Pharmacology Biochemistry & Behavior, Vol. 35, pp. 469–472. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

BRIEF COMMUNICATION

Isolate Housing Alters Ethanol Sensitivity in Long-Sleep and Short-Sleep Mice

BYRON C. JONES,[1] JOAN M. CONNELL AND V. GENE ERWIN

Alcohol Research Center, School of Pharmacy
University of Colorado, Boulder, CO 80309

Received 27 July 1989

JONES, B. C., J. M. CONNELL AND V. G. ERWIN. *Isolate housing alters ethanol sensitivity in long-sleep and short-sleep mice.* PHARMACOL BIOCHEM BEHAV 35(2) 469–472, 1990.—Beginning at 45 days of age, male long-sleep (LS) and short-sleep (SS) mice were placed into individual cages for 21–22 days. Control animals were group-housed for the same amount of time. At 65–66 days of age, animals were given anesthetic doses of ethanol, IP. Measures taken were sleep time, body temperature at 30 and 60 minutes postinjection and blood ethanol content (BEC) at regain of righting response. Compared to the same measures in group-housed animals, sleep times and hypothermia were attenuated in isolate-housed LS and SS mice. Isolate housing resulted in a 15% increase, compared to levels observed in group-housed animals, in BEC at regain of righting in LS; there was no significant difference in BEC in SS mice. The results indicated an isolation-related decrease in sensitivity to the anesthetic effects of ethanol in LS; the effect of isolation in SS may be an increased clearance rate of ethanol.

Long-sleep and short-sleep mice Social isolation Ethanol sensitivity

A highly successful program of selection for differential anesthetic sensitivity to ethanol (EtOH) was initiated more than twenty years ago in a heterozygous murine stock (8). The resultant lines of mice, long-sleep (LS) and short-sleep (SS), became completely divergent in anesthetic response, with doses of EtOH that anesthetize LS being ineffective in SS and doses that anesthetize SS being lethal to LS. There is evidence, moreover, that the genes imparting differences in EtOH sensitivity are fixed in the LS and SS lines of mice (6). A large body of literature on the neurochemistry and pharmacology of LS/SS EtOH sensitivity is extant (2); however, environmental influences on EtOH sensitivity in these mice are less well known. In a study of the circadian cyclicity in anesthetic sensitivity to EtOH in LS and SS (4), the former line demonstrated a 20–30% daily variation in anesthetic response to EtOH as measured by sleep time and BEC at regain of righting response. Alternatively, SS response to EtOH remained constant over 24 hours. The authors concluded that the 24-hour cyclicity in anesthetic response to EtOH in LS reflected a rhythmic, episodic change in brain sensitivity to EtOH. Since this cyclicity is likely to be associated with neurohumoral events and, thus, may indicate important neurochemical mechanisms involved in anesthetic response to EtOH, we conducted a study in which we applied an environmental perturbation, social isolation, which is known to effect neurochemical change in mice (11).

Among members of the rodent family, muridae, including the European housemouse (*Mus domesticus*), long-term isolation from conspecifics has been shown to alter many behavioral and neurobiological characteristics (11). Isolation-induced increase in aggression is well documented (1). Neurochemical changes, especially in the monoaminergic systems associated with social isolation (9), point to its potential importance as concerns initial sensitivity to EtOH. Indeed, Yanai and Sze (13) demonstrated an accelerated tolerance to the anesthetic effects of EtOH following six weeks of social isolation in male heterozygous mice. The following study was undertaken in order to investigate further the apparent greater lability of anesthetic response to ethanol in LS compared to SS mice.

METHOD

Animals

Eighteen male LS and twenty male SS mice served as subjects

[1]Requests for reprints should be addressed to Byron C. Jones, School of Pharmacy, Campus Box 297, University of Colorado, Boulder, CO 80309-0297.

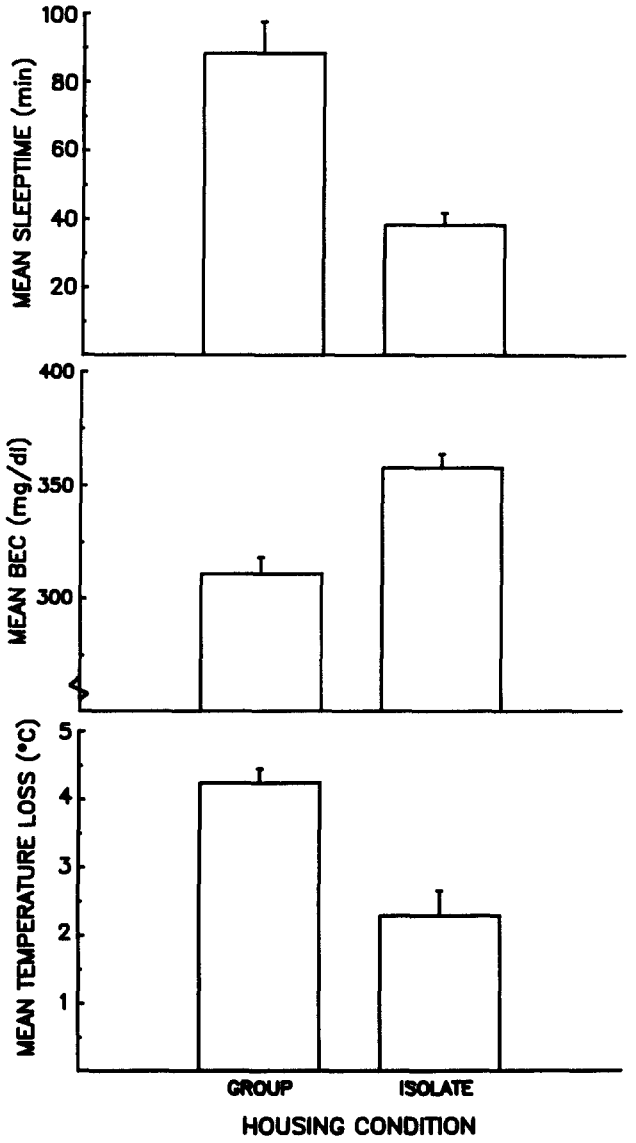

FIG. 1. Effect of social isolation on ethanol-induced sleep time (top panel), BEC at regain of righting (center panel) and core temperature change (bottom panel) in LS/Ibg male mice. Social isolation was for 21–22 days, beginning at age 45 days. Isolated (n = 9) and group-housed animals (n = 9) were injected IP with 24% (w/v) ethanol at a dose of 2.8 g/kg.

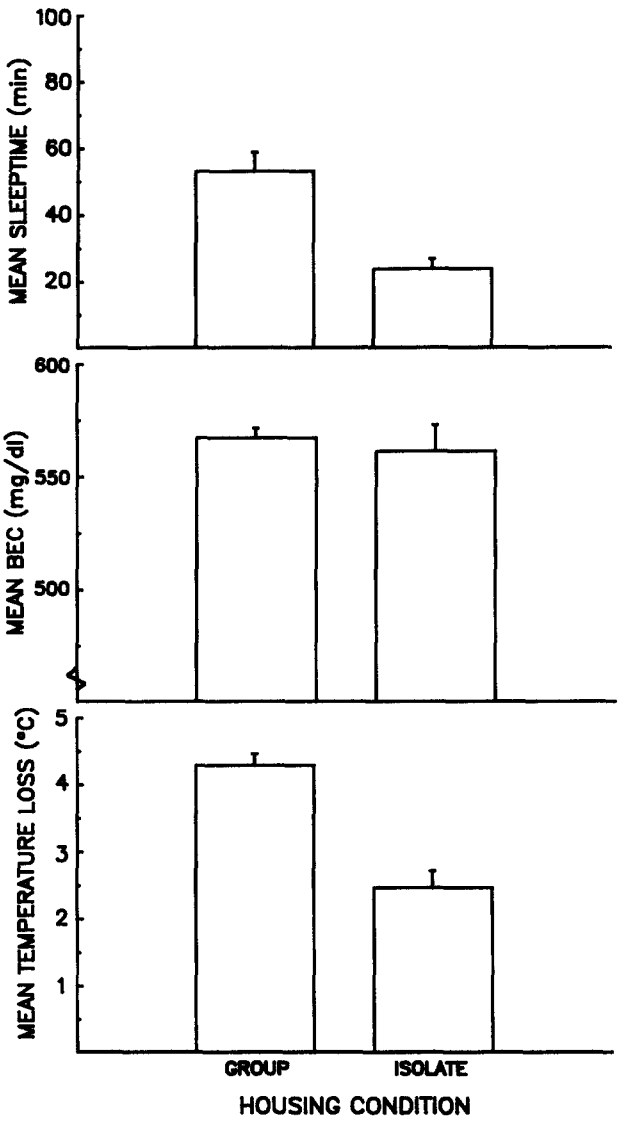

FIG. 2. Effect of social isolation on ethanol-induced sleep time (top panel), BEC at regain of righting (center panel) and core temperatures change (bottom panel) in SS/Ibg male mice. Social isolation was for 21–22 days, beginning at age 45 days. Isolated (n = 10) and group-housed animals (n = 10) were injected IP with 24% (w/v) ethanol at a dose of 5.0 g/kg.

for this study. Two replicate experiments separated by six weeks and littermate identification were used to minimize the assignment of littermates to treatment conditions. All animals were housed in the School of Pharmacy colony with constant access to food and water. Temperature and humidity were maintained at 22°C and 20%, respectively, with light cycle, 0700 L:1900 D. The animals were born and raised to 45 days of age at the Institute for Behavioral Genetics, at which time they were transported (approximately 1.5 km) to the School of Pharmacy colony.

Procedures

Isolation. Upon receipt at the School of Pharmacy, animals were selected at random for assignment to isolation or group housing conditions. Isolation housing was in stainless steel shoebox cages measuring 24.5 × 12.5 × 10.0 cm (L × W × H). Group housing, five mice per cage, was in standard polycarbonate cages measuring 29.0 × 18.0 × 12.5 cm. Bedding consisting of aspen chips was uniform between groups.

Testing. At 65–66 days of age all animals were moved to a small holding area in the laboratory for overnight acclimatization. At 0900 on the following day, all animals received an injection of EtOH (24% w/v in isotonic saline), 2.8 g/kg for LS and 5.0 g/kg for SS. These differential doses were selected to give equivalent durations of loss of righting response (3). Ambient temperatures during testing ranged from 19–21°C. Sleep time was measured as the interval between loss and regain of righting response. Operationally, the criterion for righting is the animal being able to change from supine to prone position three times in 30 sec. Other measures included rectal temperature immediately prior to injec-

tion, 30 min postinjection and BEC at regain of righting response. The latter was measured by taking a 25-μl blood sample from the retroorbital sinus followed by enzymatic-colorimetric assay for EtOH (7).

Data analysis. Because each line received a different dose of ethanol, differences between treatment conditions for mean sleep time and BEC were evaluated separately for LS and SS mice by analysis of variance for a one between-subjects variable experiment. Change in body core temperature was expressed as difference between temperature prior to injection and temperature at 30 min postinjection.

RESULTS

Because of the need to use different doses of EtOH to achieve comparable levels of anesthesia in LS and SS mice, the results are presented separately for each line.

Effect of Social Isolation on Ethanol Sensitivity in LS

Figure 1 illustrates mean sleep time (top panel), BEC at regain of righting (center panel) and temperature change (bottom panel), respectively, in group-housed and isolated LS mice injected IP with 2.8 g/kg EtOH. Isolate housing was accompanied by a highly significant (nearly 57%) reduction in sleep time, $F(1,16) = 25.09$, $p<0.0001$. Isolation also resulted in reduced (45%) temperature loss at 30 min, $F(1,16) = 21.73$, $p<0.0004$, and increased (15%) BEC at regain of righting response, $F(1,16) = 24.34$, $p<0.0003$.

Effect of Social Isolation on Ethanol Sensitivity in SS

Figure 2 illustrates mean sleep time (top panel), BEC at regain of righting (center panel) and temperature change 30 min postinjection (bottom panel), respectively, in group-housed and isolated SS mice treated with 5.0 g/kg EtOH, IP. Similar to the results observed in LS, sleep time and temperature loss were attenuated (55 and 42%, respectively) by isolation, $F(1,18) = 20.15$, $p<0.0004$; $F(1,18) = 36.67$, $p<0.0001$. BEC at regain of righting, however, was not significantly affected by isolation housing, $F(1,18)<1$.

DISCUSSION

The effects of isolate housing on responses to a hypnotic dose of EtOH in LS are clear and consistent with the hypothesis that social isolation decreased brain sensitivity to EtOH. Mean sleep time for isolated animals was 38 min compared to 88 min in group-housed subjects; hypothermia was attenuated and BEC at regain of righting increased from 310 to 360 mg/dl. If the apparent decrease in sensitivity to ethanol were due to increased clearance of ethanol from the blood, then BECs at regain of righting response would be expected to be the same for group- and isolate-housed animals. Previous work has shown EtOH clearance rates for LS and SS to be similar, at around 80 mg/dl/hr (5). The increase in BEC of 50 mg/dl in isolated compared to grouped animals, together with 50 min (0.83 hr) shorter sleep time, is consistent, therefore, with an isolation-induced difference in brain sensitivity to EtOH.

The outcome with SS mice, however, is not so clear. Isolation significantly decreased both sleep time and temperature loss in SS, but had no consistent effect on BEC at regain of righting response. A recent study reported that ethanol clearance rates were significantly altered by hypothermia in LS and SS mice (10). Thus, one would expect clearance rates in both lines to be similarly increased by isolation housing. Increased clearance of EtOH as indicated by decreased hypothermia could account for decreased sleep times in either line. Since the BEC levels (a measure of brain sensitivity) in SS mice were not significantly affected by isolation, results from this experiment indicate that change in clearance rate of EtOH alone in SS may well have accounted for the apparent change in sensitivity to EtOH. It is possible that isolate housing differentially alters rates of absorption and redistribution of ethanol in LS and SS mice. Since these processes are rapid, i.e., are complete within 5 minutes following IP injection (5), it is unlikely that isolate housing would cause sufficient changes in absorption or redistribution of ethanol to account for the effects observed.

Because different ethanol doses for LS and SS mice were used in this study, a direct test of genotype-environment interaction was not possible. Nonetheless, the differential effect of isolate housing on BECs at regaining righting response strongly suggests a gene-environment interaction with regard to brain sensitivity to ethanol.

Of interest in the present case are the results of Yanai and Sze (13). These investigators were interested in influence of isolation housing on rate of acquisition of tolerance to an anesthetic dose of ethanol. In their study, apparent initial sensitivity, as measured by sleep time, to an anesthetic dose of ethanol was also decreased in isolate-housed animals. Brain ethanol levels, however, appeared to be not significantly different on the first day of ethanol testing. These results are similar to what we observed in the SS mice.

Taken together, our findings are consistent with those of other researchers demonstrating that social isolation is capable of altering CNS response to ethanol and other sedative hypnotics (12). Furthermore, our results point to the potential usefulness in using genetically defined animals in correlating neurochemical changes with changes induced by social isolation in sensitivity to EtOH.

ACKNOWLEDGEMENTS

This work was supported in part by USPHS Grants AA 03527, AA 00079 and AA 07330.

REFERENCES

1. Brain, P. What does individual housing mean to a mouse? Life Sci. 16:187–200; 1975.
2. Deitrich, R. A. Selective breeding in mice and rats for initial sensitivity to ethanol: contributions to understanding ethanol actions. In: Deitrich, R. A., ed. Mechanisms of initial sensitivity to ethanol. NIAAA Monograph, in press; 1989.
3. Erwin, V. G.; Korte, A.; Marty, M. Neurotensin selectively alters ethanol-induced anesthesia in LS/Ibg and SS/Ibg lines of mice. Brain Res. 400:80–90; 1987.
4. Gilliam, D. M.; Collins, A. C. Circadian and genetic influences on tissue sensitivity and sleep time to ethanol in LS and SS mice. Pharmacol. Biochem. Behav. 18:303–308; 1983.
5. Heston, W. D. W.; Erwin, V. G.; Anderson, S. M. A comparison of the effects of alcohol on mice selectively bred for differences in ethanol sleep-time. Life Sci. 14:365–370; 1974.
6. Holmes, R. S.; Petersen, D. R.; Deitrich, R. A. Biochemical genetic variants in mice selectively bred for sensitivity or resistance to ethanol-induced sedation. Anim. Genet. 17:235–244; 1986.
7. Lundquist, F. The determination of ethyl alcohol in blood and tissue. Methods Biochem. Anal. 7:217–251; 1959.
8. McClearn, G. E.; Kakihana, R. Selective breeding for ethanol sensitivity: Short-sleep and long-sleep mice. In: McClearn, G. E.; Deitrich, R. A.; Erwin, V. G., eds. Development of animal models as pharmacogenetic tools. NIAAA Research Monograph No. 6. Washington, DC: U.S. Government Printing Office, DHHS Publication No. (ADM) 81-1133; 1981:147–159.
9. Oehler, J.; Jahkel, M.; Schmidt, J. Altered neurobiological responses to acute immobilization in social-isolated mice. Pharmacol. Biochem.

Behav. 25:41–44; 1986.
10. Romm, E.; Collins, A. C. Body temperature influences on ethanol elimination rate. Alcohol 4:189–198; 1987.
11. Valzelli, L. The "isolation syndrome" in mice. Psychopharmacologia 31:305–320; 1973.
12. Valzelli, L.; Bernasconi, S. Alcohol, prolonged isolation and barbiturate sedation in two strains of mice. Neuropsychobiology 4:86–92; 1978.
13. Yanai, J.; Sze, P. Y. Accelerated acquisition of ethanol tolerance in isolated mice. Neuropsychobiology 8:135–139; 1982.