Zinc Inhibition of t-[^{3}H]Butylbicycloorthobenzoate Binding to the GABA$_A$ Receptor Complex

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Abstract: The effect of Zn^{2+} on t-[^{3}H]butylbicycloorthobenzoate ([^{3}H]TBOB) binding to the GABA$_A$ receptor complex was studied autoradiographically in rat brain. Zn^{2+} inhibited [^{3}H]TBOB binding in a dose-dependent manner at physiological concentrations. Saturation analysis revealed noncompetitive inhibition in various brain regions. The inhibitory effect of Zn^{2+} had regional heterogeneity; regions showing the greatest inhibition of [^{3}H]TBOB binding were cortical laminae I–III, most areas of hippocampus, striatum, septum, and cerebellar cortex. Regions with relatively less inhibition of [^{3}H]TBOB binding included cortical laminae V–VI, thalamus, superior colliculus, inferior colliculus, and central gray matter. The effect of Zn^{2+} and those of other GABA$_A$ ligands, such as benzodiazepines, bicuculline, isoguvacine, and picrotoxin, on [^{3}H]TBOB binding seemed to be additive. Ni^{2+}, Cd^{2+}, and Cu^{2+} also inhibited [^{3}H]TBOB binding with a regional heterogeneity similar to that produced by Zn^{2+}. These results are consistent with Zn^{2+} acting at the previously detected recognition site on the GABA$_A$ receptor complex, distinct from the picrotoxin, GABA, and benzodiazepine sites. The regional heterogeneity of the Zn^{2+} effect may reflect differential regional distribution of GABA$_A$ receptor subtypes among brain regions. Other divalent cations probably act at the Zn^{2+} binding site. **Key Words:** Zinc—t-Butylbicycloorthobenzoate—GABA—GABA$_A$ receptor complex—Autoradiography. *J. Neurochem.* **62,** 602–607 (1994).

The GABA$_A$ receptor is the major mediator of synaptic inhibition in the CNS and structurally is a heterooligomeric protein comprised of five (or fewer) subunits per active complex (Burt and Kamatchi, 1991). Molecular cloning studies have provided evidence for the existence of a number of different subunits forming the GABA$_A$ receptor, and each of these subunits has a distinct regional distribution in the brain (Olsen and Tobin, 1990; Burt and Kamatchi, 1991; Lüddens and Wisden, 1991; Doble and Martin, 1992; Wisden et al., 1992). Functionally, the GABA$_A$ receptor incorporates a chloride ionophore, as well as binding sites for several classes of ligands that interact allosterically either to potentiate or to inhibit receptor function (Burt and Kamatchi, 1991; Sieghart, 1992).

Divalent cations have been shown to modulate GABA$_A$ receptors in biochemical and electrophysiological studies. Zn^{2+} and other metal cations, including Cd^{2+}, Ni^{2+}, Mn^{2+}, and Co^{2+}, inhibited the GABA response of neurons in a variety of preparations (Smart and Constanti, 1982; Kaneko and Tachibana, 1986; Celentano et al., 1991; Smart, 1992; Kilić et al., 1993). Binding studies have provided evidence for Zn^{2+} modulation of GABA ligand binding. Zn^{2+} enhanced the specific binding of [^{3}H]diazepam in the CNS (MacKerer and Kochman, 1978). Although Zn^{2+} seemed to increase [^{3}H]GABA binding to synaptic membranes at physiological concentrations, it inhibited the binding at pharmacotoxicological doses (Baraldi et al., 1984). An autoradiographic study showed that Zn^{2+} inhibited [^{3}H]GABA binding to GABA$_B$ receptors, but not significantly to GABA$_A$ receptors in any brain region (Turgeon and Albin, 1992). For the picrotoxin binding site, a binding site on the GABA$_A$ receptor complex whose binding is modulated by the activation of other ligand binding sites of the GABA$_A$ receptor complex, there are no published data on the effect of Zn^{2+} and other divalent cations.

The effects of drugs on picrotoxin ligand binding have been used frequently to assess ligand interactions with the GABA$_A$ receptor complex. The purposes of this study were to elucidate Zn^{2+} effects on picrotoxin ligand binding, to examine the regional heterogeneity of the Zn^{2+} effect on GABA$_A$ receptors, and to study the possible interaction of Zn^{2+} effects with the effects of other classes of GABA$_A$ receptor ligands. The picrotoxin binding site of the GABA$_A$ receptor complex can be assayed with t-[^{3}H]butylbicycloorthobenzoate ([^{3}H]TBOB) (Lawrence et al., 1985; O'Connor and McEwen, 1986; Sakurai et al., 1992). We used in vitro quantitative autoradiography to determine the effects of Zn^{2+} on [^{3}H]TBOB binding in rat brain.

EXPERIMENTAL PROCEDURES

Male Sprague-Dawley rats (175-199 g; Harlan Labs, Indianapolis, IN, U.S.A.), were decapitated, and their brains rapidly removed and frozen in Lipshaw embedding matrix surrounded by powdered dry ice. Twenty-micrometer-thick sections were cut horizontally on a Lipshaw cryostat. Sections were obtained at the level including the caudoputamen, thaw-mounted onto 2 × subbed gelatin-coated slides on a warming plate, and stored at −20°C until the time of assay. All assays were performed 24 h after decapitation. Sections were run in triplicate. Slide-mounted tissue sections were warmed to room temperature and prewashed for 3 × 10 min in buffer (50 mM Tris-HCl plus 1 mM EDTA, pH 7.4 at 4°C) at 4°C and dried under a stream of cool air. Binding of [^{3}H]TBOB (20 nM) was carried out in buffer (50 mM Tris-HCl plus 120 mM NaCl, pH 7.4, at room temperature) for 90 min at room temperature. The incubation was terminated by two 30-min rinses in buffer (50 mM Tris-HCl, pH 7.4, at 4°C) at 4°C, followed by a brief dip in distilled water. Each slide was then dried under a stream of hot air. Nonspecific binding was assessed in the presence of 20 μM picrotoxin. For analysis of the Zn^{2+} effect on [^{3}H]-TBOB binding, tissue sections were incubated with nine concentrations of ZnCl$_2$ ranging from 1 nM to 1 mM with 20 nM [^{3}H]TBOB. Saturation studies were performed with nine concentrations of [^{3}H]TBOB ranging from 1 pM to 100 nM. Specific [^{3}H]TBOB binding and nonspecific binding were determined at all concentrations of [^{3}H]TBOB in both the presence and the absence of 100 μM ZnCl$_2$. To determine the interaction of Zn^{2+} effects with the effects of other GABA$_A$ ligands that modulate [^{3}H]TBOB binding, other GABA$_A$ ligands were included in the incubation mixture with 20 nM [^{3}H]TBOB in both the presence and absence of 100 μM ZnCl$_2$. The effects of other divalent cations on [^{3}H]TBOB binding were analyzed in the incubation mixture with 20 nM [^{3}H]TBOB. Autoradiograms were generated by apposing the slides to ^{3}H-sensitive film (Hyperfilm, Amersham Inc., Arlington Heights, IL, U.S.A.) in light-tight cassettes along with standards containing known amounts of radioactivity. After 3 weeks, films were developed in Kodak D-19. Ligand binding was quantitated with computer-assisted densitometry using the MCID system (Imaging Research Inc., St. Catharines, Ontario, Canada). To quantify ligand binding density, the optical density of coexposed standards was determined and a standard curve generated by fitting standard values with a fourth-degree polynomial regression equation. Standards were commercial ^{14}C plastic standards (ARC, St. Louis, MO, U.S.A.). Use of the standards and derived standard curve allow conversion of areal optical density to picomoles per milligram of protein. Areas read included the cortical laminae I-III, lamina IV, and laminae V-VI, hippocampal CA1 stratum oriens, CA1 stratum radiatum, CA3 stratum oriens, CA3 stratum radiatum, and dentate gyrus, striatum, thalamus (laterodorsal thalamic nucleus and lateral posterior thalamic nucleus), septum, superior colliculus, inferior colliculus, periaqueductal gray matter, and cerebellar molecular and granule cell layers. Ten to 20 readings per area from triplicate sections were averaged. IC$_{50}$ values for Zn^{2+} were calculated by log-logit analysis. In the saturation experiment, values of specific [^{3}H]TBOB bound were quantified to construct Scatchard plots.

Materials

[^{3}H]TBOB (sp. act. 19.4 Ci/mmol) was purchased from Amersham. Clonazepam was the gift of Dr. Peter Sorter
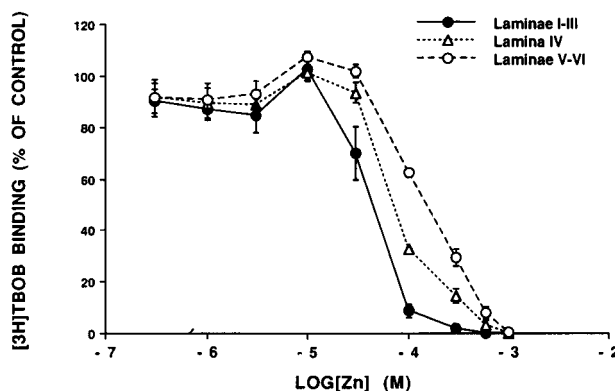

FIG. 1. Curves for the inhibition of [^{3}H]TBOB binding by Zn^{2+} in laminae I-III, lamina IV, and laminae V-VI of the cerebral cortex. Zn^{2+} inhibited TBOB binding in a dose-dependent manner. Data are means ± SEM (bars) from experiments carried out with three animals.

(Hoffmann-La Roche). Zolpidem was the gift of Synthelabo Recherche. Isoguvacine was purchased from Cambridge Research Biochemicals Ltd. (Cambridge, U.K.). Bicuculline and picrotoxin were purchased from Research Biochemicals Inc. (Natick, MA, U.S.A.). The remaining reagents were purchased from Sigma Chemical (St. Louis, MO, U.S.A.) and were of the highest possible purity.

RESULTS

Zn^{2+} modulation of [^{3}H]TBOB binding

Zn^{2+} produced dose-dependent inhibition of [^{3}H]-TBOB binding in rat brain sections (Fig. 1). Inhibition of [^{3}H]TBOB binding was observed in all areas examined (Fig. 2 and Table 1). Saturation analysis of [^{3}H]TBOB in the presence of 100 μM Zn^{2+} revealed a significant decrease in B_{max} with little change in K_D in most regions (Table 2).

Regional difference of Zn^{2+} modulation of [^{3}H]TBOB binding

The observed regional distribution of [^{3}H]TBOB binding sites was similar to that reported previously by Olsen et al. (1990). Picrotoxin displaced to the same extent all the [^{3}H]TBOB bound in all brain regions (data not shown). On the other hand, there were regional differences in Zn^{2+} inhibition of [^{3}H]TBOB binding (Fig. 1). IC$_{50}$ values for Zn^{2+} were significantly different among brain regions ($p < 0.05$ on ANOVA; Table 1), with IC$_{50}$ values lowest in cortical laminae I-III, the cerebellar molecular layer, and septum. Relatively low IC$_{50}$ values were observed also in most subfields of the hippocampus, the striatum, and cerebellar granule cell layer. Relatively high IC$_{50}$ values were found in cortical laminae V-VI, thalamus, superior colliculus, inferior colliculus, and periaqueductal gray matter.

Pharmacological profile of [^{3}H]TBOB binding

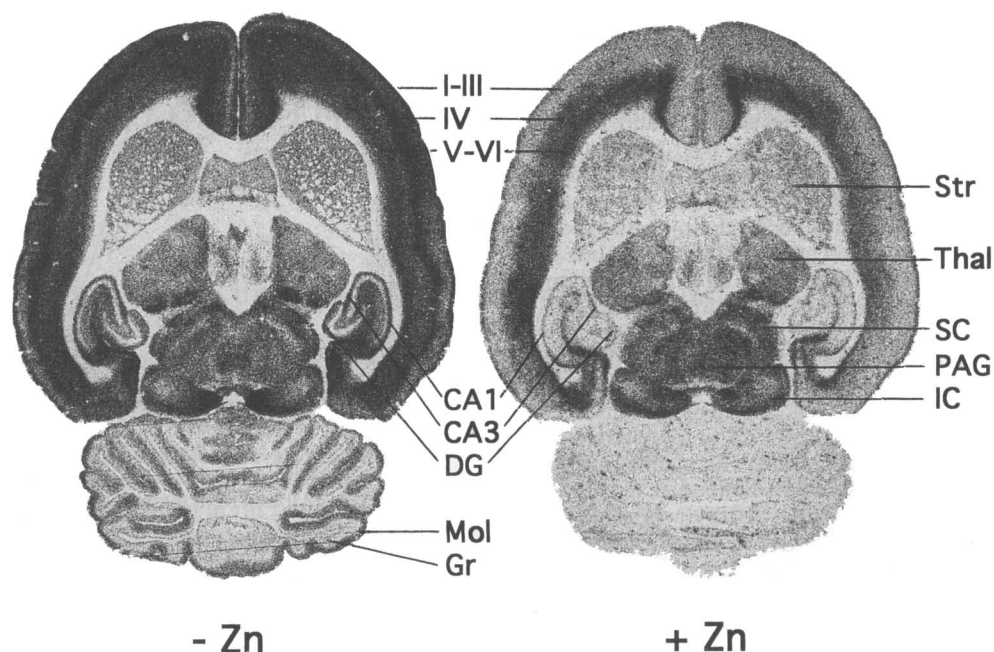Compounds that modulate the activity of the GABA$_A$ receptor complex allosterically modulate bind-

FIG. 2. Autoradiographs of [³H]TBOB binding in the absence (−Zn) and presence (+Zn) of 100 μM Zn²⁺ in horizontal sections of rat brain. Zn²⁺ inhibited [³H]TBOB binding in all areas with regional heterogeneity. Regions showing the greatest inhibition of [³H]TBOB binding were cortical laminae I–III, most areas of hippocampus, striatum, septum, and cerebellar cortex. Regions with relatively less inhibition of [³H]TBOB binding included cortical laminae V–VI, thalamus, superior colliculus, inferior colliculus, and periaqueductal gray matter. I–III, cortical laminae I–III; IV, cortical lamina IV; V–VI, cortical laminae V–VI; CA1, CA1 stratum oriens; CA3, CA3 stratum oriens; DG, dentate gyrus; Gr, cerebellar granule cell layer; IC, inferior colliculus; Mol, cerebellar molecular layer; PAG, periaqueductal gray matter; SC, superior colliculus; Str, striatum; Thal, thalamus.

ing of the picrotoxin ligands, *t*-[³⁵S]butylbicyclophos-phorothionate ([³⁵S]TBPS) and [³H]TBOB. The ben-zodiazepine agonists, clonazepam and zolpidem, en-hance TBPS and TBOB binding at concentrations between 100 n*M* and 1 μ*M* (Lloyd et al., 1990; Sa-kurai et al., 1992; Kume et al., unpublished observa-tions). GABA_A agonists, such as muscimol and isogu-vacine, inhibit TBPS and TBOB binding (Squires et al., 1983; Lawrence et al., 1985; Edgar and Schwartz, 1990; Sakurai et al., 1992), whereas the GABA_A com-petitive antagonist bicuculline enhances their binding in the cerebellar granule cell layer (Squires et al., 1983; Korpi et al., 1992; Sakurai et al., 1992). In this experiment, Zn²⁺ inhibited [³H]TBOB binding in the presence of either clonazepam or zolpidem (Fig. 3). In the presence of either isoguvacine or bicuculline, Zn²⁺ produced approximately the same percent reduction of TBOB binding as that produced in the absence of these compounds. The effects of Zn²⁺ and benzodiaze-pines, isoguvacine, and bicuculline seemed to be es-sentially additive. Picrotoxin is considered to be a competitive inhibitor of [³H]TBOB (Lawrence et al., 1985). The inhibition by picrotoxin and Zn²⁺ also seemed to be additive (Fig. 3).

Modulation of [³H]TBOB binding by other divalent cations

Ni²⁺, Cd²⁺, and Cu²⁺ at 300 μ*M* also inhibited [³H]-TBOB binding (Fig. 4). There were regional differ-

ences in the inhibition of [³H]TBOB binding similar to that produced by Zn²⁺.

DISCUSSION

The primary finding of this study is that Zn²⁺ pro-duced dose-dependent inhibition of [³H]TBOB bind-ing in rat brain sections. Zn²⁺ inhibition of [³H]TBOB binding was observed in all areas examined, and there were regional differences in the Zn²⁺ effect on [³H]-TBOB binding. Saturation analysis indicated that Zn²⁺ inhibits [³H]TBOB binding in a noncompetitive manner. Divalent cations are known to form soluble complexes with some ligands (Crawford and McBur-ney, 1977). If Zn²⁺ forms a soluble complex with [³H]-TBOB, reducing the concentration of free [³H]TBOB, Zn²⁺ would decrease the amount of free [³H]TBOB interacting with the GABA_A receptor. In this case, however, an autoradiogram obtained from the incu-bation mixture of [³H]TBOB with Zn²⁺ would show uniform decrease in regional binding and not the re-gional differences we documented. Moreover, soluble complex formation should not alter the B_{max} of satura-tion curves. Our results indicate a noncompetitive in-teraction, and these observations cannot be explained by the hypothesis of complex formation. We infer that Zn²⁺ is acting as an inhibitor of [³H]TBOB bind-ing by acting at a site on the GABA_A receptor.

TABLE 1. *Inhibition of [³H]TBOB binding in various brain regions by Zn^{2+}*

Brain region	IC_{50} (μM)
With high effect of Zn^{2+}:	
Neocortex	
Laminae I–III	42.2 ± 4.1
Hippocampus	
CA1 stratum oriens	74.8 ± 5.3
CA1 stratum radiatum	57.0 ± 16.3
CA3 stratum oriens	46.9 ± 2.8
Dentate gyrus	59.4 ± 4.5
Striatum	83.5 ± 15.7
Septum	48.3 ± 8.1
Cerebellum	
Molecular layer	47.7 ± 8.8
Granule cell layer	70.8 ± 27.6
With moderate effect of Zn^{2+}:	
Neocortex	
Lamina IV	102.5 ± 16.8
Hippocampus	
CA3 stratum radiatum	113.3 ± 10.8
Pre-/parasubiculum	123.6 ± 17.5
With low effect of Zn^{2+}:	
Neocortex	
Laminae V–VI	150.6 ± 9.4
Thalamus	167.2 ± 14.8
Superior colliculus	214.2 ± 4.7
Inferior colliculus	247.9 ± 43.3
Central gray mater	193.6 ± 12.8

IC_{50} values were derived from inhibition curves with $ZnCl_2$. Data are means ± SEM from experiments with three animals. A significant difference of the IC_{50} values among regions was noted using one-way ANOVA ($p < 0.05$).

Our results are consistent with prior electrophysiologic data and indicate that our binding data address the same phenomena described in electrophysiologic studies. Our observed IC_{50} values approximate 100 μM, which is consistent with results obtained in several functional studies (Smart and Constanti, 1982; Westbrook and Mayer, 1987; Celentano et al., 1991; Legendre and Westbrook, 1991; Smart et al., 1991; Smart, 1992; Kilić et al., 1993), and the effects of Zn^{2+} are mimicked by other divalent metal cations, a phenomenon noted also in electrophysiologic studies

(Kaneko and Tachibana, 1986; Celentano et al., 1991).

Zn^{2+} is present in many synaptic vesicles in the vertebrate CNS (Pérez-Clausell and Danscher, 1985) and is released into the extracellular space during neuronal activity (Assaf and Chung, 1984; Howell et al., 1984). Assaf and Chung (1984) demonstrated extracellular concentrations of Zn^{2+} as high as 300 μM following depolarization. The effective concentration range for Zn^{2+} effects in our study and prior electrophysiologic studies (Smart and Constanti, 1982; Westbrook and Mayer, 1987; Celentano et al., 1991; Legendre and Westbrook, 1991; Smart et al., 1991; Smart, 1992; Kilić et al., 1993) is within the probable physiological concentration range of Zn^{2+} within the synaptic cleft. Zn^{2+} may function as an endogenous neuromodulator of $GABA_A$ receptor complex function within the CNS.

Our binding data provide evidence for regional differences in the Zn^{2+} effect on the $GABA_A$ receptor complex. Regions showing the greatest inhibition of [³H]TBOB binding were cortical laminae I–III, most areas of hippocampus, striatum, septum, and cerebellar cortex. Regions with relatively less inhibition of [³H]TBOB binding included cortical laminae V–VI, thalamus, superior colliculus, inferior colliculus, and periaqueductal gray matter.

The pharmacological and molecular biological evidence for regional heterogeneity of the $GABA_A$ receptor complex has been widely reported, and regional/cellular differences in the expression of $GABA_A$ receptor subtypes probably underlie, at least in part, regional/cellular differences in receptor function and pharmacology (Olsen and Tobin, 1990; Burt and Kamatchi, 1991). Recent recombinant receptor studies have shown that $GABA_A$ receptors expressing the $\gamma2$ subunit are relatively insensitive to Zn^{2+} (Draguhn et al., 1990; Smart et al., 1991), suggesting that the presence of $\gamma2$ subunits is an important determinant of the relative Zn^{2+} sensitivity of native $GABA_A$ receptors. It is likely that each brain region expresses a variety of $GABA_A$ receptors of varying isoform composition with substantial regional differences in the expressed amount of different types of receptor heterooligomers. The $\gamma2$ subunit is expressed in vir-

TABLE 2. *Saturation binding parameters of [³H]TBOB binding in several brain regions*

Brain region	K_D (−Zn)	K_D (+Zn)	B_{max} (−Zn)	B_{max} (+Zn)
Cerebral cortex				
Laminae I–III	24.0 ± 1.5	37.0 ± 2.4[a]	2.25 ± 0.21	0.61 ± 0.07[a]
Lamina IV	22.7 ± 2.2	28.7 ± 0.6	2.84 ± 0.30	1.46 ± 0.16[a]
Laminae V–VI	30.3 ± 3.6	34.2 ± 5.3	2.60 ± 0.27	1.92 ± 0.11
Striatum	55.7 ± 11.1	35.7 ± 5.6	1.80 ± 0.26	0.74 ± 0.13[a]

K_D (nM) and B_{max} (pmol/mg of protein) values were determined in the presence and absence of 100 μM $ZnCl_2$. Data are means ± SEM from experiments with three animals.
[a] A significant difference between −Zn and +Zn was noted using two-tailed t test ($p < 0.05$).
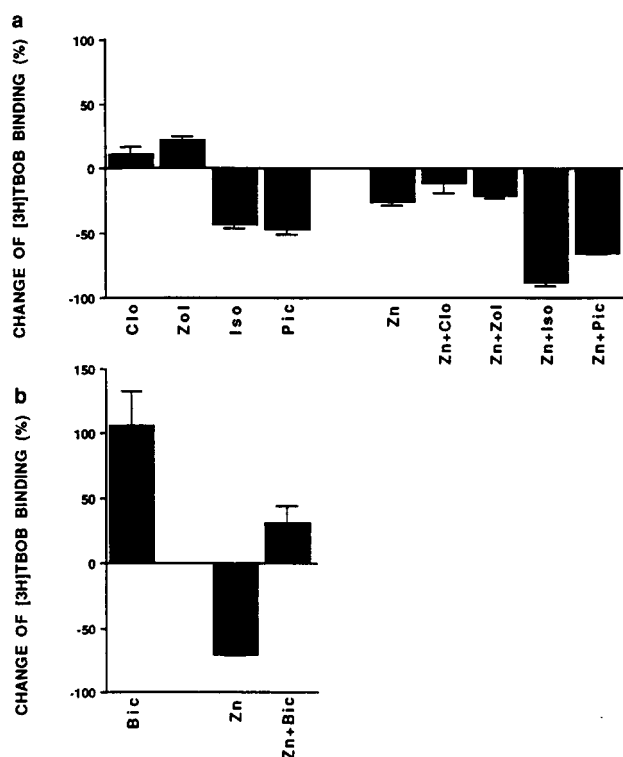
FIG. 3. Zn^{2+} effects were essentially additive to those of other $GABA_A$ ligands. The graphs show the results in the cortical laminae V–VI (a) and in the cerebellar granule cell layer (b). The experiment was performed in the presence of 500 nM clonazepam (Clo), 500 nM zolpidem (Zol), 10 μM isoguvacine (Iso), 600 nM picrotoxin (Pic), or 100 μM bicuculline (Bic), each done in the absence and presence of 100 μM Zn^{2+}. Zn^{2+} produced inhibition in the presence of either clonazepam or zolpidem (a). Zn^{2+} added another inhibitory effect to the inhibition produced by either isoguvacine or picrotoxin. Zn^{2+} decreased the enhancement of [^{3}H]-TBOB binding by bicuculline in the cerebellar granule cell layer (b). Data are means ± SEM (bars) from experiments with three animals.

tually all brain regions examined (Wisden et al., 1992), albeit at low levels in many regions, and it is likely that most, if not all, brain regions possess $\gamma 2$-containing $GABA_A$ receptors. Recombinant receptors without $\gamma 2$ subunits [$\alpha 1 \beta 1$ (Draguhn et al., 1990), $\alpha 1 \beta 2$ (Smart et al., 1991)] have IC_{50} values for Zn^{2+} approximating 1 μM. These IC_{50} values are much lower than those of either recombinant receptors containing $\gamma 2$ subunits or native $GABA_A$ receptors, and are significantly lower than the IC_{50} values of the Zn^{2+} effect on [^{3}H]TBOB binding. It is likely that the presence of $GABA_A$ receptors containing $\gamma 2$ subunits accounts for the relative insensitivity of native receptors compared with recombinant receptors lacking $\gamma 2$ receptors.

Our autoradiographic binding data, however, suggest that the relative abundance of $\gamma 2$ subunits is not the sole determinant of regional differences in the Zn^{2+} sensitivity of $GABA_A$ receptors. The neocortex, hippocampus, and cerebellar cortex are brain regions

in which abundant $\gamma 2$ mRNA has been detected, and the $\gamma 2$ subunit is considered to be a primary component of native $GABA_A$ receptor complexes (Laurie et al., 1992; Wisden et al., 1992). Therefore, those regions are predicted to be less sensitive to Zn^{2+}. In our autoradiograms, however, those brain regions showed relatively high sensitivity to the effects of Zn^{2+}. Moreover, our data show that thalamus, which contains a relatively low level of $\gamma 2$ subunit mRNA (Wisden et al., 1992), was relatively insensitive to Zn^{2+}. These results are consistent with the suggestion of Smart et al. (1991) that some combinations of $GABA_A$ receptor subunits that lack the $\gamma 2$ subunit are also likely to be insensitive to Zn^{2+}. Comparison of our data with the reported distribution of other $GABA_A$ receptor subunit mRNAs (Laurie et al., 1992; Wisden et al., 1992) reveals that the distribution of no single subunit correlates with the distribution of the Zn^{2+} effect on [^{3}H]TBOB binding. The degree of susceptibility to the effects of Zn^{2+} may be regulated by complex combinations of subunits and/or by posttranslational modification of $GABA_A$ receptor complexes.

Electrophysiologic studies (Smart and Constanti, 1982; Celentano et al., 1991; Smart, 1992) indicate that modulation of $GABA_A$ receptor function by benzodiazepines, barbiturates, neurosteroids, bicuculline, and picrotoxin does not interfere with the antagonism exerted by Zn^{2+}. These studies suggest the existence of a Zn^{2+} site distinct from other modulatory $GABA_A$ ligand binding sites. Our binding data are consistent with this inference. The effect of Zn^{2+} and those of other $GABA_A$ ligands on [^{3}H]TBOB binding seemed to be additive, a result consistent with the existence of a separate Zn^{2+} site. There is a possible discrepancy between our results and prior electrophysiologic data (Celentano et al., 1991; Smart, 1992). Our results indicate that Zn^{2+} inhibits picrotoxin ligand binding, and this suggests that Zn^{2+} might decrease the effectiveness of picrotoxin, a result not apparently
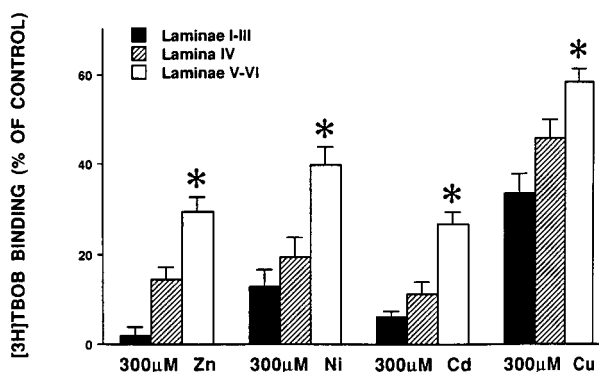

FIG. 4. Divalent metal cations inhibit [^{3}H]TBOB binding with similar regional differences in effect. [^{3}H]TBOB binding was more inhibited in laminae I–III than in laminae IV and V–VI of the cerebral cortex. Data are means ± SEM (bars) from experiments done with three animals. *A significant difference from laminae I–III and IV was noted using ANOVA ($p < 0.05$).

seen in prior studies. Zn^{2+}, however, is a noncompetitive inhibitor of [³H]TBOB binding with a predominant effect on B_{max} and little effect on binding site affinity. Prior studies of Zn^{2+}–picrotoxin interactions were done with single, subsaturating concentrations of picrotoxin, and full dose–response curves achieving saturating concentrations of picrotoxin might be necessary to document a Zn^{2+}–picrotoxin interaction.

In addition to Zn^{2+}, other divalent metal cations, such as Co^{2+}, Ni^{2+}, Mn^{2+}, and Cd^{2+}, also have been reported to inhibit GABA-induced current noncompetitively (Kaneko and Tachibana, 1986; Celentano et al., 1991). These cations probably act at a common site, but possess different intrinsic efficacies as allosteric inhibitors, such that Zn^{2+} has greater efficacy than Cd^{2+} (Celentano et al., 1991). In the present study, 300 μM Ni^{2+}, Cd^{2+}, and Cu^{2+} inhibited [³H]-TBOB binding as well as 300 μM Zn^{2+}. Moreover, there were regional differences in the inhibition of [³H]TBOB binding, similar to that produced by Zn^{2+}. These results suggest that Ni^{2+}, Cd^{2+}, and Cu^{2+} act at the Zn^{2+} binding site.

Further studies will be necessary to establish the subunit composition, location, and functional properties of cation-sensitive $GABA_A$ receptor subtypes.

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