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Behavioral Effects of Ethanol and Salsolinol in Mice Selectively Bred for Acute Sensitivity to Ethanol[1]

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SMOLEN, T. N. AND A. C. COLLINS. *Behavioral effects of ethanol and salsolinol in mice selectively bred for acute sensitivity to ethanol.* PHARMACOL BIOCHEM BEHAV 20(2) 281–287, 1984.—The effects of intracerebral injection of salsolinol, the condensation product of dopamine and acetaldehyde, were compared in LS and SS mice selectively bred for differential sleep time following an intraperitoneal injection of ethanol. Salsolinol differentially affected open-field activity in the two mouse lines. Low doses of salsolinol increased open-field activity in the SS line; no such effects were obtained in the LS line. Larger doses decreased activity in both lines but the LS mice were more sensitive to this effect. Similar effects on open-field activity have been reported for ethanol in these same lines of mice. A 40 μg dose of salsolinol increased ethanol-induced sleep time in both lines but the increase was greater in the LS line. With lower doses of salsolinol the duration of ethanol-induced sleep time decreased in SS mice and increased in LS mice. This effect of salsolinol on sleep time most likely involves some CNS mechanism since salsolinol did not alter ethanol elimation rate in the two lines of mice. A more severe and prolonged hypothermia developed in LS mice following injections of salsolinol or equal doses of ethanol; however, equi-hypnotic doses of ethanol elicited similar hypothermic responses. Taken together, these data support a possible role for salsolinol in ethanol's actions.

Salsolinol Ethanol Tetrahydroisoquinoline Sleep time Activity Hypothermia Ethanol elimination

TETRAHYDROISOQUINOLINE alkaloids (TIQs) are compounds formed by the condensation of catecholamines with aldehydes. Increased interest in TIQs has developed over the past decade following reports suggesting a linkage between TIQs and a number of disease states, including parkinsonism, phenylketonuria, and ethanol and opiate addiction [3, 8, 12, 13, 19, 20]. It has been hypothesized that the TIQ salsolinol, which is formed by the condensation of dopamine with acetaldehyde, may contribute to some of the behavioral and physiological effects of ethanol [6, 7, 10, 11, 27, 28]. Church and co-workers [6], using mice selectively bred for differences in response to ethanol, have reported differences in shuttle box activity following salsolinol administration. Additionally, salsolinol, alone and in combination with ethanol, has been shown to induce and prolong ethanol narcosis in mice [5,21].

In the present study, we tested the hypothesis that salsolinol mediates some of the behavioral and physiological effects of acute ethanol treatment. This hypothesis was tested with Long Sleep (LS) and Short Sleep (SS) mice selectively bred for differences in their behavioral response (ethanol-induced sleep time) to an acute dose of ethanol [22]. These lines of mice differ in their central nervous system (CNS) sensitivity to ethanol and thus provide a useful genetic tool for testing hypotheses concerning the actions of ethanol [14,18]. If salsolinol mediates the acute response to ethanol, then these lines of mice should respond differentially to both the behavioral and physiological effects of salsolinol. The studies which follow examined the effects of salsolinol on open-field activity, body temperature, ethanol sleep time and ethanol elimination rate, and provide additional evidence that TIQs contribute to or share ethanol-like properties.

METHOD

Animals

The subjects consisted of equal numbers of male and female LS and SS mice, 65±10 days of age which were group-housed in a room with a conventional 12 hr light:dark cycle. Ad lib food and water was available in their home cage. All mice were drug-naive prior to testing and each subject received only one test.

Synthesis of Salsolinol-HBr

Dopamine-HCl (3.8 g, Sigma Chemical Co.) and acetaldehyde (2.0 g) were dissolved in 200 ml of distilled water in a dark bottle and stirred at room temperature, in the dark, for three days. The solution was then evaporated to dryness

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TABLE 1
THE EFFECT OF LOW DOSES OF SALSOLINOL ON ETHANOL SLEEP TIME

Dose of Salsolinol	Sleep Time (min) Long Sleep Males	Long Sleep Females	Short Sleep Males	Short Sleep Females
0	84.9 ± 7.3 (20)	78.7 ± 5.9 (20)	66.7 ± 6.2 (20)	62.2 ± 4.6 (20)
40 μg	161.1 ± 9.7* (20)	149.6 ± 8.6* (20)	114.2 ± 10.4* (20)	88.9 ± 7.6* (20)
1 ng	129.7 ± 12.1* (7)	89.0 ± 30.5 (6)	43.4 ± 14.0* (5)	42.0 ± 17.8* (5)
1 pg	127.7 ± 23.3* (6)	101.3 ± 18.9* (7)	37.6 ± 8.7* (5)	40.8 ± 9.5* (6)

Tabled values represent mean sleep time in minutes ± SEM. The numbers in parentheses represent the sample size for each group. Salsolinol was administered immediately following an IP injection of ethanol (2.5 g/kg for LS mice and 4.1 g/kg for SS mice).
*Significantly different from respective 0 dose, $p<0.01$.

under a vacuum at 25°. The residual yellowish oil was dissolved in 5–10 ml of constant-boiling hydrobromic acid (J. T. Baker Co.). This solution was covered and allowed to crystallize over several days in a freezer set at −20°. The resulting precipitate was collected and dried under a vacuum at 25° using phosphorus pentoxide (J. T. Baker Co.) as a dessicant. The yield varied between 30–80%. Salsolinol prepared in this manner co-chromatographed with salsolinol samples obtained from other laboratories as tested by high performance liquid chromatography (HPLC) with electro-chemical detection and by gas chromatography with an electron capture detector. Similarly, the salsolinol synthesized as described above yielded the appropriate mass spectrum when analyzed using a gas chromatograph coupled to a mass spectrometer.

Open-Field Apparatus and Testing Procedure

Open-field activity was monitored using a white Plexiglas enclosure approximately 90 cm square. The floor was divided into 36 squares by rows of five photoelectric cells. Interruption of the photoelectric beam by the mouse engaged a two-channel counter. In order to minimize confounding of locomotor activity with Hall's [16] measure of rodent "emotionality," open-field testing in this study was conducted under red light. All activity was tested between 1300 and 1630 hours. Mice were removed from their home cages and placed in a clear enclosure in one corner of the apparatus. After a 30 sec acclimation period, the photocells were activated and the counter engaged. The number of squares crossed during a 3 min test period was used as the activity score. The open-field was cleaned thoroughly with water between tests.

Prior to measuring activity, the mice were lightly anesthetized with ether and injected with 10, 20, 25, 30 or 40 μg of salsolinol-HBr. The salsolinol-HBr was delivered intracerebrally (IC) [15] in 5 μl of artificial cerebrospinal fluid (CSF, containing 154 mM NaCl, 3.35 mM KCl, 1.33 mM $CaCl_2$, 1.15 mM $MgCl_2$, 0.12 mM NaH_2PO_4, 0.46 mM Na_2HPO_4, 0.22 mM urea, 3.39 mM glucose, adjusted to pH 7.0 with NaOH) using a 27 gauge, 3 mm needle attached to a 25 μl Hamilton syringe. Test injections of hematoxylin dye confirmed the site of injection to be in or near the lateral ventricle. Subjects were tested 25 min following injection to minimize the effects of the anesthesia. Three control groups were tested: untreated controls; etherized, non-injected; and etherized, injected with CSF.

Ethanol-Induced Sleep Time

Ethanol-induced sleep time was assessed following an intraperitoneal (IP) injection of either a 4.1 g/kg (SS mice) or 2.5 g/kg (LS mice) dose of ethanol. These are doses which we have found give nearly equal sleep times in the two lines of about 70 min duration. By adjusting the concentrations of ethanol such that SS mice were administered 0.013 ml/g body weight and LS mice 0.01 ml/g body weight, the two lines were administered similar volumes of ethanol. Following loss of the righting response (about two min), the mice were injected IC with either salsolinol-HBr (1 pg to 40 μg), or the artificial CSF, in a 5 μl volume. The mice were placed on their backs in V-shaped plastic troughs. Sleep time was judged terminated when the animals could right themselves three times within 30 sec.

Determination of Body Temperature

The drug-free basal temperature of each mouse was measured rectally with a 2.5 cm thermal probe attached to a Digitec HT-5810 digital thermometer. Immediately after, the mice were injected with either ethanol (2.5 g/kg or 4.1 g/kg, IP) or salsolinol (40 μg in 5 μl of CSF, IC, following light etherization) and returned to their home cages. Their body temperatures were recorded, as described above, at 30, 90, 150 and 210 min post injection. Thus, each subject's temperature was measured five times—once pre- and four post-treatment. Four control groups were tested: untreated controls; etherized mice; etherized and injected with CSF; and saline-injected controls.

Ethanol Elimination Studies

Ethanol elimination rates were determined under two conditions: either LS and SS mice received only an IP injec-

tion of 2.5 g/kg or 4.1 g/kg ethanol, respectively; or, in addition to the IP injection of ethanol, the mice also received an IC injection of 40 μg of salsolinol immediately following loss of the righting response. A 40 μl blood sample was obtained from the retroorbital sinus of each animal at 30 min, 1, 2 and 4 hours following ethanol or ethanol plus salsolinol treatment and was added to 0.96 ml of 0.01 mg% isopropyl alcohol, the internal standard. All samples were stored on ice in stoppered 16×100 mm Vacu-tainer® tubes (Becton Dickinson and Co.) until analyzed by "head space" gas chromatography. The ethanol content of each sample was determined by incubating the samples for 15 min at 60°. A 1 ml aliquot of the gas phase from each tube was withdrawn using an air tight gas syringe and injected onto a 6 ft column (4 mm i.d.) of Porapak Q (100-120 mesh) in a Beckman GC-45 gas chromatograph equipped with a flame ionization detector. The inlet temperature was maintained at 110°, the column and detector temperatures were 150° and 190°, respectively. The gases and their flow rates were as follows: helium, 55 ml/min; air, 300 ml/min; and hydrogen, 45 ml/min. Peak areas were determined by the method of triangulation and compared with ethanol standards which were prepared and analyzed daily. Ethanol elimination rates were estimated for each animal by calculating the slope of the best fit line derived from the data obtained for the four time points.

Statistical Analysis

Data were analyzed by analysis of variance. Post hoc comparisons were made using either the Tukey B [33] or Duncan's Multiple Range Test [4] for significant differences among means. Hartley's F_{max} Test [17] was used to determine the homogeneity of variances. Because of the large difference in sample size, the data found in Table 1 were analyzed using a *t*-test to determine the difference between two independent means [33].

RESULTS

Open-Field Activity

The effect of salsolinol on open-field activity is summarized in Fig. 1. There were no significant differences among the SS control groups, however, LS ether and ether/CSF control groups were significantly different from LS untreated controls. Ether and ether in combination with CSF caused a significant increase in open-field activity. Because of the significant effect of ether/CSF treatment on open-field activity, all drug treatments were compared to this control group in both lines. The main effects of line (genotype) and treatment (drug dose) were found to be highly significant following two-way analysis of variance, $F(1,446)=452.3$, $p<0.001$; $F(8,446)=142.4$, $p<0.001$. Clearly LS and SS mice differ in their response to salsolinol. The LS mice are more sensitive to the depressant effects of this drug on open-field activity: a 25 μg dose produced a 96% drop in LS activity relative to their ether/CSF controls, while SS mice experienced only a 63% decrease in activity. At the highest doses (30 and 40 μg salsolinol) LS and SS mice showed little activity. However, they were neither ataxic nor catatonic and would move about briefly during attempted transfers into and out of the apparatus.

Sleep Time

A three-way analysis of variance of sleep time scores revealed significant effects of line and treatment which are summarized in Fig. 2. There were no significant differences between males and females, thus scores were collapsed across sex for the sake of clarity. As can be seen from Fig. 2, there were no significant differences between the ethanol and ethanol/CSF controls. However, the increase in sleep time following ethanol plus salsolinol treatment was highly significant, $F(4,380)=28.98$, $p<0.001$. Although sleep time was potentiated in both lines, the increase was greater for LS than for SS mice; an 89% increase vs. 57% increase, respectively, $F(1,380)=49.38$, $p<0.001$.

Table 1 presents preliminary results on the effects of lower doses of salsolinol on ethanol-induced sleep time. Two interesting observations emerged from this study: first, comparable sleep times were observed within each line for these lower doses, thus a clear dose-response relationship was not evident; and second, LS and SS mice were differentially sensitive to these low doses, i.e., ethanol-induced sleep time decreased in SS mice and increased in LS mice when compared to the sleep time of controls.

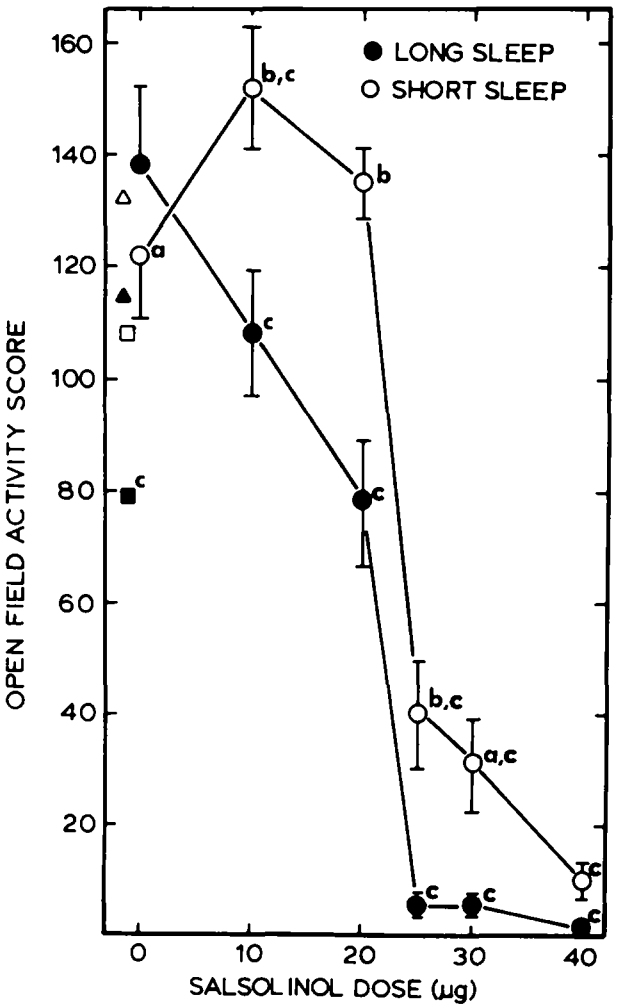

FIG. 1. The effect of salsolinol on open-field activity. Each value is the mean±SEM of 24 mice per group except the basal activity scores which are based on 40 mice per group; (□) untreated, basal activity scores; (△) ether controls; (○) at the 0 μg dose, represents the ether/CSF controls. [a]Significantly different from LS, $p<0.05$; [b]Significantly different from LS, $p<0.01$; [c]Significantly different from respective ether/CSF treated control, $p<0.05$.

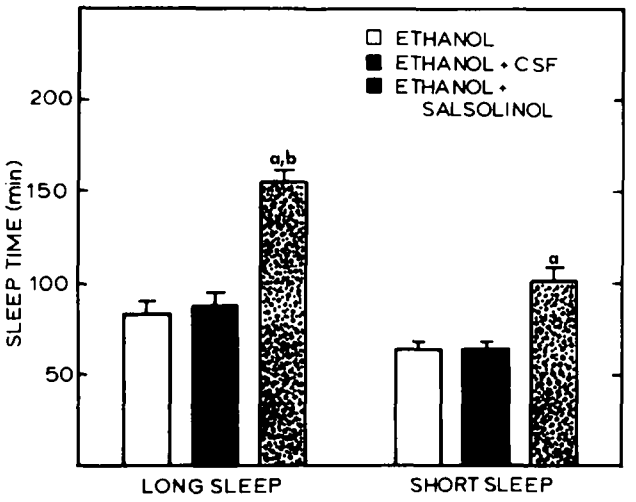

FIG. 2. The effect of salsolinol on ethanol-induced sleep time. Each bar is the mean sleep time score in minutes ± SEM of 40 mice. LS mice were injected with 2.5 g/kg ethanol and SS mice, 4.1 g/kg. Salsolinol (40 μg) and CSF were administered, IC, immediately following loss of the righting response. [a]Significantly different from respective ethanol group, $p<0.001$; [b]Significantly different from SS mice, $p<0.001$.

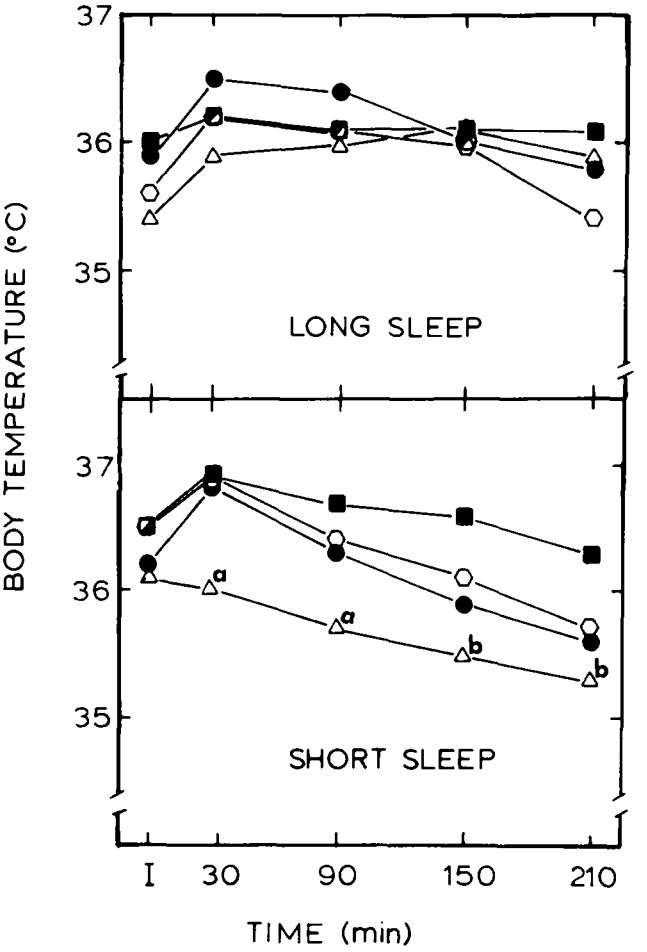

FIG. 3. Hypothermia control comparisons. (hexagon) untreated controls; (△) ether/CSF controls; (●) ether controls; (■) saline controls. Saline control groups contained 10 mice/line, all other groups contained 20 mice/line. [a]Significantly different from respective controls, $p<0.05$; [b]Significantly different from saline controls only, $p<0.05$.

Body Temperature

With respect to body temperature, sex differences were not consistent or marked among treatments or time points, therefore, the data were collapsed across sex for analysis. Figures 3 and 4 summarize the results of this experiment. Four control groups were tested: untreated controls; etherized; etherized and injected with CSF; and saline-injected controls. Although there were slight differences among the control groups in the initial body temperature, there were no significant differences among controls in the change in body temperature seen after the various control treatments, with one exception. The SS etherized/CSF-injected control group had significantly ($p<0.05$) lower body temperatures at 30 and 90 min compared to the remaining three control groups but was significantly different ($p<0.05$) from the saline controls only at 150 and 210 min. Because of this variability, the etherized/CSF-injected control group was used in all subsequent salsolinol comparisons for both LS and SS mice. Similarly, the saline-injected control was used in all ethanol comparisons.

One of the major findings of this study was that LS mice are more sensitive to salsolinol than are SS mice (Fig. 4). The LS mice lost an average of 3.7°, and the SS mice 2.5°, 30 min following a IC injection of salsolinol, $F(1,76)=11.4$, $p<0.01$. Also, the time course of the return to pre-drug body temperature took longer in the LS compared to the SS line.

In addition to differential sensitivity to the hypothermic effects of salsolinol, the LS and SS lines of mice also differ in sensitivity to the hypothermic effects of ethanol. This result is in agreement with previous reports for these same lines of mice [2,25]. When the mice are given doses of ethanol which yield almost equivalent sleep times (2.5 g/kg for LS and 4.1 g/kg for SS) they do not differ in their hypothermic response (Fig. 4). However, the fact that such drastically different doses are required to produce nearly identical responses underscores the tremendous difference in sensitivity apparent in this hypothermic response. Interestingly, when given equivalent doses of ethanol (4.1 g/kg for both LS and SS mice) the two lines still do not differ in the initial hypothermic response seen at 30 min but dramatic differences are seen at later time points. The thermoregulatory ability of the LS mice breaks down completely and may partially explain the high mortality rate observed at this dose.

Ethanol Elimination

Salsolinol, given in conjunction with ethanol, did not alter ethanol elimination in any of the treatment groups (Fig. 5). This suggests that the increase in ethanol-induced sleep time seen following salsolinol administration is due to a CNS effect. Examination of the data in Fig. 5 reveals a sex difference in both lines of mice. Females eliminated ethanol more rapidly than did males, although this attained statistical significance only among LS mice. This finding agrees with a previous report on inbred mice [9] but not for heterogeneous stock mice [1].

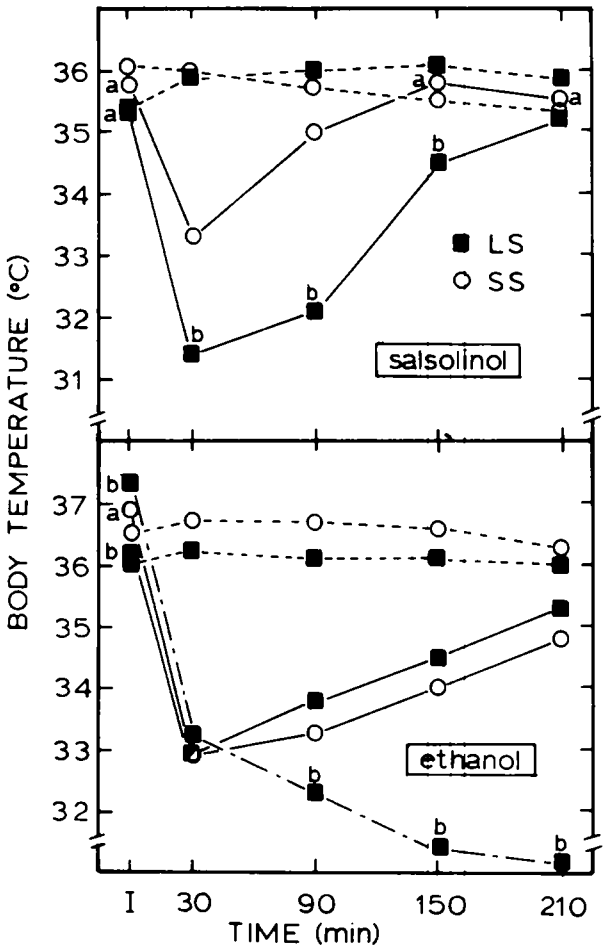

FIG. 4. The effect of salsolinol and ethanol on body temperature. Each time point represents the mean of 20 mice/line except for the saline controls which are based on 10 mice/line/time point. Standard errors are uniformly ±1% of the graphed values. (— — —) ether/CSF (upper graph) *or* saline (lower graph) controls; (—) respective experimental groups; (— · — · —) LS mice administered 4.1 g/kg ethanol. Except where noted, all drug-treated mice were significantly different from the respective controls, $p<0.05$. [a]*Not* significantly different from respective controls. [b]Significantly different from SS mice, $p<0.05$.

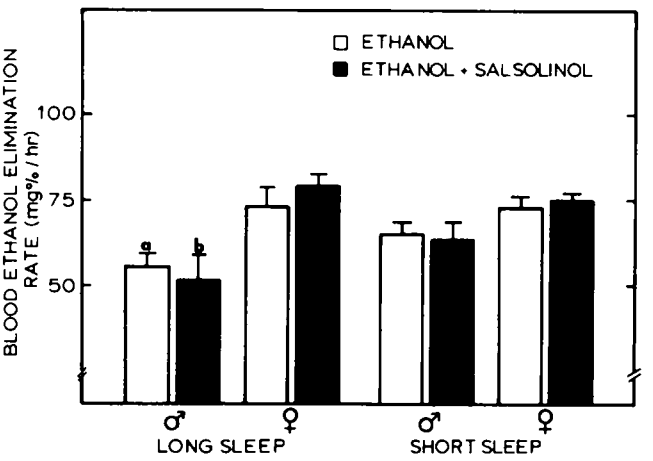

FIG. 5. The effect of salsolinol on blood ethanol elimination rate. The doses administered were identical to those outlined in Fig. 2. The elimination rate in mg%/hour was calculated from the slope of the regression line for each subject. The graphed values represent the average of 12 subjects/sex/line/treatment ± SEM.

The selection pressure applied to the LS mice has resulted in tremendous CNS sensitivity to the 4.1 g/kg dose of ethanol. Under the conditions of this study there was virtually 100% mortality four hours following ethanol injection. Estimation of ethanol elimination rates in these dying animals proved to be unreliable and thus are not reported.

DISCUSSION

The results of this investigation extend earlier reports of a differential sensitivity of the LS and SS mice to salsolinol [5,6]. We found, in agreement with Church *et al.* [6], that salsolinol treatment results in a dose-dependent decrease in open-field activity. More importantly, differential effects which are genotype-dependent have been demonstrated. The LS mice are more sensitive to the depressant effects of salsolinol while a biphasic effect is clearly apparent in the SS line: low doses of salsolinol stimulate open-field activity while higher doses depress activity. The biphasic effect is similar to that seen with ethanol and several other depressants which produce activation rather than depression following administration of low doses [26,30]. Sanders and co-workers [30] demonstrated that SS mice are more sensitive to the activating properties of ethanol, while LS mice appear to be more sensitive to the depressant effects. The SS line of mice were more active in an open-field arena following an IP injection of a low dose of ethanol than were LS mice. Thus, these data support the hypothesis that qualitative differences exist in salsolinol sensitivity in LS and SS mice which are not unlike those demonstrated for ethanol.

Qualitative differences in salsolinol sensitivity were also observed in the ethanol sleep time experiments. For the LS mice, a synergistic action was observed between salsolinol and ethanol in the potentiation of ethanol-induced narcosis. In the SS mice, low doses of salsolinol blocked the effect of ethanol and reduced sleep time (Table 1). At the 40 μg dose of salsolinol the difference between LS and SS mice does not appear to be striking—a mere 30%—until one remembers that LS mice were treated with a much lower dose of ethanol (2.5 g/kg for LS compared to 4.1 g/kg for SS mice). Administration of 4.1 g/kg ethanol in conjunction with 40 μg of salsolinol led to virtual 100% mortality among LS mice. Similarly, differences between the LS and SS lines were much greater at the lower salsolinol doses. These data imply that differential sensitivity to salsolinol formed as a consequence of ethanol administration could explain the differences in sleep time seen in the LS and SS mice. This supports the hypothesis of a role for salsolinol in the mediation of the acute effects of ethanol.

The rate of ethanol elimination in LS and SS mice was not altered by the co-administration of salsolinol. This finding suggests that the increase in ethanol sleep time following salsolinol treatment may be due to an increase in CNS sensitivity to the depressant effects of these drugs. However, we have not ruled out the possibility of an affect of salsolinol on pharmacokinetic or physiological parameters, such as volume of distribution or cerebral blood flow, which could also explain our results.

A sex difference in the rate of ethanol elimination was

detected in LS but not SS mice. Male LS mice metabolized ethanol more slowly than LS females. Gender differences in ethanol elimination rates have been previously reported for inbred mice [9] but not for heterogeneous stock (HS) mice [1]. This is thought to be due to linkage or random fixation of genes affecting ethanol elimination rates during the course of inbreeding. It is interesting that the inbred strains which show sex differences were used in the development of the HS mice [23], and the HS mice were subsequently used as the foundation population from which the LS and SS mice were developed. The LS and SS mice are maintained as a closed breeding population. The inbreeding which occurs as a result of this breeding paradigm could have resulted in linkage or fixation of genes in the LS line of mice.

The major finding of the hypothermia experiment was that the LS mice are more affected by both salsolinol and ethanol than are SS mice. These results suggest that the two drugs are acting via similar mechanisms. However, ethanol produced a far more severe and long-lasting depression of body temperature than did salsolinol: by 210 min post-injection the body temperature of ethanol treated LS mice continued to decline. Although the difference in the response to these two drugs could be a reflection of differences in their sites of action, a more likely explanation is that the differences in the time course of the response is related to the difference in metabolism of these drugs. Ethanol is more slowly eliminated than is salsolinol [24].

The LS and SS mice have been selectively bred for differential sensitivity to ethanol, thus genetic theory predicts that these lines of mice should differ only with regard to those traits related to their sensitivity to ethanol. The observation that these two lines of mice also differ in response to salsolinol suggests that some of the effects of acute ethanol treatment could arise as a result of salsolinol formation. Alternatively, salsolinol might affect the same neurochemical events or neuronal pathways as does ethanol. If so, the difference in response between the LS and SS mice could arise because of a differential rate of salsolinol metabolism following intracerebral injection. However, data currently available (Smolen and Collins, submitted) does not suggest a difference in salsolinol elimination under these conditions. Therefore, the results reported here are supportive of a role for salsolinol in the actions of ethanol. The major issue as to whether or not salsolinol formation occurs *in vivo* in "physiologically relevant" amounts as a consequence of ethanol administration remains controversial. Furthermore, it has been impossible to predict with any degree of certainty the exact concentration of salsolinol that might be formed following ethanol administration. Weiner [32] has calculated the upper limit to be approximately 1 ng/g of brain tissue. This value is based on estimated concentrations of dopamine and acetaldehyde available in brain for formation of salsolinol and on the assumption that a steady-state condition exists. The 1 pg dose of salsolinol, found to have behavioral effects in the present study, is well below Weiner's calculated upper limit. Our lack of knowledge concerning the site of drug action precludes our ability to measure the concentration of salsolinol at this site. Nevertheless, studies of salsolinol's actions may provide insights into the mechanisms by which ethanol exerts its effects.

REFERENCES

1. Allen, D. L., R. G. Little, J. E. Theotokatos and D. R. Petersen. Ethanol elimination rates in mice: Effects of gender, nutrition, and chronic ethanol treatment. *Pharmacol Biochem Behav* **16:** 757–760, 1982.
2. Baker, R., C. Melchior and R. Deitrich. The effect of halothane on mice selectively bred for differential sensitivity to alcohol. *Pharmacol Biochem Behav* **12:** 691–695, 1980.
3. Blum, K., M. G. Hamilton, M. Hirst and J. E. Wallace. Putative role of isoquinoline alkaloids in alcoholism: A link to opiates. *Alcoholism: Clin Exp Res* **2:** 113–120, 1973.
4. Bruning, J. L. and B. L. Kintz. *Computational Handbook of Statistics*. Glenview: Scott, Foresman and Company, 1977.
5. Church, A. C., J. L. Fuller and B. C. Dudek. Salsolinol differentially affects mice selected for sensitivity to alcohol. *Psychopharmacology (Berlin)* **47:** 49–52, 1976.
6. Church, A. C., J. L. Fuller and B. C. Dudek. Behavioral effects of salsolinol and ethanol on mice selected for sensitivity to alcohol-induced sleep time. *Drug Alcohol Depend* **2:** 443–452, 1977.
7. Cohen, G. Tetrahydroisoquinoline alkaloids in the adrenal medulla after perfusion with "blood concentrations" of [^{14}C]acetaldehyde. *Biochem Pharmacol* **20:** 1757–1761, 1971.
8. Cohen, G. and M. A. Collins. Alkaloids from catecholamines in adrenal tissue: Possible role in alcoholism. *Science* **167:** 1749–1751, 1970.
9. Collins, A. C., T. N. Yeager, M. E. Lebsack and S. S. Panter. Variations in alcohol metabolism: Influence of sex and age. *Pharmacol Biochem Behav* **3:** 973–978, 1975.
10. Collins, M. A. and M. G. Bigdeli. Tetrahydroisoquinolines *in vivo*. I. Rat brain formation of salsolinol, a condensation product of dopamine and acetaldehyde, under certain conditions during ethanol intoxication. *Life Sci* **16:** 585–590, 1975.
11. Collins, M. A., W. P. Nijm, G. F. Borge, G. Teas and C. Goldfarb. Dopamine related tetrahydroisoquinolines: Significant urinary excretion by alcoholics after alcohol consumption. *Science* **206:** 1184–1186, 1979.
12. Davis, V. E. and M. J. Walsh. Alcohol, amines and alkaloids: A possible biochemical basis for alcohol addiction. *Science* **167:** 1005–1007, 1970.
13. Dougan, D., D. Wade and P. Mearrick. Effects of *l*-dopa metabolites at dopamine receptor suggest a basis for "on-off" effect in Parkinson's disease. *Nature* **254:** 70–72, 1975.
14. Erwin, V. G., W. D. Heston, G. E. McClearn and R. A.Deitrich. Effect of hypnotics on mice genetically selected for sensitivity to ethanol. *Pharmacol Biochem Behav* **4:** 679–683, 1976.
15. Haley, T. J. and W. G. McCormick. Pharmacological effects produced by intracerebral injection of drugs in the conscious mouse. *Br J Pharmacol* **12:** 12–15, 1957.
16. Hall, C. S. The inheritance of emotionality. *Sigma Xi Quart* **26:** 17–27, 1938.
17. Hartley, H. O. The maximum F-ratio as a short-cut test for heterogeneity of variance. *Biometrika* **37:** 308–312, 1950.
18. Heston, W. D. W., V. G. Erwin, S. M. Anderson and H. Robbins. A comparison of the effects of alcohol on mice selectively bred for differences in ethanol sleep time. *Life Sci* **14:** 365–370, 1974.
19. Hornykiewicz, O. The mechanisms of action of *l*-dopa in Parkinson's disease. *Life Sci* **15:** 1249–1259, 1974.
20. Lasala, J. M. and C. J. Coscia. Accumulation of a tetrahydroisoquinoline in phenylketonuria. *Science* **203:** 283–284, 1979.
21. Marshall, A. and M. Hirst. Potentiation of ethanol narcosis by dopamine- and L-DOPA-based isoquinolines. *Experientia* **32:** 201–203, 1976.

22. McClearn, G. E. and R. Kakihana. Selective breeding for ethanol sensitivity: Short-Sleep and Long-Sleep mice. In: *Development of Animal Models as Pharmacogenetic Tools*. NIAAA Monograph-6, edited by G. E. McClearn, R. A. Deitrich and V. G. Erwin. Washington, DC: U.S. Government Printing Office, 1981, pp. 147–160.
23. McClearn, G. E., J. R. Wilson and W. Meredith. The use of isogenic and heterogenic mouse stocks in behavioral research. In: *Contributions to Behavior-Genetic Analysis: The Mouse as a Prototype*, edited by G. Lindzey and D. D. Thiessen. New York: Appleton-Century-Crofts, 1970, pp. 3–22.
24. Melchior, C. L., A. Mueller and R. A. Deitrich. Half-lives of salsolinol and tetrahydropapaveroline hydrobromide following intracerebroventricular injection. *Biochem Pharmacol* **29:** 657–658, 1980.
25. Moore, J. A. and R. Kakihana. Ethanol-induced hypothermia in mice: Influence of genotype on development of tolerance. *Life Sci* **23:** 2331–2338, 1978.
26. Murphee, H. B. Electroencephalographic and other evidence for mixed depressant and stimulant actions of alcoholic beverages. *Ann NY Acad Sci* **215:** 325–331, 1973.
27. Myers, R. D. and C. L. Melchior. Alcohol drinking: Abnormal intake caused by tetrahydropapaveroline in brain. *Science* **196:** 554–556, 1977.
28. Myers, R. D. and M. M. Oblinger. Alcohol drinking in the rat induced by acute and intracerebral infusion of two tetrahydroisoquinolines and a β-carboline. *Drug Alcohol Depend* **2:** 469–483, 1977.
29. Riley, V. Adaption of orbital bleeding technique to rapid serial blood studies. *Proc Soc Exp Biol Med* **104:** 751–754, 1960.
30. Sanders, B., S. K. Sharpless, A. C. Collins, G. E. McClearn and C. Flanagan. Activating and anesthetic effects of general depressants. *Psychopharmacology (Berlin)* **56:** 185–189, 1978.
31. Sharpless, S. K. Hypnotics and sedatives: I. The barbiturates. In: *The Pharmacological Basis of Therapeutics* (4th edition), edited by L. Goodman and A. Gilman. New York: MacMillan Press, 1970, pp. 98–121.
32. Weiner, H. Possible steady-state concentrations of tetrahydroisoquinolines in brain after the consumption of ethanol. *Fed Proc* **40:** 2082–2085, 1981.
33. Wike, E. L. *Data Analysis: A Statistical Primer for Psychology Students*. Chicago: Aldine-Atherton, 1971.