Pergamon

Pharmacology Biochemistry and Behavior, Vol. 49, No. 3, pp. 477–482, 1994

0091-3057/94 $6.00 + .00

0091-3057(94)E0112-U

Zinc Inhibition of GABA-Stimulated Cl^- Influx in Rat Brain Regions Is Unaffected by Acute or Chronic Benzodiazepine

MING LI, HOWARD C. ROSENBERG[1] AND TED H. CHIU

Department of Pharmacology, Medical College of Ohio, Toledo, OH 43699-0008

Received 15 November 1993

LI, M., H. C. ROSENBERG AND T. H. CHIU. *Zinc inhibition of GABA-stimulated Cl^- influx in rat brain regions is unaffected by acute or chronic benzodiazepine.* PHARMACOL BIOCHEM BEHAV **49**(3) 477–482, 1994.—Zinc modulation of $GABA_A$ receptor function was studied using GABA-stimulated $^{36}Cl^-$ influx into microsacs prepared from rat cerebral cortex, cerebellum and hippocampus. Zinc (10–100 μM) did not affect the basal influx, but significantly inhibited GABA-stimulated $^{36}Cl^-$ influx. The inhibition appeared to be noncompetitive. Zinc produced differing degrees of inhibition of GABA-stimulated $^{36}Cl^-$ influx in different brain regions. The order of sensitivity to zinc inhibition of GABA-stimulated $^{36}Cl^-$ influx was hippocampus > cerebral cortex > cerebellum. These regional differences may reflect the structural heterogeneity of $GABA_A$ receptors among brain areas. Zinc inhibition was not affected by the short-term addition of three benzodiazepines, diazepam, bretazenil and triazolam. The effect of diazepam and bretazenil to potentiate GABA-stimulated $^{36}Cl^-$ influx was not affected by zinc, but the effect of triazolam was decreased by zinc. In brain tissue prepared from flurazepam-treated rats, there was no difference compared with controls in zinc inhibition of GABA-stimulated $^{36}Cl^-$ influx. The results indicate that the effects of zinc on the $GABA_A$ receptor are largely independent of drugs acting on the benzodiazepine binding site.

Cl^- influx Zinc Benzodiazepines Tolerance

THE γ-AMINOBUTYRIC $acid_A$ ($GABA_A$) receptor is a ligand-gated Cl^- channel possessing numerous binding sites for several classes of drugs that interact allosterically to either potentiate or inhibit receptor function (22,27). A specific zinc interaction with the $GABA_A$ receptor has also been demonstrated (28). The current interest in zinc results from the observation that Zn^{2+} is concentrated in synaptic terminals and released upon electrical activation in sufficient quantities to possibly act as a neuromodulator (35,39). Zinc has been found to inhibit specific binding of GABA to brain membranes (2). Zinc also inhibited the $GABA_A$ receptor-mediated inhibitory responses in cultured hippocampal neurons (12,35), cultured spinal cord neurons (4), frog dorsal root ganglion neurons (40), and lobster muscle (29). However, it also has been reported that zinc had no effect on GABA inhibition of dorsal horn interneurons (5), and potentiated the effect of GABA on guinea pig prepyriform neurons (30). In primary cultures of rat superior cervical ganglion cells, zinc antagonized the GABA-stimulated currents in cells from embryonic and young animals, whereas neurons cultured from adults were insensitive to zinc (28,31). Such heterogeneous actions of zinc could reflect the structural heterogeneity of $GABA_A$ receptors in different cell types and at differing developmental stages.

Molecular cloning studies have established that $GABA_A$ receptors contain various combinations of several subunit proteins (α, β, γ, δ, ρ), most of which exist in multiple forms, each determined by separate genes (3,22). Expression studies showed that GABA currents mediated by $GABA_A$ receptor channels containing the α and β subunits were blocked by zinc, whereas channels containing the γ_2 subunit in combination with α and/or β subunits were almost insensitive to zinc (7,32). However, only a limited number of the many $GABA_A$ receptor subunits were studied, and the results may have been partly determined by the particular expression system. In comparison with these expression experiments, the subunit composition of $GABA_A$ receptors in the brain is more complex and more varied, with differences among brain regions (22,36,37). In the present study, we examined zinc action on $GABA_A$ receptor function in brain microsacs freshly prepared from different brain regions using the $^{36}Cl^-$ influx technique.

[1] To whom requests for reprints should be addressed.

Several studies, such as those showing that chronic exposure to benzodiazepines produced changes in the benzodiazepine binding site (1,17,24,38) and in levels of the mRNA encoding some of the subunits of the $GABA_A$ receptor (9,10, 21,42), suggested that changes in the $GABA_A$ receptor occur during chronic benzodiazepine treatment. To determine whether such changes in the $GABA_A$ receptor would affect zinc inhibition of $GABA_A$ receptor function, we also evaluated the zinc action on $GABA_A$ receptor function in brain tissue from rats after treatment with flurazepam for 1 week according to a method known to produce tolerance in this assay (14,20).

METHOD

Preparation of Brain Microsacs

Rats were decapitated and brains were quickly removed and put into ice-cold buffer (145 mM NaCl, 2.5 mM K_2SO_4, 1 mM $MgSO_4$, 2.5 mM $CaCl_2$, 10 mM D-glucose, and 10 mM HEPES adjusted to pH 7.4 with Tris base). Cerebral cortex, hippocampus, and cerebellum were isolated, cleaned of superficial blood vessels, and gently hand-homogenized in 30 ml of ice-cold buffer (15 ml for hippocampus) in a glass homogenizer fitted with a Teflon pestle (Thomas B, 15 up and down strokes). The homogenate was centrifuged at 1000 × *g* for 15 min at 4°C. The resultant pellet was washed twice by resuspension in the same volume of buffer using high-speed vortex, and centrifuged again. The final pellet was resuspended in buffer to give a protein concentration of 6–7 mg/ml per assay. Protein concentration was determined by the bicinchoninic acid protein assay method (Pierce Chemical Company, Rockford, IL) using bovine serum albumin as standard.

Measurement of $^{36}Cl^-$ Influx

Aliquots of microsacs (200 μl) were preincubated for 15 min at 30°C in a temperature-regulated water bath, then mixed with the GABA mixture for 3 s. The GABA mixture, also preincubated at 30°C, contained GABA (Sigma Chemical Co., St. Louis, MO) and 0.4 μCi $^{36}Cl^-$ per assay as $Na^{36}Cl$ in water (ICN Biomedicals, Irvine, CA). The reaction was terminated by adding 5 ml of ice-cold buffer followed by rapid filtration through a Schleicher and Schuell (No. 32) glass fiber filter on a vacuum filtration unit (no. XX10-025-000; Millipore Corp. Bedford, MA) modified as described previously (14). The filter was washed twice more with 5 ml ice-cold buffer and the final radioactivity was counted with 5 ml of Cytoscint (ICN Biomedicals) in a Beckman liquid scintillation counter. All experiments were performed in triplicate.

Long-Term Treatment of Rats

Male Sprague-Dawley rats (initial weight 150–174 g, 250–300 g at sacrifice) were treated as previously described (14,24). Flurazepam was administered in 0.02% saccharin solution as the only drinking water source. Based on the volume consumed over the previous 24 h, the drug concentration was adjusted to provide 100 mg/kg daily for the first 3 days and 150 mg/kg daily for the next 4 days. Rats were treated for 1 week and sacrificed without withdrawing flurazepam treatment. Control rats were given 0.02% saccharin and handled identically to the treated rats.

Data Analysis

Data were analyzed by analysis of variance (ANOVA), as described subsequently. $p < 0.05$ was considered to be statistically significant.

RESULTS

Similar to earlier studies (14), both basal (GABA-independent) and GABA-stimulated $^{36}Cl^-$ influx into brain microsacs were stable, with no indication of a consistent increase or decrease during the course of the experiment. Comparing the values obtained at the beginning of the assay with those from the end showed that 10 μM GABA-stimulated $^{36}Cl^-$ influx changed no more than ±3%.

To examine the zinc action on GABA-stimulated $^{36}Cl^-$ influx, $ZnCl_2$ was added 1 min before the addition of the GABA mixture. The final GABA concentration used for this experiment was 30 μM, which produces a half-maximal response. Zinc (10–100 μM) had no effect on the basal influx (results not shown), but significantly inhibited, as a function of concentration, the 30 μM GABA-stimulated $^{36}Cl^-$ influx in cerebral cortical membrane vesicles (Fig. 1). The 30-μM concentration of zinc inhibited 30-μM GABA-stimulated $^{36}Cl^-$ influx approximately 50%, and this concentration was used to examine the effect of zinc on the GABA concentration–response relation. It was found that 30 μM zinc inhibited GABA-stimulated $^{36}Cl^-$ influx over a wide range of GABA concentrations (Fig. 2). However, the fractional inhibition was similar over the entire range of GABA concentrations used (5–500 μM), and the interaction appeared to be noncompetitive (inset, Fig. 2). The inhibition by zinc could not be reversed by increasing the GABA concentration to 500 μM, which is 5 times the concentration required for maximum $^{36}Cl^-$ influx. Essentially identical results were found in cerebral cortical microsacs prepared from control rats (Fig. 2A) and flurazepam treated rats (Fig. 2B). The GABA concentration–response curves shown in Fig. 2 were analyzed by ANOVA with GABA concentration used as a repeated measure. There was a significant effect of GABA concentration [$F(5, 110) = 319$, $p < 0.0001$], and a significant effect of zinc [$F(1, 22) = 52$, $p < 0.0001$]. There was no significant effect of flurazepam treatment [$F(1, 22) = 1.44$, $p = 0.24$], and no significant interaction between flurazepam treatment and the other grouping factors.

The inhibitory effect of zinc on 30 μM GABA-stimulated $^{36}Cl^-$ influx was compared in membranes from three brain

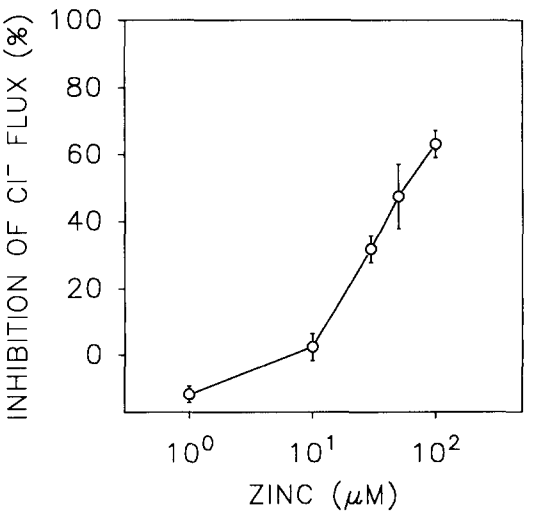

FIG. 1. Zinc inhibition of 30 μM GABA-stimulated $^{36}Cl^-$ flux into microsacs prepared from cerebral cortex. Values are means ± SEM of four experiments.

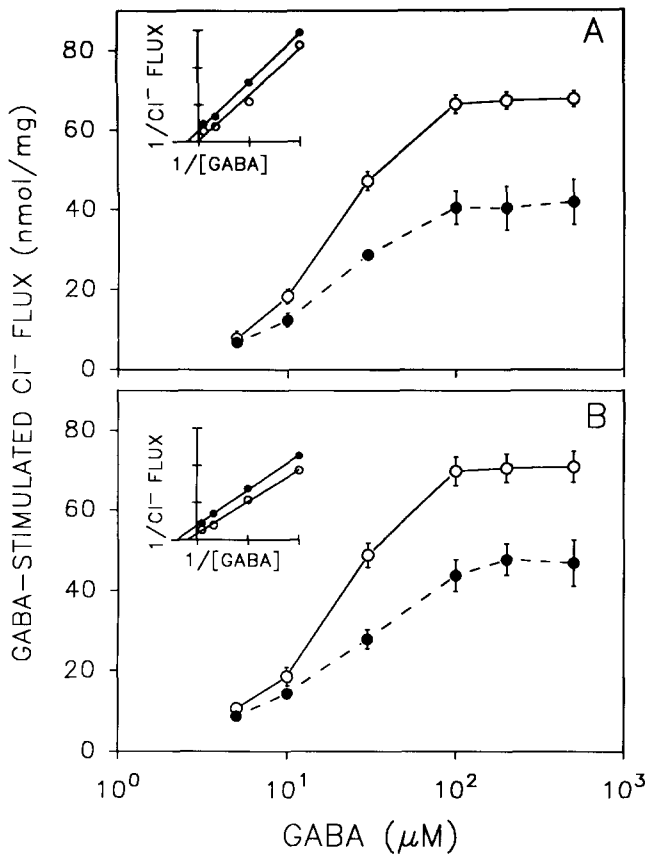

FIG. 2. GABA-stimulated $^{36}Cl^-$ flux in cerebral cortical microsacs in the absence (open circles) and in the presence (closed circles) of 30 μM $ZnCl_2$. Insets show double reciprocal plots of the data using the data points for the four lowest GABA concentrations, with calculated linear regression lines. (A) Results from six vehicle-treated rats. (B). Results from seven flurazepam-treated rats.

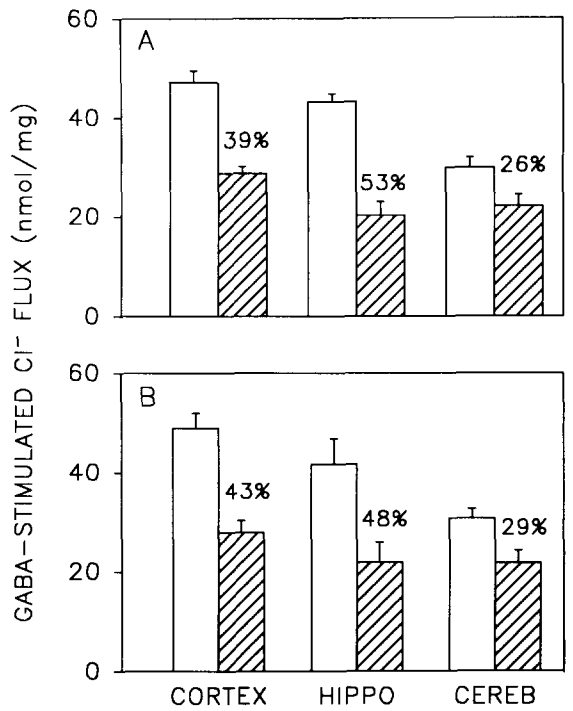

FIG. 3. 30 μM GABA-stimulated $^{36}Cl^-$ flux in microsacs prepared from cerebral cortex (CORTEX), hippocampus (HIPPO), and cerebellum (CEREB) in the absence (open bars) or in the presence of 30 μM $ZnCl_2$ (cross-hatched bars). Values indicate the mean percent inhibition caused by zinc. (A) Results from six vehicle-treated rats. (B) Results from seven flurazepam-treated rats.

regions (Fig. 3). In the absence of zinc, it was noted that 30 μM GABA-stimulated $^{36}Cl^-$ influx in rat brain vesicles varied regionally. The relative order of magnitude of $^{36}Cl^-$ influx (cortex ≈ hippocampus > cerebellum) was similar to results reported by Tietz and Chiu (33) in the same preparation, and by Luu et al. (16) in synaptoneurosomes. Inhibition of GABA-stimulated $^{36}Cl^-$ influx also varied regionally, being greatest in hippocampus and least in cerebellum. In each brain region studied, essentially identical results were found in microsacs prepared from control rats (Fig. 3A) and flurazepam treated rats (Fig. 3B). The results in Fig. 3 were analyzed by ANOVA. There was a significant effect of zinc [$F(1, 32) = 298$, $p < 0.0001$] and among brain regions [$F(2, 32) = 9.5$, $p < 0.001$], but no significant effect of flurazepam treatment [$F(1, 32) = 0.006$, $p = 0.9$]. A significant interaction between zinc and brain region [$F(2, 32) = 18$, $p < 0.0001$] demonstrated the regional difference in susceptibility to zinc.

The acute interactions of zinc with three benzodiazepines (triazolam, diazepam, and bretazenil) were also examined. As shown in Table 1, all three benzodiazepines enhanced GABA-stimulated $^{36}Cl^-$ influx. Triazolam and diazepam produced a greater maximum effect than the partial agonist, bretazenil. In the presence of these benzodiazepines, zinc still produced the same relative inhibition of $^{36}Cl^-$ influx, as shown in the far right column of Table 1. The effects of benzodiazepines, expressed as the percent increase over the corresponding baseline GABA-stimulated $^{36}Cl^-$ influx (i.e., at 0 or 30 μM zinc) produced by the 1.0 μM concentration of each drug (means shown in parentheses in Table 1) was used to analyze the

TABLE 1

BENZODIAZEPINE POTENTIATION OF 10 μM GABA-STIMULATED $^{36}Cl^-$ INFLUX INTO CEREBRAL CORTICAL MICROSACS ($n = 6$)

Agent	0 Zn^{2+}	30 μM Zn^{2+}	% Inhibition
None	18.2 ± 0.6	10.4 ± 0.9	43%
Diazepam, 0.1 μM	24.1 ± 2.4	14.2 ± 1.9	41%
Diazepam, 1 μM	30.0 ± 2.1	15.3 ± 1.4	49%
(% Control)*	(164 ± 6)	(147 ± 4)	
Bretazenil, 0.1 μM	20.7 ± 1.3	13.1 ± 1.3	37%
Bretazenil, 1 μM	24.1 ± 1.8	13.4 ± 2.1	44%
(% Control)	(132 ± 8)	(129 ± 14)	
Triazolam, 0.1 μM	25.9 ± 1.5	12.6 ± 1.9	51%
Triazolam, 1 μM	28.9 ± 2.1	12.5 ± 1.4	57%
(% Control)	(153 ± 10)	(128 ± 3)†	

*Potentiation of GABA-stimulated $^{36}Cl^-$ flux by 1 μM benzodiazepine, expressed as a percent of the corresponding control.

†Significantly less than the triazolam effect in the absence of Zn^{2+}.

zinc-benzodiazepine interaction. These data were analyzed by ANOVA with specific planned comparisons for each drug. For diazepam and bretazenil, the enhancement of GABA-stimulated $^{36}Cl^-$ influx, expressed as a percent of the appropriate baseline, was similar in the presence or absence of zinc. However, zinc significantly reduced the effect of triazolam [$F(1, 15) = 5.5, p < 0.05$].

DISCUSSION

The $GABA_A$ receptor is a multisubunit complex containing a number of allosteric binding sites that can modulate receptor-ion channel function (22,27). Zinc has been shown to inhibit $GABA_A$ receptor function (2,4,26,31,35). Previous reports were obtained mainly from cultured neurons using electrophysiologic techniques. The present results have provided further evidence of zinc antagonism of $GABA_A$ receptor function in brain membrane vesicles using the $^{36}Cl^-$ influx technique. Our data were consistent with the report of Legendre and Westbrook (12) from cultured hippocampal neurons, which indicated that zinc is a noncompetitive inhibitor of $GABA_A$ receptor function, but differed from other data that showed competitive inhibition by zinc of GABA action on frog dorsal root ganglion neurons (40).

The noncompetitive nature of zinc inhibition suggests that zinc does not compete with GABA for the recognition site. Previous electrophysiologic studies revealed that the effect of zinc was apparently independent of the binding and effects of other agents acting at the $GABA_A$ receptor, including benzodiazepines, barbiturates, steroids, and convulsant antagonists (4,28). This is further supported by the present experiments. Assays of the acute interaction between zinc and benzodiazepines revealed that although diazepam, triazolam, and bretazenil increased the potency of GABA, they had no effect on the action of zinc. In addition, zinc had no significant effect on the ability of diazepam or bretazenil to enhance GABA-stimulated $^{36}Cl^-$ influx. These findings point to a lack of interaction between zinc and benzodiazepine sites on the $GABA_A$ receptor. Unlike the other two benzodiazepines, triazolam potentiation of GABA-stimulated $^{36}Cl^-$ influx was inhibited by zinc. This may indicate some difference in the interaction of triazolam, as compared with other benzodiazepines, with $GABA_A$ receptors, or may suggest some other unexpected interaction between zinc and triazolam in the assay.

The effect of long-term benzodiazepine administration was also studied. It has been reported that long-term treatment with benzodiazepines can reduce their binding (17,24,34), and tolerance can be shown by a reduced ability to enhance GABA-stimulated Cl^- influx (1,14,20,41), suggesting structural and functional changes of $GABA_A$/benzodiazepine receptors after long-term treatment with benzodiazepines. Even if zinc and benzodiazepines do not share a common recognition site on the $GABA_A$ receptor, there could still be alterations in the receptor during long-term treatment that might affect zinc inhibition of $GABA_A$ receptor function. Although the flurazepam treatment used is known to produce tolerance in this Cl^- influx assay (14,20), there was no difference in zinc effect between control and flurazepam-tolerant rats in any of the three brain areas studied. Like the short-term interaction, long-term benzodiazepine administration also indicated independence of zinc and benzodiazepine effects on the $GABA_A$ receptor.

The regional variation of GABA-stimulated Cl^- influx and zinc antagonism of GABA response could be due to regional differences in $GABA_A$ receptors. There is ample evidence for such $GABA_A$ receptor heterogeneity. The existence of multiple $GABA_A$ receptor protein subunits provides a theoretical basis for multiplicity of $GABA_A$ receptors (3,15). Regional differences in $GABA_A$ receptors have been found by comparing immunohistochemical localization of receptors with a monoclonal antibody and autoradiographic distribution of [^{3}H]-muscimol and [^{3}H]RO15-1788 binding sites (25). Several studies have provided evidence for varying regional distribution of receptor subunits using cDNA or cRNA hybridization (11, 13,19,26,37). Further investigation might address the possible role of differences in $GABA_A$ receptor subunit composition in determining regional variability in GABA-stimulated Cl^- flux and its sensitivity to zinc.

Expression studies showed that hetero-oligomeric $GABA_A$ receptor channels composed of only α plus β subunits were sensitive to zinc inhibition but were not affected by benzodiazepines, whereas channels that contained the γ subunit in combination with α and β subunits were sensitive to typical benzodiazepine potentiation (23), but rather insensitive to zinc (7). In addition, Smart et al. (32) also demonstrated that the recombinant $GABA_A$ channels expressed in kidney cells could be differentiated in terms of sensitivity to zinc: receptors containing the γ_2 subunit were relatively less sensitive to zinc inhibition. From these observations, it might be surmised that $GABA_A$ receptors exist in forms that are sensitive to benzodiazepine but not zinc, and others sensitive to zinc but not benzodiazepine. Thus, in a system containing a heterogeneous population of $GABA_A$ receptors (such as intact neurons in the brain or the microsac preparation), the zinc sensitivity would depend on the complement of γ_2 subunit-containing $GABA_A$ receptors. The greater the fraction of receptors with the γ_2 subunit, the greater the benzodiazepine effect, and the greater the resistance to zinc. If this hypothesis were correct, inhibiting $GABA_A$ receptors that did not include γ_2 subunits would leave only those at which benzodiazepines could act, and the relative effect of benzodiazepines would be magnified. Recent work by Davies et al. (6), who investigated the potentiation of Cl^- influx by flunitrazepam in rat brain microsacs, found that 100 μM zinc inhibited a constant amount of 10 μM GABA-stimulated $^{36}Cl^-$ influx over a range of flunitrazepam concentrations, which supports the hypothesis. However, our data did not support the hypothesis. We found that a constant fraction of Cl^- flux was inhibited by zinc, and that the percent enhancement of 10 μM GABA-stimulated Cl^- influx produced by diazepam or bretazenil was not affected by zinc, whereas the percent enhancement by triazolam was actually decreased in the presence of zinc. The regional sensitivity to zinc inhibition of GABA response also did not support the idea that only receptors devoid of the γ subunit would be sensitive to zinc. We found that zinc had the greatest effect in hippocampus and least in cerebellum. However, the relative abundance of the γ_2 subunit mRNA is no less in hippocampus than in cerebellum (18,26,36). The hypothesis that sensitivity to zinc inhibition and benzodiazepine responsiveness are inversely correlated was also not supported by experiments on cultured hippocampal (12) and superior cervical ganglion neurons (32), in which benzodiazepines potentiated and Zn^{2+} inhibited GABA-mediated currents in the same neurons. It should be noted that these experiments differ from those in which recombinant receptors are expressed, in that heterogenous populations of receptors likely contributed to the response. In the microsac preparation used in the present Cl^- flux experiments, an even greater degree of receptor heteroge-

neity is likely. Recently, Gurley et al. (8) reported that blocking γ expression in the presence of cortical mRNA does not alter sensitivity to zinc. These findings, as well as those presented here, suggest that the zinc sensitivity of neurons is determined by factors other than, or in addition to, the presence or absence of a γ subunit in the $GABA_A$ receptors.

ACKNOWLEGEMENTS

The authors wish to thank Dr. Peter Sorter and Hoffmann-LaRoche, Nutley, New Jersey, for the gifts of diazepam and bretazenil. Triazolam was a gift from Warren King (Upjohn Co., Kalamazoo, MI). This work was supported by Research Grant RO1-DA02194 from the National Institute on Drug Abuse.

REFERENCES

1. Allan, A. M.; Baier, L. D.; Zhang, X. Effects of lorazepam tolerance and withdrawal on $GABA_A$ receptor-operated chloride channels. J. Pharmacol. Exp. Ther. 261:395–402; 1992.
2. Baraldi, M.; Caselgrandi, E.; Santi, M. Effect of zinc on specific binding of GABA to rat brain membranes. In: Frederickson, C. J.; Howell, G. A.; Kasarskis, E. J. eds. The neurobiology of zinc. Part A: Physiochemistry, anatomy, and techniques. New York: Alan R. Liss; 1984: 59–71.
3. Burt, D. R.; Kamatchi, G. L. $GABA_A$ receptor subtypes: from pharmacology to molecular biology. FASEB J. 5:2916–2923; 1991.
4. Celentano, J. J.; Gyenes, M.; Gibbs, T. T.; Farb, D. H. Negative modulation of the γ-aminobutyric acid response by extracellular zinc. Mol. Pharmacol. 40:766–773; 1991.
5. Curtis, D. R.; Gynther, B. D. Divalent cations reduce depolarization of primary afferent terminations by GABA. Brain Res. 422: 192–195; 1987.
6. Davies, M. F.; Maguire, P. A.; Loew, G. H. Zinc selectively inhibits flux through benzodiazepine-insensitive γ-aminobutyric acid chloride channels in cortical and cerebellar microsacs. Mol. Pharmacol. 44:876–881; 1993.
7. Draguhn, A.; Verdoorn, T. A.; Ewert, M.; Seeburg, P. H.; Sakmann, B. Functional and molecular distinction between recombinant rat $GABA_A$ receptor subtypes by Zn^{2+}. Neuron 5:781–788; 1990.
8. Gurley, D. A.; Stirling, V.; Hartnett, C.; Ross, P. C.; White, G. Interactions between Zn and the $GABA_A$ receptor/ionophore complex expressed in Xenopus oocytes. Soc. Neurosci. Abstr. 19: 1140; 1993.
9. Heninger, C.; Saito, N.; Tallman, J. F.; Garrett, K. M.; Vitek, M. P.; Duman, R. S.; Gallager, D. W. Effect of continuous diazepam administration on $GABA_A$ subunit mRNA in rat brain. J. Mol. Neurosci. 2:101–107; 1990.
10. Kang, I.; Miller, L. G. Decreased $GABA_A$ receptor subunit mRNA concentrations following chronic lorazepam administration. Br. J. Pharmacol. 103:1285–1287; 1991.
11. Kato, K. Novel $GABA_A$ receptor α subunit is expressed only in cerebellar granule cells. J. Mol. Biol. 214: 619–624, 1990.
12. Legendre, P.; Westbrook, G. L. Noncompetitive inhibition of γ-aminobutyric $acid_A$ channels by Zn. Mol. Pharmacol. 39:267–274; 1991.
13. Levitan, E. S.; Schofield, P. R.; Burt, D. R.; Rhee, L. M.; Wisden, W.; Köhler, M.; Fujita, N.; Rodriguez, H. F.; Stephenson, A.; Darlison, M. G.; Barnard, E. A.; Seeburg, P. H. Structural and functional basis for $GABA_A$ receptor heterogeneity. Nature 335:76–79; 1988.
14. Li, M.; Rosenberg, H. C.; Chiu, T. H. Tolerance to the effects of diazepam, clonazepam and bretazenil on GABA-stimulated Cl^- influx in flurazepam tolerant rats. Eur. J. Pharmacol. Mol. Pharmacol. 247:313–318; 1993.
15. Lüddens, H.; Wisden, W. Function and pharmacology of multiple $GABA_A$ receptor subunits. Trends. Pharmacol. Sci. 12:49–51; 1991.
16. Luu, M. D.; Morrow, A. L.; Paul, S. M.; Schwartz, R. D. Characterization of $GABA_A$ receptor-mediated ^{36}chloride uptake in rat brain synaptoneurosomes. Life Sci. 41:1277–1287; 1987.
17. Miller, L. G.; Greenblatt, D. J.; Barnhill, J. G.; Shader, R. I. Chronic benzodiazepine administration. I. Tolerance is associated with benzodiazepine receptor downregulation and decreased γ-aminobutyric $acid_A$ receptor function. J. Pharmacol. Exp. Ther. 246:170–176; 1988.
18. Mohler H.; Malherbe, P.; Draguhn, A.; Richards, J. G. $GABA_A$-receptors: Structural requirements and sites of gene expression in mammalian brain. Neurochem. Res. 15:199–207; 1990.
19. Montpied, P.; Ginns, E. I.; Martin, B. M.; Stetler, D.; O'Carroll, A.-M.; Lolait, S. J.; Mahan, L. C.; Paul, S. M. Multiple $GABA_A$ receptor α subunit mRNAs revealed by developmental and regional expression in rat, chicken and human brain. FEBS Lett. 258:94–98; 1989.
20. Ngur, D. O.; Rosenberg, H. C; Chiu, T. H. Modulation of GABA-stimulated Cl^- flux by a benzodiazepine agonist and an "inverse agonist" after chronic flurazepam treatment. Eur. J. Pharmacol. 176:351–356; 1990.
21. O'Donovan, M. C.; Buckland, P. R.; Spurlock, G; McGuffin, P. Bidirectional changes in the levels of messenger RNAs encoding γ-aminobutyric $acid_A$ receptor α subunits after flurazepam treatment. Eur. J. Pharmacol. 226:335–341; 1992.
22. Olsen, R. W.; Tobin, A. J. Molecular biology of $GABA_A$ receptors. FASEB J. 4:1469–1480; 1990.
23. Pritchett, D. B.; Sontheimer, H.; Shivers, B. D.; Ymer, S.; Kettenmann, H.; Schofield, P. R.; Seeburg, P. H. Importance of a novel $GABA_A$ receptor subunit for benzodiazepine pharmacology. Nature 338:582–585; 1989.
24. Rosenberg, H. C.; Chiu, T. H. Tolerance during chronic benzodiazepine treatment associated with decreased receptor binding. Eur. J. Pharmacol. 70:453–460; 1981.
25. Schoch, P.; Richards, J. G.; Häring, P.; Takacs, B.; Stähli, C.; Staehelin, T.; Haefely, W; Möhler, H. Co-localization of $GABA_A$ receptors and benzodiazepine receptors in the brain shown by monoclonal antibodies. Nature 314:168–171; 1985.
26. Shivers, B. D.; Kilisch, I.; Sprengel, R.; Sontheimer, H.; Köhler, M.; Schofield, P. R.; Seeburg, P. H. Two novel $GABA_A$ receptor subunits exist in distinct neuronal subpopulations. Neuron 3:327–337; 1989.
27. Sieghart, W. $GABA_A$ receptors: Ligand-gated Cl^- ion channels modulated by multiple drug-binding sites. Trends Pharmacol. Sci. 13:446–450; 1992.
28. Smart, T. G. A novel modulatory binding site for zinc on the $GABA_A$ receptor complex in cultured rat neurones. J. Physiol. 447:587–625; 1992.
29. Smart, T. G.; Constanti, A. A novel effect of zinc on the lobster muscle GABA receptor. Proc. R. Soc. Lond. B. 215:327–341; 1982.
30. Smart, T. G.; Constanti, A. Pre and postsynaptic effects of zinc on in vitro prepyriform neurones. Neurosci. Lett. 40:205–211; 1983.
31. Smart, T. G.; Constanti, A. Differential effect of zinc on the vertebrate $GABA_A$-receptor complex. Br. J. Pharmacol. 99:643–654; 1990.
32. Smart, T. G.; Moss, S. J.; Xie, X.; Huganir, R. L. $GABA_A$ receptors are differentially sensitive to zinc: dependence on subunit composition. Br. J. Pharmacol. 103:1837–1839; 1991.
33. Tietz, E. I.; Chiu, T. H. Regional GABA-stimulated chloride uptake in amygdala kindled rats. Neurosci. Lett. 123:269–272; 1991.
34. Tietz, E. I.; Rosenberg, H. C.; Chiu, T. H. Autoradiographic location of benzodiazepine receptor downregulation. J. Pharmacol. Exp. Ther. 236:284–292; 1986.
35. Westbrook, G. L.; Mayer, M. L. Micromolar concentrations of Zn^{2+} antagonize NMDA and GABA responses on hippocampal neurons. Nature 328:640–643; 1987.

36. Wisden, W.; Laurie, D. J.; Monyer, H.; Seeburg, P. H. The distribution of 13 $GABA_A$ receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon. J. Neurosci. 12:1040–1062; 1992.
37. Wisden, W.; Morris, B. J.; Darlison, M. G.; Hunt, S. P.; Barnard, E. A. Distinct $GABA_A$ receptor alpha subunit mRNAs show differential patterns of expression in bovine brain. Neuron 1:937–947; 1988.
38. Wu, Y.; Rosenberg, H. C.; Chiu, T. H.; Ramsey-Williams, V. Regional changes in [^{3}H]zolpidem binding to brain benzodiazepine receptors in flurazepam tolerant rat: Comparison with changes in [^{3}H]flunitrazepam binding. J. Pharmacol. Exp. Ther. 268:675–682; 1994.
39. Xie, X.; Smart T. G. A physiological role for endogenous zinc in rat hippocampal synaptic neurotransmission. Nature 349:521–524; 1991.
40. Yakushiji, T.; Tokutomi, N.; Akaike, N; Carpenter, D. O. Antagonists of GABA responses, studied using internally perfused frog dorsal root ganglion neurons. Neuroscience 22:1123–1133; 1987.
41. Yu, O.; Chiu, T. H.; Rosenberg, H. C. Modulation of GABA-gated chloride ion flux in rat brain by acute and chronic benzodiazepine administration. J. Pharmacol. Exp. Ther. 246:107–113; 1988.
42. Zhao, T.-J.; Chiu, T. H.; Rosenberg, H. C. Reduced expression of the γ-aminobutyric acid type A/benzodiazepine receptor γ_2 and α_5 subunit mRNAs in brain regions of flurazepam-treated rats. Mol. Pharmacol. 45:657–663; 1994.