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GAMMA-AMINOBUTYRIC ACID AND ALCOHOL ACTIONS: NEUROCHEMICAL STUDIES OF LONG SLEEP AND SHORT SLEEP MICE

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Summary

Effects of ethanol and pentobarbital on the GABA receptor-chloride channel complex were evaluated in mice selected for differential sensitivity to the hypnotic effects of ethanol (long sleep and short sleep lines). $^{36}Cl^-$ influx, [^{35}S]t-butylbicyclophosphorothionate (TBPS) and [^{3}H]muscimol binding were measured in a membrane vesicle suspension (microsacs) from cerebellum or forebrain. Muscimol was found to be a more potent stimulator of $^{36}Cl^-$ flux in the LS cerebellum, as compared to the SS cerebellum, but a similar maximal level of uptake was achieved in the two lines. Muscimol displaced [^{35}S]TBPS (a ligand for the convulsant site) from cerebellar microsacs, and LS mice were also more sensitive than SS mice to this action of muscimol. However, the number or affinity of high affinity [^{3}H]muscimol binding sites did not differ between the lines. Physiologically relevant concentrations of ethanol (15-50 mM) potentiated muscimol stimulation of $^{36}Cl^-$ uptake in LS cerebellum but had no effect in SS cerebellum. Ethanol failed to alter stimulated chloride flux hippocampal microsacs from either line. Both the LS and SS lines responded similarly to pentobarbital potentiation of muscimol stimulated chloride uptake regardless of brain region. The demonstrated difference between the LS and SS mice in muscimol stimulated chloride uptake as well as in muscimol displacement of [^{35}S]TBPS binding offers a biochemical explanation for the line differences in behavioral responses to GABAergic agents. Moreover, the findings suggest that genetic differences in ethanol hypnosis are related to differences in the sensitivity of GABA-operated chloride channels to ethanol.

Gamma amino-butyric acid (GABA), the major inhibitory neurotransmitter in the mammalian brain, increases membrane chloride conductance (1,2). It is this inhibitory hyperpolarizing action of GABA that makes it a prime candidate as a mediator of the depressant effects of ethanol. There is evidence that acute exposure to ethanol enhances GABA action. For example, presynaptic inhibition mediated by GABA-induced chloride conductance is enhanced by ethanol application (3-5). Ligand binding to the GABA receptor complex has also been reported to be modulated by ethanol. Squires et al. (6) reported ethanol inhibition of the binding of the convulsant ligand t-butylbicyclophosphorothionate (TBPS), but high concentrations (IC50 = 200mM) were required. The binding affinity of [^{3}H]Diazepam to Lubrol-solubilized

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membranes is enhanced 50% by 100mM ethanol (7). This enhancement was blocked by both bicuculline and picrotoxin (8). These results were not obtained in unsolubilized membranes (9). Radioligand studies have not demonstrated a direct effect of alcohol on the GABA receptor, although it does appear to indirectly alter the receptors for the convulsant and benzodiazepine compounds.

The majority of evidence for a GABA-ethanol interaction comes from behavioral data. In most paradigms, drugs that enhance chloride conductance ($GABA_A$ agonists and GABA mimetics) enhance most of the acute behavioral effects of ethanol, and drugs that decrease chloride conductance (GABA antagonists and picrotoxin-like convulsants) reduce the acute actions of ethanol (10-13).

Another approach to the study of the CNS actions of ethanol is the use of selected lines. The goal of selective breeding is to generate divergent lines through the intermating of subjects who display a particular phenotype. This results in a systematic shift in the gene frequencies for the character under selection pressure. Selected lines are frequently used to test hypotheses about the covariance and relationship between the selected trait and the phenotype under investigation.

The long sleep (LS) and short sleep (SS) mice developed by McClearn and Kakihana (14) were selected for differential sensitivity to the hypnotic effects of ethanol as measured by the duration of the loss of the righting reflex ("sleep time"). The lines differ in a number of other alcohol-related phenotypes, and the sleep time differences appear to be due to differences in neurosensitivity, since alcohol elimination rates are virtually identical (see ref. 15, for a review). Studies of these mice suggest that differences between the lines in the GABA neurotransmitter system may be related to the selected differences to the hypnotic effects of ethanol (16). Martz et al. (13) found that LS mice are more affected by the GABA agonists, THIP and baclofen, than are SS mice. The ED_{50}'s of the GABA agonists in the bar holding test were approximately twice as large for the LS as for the SS mice. However, whole brain GABA levels (17) and GABA uptake kinetics (18) do not differ in the lines. Taken together, these data suggest that the lines may differ in postsynaptic actions of GABA.

Recently, we demonstrated GABA-regulated chloride flux using a brain membrane preparation (2,19). This preparation, called microsacs, contains sealed membrane vesicles with both pre- and post-synaptic elements (20), and responds in a pharmacologically appropriate manner for a chloride channel coupled to a $GABA_A$ receptor (2,19). Employing this approach and receptor binding techniques, the present study was designed to directly assess GABA-operated chloride channels in brain membranes of these mice as a biochemical correlate of the differential behavioral effects produced by GABA agonists (13). Furthermore, we examined the possibility that ethanol differentially alters the GABA-operated chloride channels in the two lines. Genetic differences in chloride channel function were compared to differences in receptor binding by measuring high affinity muscimol binding and the allosteric modulation of TBPS binding by muscimol, ethanol and pentobarbital.

Materials and Methods

Drugs. Muscimol HCl was purchased from Research Biochemicals (Wayland, MA). Sodium pentobarbital and picrotoxinin were purchased from Sigma Chemicals (St. Louis, MO). Absolute ethanol was purchased from Midwest Solvents (Pekin, IL), $^{36}Cl^-$ from ICN (Irvine, CA), and [^{35}S]TBPS and