals. Cu^{2+} (Cu_2SO_4) and Zn^{2+} ($ZnCl_2$) were dissolved in the perfusate and applied extracellularly. All reagents were obtained from Sigma, Deisenhofen, Germany.

Data analysis

To quantify the inhibitory effects of Zn^{2+} upon the current induced by a constant GABA concentration the following equation was used:

$$I = 1 - I_{max}/(1 + (IC_{50}/[Zn^{2+}])^n) \quad (1)$$

where I_{max} is the maximal degree of block of the GABA-mediated response achieved by the tested ligand, IC_{50} is the concentration of the ligand producing a half-maximal block of GABA-mediated responses, and n is the Hill coefficient. The removal of Cu^{2+}-induced block of GABA responses by preapplication of Zn^{2+} was fitted by the equation

$$I = (I_0 - I_{Cu})/(1 + (EC_{50}/[Zn^{2+}])^n) \quad (2)$$

where I_{Cu} is the amplitude of GABA responses blocked by Cu^{2+} and I_0 the amplitude of the control GABA response and EC_{50} is the concentration of Zn^{2+} producing a half-maximal restoration of the GABA response. Other values have the meaning noted above. Data values are presented as mean ± s.e.mean.

Results

Blocking actions of Cu^{2+} and Zn^{2+}

We studied the effect of Zn^{2+} on GABA-induced current in control and in the presence of Cu^{2+} in the bathing medium. In the absence of copper in the outer solutions, Zn^{2+}, applied together with GABA, suppressed the responses to GABA in a concentration-dependent manner (Figure 1A). The Zn^{2+} induced block was fitted by the logistic equation with the following parameters: IC_{50} 36 ± 5 μM with an I_{max} of 0.66 ± 0.05 and a Hill slope of 1 ± 0.2 ($n = 5$). The presence of 100 nM Cu^{2+} in the bath solution – which itself reduced GABA induced currents to about 40% of control – markedly changed the concentration-response curve for inhibition of GABA induced currents by Zn^{2+} (Figure 1B). Figure 1C presents the analysis of six experiments carried out in Cu^{2+}-free solutions and in the presence of 0.1 μM Cu^{2+} in the bath solution. Inhibition of GABA-mediated responses by Zn^{2+} was strongly attenuated in the presence of Cu^{2+}. Moreover, at Zn^{2+} concentrations of 10 and 30 μM the amplitude of GABA-mediated currents was larger than in the presence of Cu^{2+} alone. Therefore, the dose-response curve for Zn^{2+} in the presence of Cu^{2+} was biphasic. Although the complex nature of the dose-response curve makes a quantitative analysis difficult, inspection of Figure 1C shows that the apparent IC_{50} for inhibition of the GABA mediated response by Zn^{2+} is increased in the presence of Cu^{2+}. Besides this, after coapplication of GABA and 100 μM Zn^{2+} in the presence of 0.1 μM Cu^{2+} the subsequent response to GABA was slightly enhanced (Figure 1B).

Relief of Cu^{2+} inhibition by Zn^{2+}

The increase of GABA current during coapplication with Zn^{2+} suggests a partial rescue from Cu^{2+} block by Zn^{2+}. We therefore examined the effect of Zn^{2+}-preapplication on Cu^{2+}-induced inhibition of GABA responses. Preapplication of Zn^{2+} for 10 s was terminated 1 s before GABA exposure. Under control conditions (Cu^{2+}-free solution) preapplication of Zn^{2+} (10–300 μM) for 10 s terminated 1 s before GABA exposure did not affect the GABA current (data not shown). The one second (1 s) interval between applications of Zn^{2+} and GABA was essential to minimize the direct blocking effect of Zn^{2+} on GABA responses (Figure 2B). When GABA currents were suppressed in the presence of 0.1 μM Cu^{2+} in the bath solution preapplication of 100 μM Zn^{2+} increased the amplitude of GABA-mediated response, in contrast to the inhibitory effect of high Zn^{2+} concentrations applied together with GABA (Figure 3B). In this set of experiments Zn^{2+} containing solutions were Cu^{2+}-free.

The copper-induced block of GABA current can be easily and reversibly removed by short exposure to a metal chelator such as histidine (Sharonova *et al*., 1998). In order to determine whether Zn^{2+} augments the GABA responses by removing Cu^{2+} from the receptor molecule, we compared the effects of Zn^{2+} and histidine on GABA responses suppressed by Cu^{2+}. To avoid the formation of histidine Zn^{2+} was preapplied during 10 s as before. Figure 3A demonstrates that preapplication of 100 μM Zn^{2+} (at vertical arrows) increases the GABA current suppressed by Cu^{2+}. However, when the Cu^{2+} block was removed by coapplication of GABA with 50 μM histidine, preapplication of Zn^{2+} failed to further augment the response to GABA. Thus the potentiating effect of Zn^{2+} is occluded by histidine-chelation of Cu^{2+}. Figure 3B illustrates that a GABA response relieved from copper block

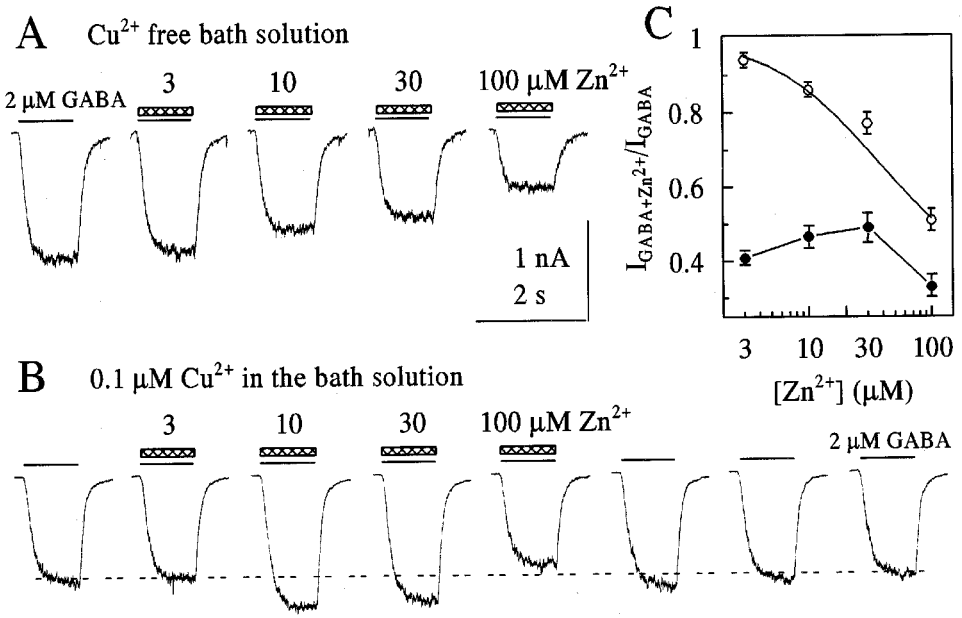

Figure 1 Copper and zinc block GABA mediated currents. (A) illustrates the block of currents evoked by sequential application of 2 μM GABA + 0, 3, 10, 30, 100 μM Zn^{2+} in the absence of Cu^{2+} in the bath solution. (B) shows results from another cell to which the same set of solutions was applied, however in the presence of 100 nM Cu^{2+} in the bath solution. Applications were made every 30 s and are marked by thin lines (GABA) and hatched bars (Zn^{2+}). The response following trace 5 (coapplication of GABA and 100 μM Zn^{2+}) displays still a partial removal of Cu^{2+}-block by the preceding Zn^{2+}-application. (C) In Cu^{2+}-free bath solution Zn^{2+} blocks GABA mediated currents in a concentration-dependent manner (open circles). Data points are fitted in equation 1 with $I_{max} = 0.66 \pm 0.05$, $IC_{50} = 36 \pm 5$ μM; $n = 1 \pm 02$. In the presence of 100 nM Cu^{2+} the concentration-response curve is biphasic (filled circles). Data points are normalized to the response elicited by 2 μM GABA in Cu^{2+}-free solution. Each data point averages the responses of six cells.

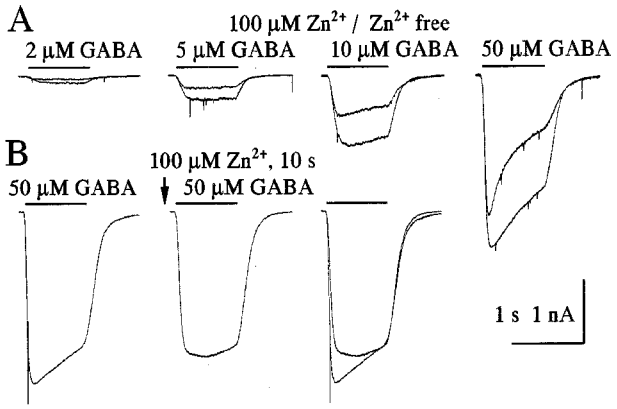

Figure 2 Properties of zinc inhibition of GABA-induced currents in Purkinje cells. (A) The inhibitory effect of Zn^{2+} does not depend on GABA concentration. Responses to application of different concentrations of GABA and GABA + 100 μM Zn^{2+} obtained from a single cell. (B) Rapid reversal of Zn^{2+} inhibition. Responses to 50 μM GABA in control, after preapplication of 100 μM Zn^{2+} and superposition of two traces. Interval between GABA applications was 90 s, duration of GABA exposure 1 s, zinc application was for 10 s (arrow) terminating at the beginning of trace 2. Recovery from the zinc effect occurs within less than 1 s.

by preapplied Zn^{2+} can be blocked by Zn^{2+} when coapplied with GABA. The further traces are sequential GABA responses showing that, without Zn^{2+} preapplication (without removal of copper block), the GABA response is only slightly reduced by 100 μM Zn^{2+}. Thus, the effect of Zn^{2+} on the GABA response in the presence of 0.1 μM Cu^{2+} depends on the way of Zn^{2+} administration. Preapplication of Zn^{2+} during 10 s increased subsequent responses to GABA in a dose-dependent way with an EC_{50} of 72 ± 13 μM and a Hill slope of 1.35 ± 0.3 (five cells, Figure 4).

Zn-induced block at different GABA concentrations

We have previously shown that an increasing GABA concentration decreased the blocking effect of Cu^{2+} (Sharonova *et al*., 1998). In contrast, the Zn^{2+}-induced block of GABA responses could not be surmounted by increasing the GABA concentration (Figure 2A). The block induced by 100 μM Zn^{2+} was about the same at 2 μM GABA (to 0.55 ± 0.05) and at 50 μM GABA (to 0.49 ± 0.07) ($n = 4$ cells). Thus, Zn^{2+} antagonizes GABA responses in cerebellar Purkinje cells in a non-competitive way.

Discussion

Do Cu^{2+} and Zn^{2+} bind to a common site?

The inhibitory action of Zn^{2+} on GABA-activated current is well documented (Celentano *et al*., 1991; Draguhn *et al*., 1990; Kilic *et al*., 1993; Smart *et al*., 1991; White & Gurley, 1995) as a direct interaction of Zn^{2+} with the $GABA_A$ receptor/ion channel complex. Studies on recombinant $GABA_A$ receptors have demonstrated that the action of Zn^{2+} requires presence of the γ subunit. A Zn^{2+} binding site seems to be present on channels that contain only $\alpha 1$ or $\beta 2$ or a combination of these subunits. Zn^{2+} can block the current evoked by GABA in these channels with high potency (IC_{50} about 1 μM). When either the α or the α and β subunits were co-expressed with the γ subunit, $GABA_A$ receptors were rather insensitive to Zn^{2+} (Draguhn *et al*., 1990; Smart *et al*., 1991). Purkinje cells seem to contain only $\alpha 1$, $\beta 2$, $\beta 3$ and $\gamma 2$ subunit mRNAs (Laurie *et al*., 1992; Persohn *et al*., 1992). In spite of the presence of the $\gamma 2$ subunit in $GABA_A$ receptors on Purkinje cells, their sensitivity to Zn^{2+} was not as low (IC_{50} 36 μM) as might have been expected from studies on recombinant $GABA_A$ receptors

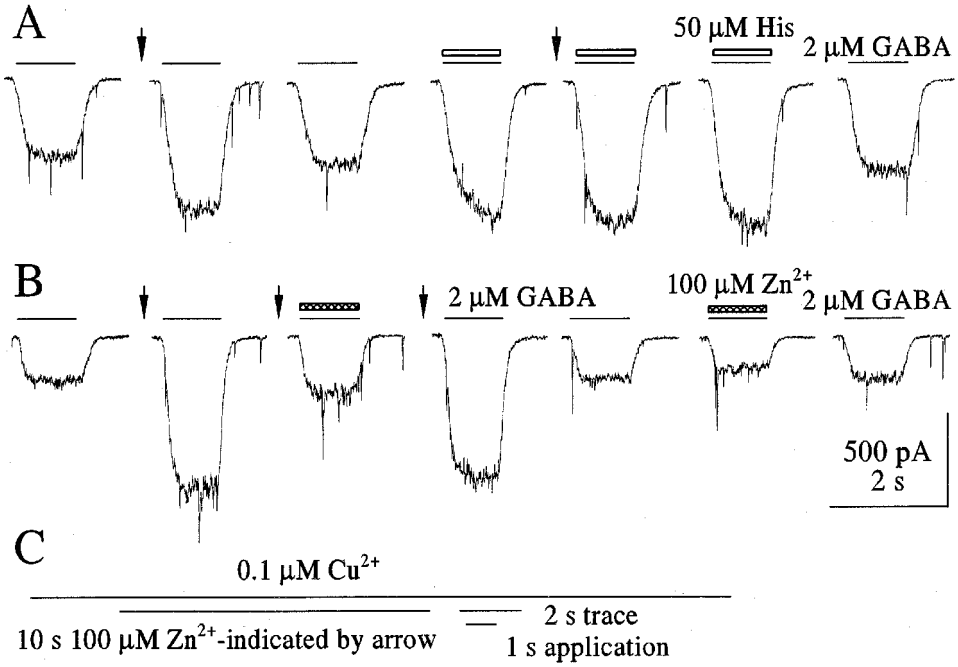

Figure 3 Brief Zn^{2+} exposure reduces copper induced block of GABA mediated currents. (C) Real-time-scheme of drug exposures: 100 nM Cu^{2+} was present throughout, the times of exposure to Zn^{2+}, GABA, GABA + histidine and GABA + Zn^{2+} are indicated. The time for the two second traces illustrated in A and B is also given. The 10 s preapplication of 100 μM Zn^{2+} (at vertical arrows in A and B) which was terminated 1 s before GABA application removed the Cu^{2+} block. Two μM GABA (lines above traces) was applied for 1 s with 30 s intervals. (A) Sequential traces illustrate that the Zn^{2+} preapplication enhances the amplitude of GABA-induced responses. This effect is occluded by 50 μM histidine (open bars) applied together with GABA. (B) Illustrates sequential traces in another cell. Zn^{2+} preapplication enhanced the GABA response. Zn^{2+} applied together with GABA (filled bar above trace) markedly reduced this enhancement (third trace). Without Zn^{2+} preapplication Zn^{2+} did not alter the response significantly (sixth trace).

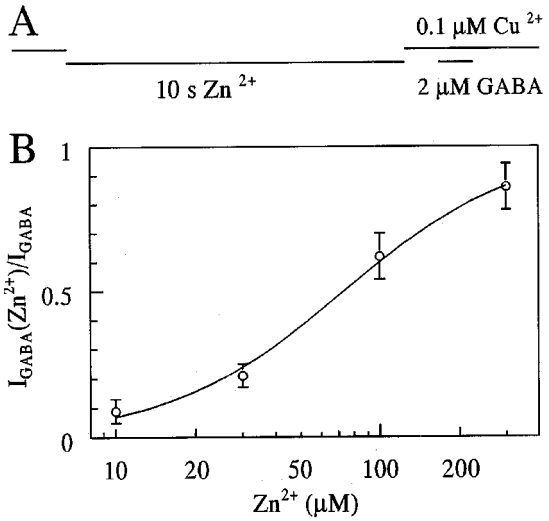

Figure 4 Zn^{2+} reduces Cu^{2+}-block in a concentration dependent manner. (A) Real-time-scheme of experiments; upper line: Cu^{2+}, lower lines Zn 10–300 nM for 10 s and GABA 2 μM for 1 s. data points in the dose response curve are normalized to control GABA responses and represent the mean responses of five cells. The data are fitted by equation 2 with $EC_{50} = 72 \pm 13$ μM, $n = 1.35 \pm 0.3$.

(Draguhn *et al*., 1990; White & Gurley, 1995). This may result from the influence of some other factors determining the sensitivity of the $GABA_A$ receptor to Zn^{2+} (Berger *et al*., 1998).

The mechanism of Zn^{2+} antagonism varies in different cell types. Zn^{2+} noncompetitively inhibits GABA-activated current from neurons of rat superior cervical ganglion (Smart & Constanti, 1990), hippocampus (Legendre & Westbrook, 1991; Mayer & Vyklicky, 1989), cerebellar granule cells (Kilic *et al*., 1993), from dorsal root ganglion neurons (Ma & Narahashi, 1993) and from recombinant $GABA_A$ receptors composed from α and β subunits (Draguhn *et al*., 1990; Smart *et al*., 1991). Zn^{2+} inhibition of some recombinant $GABA_A$ receptors is surmountable by GABA, indicating competitive antagonism (White & Gurley, 1995). In spinal cord neurons the Zn^{2+} block was partially surmountable when the agonist concentration was raised (Celentano *et al*., 1991). A general model of Zn^{2+} inhibition of $GABA_A$ receptors proposes that Zn^{2+} binds to a single site that allosterically induces two unconducting states; the site affinity is state dependent and controlled by the γ-subunit (Gingrich & Burkat, 1998). The inability to overcome Zn^{2+} inhibition at high GABA concentration in our study is consistent with a noncompetitive mechanism.

While the mechanisms of Zn^{2+} modulation of GABA mediated responses have been widely studied, information regarding the modulation of GABA-induced currents by Cu^{2+} is limited (Yakushiji *et al*., 1987; Trombley & Shepherd, 1996). Recently, we have described the properties of Cu^{2+}-induced inhibition of GABA receptor currents in cerebellar Purkinje cells (Sharonova *et al*., 1998). The blocks of GABA responses by Zn^{2+} and Cu^{2+} ions in these neurons differ in several respects. Unlike Zn^{2+} inhibition, the effect of Cu^{2+} decreased with increasing GABA concentration, suggesting a competitive mode of interaction. In addition, Cu^{2+} acts with a higher apparent affinity than Zn^{2+} (IC_{50} 0.035 μM for Cu^{2+} *versus* 36 μM for Zn^{2+}). On the other hand, we have found an obvious interaction between the blocking effects of these metal ions. First, suppression of GABA responses by Zn^{2+} is diminished in the presence of Cu^{2+}. Second, preapplication of Zn^{2+} can completely relief the block of $GABA_A$ receptors induced by Cu^{2+}. When Cu^{2+}-induced block was removed by histidine, preapplication of Zn^{2+} did not further increase the

GABA-current, suggesting that the interaction of Zn^{2+} with the $GABA_A$ receptor results in the removal of Cu^{2+} from its binding site during this brief exposure. The decrease of the relative degree of Zn^{2+} inhibition in the presence of Cu^{2+} is explained by a model assuming that these two metal ions compete for the same binding site on the $GABA_A$ receptor. However, the relief of Cu^{2+} block by Zn^{2+} preapplication is not consistent with a passive substitution of Cu^{2+} by Zn^{2+} because, as we have found previously (Sharonova *et al*., 1998), Cu^{2+}, in contrast to Zn^{2+} ions dissociate too slowly ($\tau \leqslant 3$ min) from the $GABA_A$ receptor to allow their unbinding from the protein just for the period of Zn^{2+} application (maximally 10 s); the copper binding sites remain occupied and unavailable for zinc during this time.

On the other hand, Cu^{2+}-induced block can easily be removed by exposure to histidine for 10 s (Sharonova *et al*., 1998). To explain the fast recovery of the GABA response from Cu^{2+} block we have to assume that histidine chelates not only free Cu^{2+}, but also Cu^{2+} bound on the protein, and removes it from this site. When we preapplied Zn^{2+}, we observed a similar recovery from Cu^{2+} block, which was much faster than passive dissociation of Cu^{2+} from the $GABA_A$ receptor. Zn^{2+} seems to accelerate Cu^{2+} dissociation. The concentration response curve (Figure 4) shows that no removal of block occurs with only 10 μM Zn^{2+}. The occupation of the Cu^{2+} binding site can be qualified as quasistationary; no turnover of Cu^{2+} occurs as the medium does not contain Cu^{2+} which could occupy vacant sites. The relief of Cu^{2+} block is only seen with higher Zn^{2+} concentrations. Since Zn^{2+} cannot interact with the binding site occupied by Cu^{2+}, it has to bind to another site to facilitate Cu^{2+} dissociation. Thus, our results are consistent with the existence of different, but conformationally linked binding sites for Zn^{2+} and Cu^{2+} ions on the $GABA_A$ receptor molecule. We propose that the binding of Zn^{2+} lowers the affinity for Cu^{2+} and causes subsequent dissociation of Cu^{2+} from its binding site as a result of negative cooperativity between the binding sites for Cu^{2+} and Zn^{2+}.

The existence of distinct sites for zinc and copper cations on the $GABA_A$ receptor is supported by a study (Fisher & Macdonald, 1998) demonstrating that the His residue located in the M2–M3 extracellular domain of the α6 subtype (rat α6 H273) plays an important role in determining the sensitivity of recombinant $GABA_A$ receptors to zinc, but not to copper. The α6-subtype confers a relatively low sensitivity to copper, and replacement of the H273 with Asn did not affect inhibition by copper. Studies on rat α1 and α6 chimeras also suggest that the extracellular N-terminal domain of the α1 subunit contributes to a regulatory site(s) for divalent cations, conferring high sensitivity to inhibition by copper and cadmium. These findings are in keeping with our suggestion that inhibitory effects of Cu^{2+} and Zn^{2+} on GABA-induced currents result from interaction of these metal ions with distinct, but allosterically connected binding sites on the $GABA_A$-receptor.

Functional significance

Release of Cu^{2+} and Zn^{2+} into the extracellular space from cortical and hypothalamic synaptosomes was observed upon depolarization (Hartter & Garnea, 1988; Kardos *et al*., 1989). According to the estimate made by Kardos *et al*. (1989), the concentration of Cu^{2+} in the synaptic cleft is in the range of 100–250 μM. Most of the extracellular Cu^{2+} in the brain is not free copper, it is bound to protein. But under reduced amounts of Cu-binding proteins such as caerulopasmin or the PrPc (Brown *et al*., 1997) increased free copper levels and their pathogenic consequences are to be expected. Synaptically released Zn^{2+} may, depending on the free copper level or the occupancy of proteins with copper, have potentiating or inhibiting effects on gabaergic transmission. In physiological conditions Zn^{2+} would liberate Cu^{2+} from $GABA_A$ receptors, thus facilitating Cu^{2+} turnover and its binding by other endogenous molecules which are more effective in chelating free Cu^{2+} than Cu^{2+} bound to protein. In this way many copper chelating substances may participate in the regulation of GABAergic transmission.

Furthermore, this mechanism may contribute to the beneficial effects of chelating agents and Zn^{2+} when they are used for treating Wilson's disease, an inherited disorder related with copper imbalance. Disturbances in copper metabolism result in its accumulation in the liver, the basal ganglia and cause hepatolenticular degeneration. Zinc is the treatment of choice for maintenance therapy because of its high efficacy and lack of toxicity, it blocks copper absorbtion (Brewer & Yuzbasiyan Gurkan, 1992). Our results raise the possibility that interaction with copper binding to neuronal proteins including the $GABA_A$-receptor may be partially responsible for the therapeutic effect of zinc in Wilson's disease.

Supported by Deutsche Forschungsgemeinschaft (SFB 194, B13).

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(Received January 17, 2000
Revised March 20, 2000
Accepted March 24, 2000)