Brain Research, 30 (1992) 30–34

BRES 18246

Zinc modulates $GABA_B$ binding in rat brain

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(Accepted 16 June 1992)

Key words: $GABA_A$; $GABA_B$; Zinc; Calcium; Neuromodulation; Hippocampus; Cerebellar molecular layer; Thalamus

The effects of $ZnCl_2$ on [^{3}H]GABA binding to $GABA_A$ and $GABA_B$ binding sites were investigated using receptor autoradiography. At concentrations exceeding 100 μM, zinc non-competitively inhibited $GABA_B$ binding in a dose dependent fashion. $GABA_A$binding was not inhibited significantly by zinc eliminating the possibility of a non-specific effect of zinc. Increased calcium concentrations up to 10 mM enhanced total $GABA_B$ binding but did not prevent zinc induced inhibition of $GABA_B$ binding, indicating a separate site of action for these cations at the $GABA_B$ binding site. In some regions, zinc modulates $GABA_B$ binding in a biphasic manner as concentrations of 10–100 μM zinc significantly enhanced $GABA_B$ binding in the hippocampus and the molecular layer of the cerebellum but not in the thalamus. These results provide further evidence for a neuromodulatory role for zinc in the central nervous system.

INTRODUCTION

Zinc is widely distributed throughout the mammalian central nervous system and may function as a neuromodulator. Zinc-containing neuronal pathways[10], zinc-containing synaptic vesicles[18], calcium-dependent zinc release[1,11], and zinc uptake mechanisms[23] have been identified. Zinc modulates the function of various neurotransmitters. Zinc non-competitively inhibits NMDA receptors[17,19,24] by either non-competitive antagonism of glycine binding[26] or direct antagonism of NMDA receptors[9]. Zinc interacts biphasically with non-NMDA excitatory amino acid receptors, exciting them at low zinc concentrations and inhibiting them at higher concentrations[17]. NMDA receptor-mediated glutamate neurotoxicity is also inhibited by zinc[8,13] while zinc enhances non-NMDA receptor-mediated glutamate neurotoxicity[13]. Zinc also inhibits $GABA_A$ receptor-mediated inhibitory responses in cultured neurons[4,15,16,21,22,24]. Binding studies have revealed inhibition of [^{3}H]GABA binding to synaptic membranes by zinc[2]. While much of the research concerning zinc and GABA transmission has focused on the $GABA_A$ receptor, there is also some evidence for zinc modulation of $GABA_B$ receptor activity. Xie and Smart have proposed that zinc induces giant depolarizing potentials (GDPs) in hippocampal neurons by inhibition of pre- and postsynaptic $GABA_B$ receptors[25]. Drew et al. have shown that 100 μM zinc inhibits [^{3}H]baclofen binding to cerebellar membranes[7] but there has been no further characterization of zinc effects on $GABA_B$ binding. We now report dose-dependent modulation of $GABA_B$ binding by zinc in several brain regions.

MATERIALS AND METHODS

Quantitative autoradiography was used to analyze the effect of varying concentrations of $ZnCl_2$ on [^{3}H]GABA binding to $GABA_A$ and $GABA_B$ binding sites, to assess the effect of [Ca^{2+}] on zinc–$GABA_B$ receptor interactions, and to determine the nature of the interaction between zinc and $GABA_B$ binding by performing saturation analysis of $GABA_B$ binding in the presence and absence of zinc. Male Sprague–Dawley rats (175–225 g; Harlan Industries, Indianapolis) were decapitated and brains were rapidly dissected and frozen in Lipshaw embedding matrix surrounded by powdered dry ice. Twenty-μm sections were cut in the horizontal plane on a Lipshaw cryostat at −20°C and thaw mounted onto gelatin-coated slides. Sections were stored at −20°C for no longer than 24 h. Sections were run in triplicate. Sections underwent a 30-min pre-wash in buffer containing 50 mM Tris-HCl and 2.5 mM $CaCl_2$ (pH 7.4 at +4°C) and were then dried under a stream of cool air. $GABA_B$ binding sites were examined with [^{3}H]GABA (Amersham, Arlington Heights, IL) in the presence of 10 μM isoguvacine (Cambridge Research Chemicals, Cambridge, UK) and non-specific binding was determined in the presence of 100 μM baclofen (gift of Ciba-Geigy, Basel, Switzerland) while $GABA_A$ binding sites were examined with [^{3}H]GABA in the presence of 100 μM baclofen and non-specific

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binding was determined in the presence of 10 μM isoguvacine[6]. Assay conditions included a 45-min incubation at +4°C in 50 mM Tris-HCl and 2.5 mM $CaCl_2$. Following incubation, slides were removed individually and rinsed 3 times with 3 ml buffer and once with 3 ml 2.5% gluteraldehyde in acetone and immediately blown dry with warm air. Slides were mounted in an X-ray cassette and opposed to tritium sensitive film (^{3}H-Hyperfilm, Amersham) along with [^{14}C] plastic standards containing known amounts of radioactivity (ARC, Inc., St. Louis, MO) for 3 weeks (competition analysis) or 6 weeks (saturation analysis) at +4°C. Films were developed in Kodak D-19, fixed and dried. The optical densities of the images were determined with computer-assisted densitometry using an MCID system (Imaging Research; St. Catherine's, Ont.). Ten to twenty-five readings were taken with a variable size cursor in each region examined and averaged together. Bound radioactivity was calculated from film optical densities with a standard curve obtained by fitting the optical density of the standards against their radioactivity with a fourth degree polynomial equation.

Zinc modulation of [^{3}H]GABA binding to $GABA_A$ and $GABA_B$ binding sites was analyzed in 3 separate experiments. Two experiments included concentrations of $ZnCl_2$ from 1 μM to 3 mM in the incubation mixture with 20 nM [^{3}H]GABA (91.7 Ci/mmol; $n = 3$ animals) and the third included concentrations of $ZnCl_2$ from 1 nM to 1 mM with 20 nM [^{3}H]GABA (61 Ci/mmol; $n = 3$ animals). The effect of calcium concentration on the modulation of $GABA_B$ binding by 500 μM zinc was analyzed at 5 concentrations of $CaCl_2$ from 1.0 mM to 10.0 mM ([^{3}H]GABA = 91 Ci/mmol; $n = 3$ animals). Saturation studies were performed using the method of isotopic dilution of 10 nM [^{3}H]GABA (91.7 Ci/mmol) with non-radioactive GABA (Sigma; $n = 3$ animals). The range of total GABA concentrations was 10–650 nM. $GABA_B$ binding and non-specific binding were determined at all concentrations of GABA in both the presence and the absence of 400 μM $ZnCl_2$.

For analysis of zinc modulation of [^{3}H]GABA binding, specific binding was assessed in the neocortex, dentate gyrus and CA1 regions of the hippocampus, striatum, thalamus, and granule cell layer and molecular layer of the cerebellum. IC_{50} values for each ligand were calculated from standard dose–response semi-log plots. For analysis of the effect of calcium concentration on zinc modulation of $GABA_B$ binding, specific binding was assessed in the molecular layer of the cerebellum. In the saturation experiments, values of specific [^{3}H]GABA bound were quantified in the neocortex, thalamus and the molecular layer of the cerebellum to construct Scatchard plots.

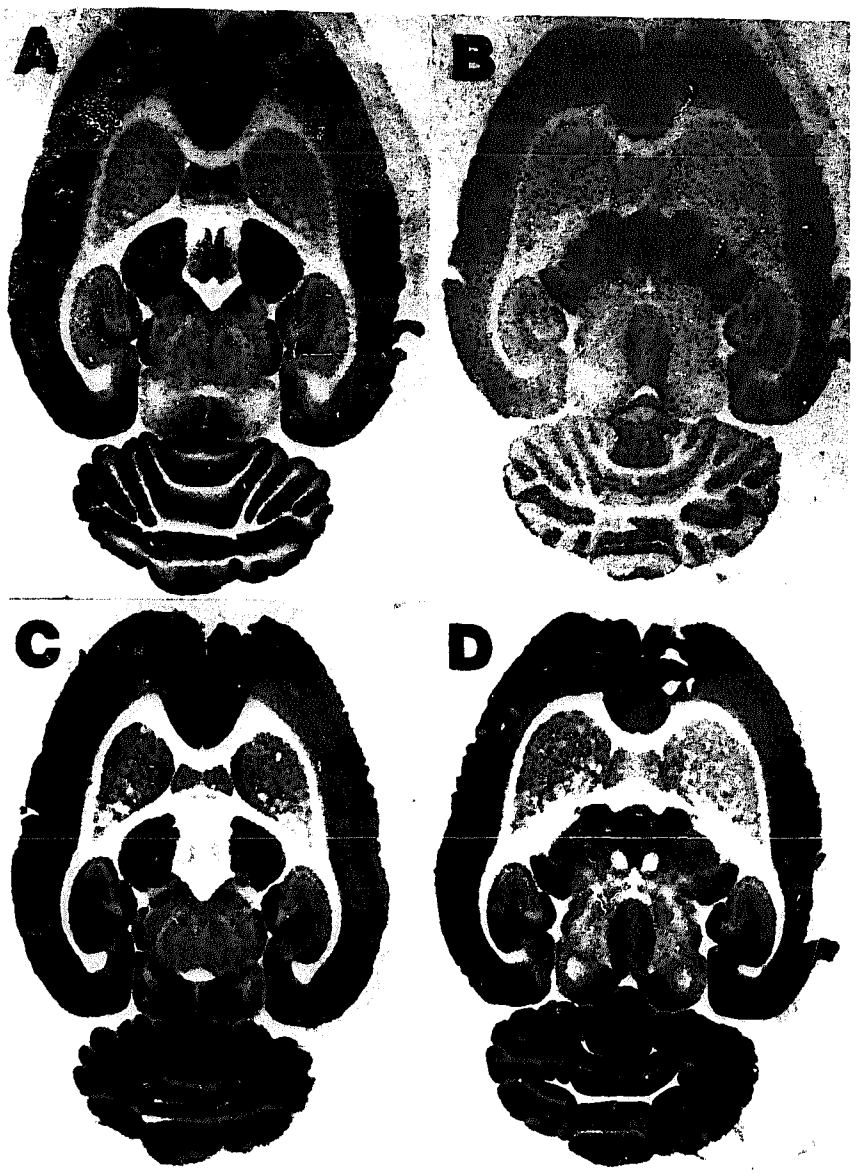


Fig. 1. Zinc inhibits $GABA_B$ binding with little effect on $GABA_A$ binding. Autoradiographs of [^{3}H]GABA binding to $GABA_B$ binding sites in the absence (A) and presence (B) of 1 mM zinc and to $GABA_A$ binding sites in the absence (C) and presence (D) of 1 mM zinc. Side to side variations in binding are due to a rinse effect and are accounted for in the data analysis by measuring and averaging data from both sides of the sections.

RESULTS

Zinc displayed a dose-dependent biphasic effect on [^{3}H]GABA binding to the $GABA_B$ binding site. At concentrations higher than 100 μM, zinc inhibited $GABA_B$ binding in all areas examined with IC_{50} values approximating 500 μM (Table I). $GABA_A$ binding was inhibited slightly by zinc but 50% inhibition was not reached by the highest concentration of zinc in the assay (3 mM), indicating that the effect on $GABA_B$

TABLE I

Inhibition of [^{3}H]GABA binding to $GABA_B$ binding sites in various brain regions by zinc

IC_{50} values were derived from inhibition curves with $ZnCl_2$ (see text). K_D (nM) and B_{max} (fmol/mg protein) values were determined in the presence and absence of 400 μM $ZnCl_2$ (see text).

Brain Region	*IC_{50} (μM)*	*K_D(−Zn)*	*K_D(+Zn)*	*B_{max}(−Zn)*	*B_{max}(+Zn)*
Cerebellum					
Molecular layer	505	98.5	113.0	1969.3	1353.3 *
Granule cell layer	400				
Striatum	710				
Thalamus	515	119.6	120.6	1585.8	1222.7
Hippocampus					
Dentate	1020				
CA1	1105				
Neocortex	620	127.9	114.8	1189.0	807.1 *

* $P < 0.05$ (two-tailed t-test).

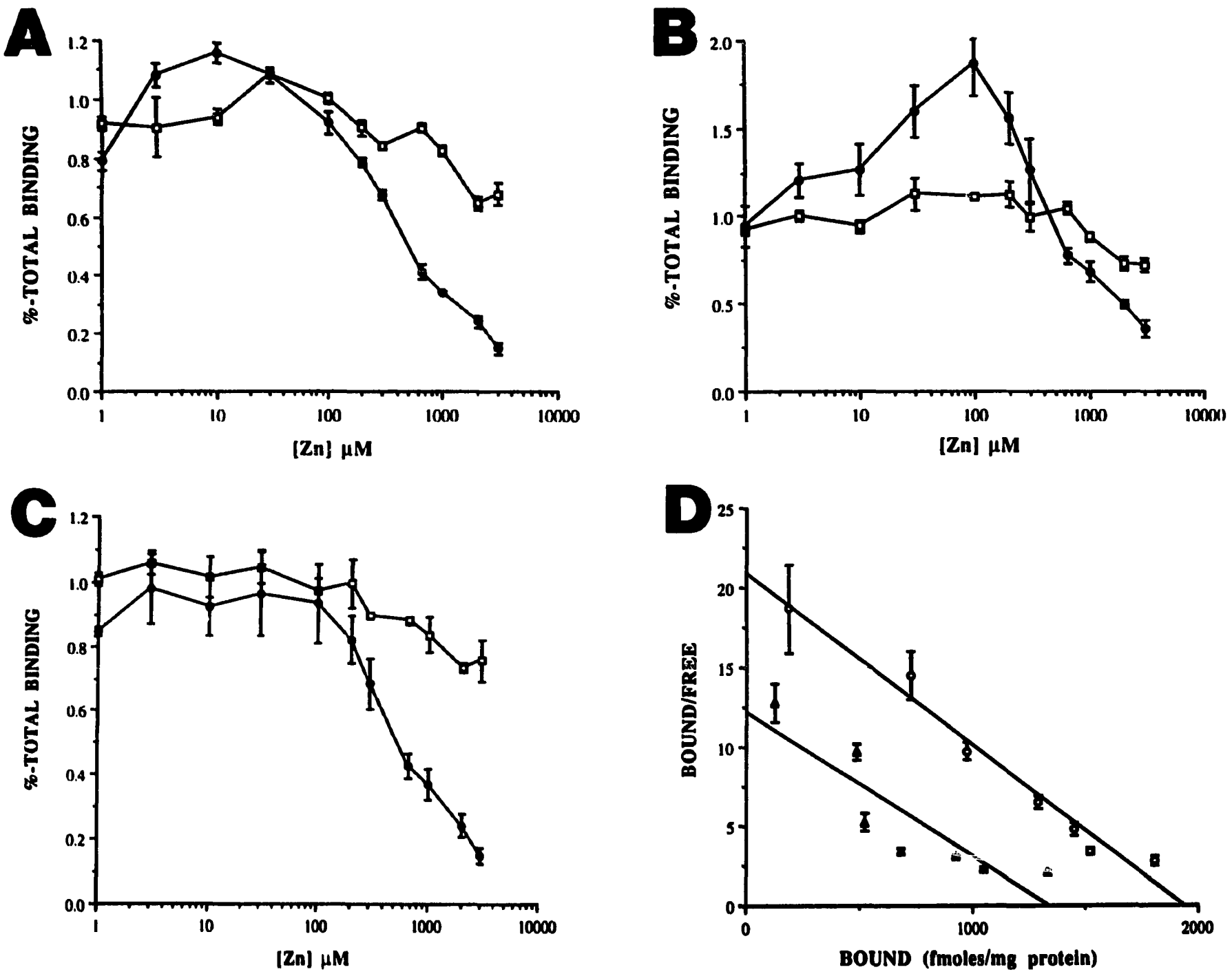


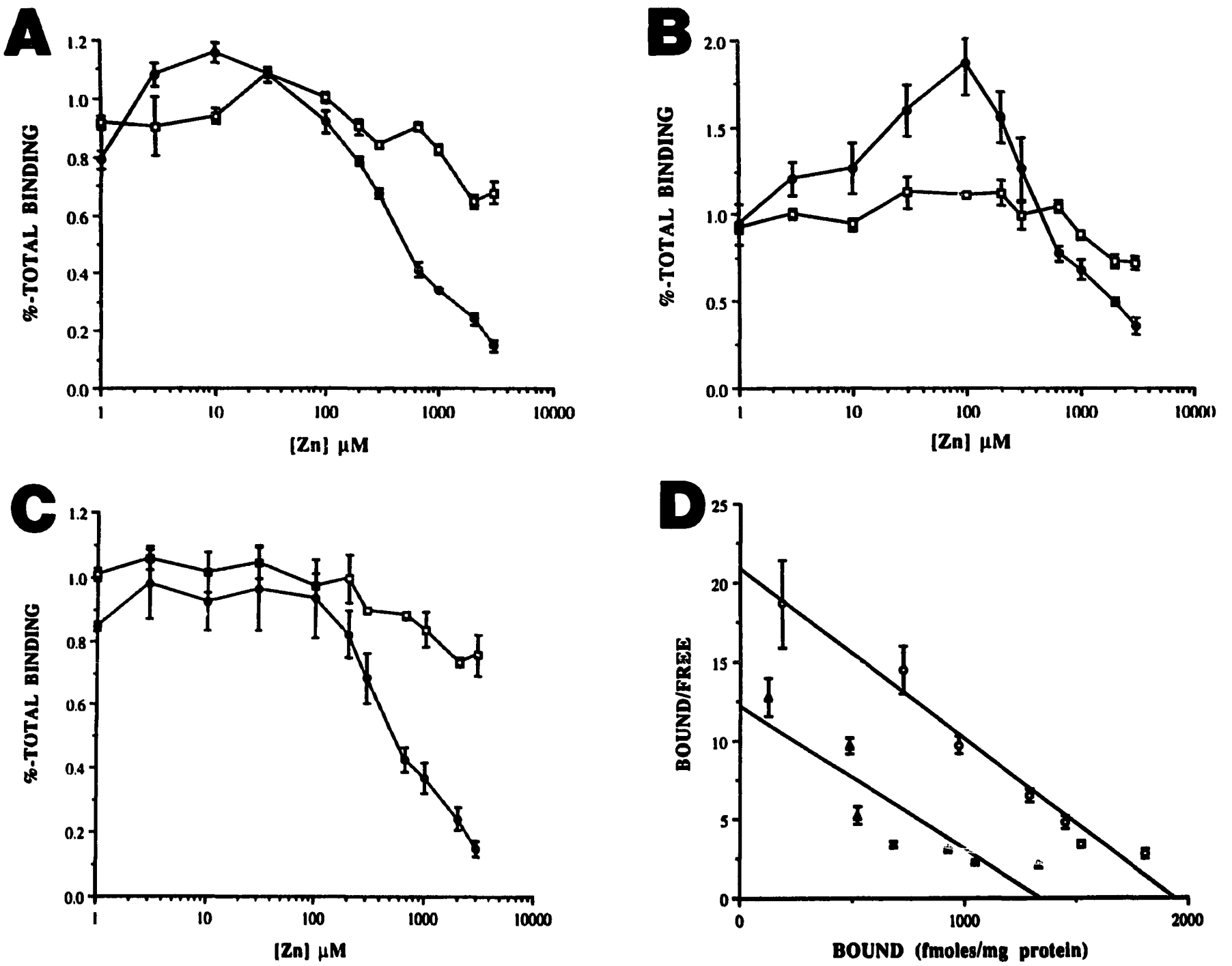
Fig. 2. Modulation of [^{3}H]GABA binding to $GABA_A$ (□) and $GABA_B$ (●) binding sites by increasing concentrations of zinc in the granule cell layer ($GABA_A$) and the molecular layer ($GABA_B$) of the cerebellum (A), in CA1 (B) and in thalamus (C). At concentrations between 10 and 100 μM, zinc enhances $GABA_B$ binding in cerebellum and hippocampus, while no enhancement is seen in thalamus. Saturation analysis of [^{3}H]GABA binding to $GABA_B$ binding sites in the molecular layer of the cerebellum in the presence (▲) and absence (○) of 400 μM zinc (D).

binding is not a non-specific effect (Figs. 1 and 2). Increased calcium concentration up to 10 mM $CaCl_2$ did not prevent inhibition of $GABA_B$ binding by 500 μM zinc (Fig. 3). At zinc concentrations of 10–100 μM, $GABA_B$ binding was enhanced in some areas. In comparison to $GABA_B$ binding in the absence of zinc, 10 μM zinc enhanced $GABA_B$ binding by 15% in the molecular layer of the cerebellum ($P < 0.05$, two-tailed t-test; Fig. 2A), 30 μM zinc enhanced $GABA_B$ binding by 27% in the dentate gyrus ($P < 0.005$, two-tailed t-test) and 82% in CA1 of the hippocampus ($P < 0.005$, two-tailed t-test), and 100 μM zinc enhanced $GABA_B$ binding by 33% in the dentate gyrus ($P < 0.005$, two-tailed t-test) and 56% in CA1 of the hippocampus ($P < 0.05$, two-tailed t-test; Fig. 2B). However, $GABA_B$ binding in the thalamus was not increased by 10–100 μM zinc (Fig. 2C).

Saturation analysis revealed a non-competitive inhibition of $GABA_B$ binding by 400 μM zinc (Fig. 2D). K_D values for [^{3}H]GABA binding in the presence and in the absence of zinc did not differ significantly in either the cerebellar molecular layer or in the neocortex while the B_{max} in the presence of zinc was significantly lower (Table I). The difference in the B_{max} values in the thalamus approached significance at the 0.05 level (Table I) and the plot resembled that of a non-competitive inhibition of [^{3}H]GABA binding to the $GABA_B$ binding site by zinc.

DISCUSSION

Our results provide further evidence for a neuromodulatory role for zinc in the central nervous system. Our findings are consistent with the physiological data



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of zinc are not mediated by the same site responsible for Ca^{2+} stimulation of $GABA_B$ binding. Our results are consistent with a hypothesis that zinc at low concentrations might enhance $GABA_B$ binding by acting at one site where calcium also acts, while at higher concentrations zinc would inhibit $GABA_B$ binding by acting at a second site. Interestingly, zinc also affects the electrophysiological responses of non-NMDA receptors in a biphasic manner, potentiating these responses at 50 μM zinc and inhibiting the responses at 1 mM zinc[17].

The regional heterogeneity of zinc effects on $GABA_B$ binding also suggests $GABA_B$ receptor heterogeneity within the central nervous system. Evidence is accumulating in support of $GABA_B$ receptor heterogeneity[3,20]. We have shown that zinc modulates $GABA_B$ binding biphasically in some regions but only inhibits $GABA_B$ binding in others, suggesting regional differences in $GABA_B$ receptors.

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