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GABAergic Drugs can Enhance or Attenuate Chlordiazepoxide-Induced Sleep Time in a Heterogeneous Strain of Mice

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McINTYRE, T. D. AND H. P. ALPERN. *GABAergic drugs can enhance or attenuate chlordiazepoxide-induced sleep time in a heterogeneous strain of mice.* PHARMACOL BIOCHEM BEHAV **25**(5) 1077–1081, 1986.—Evidence supports the notion that differences between the Long-Sleep and Short-Sleep selectively-bred lines of mice are attributable to differences in brain excitability and that these differences are mediated by activity of the GABAergic system. The general applicability of this hypothesis to other populations of mice was tested by using an outbred strain of mice. Specifically, a heterogeneous strain of mice was administered several doses of the hypnotic chlordiazepoxide. Additionally, the indirect GABA agonist AOAA, and the GABA antagonists bicuculline, picrotoxin and pentylenetetrazol were administered to independent groups in conjunction with chlordiazepoxide. The results clearly demonstrate that chlordiazepoxide dose-dependently increased hypnosis, while AOAA enhanced, and the antagonists attenuated sleep time. These findings can be used to support the contention that GABA mediates the bidirectional response of Long-Sleep and Short-Sleep mice to CNS hypnotic-depressants; and, further, show that GABA mediation of sleep time in mice is a general phenomenon.

Heterogeneous mice Pharmacogenetics GABA AOAA Bicuculline Picrotoxin Pentylenetetrazol

A useful strategy for establishing animal models (i.e., particular phenotypes) is the selective breeding method [23,24]. Typically, selective breeding involves the testing of numerous animals for some phenotype of interest, and the subsequent mating of those animals of high phenotypic value with others of like phenotypic value, and similarly for animals of low phenotypic value. Unlike inbreeding, though, sib and cousin matings are avoided. Then, as selection pressure is maintained, the offspring of each succeeding generation will exhibit a corresponding increase in the number of "high" and "low" alleles at the pertinent loci. This increase in the number of high and low alleles is a function of the degree to which the particular phenotype is heritable [24]. Most importantly, because inbreeding is proscribed, all other alleles not influencing the selected trait should assort randomly.

One such selection study, which was initiated to establish heritability of factors contributing to alcohol sensitivity, was begun by McClearn and colleagues [22,24]. Briefly, two lines of mice that differ bidirectionally in their sensitivity to a hypnotic dose of ethanol were derived from a genetically heterogeneous strain (HS). The HS was created by systematically crossing eight inbred strains of mice (A, AkR, BALB/c, C3H/2, C57/BL, DBA, Is/Bi, and RIII) [21]. Animals from this population were examined with respect to loss of righting reflex (sleep time) subsequent to an intraperitoneal injection of ethanol. Males and females that exhibited long sleep times were mated, as were males and females exhibiting minimal or no loss of the righting reflex. After 16 generations, no overlap in the distributions of sleep time was discernible. Those demonstrating extended sleep times were designated the Long-Sleep (LS) line, while those that did not lose the righting reflex, or that lost it for only a brief period, were termed the Short-Sleep (SS) line.

Initially it was proposed that these lines were selected for specific sensitivity to alcohol [10, 13, 14, 31], but recent evidence shows that this is not the case [1, 26, 27], and we have proposed a unifying hypothesis which accounts for the many differences between the LS and SS selected lines [26]. Since the LS line is more sensitive than the SS line to the soporific effects of alcohols [10,14], barbiturates [1, 2, 25, 27], benzodiazepines [27], and miscellaneous hypnotics (e.g., adenosine and nitrous oxide; [9,17]), it seemed reasonable to conclude that these lines were originally selected for differences in tonic brain excitability, and that these differences are related to activity at the GABA synapse.

If our hypothesis concerning GABAergic mediation of differences between LS and SS selected lines is generally applicable, then drug effects observable in the LS and SS lines should also be found in non-selected outbred animals such as the HS strain. Since the HS strain was maintained during the selection program as a control for evaluating random intergenerational variability, they are ideal for analyzing the relationship between the original operational selection criterion (ethanol-induced hypnosis) and whatever CNS mechanism is mediating the bidirectional response.

There is a growing body of evidence implicating the

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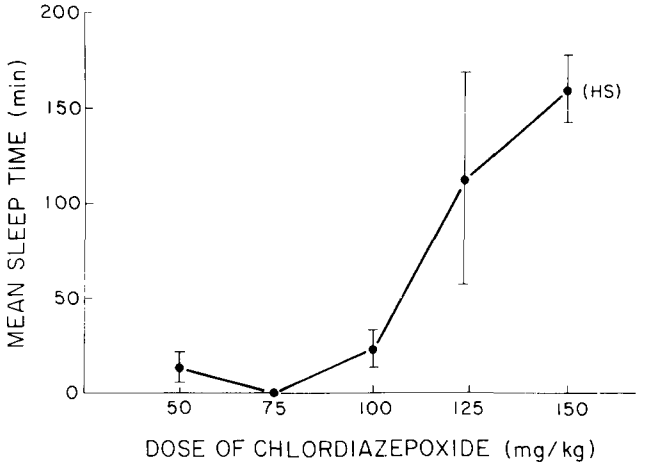

FIG. 1. Mean sleep time±S.E. for each of the independent groups of HS mice administered chlordiazepoxide.

TABLE 1

MEAN SLEEP TIME (MIN) ± S.E. FOR THE CONTROL GROUP (0 mg/kg AOAA) AND THE GROUPS TREATED WITH 10 or 20 mg/kg OF AOAA 2 HR PRIOR TO 100 mg/kg CHLORDIAZEPOXIDE (N=8 FOR EACH GROUP)

AOAA (mg/kg, IP)	Sleep Time	Significance (when compared with 0 mg/kg group)
0.0	22.7 ± 9.5	
10.0	16.7 ± 8.3	ns
20.0	182.6 ± 65.2	$p<0.01$

GABAergic system in the mediation of the hypnotic effects of most CNS depressants [6, 7, 29, 33, 34, 37, 39, 43], and the evidence for benzodiazepines is the most convincing. Consequently, this paper reports experiments that evaluate chlordiazepoxide-induced sleep time, as well as the effects of GABAergic agents on induced sleep time in HS mice. Since the effects of chlordiazepoxide on the LS and SS lines has already been established, it was selected to test the generality of our hypothesis. Initially a dose-response function was constructed, and then the effect of the indirect GABA agonist amino-oxyacetic acid (AOAA) on chlordiazepoxide-induced sleep time was determined. It was our prediction that this GABA agonist, which blocks the GABA catabolic enzyme GABA-transaminase, would enhance sleep time in these mice. To the contrary, we speculated that GABA antagonists would attenuate chlordiazepoxide-induced hypnosis. To test this, we first evaluated the effect that two doses of the classical GABA antagonist bicuculline had on induced sleep time. Since bicuculline is a direct GABA receptor antagonist [32], and since benzodiazepines bind at different locations on the GABA receptor complex [39], we also employed two analeptics (picrotoxin and pentylenetetrazol) which exert their convulsive effects via an allosteric subsite on the benzodiazepine-GABA receptor-chloride ionophore [32,42].

METHOD

Animals

One hundred and four HS mice 100–150 days old served as subjects. These mice were descendants of animals from the 39th production generation, obtained from the Institute for Behavioral Genetics, Boulder, CO 80309. Littermates were housed together, but randomly distributed across experimental groups. All animals were maintained on a 12 hr light/dark cycle with food and water continuously available. Each independent group in the four experiments consisted of eight males, except the final experiment, where each group consisted of four males and four females.

Procedure

Each group in Experiment 1 received either 50, 75, 100, 125, or 150 mg/kg chlordiazepoxide (Librium) in an injection volume of 0.1 ml/g body weight. For Experiment 2, each group received either 0.0, 10.0, or 20.0 mg/kg amino-oxyacetic acid hemihydrochloride, followed 2 hr later by 100 mg/kg of chlordiazepoxide. Each group in Experiment 3 received 150 mg/kg chlordiazepoxide. Then 15 min after sleep time began, groups were administered either saline, 1.0 or 2.0 mg/kg (+)-bicuculline dissolved in saline adjusted to a pH of 2.5. For Experiment 4, each group received 150 mg/kg chlordiazepoxide, and then 15 min later, either saline, 7.0 mg/kg picrotoxin or 50 mg/kg pentylenetetrazol. All injections were made intraperitoneally and it should be noted that with this route of administration the doses of bicuculline, picrotoxin and pentylenetetrazol selected were subconvulsive. Sleep time was assessed in the following manner. After injection of chlordiazepoxide, an animal was placed on its back in a V-shaped (90° angle) Plexiglas sleep trough until it was unable to right itself four times within sixty seconds, at which time it was considered to have lost its righting reflex. An animal was considered to have regained its righting reflex when it could right itself four times within a sixty second period.

RESULTS

Figure 1 displays the dose-response function for chlordiazepoxide. As can be seen, the animals began sleeping to a significant degree at 100 mg/kg, and the animals slept progressively longer at the two higher doses (125 and 150 mg/kg). A one-way analysis of variance was performed on the three highest doses, which constitute the positively accelerating portion of the dose-response function. The analysis was significant $F(2,21)=4.04$, $p<0.05$, and group comparisons using the protected t-test indicate that only the 100 and 150 mg/kg groups are significantly different from each other ($p<0.05$).

As can be seen in Table 1, which displays the results of administering different doses of AOAA prior to chlordiazepoxide, the highest dose of AOAA greatly enhanced sleep time. A one-way analysis of variance was used to analyze these data, $F(2,21)=6.01$, $p<0.01$, and group comparisons using the protected t-test indicate that only the 20 mg/kg dose is significantly different ($p<0.01$) from the control dose (0 mg/kg).

TABLE 2

MEAN SLEEP TIME (MIN) ± S.E. FOR THE GROUPS RECEIVING 150 mg/kg CHLORDIAZEPOXIDE AND THEN 15 MIN AFTER HYPNOSIS BEGAN, EITHER SALINE OR 1.0 OR 2.0 mg/kg OF BICUCULLINE HYDROCHLORIDE (N=8 FOR EACH GROUP)

Bicuculline (mg/kg, IP)	Sleep Time	Significance (when compared with 0 mg/kg group)
0.0	140.5 ± 13.5	
1.0	62.7 ± 18.2	$p<0.01$
2.0	26.6 ± 2.3	$p<0.01$

TABLE 3

MEAN SLEEP TIME (MIN) ± S.E. FOR THE GROUPS RECEIVING 150 mg/kg CHLORDIAZEPOXIDE AND THEN 15 MIN AFTER HYPNOSIS BEGAN, EITHER SALINE OR 7.0 mg/kg PICROTOXIN OR 50 mg/kg PENTYLENETETRAZOL (N=8 FOR EACH GROUP)

Treatment	Sleep Time	Significance (when compared with 0 mg/kg group)
Saline	195.0 ± 17.2	
Picrotoxin	33.0 ± 1.6	$p<0.01$
Pentylenetetrazol	23.1 ± 1.6	$p<0.01$

Table 2 illustrates the results of administering different doses of bicuculline after the induction of chlordiazepoxide sleep time. As can be seen, both doses of bicuculline attenuated sleep time, and a one-way analysis of variance used to analyze these data is highly significant, $F(2,21)=19.59$, $p<0.01$. Group comparisons made with the protected t-test confirm that both groups administered bicuculline are significantly different than the saline control group ($p<0.01$).

Finally, Table 3 displays the results of administering either picrotoxin or pentylenetetrazol subsequent to the induction of chlordiazepoxide hypnosis. As can be readily seen, both the these analeptics also significantly attenuated sleep time. This observation was verified with a one-way analysis of variance, $F(2,21)=92.85$, $p<0.01$, and protected t-tests confirm that each analeptic significantly attenuated chlordiazepoxide-induced sleep time ($p<0.01$).

DISCUSSION

The results of these experiments indicate that nonselected outbred mice (HS) react to GABA drugs in a manner that is similar to the LS and SS lines. We have previously demonstrated that the LS and SS lines exhibit differential hypnotic reactions to the anxiolytic chlordiazepoxide, and further, that the indirect GABA agonist AOAA dose-dependently increased the induced sleep time [25, 27]. These results, therefore, support the generality of a GABA hypothesis for the mediation of sleep time in mice. One result obtained deserves further comment. In the second experiment the 10 mg/kg dose of AOAA did not affect induced sleep time. This dose was apparently too low and it should be mentioned that the AOAA doses were selected on the basis of previous work with LS and SS mice, where it was found that doses higher than 20 mg/kg did not further enhance sleep time for LS mice. A final comment concerning experimental design should be made. The final experiment employed only one dose of picrotoxin and pentylenetetrazol since previous work had shown that these doses produce effects on convulsive threshold that are similar to 2 mg/kg of bicuculline. As noted earlier these doses are subconvulsive and the clonus ED_{25}s for the three drugs are: bicuculline, 4 mg/kg; picrotoxin, 10 mg/kg; pentylenetetrazol, 65 mg/kg.

As previously outlined, the LS and SS display a bidirectional response to alcohols, barbiturates, benzodiazepines and adenosine, all of which have had GABA transmission implicated in their mode of action. More direct evidence also exists. For instance, the GABA agonists THIP and baclofen were significantly more effective in inhibiting bar-holding in LS mice than they were in SS mice [19]. Further, in a grid-test of locomotor coordination, several alcohols and pentobarbital all produce greater coordination difficulties in LS mice than in SS mice [8]. It has been demonstrated recently that intraventricularly administered picrotoxin dramatically reduced ethanol-induced sleep time in both lines [20], and it was concluded that this demonstrates a non-specific excitatory effect and is not necessarily linked to GABA. The argument is that if a specific neurochemical deficit exists in LS and SS mice, then correcting such a deficit with an agent like picrotoxin should produce decreased sleep time for LS mice, but increased sleep time for SS mice. This appealing explanation overlooks the fact that GABA works by increasing chloride conductance, while various GABA blockers-analeptics block this increase in conductance [32,43]. It is not apparent how a particular drug would decrease chloride conductance in one selected line, while increasing this conductance in the other line.

Benzodiazepines enhance primary afferent depolarization [5] in a bicuculline-sensitive fashion [35]. Further, benzodiazepines antagonize the increase in cerebellar cGMP caused by depletion of GABA [7]. Rapid, reversible, saturable, and stereospecific binding of [^{3}H]-diazepam has been demonstrated in brain [3, 40, 41], while high correlations between the ability of benzodiazepines to displace diazepam and their efficacy in several behavioral tests has also been reported [41]. It may be more appropriate to envision "benzodiazepine receptors" as recognition sites for which a wide variety of agents have an affinity (i.e., benzodiazepines, triazolopyradazines, cyclopyrolones, quinolines, β-carbolines, and pyrazoloquinolinones). This finding has engendered a classification of ligands as either full agonists, partial agonists, inverse agonists or antagonists [4, 11, 12, 15, 28, 32, 34, 37–39]. Finally, in addition to peripheral binding sites [36], evidence exists for two high-affinity neuronal sites termed Type I and Type II [16,18], which have a heterogeneous CNS distribution [44].

Overall, the evidence reported here supports our original contention that GABA mediates the bidirectional reactions of LS and SS mice to CNS hypnotic-depressants and shows that GABA mediation of depressant-induced sleep time is applicable to mice in general. Several rather complex models of the benzodiazepine-GABA receptor-chloride ionophore have been proposed [12, 28, 30, 32, 34, 37, 39], and one of these may eventually be useful in elucidating the mechanisms responsible for the behavioral results that have been reported with hypnotic-depressants.

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