Pharmacology Biochemistry and Behavior, Vol. 44, pp. 133–139, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Solubilization of Benzodiazepine Receptors From Long- and Short-Sleep Mice

JEANNE M. WEHNER,[1] THOMAS Z. BOSY AND LEA A. WALTRIP

Institute for Behavioral Genetics and School of Pharmacy, Box 447, University of Colorado, Boulder, CO 80309

Received 6 April 1992

WEHNER, J. M., T. Z. BOSY AND L. A. WALTRIP. *Solubilization of benzodiazepine receptors from long- and short-sleep mice.* PHARMACOL BIOCHEM BEHAV 44(1) 133–139, 1993.—Previous studies have shown that long-sleep (LS) and short-sleep (SS) mice, which were selectively bred for differential responses to the sedative-hypnotic actions of ethanol, also differ in response to several other agents that act at the GABAergic receptor. Binding at cortical benzodiazepine receptors is enhanced differentially by GABA and ethanol in membranes prepared from the two lines of mice with SS receptors enhanced to a greater extent. Heat denaturation studies and other biochemical characterizations of these receptors suggest that the GABAergic receptor complex from the two lines of mice differs. The present study examined whether perturbation of receptor-membrane interactions by treatment with detergent altered either GABA enhancement of [^{3}H]flunitrazepam binding or ethanol enhancement of this binding. Octylglucopyranoside (OCTG), 3-[(3-cholamidopropyl-dimethylammonio]-1-propanesulfonate (CHAPS), or deoxycholate solubilization of cortical membranes resulted in a loss of the LS/SS difference in GABA enhancement. Ethanol's effects on binding were altered differently from those of GABA by treatment with OCTG as an increase, not a decrease, in enhancement was observed in both lines of mice. These results suggest that protein-membrane interactions play an important role in mediating LS/SS differences in the allosteric interactions within the GABAergic receptor complex.

Genetics Alcohol GABAergic receptors Membrane-protein interactions

LONG-sleep (LS) and short-sleep (SS) mice are genetically selected lines of mice bred for differential sensitivity to the sedative-hypnotic effects of ethanol (21). This bidirectional selection study resulted in LS mice being extremely sensitive to high doses of ethanol as measured by duration of loss of the righting response (sleep time) and SS mice being relatively insensitive to this effect of ethanol (21). Differences in CNS sensitivity to ethanol underlie LS/SS response differences as only small differences in ethanol absorption or metabolism have been observed (30). Because ethanol can potentiate GABAergic function (2,34,35), potential differences in the GABAergic system have been explored extensively in these mouse lines.

Behavioral studies of responses of the LS and SS selected lines of mice to agents that can interact at the GABAergic receptor have indicated that regulation of differential responsiveness is not simple but rather is dependent upon the particular agent and behavioral or physiological response (7,14, 29,33). For example, LS mice are more sensitive to the sedative-hypnotic effects of not only ethanol but also benzodiazepines (14,22). Differential responsiveness is also observed to some, but not all, barbiturates; these differential effects of barbiturates appear to depend upon lipid solubility of the barbiturate (15). Extensive data suggest that LS/SS differences in sleep-time response to ethanol and other GABAergic agents are associated with differential sensitivity of the GABA-coupled Cl^- channel in the cerebellum (2,3). GABA-potentiated Cl^- flux is increased by ethanol and flunitrazepam in LS cerebellar tissue while ethanol has little effect in SS cerebellar tissue (2,10). Total brain mRNA injected into *Xenopus* oocytes can reproduce the LS/SS difference in potentiation of GABA-stimulated Cl^- flux (34).

In addition to differential responsiveness to the sedative-hypnotic actions of drugs in LS/SS mice, other behavioral differences in response to GABAergic drugs have also been described (14,19,29,33) that are likely mediated by brain regions other than the cerebellum. LS mice are more susceptible to seizures produced by the glutamic acid decarboxylase (GAD) inhibitor 3-mercaptopropionic acid than are SS mice (18). LS mice are also more sensitive to the anxiolytic actions of diazepam (33), but SS mice are more sensitive to the anticonvulsant effects of benzodiazepines (14). To determine whether differences in the GABAergic receptor complex may mediate some of these behavioral differences, analyses of differing components of the GABAergic receptor have been performed.

For the most part, either no difference, or only modest differences, have been observed in GABA receptor number

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

(2), benzodiazepine receptor number (16,17), or binding at the barbiturate site (2,23). However, fundamental differences in properties of the cortical GABAergic receptor complex in LS and SS mice are supported by measurements of GABA enhancement of [^{3}H]flunitrazepam binding (^{3}H-FNZ) (17,24), temperature effects on the potency of GABA to enhance ^{3}H-FNZ binding (24), heat denaturation patterns of ^{3}H-FNZ binding (16,24), and the effects of GABA and barbiturates on rates of dissociation of *t*-butylbicyclophosphorothionate (TBPS) from its binding sites (23). Further, in a more extensive genotypic survey including LS and SS mice a significant correlation between GABA enhancement of benzodiazepine binding in forebrain-washed membranes and susceptibility to seizures produced by a GAD inhibitor was observed (18). These data suggest that one or more of the subunits of the GABAergic receptor complex may be physically different or that membrane-receptor interaction may differ between LS and SS cortical receptors. Clearly, because of the heterogeneity of GABAergic receptors it will be important to characterize potential differences in this receptor system to understand the nature of the biochemical variability that determines genetic variability in behavioral response. In the present study, we examined whether detergent solubilization and perturbation of normal receptor-membrane interactions in LS and SS cortical tissue have any effect on LS/SS differences in GABA or ethanol potentiation of ^{3}H-FNZ binding in the cortex, a measure correlated with seizure susceptibility to GAD inhibitors in these mice. The GABAergic receptor complex has been solubilized and purified using several detergents (31,32). A number of these detergents have allowed extraction of the receptor complex from the membrane while retaining the allosteric properties of the receptor complex (5,9,32), thus allowing a comparison of LS and SS receptors in membrane preparations with solubilized preparations.

METHOD

Animals

Female LS and SS mice were obtained from the Institute for Behavioral Genetics (Boulder, CO). All mice were 60–90 days of age when sacrificed by cervical dislocation. Animals were maintained on a 12 L : 12 D cycle (light 0700–1900) and permitted free access to food and water.

Chemical

^{3}H-FNZ was purchased from New England Nuclear Products (Boston, MA); specific activity = 85.3 Ci/mmol. All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Preparation of Membranes

In each experiment, the enhancement was compared in membrane vs. solubilized tissue. These control membranes were assayed to examine whether various buffer and ionic conditions, as well as incubation conditions, affected the previously described LS/SS differences. For each experiment, 13–15 cortices from each mouse line were dissected on ice and hippocampi removed. The pooled cortical tissue was homogenized in 30 ml 20 mM Tris citrate, pH = 7.1, at 4°C, using a motorized Teflon-glass homogenizer. Homogenates were centrifuged at 1,000× *g* for 10 min. The resulting supernatant was centrifuged at 20,000× *g* for 20 min. The resulting P_2 pellet was washed five times in 30 ml buffer by repeated centrifugations at 20,000× *g* for 20 min. After resuspension of the final pellet, approximately one third of the preparation was used to assay enhancement in membranes as described below. The remaining portion was recentrifuged and the pellet used for solubilization of receptors.

Solubilization of Receptors

In general, the procedures of Hammond and Martin were modified for preparation of solubilized membranes (9). The washed membrane pellet was resuspended in 5 mM Tris citrate, pH = 7.1, at 4°C. Protein concentration was determined by the method of Lowry et al. (11). The sample was diluted to obtain a protein concentration of 8.0 mg/ml; this solution was then diluted to a final concentration of 4.0 mg/ml in buffer (100 mM Tris citrate, pH = 7.1, at 4°C) containing: EDTA (325.5 mg/l), soybean trypsin inhibitor (50 mg/l), bacitracin (50 mg/ml), and phenylmethysulfonyl fluoride (17.5 mg/l) containing detergent. The detergents tested included octylglucopyranoside (OCTG; 1% final concentration) (9), 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS; 1.6% final concentration) (9), or deoxycholate (0.5% final concentration) (12). After addition of detergents, samples were incubated for 30 min on ice. Solubilized protein was subjected to centrifugation at 100,000× *g* for 60 min. The resulting supernatant was used to assay for GABA or ethanol enhancement of ^{3}H-FNZ binding as described below.

Membrane Receptor Assays

GABA and ethanol enhancement of ^{3}H-FNZ binding was performed as previously described except some modifications were necessary to provide a better comparison with solubilized receptors. Assays were conducted in a 250-μl volume containing ^{3}H-FNZ (1.0 nM), NaCl (200 mM), Tris citrate (100 mM, pH = 7.1, at 4°C), and varying concentration of GABA (10^{-7} to 10^{-4} M) or ethanol (20–60 mM). Reactions were initiated by the addition of membranes that were resuspended by hand homogenization immediately before addition to the reaction. Samples were assayed in duplicate and protein concentration ranged from 200–300 μg/assay. Tubes were incubated for 30 min at 37°C, a temperature shown to optimize LS/SS differences in GABA or ethanol enhancement (4). However, because of the known effects of temperature on LS/SS differences in enhancement of ^{3}H-FNZ binding (24) an additional incubation step was added to provide comparison with the solubilized receptors. Thus, after 30 min at 37°C test tubes were placed on ice for 30 min. Reactions were stopped by the addition of 5 mM Tris citrate, pH = 7.1, and filtered on GF/C glass fiber filters (Whatman, Clifton, NJ). Filters were washed twice with cold 5 mM Tris citrate buffer. Filters were counted by liquid scintillation methods in a Beckman LS 7000 counter (Beckman Instruments, Fullerton, CA). Nonspecific binding was determined by the addition of flurazepam (10^{-5} M) to some reaction tubes. Enhancement was calculated as the percent of specific binding in the presence of varying concentrations of GABA or ethanol.

Soluble Protein Assays

Assays were conducted as described for membrane preparations except in 5 mM Tris citrate (pH = 7.1) in 1.0-ml vol with appropriate adjustments of radioligands, salts, and modulators as described by others to give maximal enhancement in solubilized preparations (9). Two hundred microliters of

supernatant was added to start the reactions. Duplicate samples were assayed and incubated at 37°C for 30 min. Protein was precipitated by incubation on ice for 30 min after the addition of 75 μl γ-globulin (33 mg/ml) and 425 μl 35.2% PEG followed by vortexing until a white precipitate appeared. Samples were incubated on ice for 30 min, filtered over GF/B glass fiber filters (Whatman), and washed twice with ice-cold 5 mM Tris citrate buffer, pH = 7.1, containing 8% PEG. Samples were counted as above and per cent enhancement calculated.

Data Analysis

Data were analyzed first using a four-way analysis of variance (ANOVA) to compare mouse lines, the various detergents, treatment of tissue (membrane vs. solubilized preparations), and concentration effects of GABA. The experiments with ethanol were analyzed by a 3-way ANOVA to compare mouse lines, treatment of tissue, and concentration of ethanol. Significant interaction terms were further analyzed by 3-, 2-, or 1-way ANOVAs as appropriate.

RESULTS

GABA Enhancement of ^{3}H-FNZ Binding

The effects of solubilization on GABA enhancement of cortical ^{3}H-FNZ binding was evaluated in tissue from LS and SS mice using three different detergents. In general, the conditions used in this study (buffers and incubation temperatures) resulted in less robust LS/SS differences in GABA enhancement of binding in membranes than those previously reported (17,18,24); however, line differences were still significant. Results of a four-way ANOVA indicated significant main effects of mouse line, $F(1, 291) = 7.97, p < 0.01$, type of detergent, $F(2, 291) = 40.8, p < 0.0001$, and concentration of GABA, $F(3, 291) = 61.3, p < 0.0001$. Significant interactions included: line × tissue treatment × detergent, $F(2, 291) = 6.72, p < 0.001$; line × tissue treatment, $F(1, 291) = 14.99, p < 0.0001$; tissue treatment × detergent, $F(2, 291) = 59.3, p < 0.0001$; tissue treatment × GABA, $F(3, 291) = 3.62, p < 0.05$; and detergent × GABA concentration, $F(6, 291) = 4.36, p < 0.001$. While all these interactions were further analyzed, only those results that provided insights into mouse line differences or differential effects of detergents in the two tissue preparations are presented here.

The line × tissue interaction was further analyzed and the results indicated that enhancement by GABA was different in membranes prepared from LS and SS mice such that SS mice showed a greater enhancement, $F(1, 147) = 17.7, p < 0.001$. However, in solubilized tissues the LS/SS difference was lost. It was apparent from the interaction terms that all the detergents may not be producing the same effects in the mouse lines or in the two tissue preparations. This possibility was tested by analyzing each detergent treatment separately. The data for each detergent is presented in separate figures.

The results of solubilization with OCTG are shown in Fig. 1. A two-way ANOVA indicated that there was no significant difference between LS and SS tissue but there was a significant effect of tissue treatment, $F(1, 101) = 17.6, p < 0.001$, and a significant line × tissue interaction, $F(1, 101) = 9.58, p < 0.01$. This interaction appears to be due to the fact that SS tissue was enhanced to a greater degree than LS tissue in the membrane fraction prior to solubilization, $F(1, 51) = 8.82, p < 0.01$, but in the OCTG-solubilized preparation the LS/SS difference was lost. Further, as shown in Fig. 1, LS tissue showed no change in GABA enhancement after solubilization, $F(1, 48) = 0.9, p < 0.33$, while enhancement in SS tissue was affected more by solubilization, $F(1, 38) = 3.26, p < 0.07$. Any difference in how OCTG solubilization altered binding in LS and SS tissue was not due to differential recovery of protein because 70–80% of the starting membrane protein was recovered after solubilization in both LS and SS tissue.

The effects of solubilization on GABA enhancement of benzodiazepine binding in LS and SS cortex with the detergent CHAPS is shown in Fig. 2. With CHAPS, there was no main effect of mouse line when data was collapsed across GABA concentration. There was a significant effect of tissue treatment, $F(1, 110) = 32.7, p < 0.0001$, such that in solubilized tissues from both lines GABA enhancement was greater than in the original membranes. There was no significant line × tissue treatment interaction, indicating that LS and SS tissue was not differentially affected by CHAPS solubilization.

The effects of solubilization by deoxycholate on GABA-enhanced ^{3}H-FNZ binding are shown in Fig. 3. In general, deoxycholate solubilization produced the smallest changes in GABA enhancement of binding of the three detergents. There

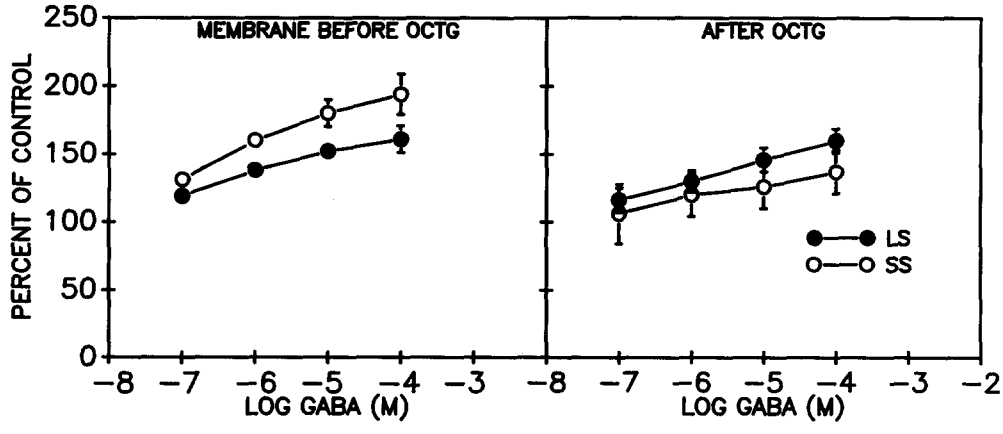

FIG. 1. GABA enhancement of [^{3}H]flunitrazepam (^{3}H-FNZ) binding and the effects of octylglucopyranoside (OCTG) solubilization. Cortical tissue was prepared and assayed as described in the Method section; each point represents mean ± SEM for n = 5–7 experiments. LS, long-sleep mice; SS, short-sleep mice.

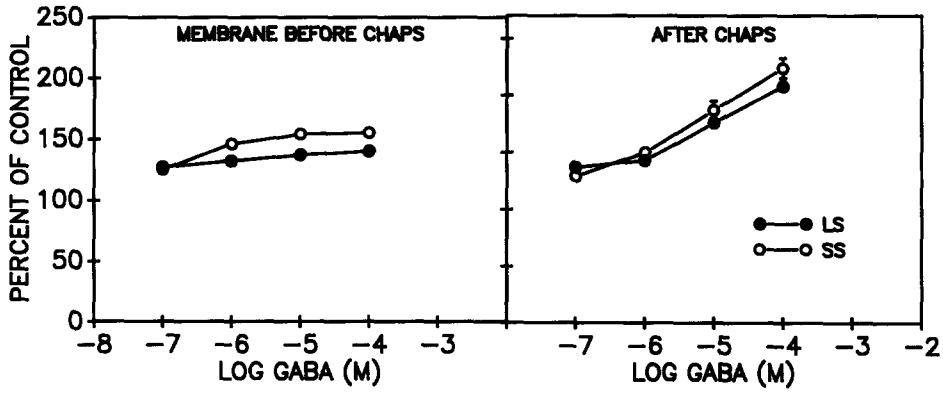

FIG. 2. GABA enhancement of [^{3}H]flunitrazepam (^{3}H-FNZ) binding and the effects of 3-[(3-cholamidopropyl-dimethylammonio]-1-propanesulfonate (CHAPS) solubilization. Cortical tissue was prepared and assayed as described in the Method section; each point represents mean ± SEM for n = 6–7 experiments. LS, long-sleep mice; SS, short-sleep mice.

was a significant effect of tissue treatment, $F(1, 78) = 4.98$, $p < 0.05$, but no significant main effect of mouse line or a significant line × tissue interaction. The recovery of protein after deoxycholate was lower than for the other detergents but was not different between the two lines (LS = 42.3 ± 0.98%, SS = 41.5 ± 0.75%).

Ethanol Effects on ^{3}H-FNZ Binding

The effects of treatment with OCTG on ethanol enhancement of ^{3}H-FNZ binding were also examined in cortical membranes from LS and SS mice. We and others reported previously that ^{3}H-FNZ binding is enhanced to a greater extent by ethanol in the cortex of SS mice than in LS mice (4,25), but detecting this difference in membrane preparations is dependent upon incubation temperature such that a line difference is observed at 37 but not at 4°C (4). Therefore, because of the necessity of incubating the membranes at 4°C after equilibrium binding was performed the differences between ethanol enhancement in washed membranes were smaller than observed in our previous studies and only evident at the 60-mM concentration. LS and SS tissues were not different at all ethanol concentrations using the conditions employed here. Nevertheless, it was of interest to determine whether solubilization with OCTG produced the same effects on ethanol enhancement as were observed on GABA enhancement.

A three-way ANOVA comparing mouse line, tissue treatment, and ethanol concentration showed no main effect of mouse line. Solubilized receptors were enhanced to a greater degree than membranes, as indicated by the significant main effect of tissue treatment, $F(1, 88) = 24.2$, $p < 0.0001$. A significant effect of ethanol concentration, $F(2, 88) = 4.65$, $p < 0.05$, was observed, but the line × ethanol concentration interaction only approached significance, $F(2, 88) = 2.63$, $p = 0.078$.

Therefore, unlike the effects of OCTG solubilization on GABA enhancement of binding (see Fig. 1), where GABA enhancement was decreased after OCTG solubilization and LS and SS tissue were differentially altered by solubilization,

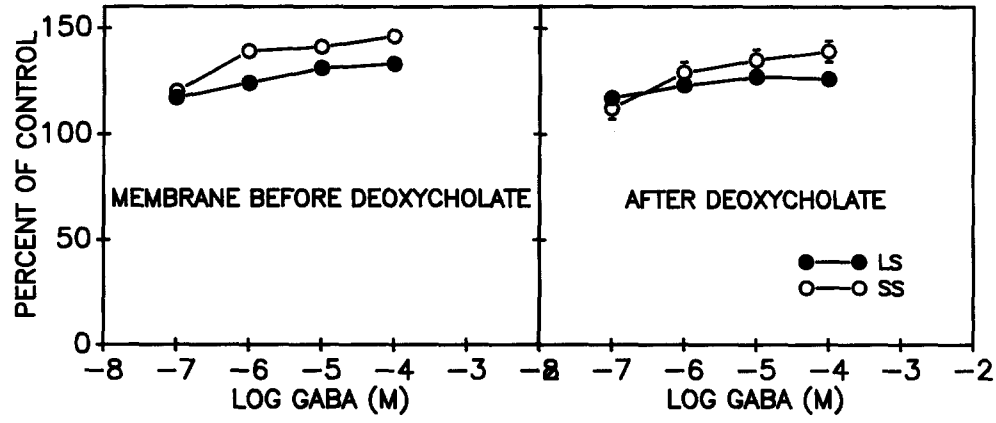

FIG. 3. GABA enhancement of [^{3}H]flunitrazepam (^{3}H-FNZ) binding and the effects of deoxycholate solubilization. Cortical tissue was prepared and assayed as described in the Method section; each point represents mean ± SEM for n = 4–5 experiments. LS, long-sleep mice; SS, short-sleep mice.

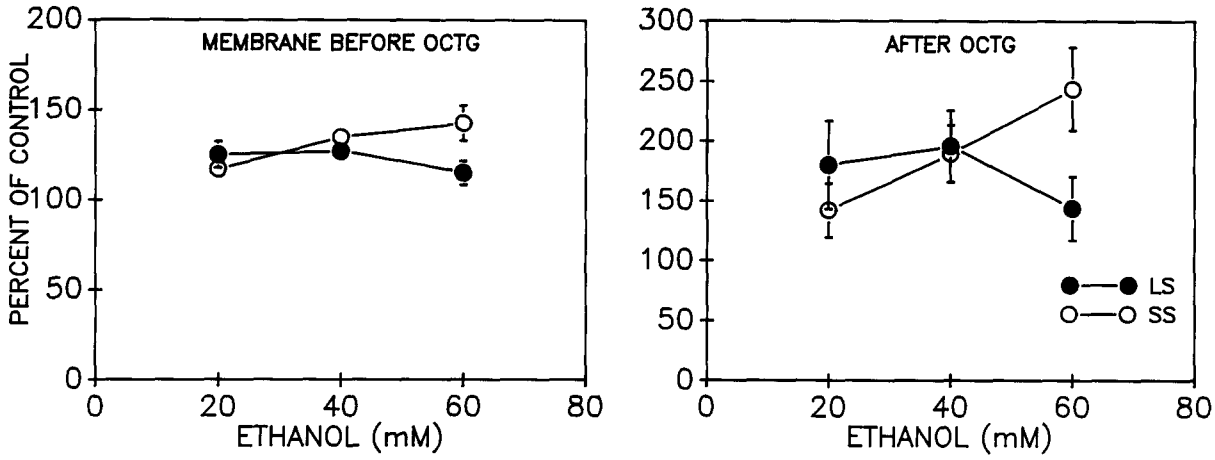

FIG. 4. Effects of ethanol on [^{3}H]flunitrazepam (^{3}H-FNZ) binding in membranes and octylglucopyranoside (OCTG)-solubilized membranes. Cortical tissue was prepared and assayed as described in the Method section; each point represents mean ± SEM for n = 6–8 experiments. LS, long-sleep mice; SS, short-sleep mice.

ethanol enhancement of binding was increased but not differentially in tissue from the two lines of mice (Fig. 4).

DISCUSSION

In general, solubilization of membrane preparations from LS and SS cortical tissue resulted in a loss of differences in GABA enhancement between these two lines of mice. In some cases, such as with OCTG solubilization and GABA enhancement of binding, the SS cortical tissue was affected more by interruptions of normal receptor-membrane interactions than was tissue from LS mice. Such a differential effect on membrane properties is not unique to this study. McIntyre et al. (24) showed that GABA enhancement of ^{3}H-FNZ binding was decreased in LS membranes by lowering the incubation temperature and presumably altering membrane fluidity. Thus, it appears that various methods of changing receptor-membrane interactions result in differential changes in the GABAergic receptor complex from the two lines of mice.

The effects of OCTG solubilization on allosteric interactions in the GABAergic complex is at least partially dependent upon the enhancing drug. While GABA enhancement of ^{3}H-FNZ binding was decreased after solubilization, ethanol effects were increased, suggesting that the conformation of the receptor may change as a function of solubilization. Alternatively, perhaps because of micelle formation, solubilization may lead to differential accessibility of the two enhancing agents to the receptor.

The degree to which GABA enhanced binding in the various solubilized preparations was dependent upon the specific detergent. Others observed that barbiturate enhancement of muscimol and benzodiazepine binding is not reproducible in deoxycholate-treated membrane preparations (28). We observed little enhancement by GABA in deoxycholate-treated membranes. Likewise, the detergent CHAPS, which has characteristics of both the polar sulfobetaine-containing detergents and the hydrophobic bile salt anionic detergents, allows retention of barbiturate enhancement of ^{3}H-FNZ and [^{3}H]muscimol binding in solubilized preparations (28,32). Consistent with the properties of CHAPS, a substantial increase in GABA enhancement of ^{3}H-FNZ binding was observed here.

Our previous studies (16), as well as those of others (23,24), suggested that LS and SS cortical receptors may be different physical entities. However, there have been no reports thus far of differences in the cloned DNA sequences or expression of the GABAergic receptor subunits from LS and SS mice. Presuming that receptor-membrane interactions are disrupted by detergent solubilization due to the removal of lipid, these data suggest that subtle differences in the protein-lipid interactions may exist between the two lines. The results of studies by Collins et al. (6) suggested that such differences may exist between LS and SS brain membranes. Their investigations examined the effects of ethanol and temperature on transition temperatures by constructing Arrhenius plots for several membrane-bound enzymes. Only the activity of the low K_i NaK-ATPase, an integral membrane protein, was affected differentially in LS and SS membranes. Neither another integral glycoprotein (5′-nucleotidase) nor a peripheral membrane protein (acetylcholinesterase) were altered differentially. Further biochemical characterization of the LS/SS difference in NaK-ATPase by heat inactivation and electrophoretic comparisons indicated no intrinsic differences in the enzyme from the two lines (13). Thus, the LS/SS differences in the effects of alcohol on NaK-ATPase activity at various temperatures suggest that unique protein-membrane interactions may exist that are specific to certain transmembrane proteins.

The site of action for alcohols and anesthetics in CNS membranes is the subject of considerable debate. Some investigators favor lipids as the site of interaction (20), while others believe that alcohols and anesthetics interact directly with proteins (8,26). The simplest interpretation of the data presented here is that detergents are removing lipids essential in maintaining greater enhancement of benzodiazepine in SS membranes. However, it is also possible that solubilization releases some other modulator, such as a protein or peptide, that is important for maintenance of some LS/SS differences in GABAergic receptors. We believe that enhancement of benzodiazepine binding by GABA and ethanol in washed cortical membranes from LS and SS mice does not relate to the anesthetic actions of drugs at the GABAergic receptor because differences in enhancement do not correlate to sleep-time differences in these lines but rather correlate to differences in seizure susceptibility (18).

The results of other studies of the GABAergic system in LS and SS mice favor a role for proteins in mediating differential anesthetic responses to ethanol. Ethanol potentiation of GABA-stimulated Cl^- flux in LS mice and several other ethanol-sensitive lines of mice is greater than in comparable selected lines of mice that are insensitive to the sleep-time-inducing properties of ethanol (1-3). It would appear that the argument for proteins as the site of action mediating ethanol's anesthetic effects is supported by the fact that oocytes injected with total brain mRNA from LS and SS mice reproduce the line differences in ethanol's effects on GABAergic currents (34). Thus, it appears that the LS/SS difference most closely correlated to sleep-time differences can be reconstituted even in a foreign membrane system. Because of the diverse actions of ethanol and benzodiazepines in the CNS and the known heterogeneity of GABAergic receptors (27), there may be multiple ways in which LS and SS GABAergic receptors differ.

In summary, the results of these studies suggest that differences in allosteric interactions as measured by enhancement of benzodiazepine binding within the cortical GABAergic receptor system of LS and SS mice may be due to subtle differences in protein-membrane interactions that are important for maintenance of benzodiazepine receptor conformation.

ACKNOWLEDGEMENTS

This work was supported by AA-03527 and a Research Scientist Development Award (AA-00141) to J.M.W. The authors thank Drs. Allan C. Collins, Barbara J. Bowers, and Richard Paylor for helpful discussions.

REFERENCES

1. Allan, A. M.; Gallaher, E. J.; Gionet, S. E.; Harris, R. A. Genetic selection for benzodiazepine ataxia produces functional changes in the gamma-aminobutyric acid receptor chloride channel complex. Brain Res. 452:118-126; 1988.
2. Allan, A. M.; Harris, R. A. Gamma-aminobutyric acid and alcohol actions: Neurochemical studies of long sleep and short sleep mice. Life Sci. 39:2005-2015; 1986.
3. Allan, A. M.; Spuhler, K. P.; Harris, R. A. Gamma-aminobutyric acid-activated chloride channels: Relationship to genetic differences in ethanol sensitivity. J. Pharmacol. Exp. Ther. 244:866-870; 1988.
4. Bowers, B. J.; Wehner, J. M. Interaction of ethanol and stress with the GABA/BZ receptor in LS and SS mice. Brain Res. Bull. 23:53-59; 1989.
5. Bristow, D. R.; Martin, I. L. Solubilization of the gamma-aminobutyric acid/benzodiazepine receptor from rat cerebellum: Optimal preservation of the modulatory responses by natural brain lipids. J. Neurochem. 49:1386-1392; 1987.
6. Collins, A. C.; Smolen, A.; Wayman, A. L.; Marks, M. J. Ethanol and temperature effects on five membrane bound enzymes. Alcohol 1:237-246; 1984.
7. Dudek, B. C.; Phillips, T. J. Genotype-dependent effects of GABAergic agents on sedative properties of ethanol. Psychopharmacology (Berl.) 98:518-523; 1989.
8. Franks, N. P.; Lieb, W. R. Are the biological effects of ethanol due to primary interactions with lipids or with proteins? Alcohol Alcohol. 1(suppl.):139-145; 1987.
9. Hammond, J. R.; Martin, I. L. Solubilization of the benzodiazepine/gamma-aminobutyric acid receptor complex: Comparison of the detergents octylglucopyranoside and 3-[(3- cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS). J. Neurochem. 47:1161-1171; 1986.
10. Harris, R. A.; Allan, A. M. Genetic differences in coupling of benzodiazepine receptors to chloride channels. Brain Res. 490: 26-32; 1989.
11. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin reagent. J. Biol. Chem. 193: 265-275; 1951.
12. Mamalake, C.; Barnard, E. A.; Stephenson, F. A. Molecular size of the gamma-aminobutyric $acid_A$ receptor purified from mammalian cerebral cortex. J. Neurochem. 52:124-134; 1989.
13. Marks, M. J.; Smolen, A.; Collins, A. C. Brain NaK-ATPases in mice differentially sensitive to alcohols. Alcohol. Clin. Exp. Res. 8:390-396; 1984.
14. Marley, R. J.; Freund, R. K.; Wehner, J. M. Differential response to flurazepam in long-sleep and short-sleep mice. Pharmacol. Biochem. Behav. 31:453-458; 1988.
15. Marley, R. J.; Miner, L. L.; Wehner, J. M.; Collins, A. C. Differential effects of central nervous system depressants in long-sleep and short-sleep mice. J. Pharmacol. Exp. Ther. 238:1028-1033, 1986.
16. Marley, R. J.; Stinchcomb, A.; Wehner, J. M. Further characterization of benzodiazepine receptor differences in long-sleep and short-sleep mice. Life Sci. 43:1223-1231; 1988.
17. Marley, R. J.; Wehner, J. M. GABA enhancement of flunitrazepam binding in mice selectively bred for differential sensitivity to ethanol. Alcohol Drug Res. 7:25-32; 1986.
18. Marley, R. J.; Wehner, J. M. Correlation between the enhancement of flunitrazepam binding and seizure susceptibility in mice. Life Sci. 40:2215-2224; 1987.
19. Martz, A.; Deitrich, R. A.; Harris, R. A. Behavioral evidence for the involvement of gamma-aminobutyric acid in the actions of ethanol. Eur. J. Pharmacol. 98:53-62; 1983.
20. Mason, R. P.; Moring, J.; Herbette, L. E.; Meyer, R. E.; Shoemaker, W. J. Probing molecular sites of action for alcohol's acute and chronic effects on synaptosomal membranes: A potential tool for studying drug-receptor interactions. In: Meyer, R. G.; Koob, G. F.; Lewis, M. J.; Paul, S. M., eds. Neuropharmacology of ethanol. Boston, MA: Burkhauser; 1991:21-47.
21. McClearn, G. E.; Kakihana, R. Selective breeding for ethanol sensitivity: Short-sleep and long-sleep mice. In: McClearn, G. E.; Dietrich, R. A.; Erwin, V. G., eds. Development of animal models as pharmacogenetic tools. Washington, DC: U.S. Government Printing Office; 1974:147-159.
22. McIntyre, T. D.; Alpern, H. P. Thiopental, phenobarbital, and chlordiazepoxide induce the same differential sensitivities of long-sleep and short-sleep selectively-bred mice. Pharmacol. Biochem. Behav. 24:895-898; 1986.
23. McIntyre, T. D.; Skolnick, P. *t*-[^{35}S]Butylbicyclophosphorothionate binding under equilibrium and nonequilibrium conditions: Differential effects of barbiturates and gamma-aminobutyric acid in the long-sleep and short-sleep mouse lines. J. Neurochem. 56:287-293; 1991.
24. McIntyre, T. D.; Trullas, R.; Skolnick, P. Differences in the biophysical properties of the benzodiazepine/gamma- aminobutyric acid receptor chloride channel complex in the long-sleep and short-sleep mouse lines. J. Neurochem. 51:642-647; 1988.
25. Miller, L. G.; Greenblatt, D. J.; Barnhill, J. G.; Shader, R. I. Differential modulation of benzodiazepine receptor binding by ethanol in LS and SS mice. Pharmacol. Biochem. Behav. 29:471-477; 1988.
26. Moss, G. W.; Curry, S.; Franks, N. P.; Lieb, W. R. Mapping the polarity of general anesthetics target sites using *n*-alkane-(alpha, omega)-diols. Biochemistry 43:10551-10557; 1991.
27. Olsen, R. W.; Tobin, A. J. Molecular biology of $GABA_A$ receptors. FASEB J. 4:1469-1480; 1990.
28. Olsen, R. W.; Yang, J.; King, R. G.; Dilber, A.; Stauber, G. B.; Ransom, R. W. V. Barbiturate and benzodiazepine modulation of GABA receptor binding and function. Life Sci. 39:1969-1976; 1986.
29. Phillips, T. J.; Dudek, B. C. Modifications of ethanol effects by bicuculline: Genotype-dependent responses and inheritance. Psychopharmacology (Berl.) 98:549-555; 1989.

30. Phillips, T. J.; Gilliam, D. M.; Dudek, B. C. An evaluation of the role of ethanol clearance rate in the differential response of long-sleep and short-sleep mice to ethanol. Alcohol 1:373–378; 1984.
31. Sigel, E.; Barnard, E. A. A gamma-aminobutyric acid/benzodiazepine receptor complex from bovine cerebral cortex. J. Biol. Chem. 259:7219–7223; 1984.
32. Stephenson, F. A.; Olsen, R. W. Solubilization by CHAPS detergent of barbiturate-enhanced benzodiazepine–GABA receptor complex. J. Neurochem. 39:1579–1586; 1982.
33. Stinchcomb, A.; Bowers, B. J.; Wehner, J. M. The effects of ethanol and RO 15-4513 on elevated plus-maze and rotarod performance in long-sleep and short-sleep mice. Alcohol 6:369–376; 1989.
34. Wafford, K. A.; Burnett, D. M.; Dunwiddie, T. V.; Harris, R. A. Genetic differences in the ethanol sensitivity of $GABA_A$ receptors expressed in *Xenopus* oocytes. Science 249:291–293; 1990.
35. Wafford, K. A.; Burnett, D. M.; Leidenheimer, N. J.; Burt, D. R.; Wano, J. B.; Kofuii, P.; Dunwiddie, T. V.; Harris, R. A.; Sikela, J. M. Ethanol sensitivity of the GABA-A receptor expressed in *Xenopus* oocytes requires 8 amino acids contained in the gamma 21 subunit. Neuron 7:27–33; 1991.