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Effects of Ethanol and Salsolinol on Catecholamine Function in LS and SS Mice[1]

TONI NESS SMOLEN,*[2] THOMAS C. HOWERTON†[3]
AND ALLAN C. COLLINS†

*Institute for Behavioral Genetics, *Department of Psychology, †School of Pharmacy and Alcohol Research Center, University of Colorado, Boulder, CO 80309*

SMOLEN, T. N., T. C. HOWERTON AND A. C. COLLINS. *Effects of ethanol and salsolinol on catecholamine function in LS and SS mice*. PHARMACOL BIOCHEM BEHAV **20**(1)125–131, 1984.— Long Sleep (LS) and Short Sleep (SS) mice differ in duration of ethanol-induced sleep time because of differences in brain sensitivity to the depressant effects of alcohols. These lines of mice also differ in their sensitivity to salsolinol, the condensation product of acetaldehyde with dopamine. Some of ethanol's acute effects may be due to salsolinol interactions with catecholamine systems. In the present study, the half-lives of salsolinol were found to be 12.8 min (LS) and 12.3 min (SS). Salsolinol administration resulted in a decrease in brain norepinephrine content in LS but not SS mice. Dopamine levels were not altered by salsolinol. Ethanol or salsolinol, *in vitro*, inhibited dopamine uptake by striatal synaptosomes. The IC_{50} values for ethanol were 491 mM (LS) and 514 mM (SS), and for salsolinol, 300 μM (LS) and 1000 μM (SS). Thus, the mouse line which is most sensitive to the behavioral effects of salsolinol is also most sensitive to salsolinol's effects on norepinephrine levels and inhibition of dopamine uptake. However, much higher concentrations are required to alter dopamine uptake *in vitro* than are required to alter behavior *in vivo*.

Ethanol Salsolinol Tetrahydroisoquinoline Catecholamine Dopamine uptake

THE hypothesis that some of the acute and chronic effects of ethanol are due to the formation of tetrahydroisoquinoline (TIQ) alkaloids has been studied by a number of investigators [3, 7, 10, 16]. The TIQ alkaloids are condensation products of aldehydes and the catecholamine neurotransmitters dopamine (DA) or norepinephrine (NE). One of the more frequently studied TIQs, salsolinol, is formed by the condensation of acetaldehyde (the proximate metabolite of ethanol) with DA. There has been considerable speculation whether TIQs elicit behavioral effects which could potentially explain the effects of ethanol, and whether TIQ alkaloids alter any of the neurochemical systems which are also altered by ethanol. Several reviews of these topics are currently available [4, 9, 18, 36].

Recent studies in our laboratory have been concerned with the behavioral and physiological effects of salsolinol. These studies utilized the Long Sleep (LS) and Short Sleep (SS) lines of mice which were selectively bred by McClearn and Kakihana [32] for differences in duration of ethanol-induced sleep time (loss of the righting response). These lines of mice differ in duration of ethanol-induced sleep time because of differences in brain sensitivity to the depressant effects of alcohols [20]. This genetically determined difference in central nervous system (CNS) sensitivity makes the LS and SS lines of mice useful genetic tools for testing hypotheses concerning mechanisms of ethanol action. Two reports by Church *et al.* [7,8] have suggested that the LS line is more sensitive to the depressant effects of salsolinol, following intracisternal injection of very large doses of this TIQ, than are the SS mice. The behavioral effects measured in these studies were activity in an hour-glass shuttle chamber and salsolinol sleep time. The activity of LS mice in the shuttle chamber was depressed at a lower dose than was SS activity, and LS mice lost the righting response for a longer period than did SS mice following injection of a 240 μg dose of salsolinol. We have extended these observations by demonstrating that salsolinol prolongs ethanol-induced sleep time, depresses open-field activity and decreases body temperature to a greater degree in LS mice than in SS mice (Smolen and Collins, submitted). These observations suggest the possibility that ethanol's acute actions may be mediated, at least in part, by TIQ alkaloids such as salsolinol. Among the ways this might occur include differential *in vivo* formation of salsolinol as a consequence of ethanol administration. Current methodologies and limits of detection available to us have precluded our demonstrating *in vivo* formation. However, several research groups have addressed this problem with varying degrees of success [14, 15, 34, 38].

It has been suggested that the failure to detect TIQ alkaloids in brain may be due to their rapid metabolism to other compounds. In support of this hypothesis, O-methylsalsolinol has been detected in the brains of

[1]Supported in part by grants GM-07305, AA-00029 and AA 003527.
[2]Requests for reprints should be addressed to Dr. T. Smolen at her current address: Institute for Behavioral Genetics, University of Colorado, East Campus—Box 447, Boulder, CO 80309, Telephone: (303) 492-8844.
[3]School of Pharmacy, University of Kansas, Lawrence, KS 66045.

alcohol-treated rats [24] and in the urine of alcoholics [14]. The metabolism of salsolinol to O-methylsalsolinol occurs very rapidly in rodents. Melchior and co-workers [33] calculated a half-life of 12 min for the disappearance of salsolinol from rat brain following intraventricular injection.

Other ways by which ethanol's acute actions could be mediated by salsolinol include interactions with catecholaminergic systems. Ethanol and salsolinol interact with catecholamine systems in a highly complex manner. Several studies have hypothesized that many of the behavioral effects produced by these drugs are attributable to alterations in catecholaminergic functioning [2, 8, 12]. This hypothesis has direct bearing on the response of LS and SS mice to salsolinol because marked differences in whole brain concentrations of NE and DA and in catecholamine turnover rates have been reported for these lines of mice [12].

Heikkila and co-workers [25] were the first to report inhibition of catecholamine uptake into nerve terminals by TIQs. These researchers incubated synaptosomal preparations from rat brain with varying concentrations (0.1 mM–1.0 mM) of salsolinol. They found that salsolinol inhibited the uptake of ^{3}H-NE and ^{3}H-DA in a dose-dependent manner. More recently, Alpers *et al.* [1] have demonstrated that salsolinol inhibits DA uptake by synaptosomes competitively with an inhibition constant, K_i, of 125 μM.

In the present study we tested the hypothesis that LS and SS mice differ in their behavioral and physiological response to salsolinol due either to a difference in the rate of disappearance of salsolinol from brain or to changes in catecholamine levels following salsolinol treatment. Several lines of evidence have suggested that, compared to other neurotransmitter systems, the dopaminergic system is quite sensitive to ethanol [6, 17, 26, 29, 37]. Thus we examined the effects of ethanol and salsolinol on DA uptake by striatal synaptosomes.

METHOD

Animals

The subjects used in the following experiments were male LS and SS mice 72±10 days of age. The sample sizes varied between experiments and are noted where appropriate. Prior to testing the mice were housed at 23±2° under a 12:12, light:dark photoperiod. Food and water were available ad lib. Each animal was tested only once.

Materials

The materials used in these experiments and their sources were as follows: 3,4-dihydroxybenzylamine-HBr (Aldrich Chemical Co.); norepinephrine-HCl and dopamine-HCl (Sigma Chemical Co.); 3,4-(7-^{3}H(N))-dihydroxyphenylethylamine, specific activity = 26.8 Ci/mmole (New England Nuclear); 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES, Sigma Chemical Co.); Tris(hydroxymethyl)aminomethane hydrochloride (Tris, Sigma Chemical Co.); sodium octyl sulfate (Eastman Kodak Co.); acid-washed aluminum oxide (ICN Nutritional Biochemicals); centrifugal filter assemblies (Bioanalytic Systems); salsolinol-HBr was synthesized from dopamine and acetaldehyde as previously described (Smolen and Collins, submitted).

Salsolinol and Catecholamine Determinations

Male LS and SS mice (five/line/time point) were lightly anesthetized with ether and administered 1.0 μg of salsolinol-HBr 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) by intracerebral (IC) injection. Test injections of hematoxylin dye have confirmed the site of injection to be in or very near the lateral ventricle. The animals were killed by cervical dislocation at 0, 5, 10 or 20 min after the injection. The whole brain was rapidly removed, cooled on dry ice and weighed.

Two ml of 0.05 M perchloric acid containing 50 ng of the internal standard, dihydroxybenzylamine, were added to each brain sample which was then homogenized and centrifuged at 15,000 g for 15 min. The resulting supernatant fluids were transferred to 12 ml conical screw cap vials containing 1 ml of 3.0 M Tris buffer, pH 8.6 at room temperature, and 90 mg of alumina and shaken for 10 min. Following this step, the alumina was allowed to settle and the supernatant fluids were removed by aspiration. The alumina was washed once with 6 mM Tris buffer (pH 8.6) and twice with distilled water. After adding 1 ml of 0.05 M perchloric acid, the samples were mixed for 5 sec and centrifuged at 500 g for 1 min to remove the alumina particles. The resulting supernatant fluid was filtered using a centrifugal filter assembly with a 0.22 μm filter. A 20 μl aliquot was used for the determination of salsolinol and catecholamine content which were assayed the same day by a high performance liquid chromatography (HPLC) procedure [21].

The HPLC consisted of an Altex Model 110 solvent metering pump attached to a 25 cm×4.6 mm, i.d., 5 μ, Ultrasphere-ODS reverse phase (C_{18}) column (Altex Corp.). A 40 mm × 3.2 mm, i.d. stainless steel precolumn packed with 10 μ ODS reverse phase packing material (Altex Corp.) was used as a guard column. Samples were injected into a six-port valve equipped with a 20 μl sample loop. Catecholamines and salsolinol were measured using an LC-2A electrochemical detector attached to a TL-3 carbon paste amperometric electrode (Bioanalytic Systems). The mobile phase was composed of three parts 0.1 M citric acid, two parts 0.1 M NaH_2PO_4 and contained 0.03 mM sodium octyl sulfate. The detector potential was set at +0.72 V vs. Ag/AgCl reference electrode. The mobile phase was pumped at a rate of 1 ml/min.

Norepinephrine and DA stock solutions (100 μg free base/ml of 0.05 M perchloric acid) were stored at −20°. Fresh solutions were prepared every two weeks. Salsolinol was similarly prepared immediately before use. Working standards (0.25–1.0 μg/ml) were prepared daily by diluting the catecholamine and salsolinol stock solutions prior to processing the samples. In order to calculate the concentration of salsolinol and catecholamines in the samples, additional tissue samples were prepared to calibrate the procedure. Two whole brains were combined with 4 ml of 0.05 M perchloric acid and 100 ng of internal standard. The tissue was homogenized and divided into two equal parts; one part served as a blank and to the second was added a known amount of salsolinol and catecholamine working standard. These two homogenates were processed along with the samples. The ratio of the peak height for salsolinol and each catecholamine to that of the internal standard was determined. The amount of salsolinol and amines for each sample was calculated according to the method of Felice *et al.* [21] All samples and standards were run in duplicate.

Dopamine Uptake Studies

Dopamine uptake was measured in synaptosomes from mouse striatum. Mice were killed by cervical dislocation.

The brains were removed, cooled to 4°, and dissected. Three pairs of striata were placed in 20 ml of 0.32 M sucrose and samples were gently homogenized by hand in a Potter-Elvehjem homogenizer using ten strokes. Synaptosomes were isolated using a modification of the method of Gray and Whittaker [22]. This homogenate was centrifuged at 1475 g for 10 min to remove unbroken cells, nuclei and tissue debris. The resulting supernatant was centrifuged at 22,000 g for 20 min to obtain a P2 pellet containing the crude synaptosomal fraction.

The P2 pellet was resuspended in a volume of 0.32 M sucrose (550–1600 μl) to yield a protein concentration of approximately 0.4 mg/ml as determined by the method of Lowry *et al.* [31]. A 50 μl aliquot was added to 400 μl of a modified Krebs-Ringer buffer (15.8 mM HEPES, 0.01 mM glucose, 1.4 mM $MgSO_4$, 1.3 mM $CaCl_2$, 4.7 mM KCl, 126 mM NaCl and 0.1 mM ascorbic acid). The total volume was 500 μl.

Dopamine uptake was measured by the procedure established by Komiskey and Miller [28]. The synaptosomes were incubated in stoppered test tubes at 37° in the presence or absence of ethanol, salsolinol or the uptake inhibitor, desmethylimiprimine (DMI; all drugs made up in buffer). Uptake was initiated by the addition of 50 μl of radiolabeled substrate (ca. 400,000 cpm; final concentration 1 μM). Initial time course experiments revealed that 2–3 min incubations were within the linear phase of uptake, thus a 2 min incubation period was used. Less than 10% of the substrate was taken up in 3 min. Blanks contained buffer, synaptosomes and labeled DA. Dopamine uptake was terminated by rapid filtration through Whatman GF/A glass microfiber filters, 24 mm in diameter, mounted in a Model 1225 Millipore Sampling Manifold (Millipore Filter Corp.). After collecting the synaptosomes with mild suction, the filters were washed with 12 ml of cold 0.9% NaCl. Prior to filtration the filters were soaked with 10 mM DA to reduce nonspecific binding.

After filtration and washing, the filters were transferred to 10 ml Nalgene filmware bags (Nalgene Corp.) Three ml of scintillation fluid (10.6 g of 2,5-diphenyloxazole, 1260 ml toluene and 900 ml Triton X-100) were added to each bag. The bags were heat-sealed, the filters were disintegrated by mechanical pressure, and the amount of radioactivity in the samples was determined by liquid scintillation spectrometry with a Beckman Model LS 7000 liquid scintillation counter. The amount of DA accumulated by the synaptosomes was expressed as the percentage of the radioactivity added to the incubation mixture. IC_{50} values were calculated as the concentration of drug (either ethanol or salsolinol) inhibiting the specific uptake of ^{3}H-DA by 50%. At least five different concentrations were assayed for each drug. In preliminary experiments the uptake of DA was found to be sodium dependent and responsive to the classical inhibitor DMI (data not shown).

Statistical Analyses

The data were analyzed by analysis of variance (ANOVA). Following a significant overall F test ($p<0.05$) differences in individual group means were detected using the Tukey B test [41] or, where noted, Duncan's Multiple Range Test [5].

RESULTS

Half-Life of Salsolinol

The disappearance of salsolinol from brain following IC injection follows first order reaction kinetics (Fig. 1). The half-lives of this drug in LS and SS mice were calculated from the first order kinetic constant and found to be 12.8 min for LS and 12.3 min for SS mice. These half-lives are virtually identical to that calculated by Melchior and co-workers [33] for the disappearance of salsolinol from rat brain following intraventricular injection.

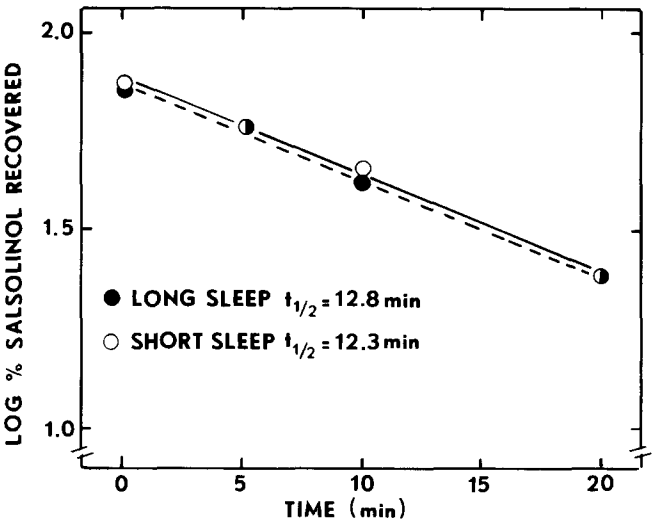

FIG. 1. Salsolinol elimination from brain following intracerebral injection. LS and SS mice were administered 1.0 μg salsolinol-HBr in 5 μl of artificial CSF and sacrificed at 0, 5, 10 or 20 min after injection. The whole brain was removed and assayed for salsolinol by HPLC as described in the Method section.

Catecholamine Levels Following Salsolinol Injection

Several investigators have suggested that the behavioral and physiological effects of salsolinol may be related to interactions between salsolinol and catecholamine systems ([2, 8, 12], Smolen and Collins, submitted). The HPLC assay for determining salsolinol content in brain allowed us to concurrently monitor catecholamine levels following salsolinol injection. The catecholamine data are summarized in Table 1. The data from salsolinol-treated mice and the untreated controls were analyzed by ANOVA. The data from CSF-treated mice are presented for comparison but were not included in the analyses because of the disparity in sample sizes. However, one can see by inspection that these values do not differ among time points. Injections of CSF had no effect on NE content. Similarly, there was no difference in NE level between untreated LS and SS controls. Following injection of salsolinol, NE levels decreased in LS but not in SS mice. This decrease in NE content was significant, $F(3,32)=3.67$, $p<0.05$ for LS mice at the 20 min time point. The reason for this decline is unclear but it may represent an interesting line difference in response to salsolinol.

In the present study, male LS and SS untreated control mice exhibited similar DA levels (bottom of Table 1). This finding is inconsistent with previously published reports from this laboratory and others which have shown SS mice to have greater whole brain levels of DA than LS mice [11, 12, 19]. However, untreated female LS and SS mice measured and analyzed contemporaneously with the males of this study were significantly different from each other with respect to brain DA levels (female LS mice, 849±43 ng/g, n=7;

TABLE 1

WHOLE BRAIN LEVELS OF CATECHOLAMINES IN MALE LONG SLEEP AND SHORT SLEEP MICE FOLLOWING SALSOLINOL OR CSF INJECTION

Time After Injection (min)	Norepinephrine (ng/g tissue)				Dopamine (ng/g tissue)			
	Long Sleep		Short Sleep		Long Sleep		Short Sleep	
	Salsolinol	CSF	Salsolinol	CSF	Salsolinol	CSF	Salsolinol	CSF
0	452 ± 19 (5)	475 ± 27 (2)	475 ± 15 (5)	469 ± 15 (3)	1033 ± 47 (5)	1066 ± 125 (3)	940 ± 90 (5)	1010 ± 63 (3)
5	462 ± 60 (5)	465 ± 6 (3)	483 ± 21 (5)	465 ± 8 (3)	1013 ± 64 (5)	1005 ± 7 (2)	1027 ± 33 (5)	1028 ± 95 (3)
10	430 ± 29 (5)	457 ± 16 (3)	499 ± 43 (5)	482 ± 15 (3)	999 ± 72 (5)	888 ± 73 (3)	1028 ± 72 (5)	1022 ± 40 (2)
20	335 ± 40* (5)	453 ± 10 (3)	413 ± 24 (5)	474 ± 17 (2)	1058 ± 123 (5)	931 ± 41 (3)	1001 ± 92 (5)	992 ± 34 (3)
Untreated Controls	(445 ± 20) (7)		(484 ± 12) (7)		(887 ± 67) (7)		(978 ± 50) (7)	

Mice were injected with salsolinol (1.0 μg/5 μl CSF, IC) or CSF (5 μl, IC) following light ether anesthesia. Tabled values are means ± S.E.M. Numbers in parentheses are the number of animals in each group. All samples were analyzed in duplicate.

*Significantly different from the respective 0 and 5 min time points, $p<0.05$, as determined by Duncan's Multiple Range Test.

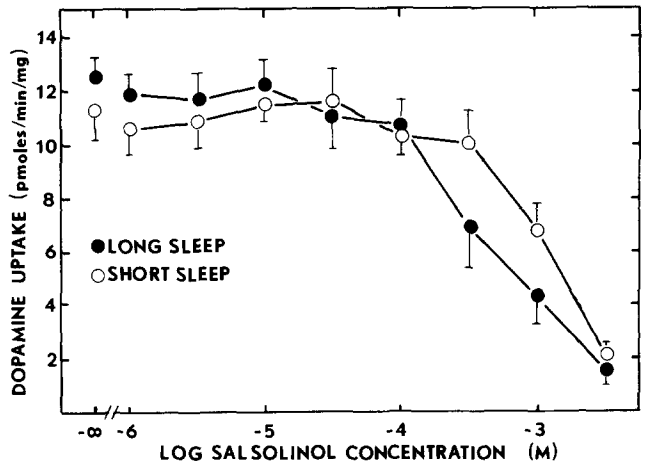

FIG. 2. The effect of ethanol on dopamine uptake in mouse striatum. Crude synaptosomal preparations from striatum were incubated for two min at 37° in the presence of 1.0 μM dopamine and varying concentrations (0.25%–5.0%, which corresponds to 43–858 mM) of ethanol as outlined under Method section.

female SS mice, 1130±77 ng/g, n=7) as determined by two-way ANOVA (line×sex) followed by post hoc comparisons using the Duncan's Multiple Range test, $F(1,24)=9.40$, $p<0.01$. The main effect of sex and the line by sex interaction were nonsignificant. Thus the finding of no difference between untreated male LS and SS DA levels is most likely due to sample bias. Dopamine levels did not vary significantly among time points following injection of salsolinol or CSF in either line.

Kinetics of ^{3}H-Dopamine Uptake

Synaptosomes were incubated with several concentrations of labeled DA ranging from 0.1 to 5.0 μM for 2 min at 37°. Kinetic constants for DA uptake were calculated from linear regression analysis of Eadie-Hofstee plots of the data and are summarized in Table 2. These data demonstrate that, under drug-free conditions, the DA uptake systems of LS and SS mice do not differ either in affinity for DA or in their maximal rate of uptake.

TABLE 2

DOPAMINE UPTAKE KINETICS IN LS AND SS MOUSE STRIATUM

Mouse Line	N	K_m (μM)	V_{max} (nmoles/min/mg)
LS	6	0.326 ± 0.05	0.086 ± 0.01
SS	6	0.276 ± 0.05	0.092 ± 0.01

Tabled values represent the mean ± S.E.M. of six separate K_m and V_{max} determinations. Three pairs of striata per line were pooled for each experiment. Dopamine concentration was varied from 0.1–5 μM and each assay was run in duplicate.

Effects of Ethanol on Dopamine Uptake

Eight concentrations of ethanol ranging from 0.25% to 5.0% (v/v, corresponding to 43 mM to 858 mM) were tested for their ability to inhibit DA uptake (Fig. 2). The IC_{50} values for ethanol were calculated to be 491 mM for LS mice and 514 mM for SS mice. These values correspond to an ethanol concentration of approximately 3% (v/v). In light of the evidence which suggests that dopaminergic systems are more affected by ethanol [6, 17, 26, 29, 37] compared to other

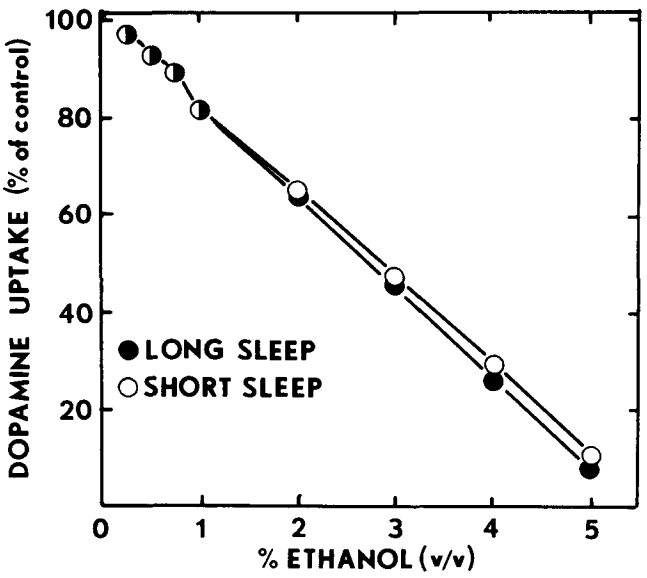

FIG. 3. The effect of salsolinol on dopamine uptake in mouse striatum. Crude synaptosomal preparations from striatum were incubated for two min at 37° in the presence of (1.0 μM–3000 μM) of salsolinol as outlined under the Method section.

neurotransmitter systems, it is interesting to note that the effects of ethanol on DA uptake is nearly identical in LS and SS mice. However, it should be noted that, within the physiological range of ethanol concentrations (0.25–1.0%, corresponding to 43–174 mM), ethanol significantly inhibited the uptake of DA by 10–20%, $F(1,70)=2.8$, $p<0.05$, in both lines of mice. Since the two lines of mice did not differ significantly within physiologically relevant concentrations, it is unlikely that ethanol elicits its behavioral and physiological effects in these lines of mice via an inhibition of DA uptake.

Effects of Salsolinol on Dopamine Uptake

Eight concentrations of salsolinol spanning the range of 1 μM to 3000 μM were evaluated for their ability to inhibit DA uptake. As can be seen from Fig. 3, DA uptake by striatal synaptosomes was inhibited by salsolinol. The IC_{50} values were calculated to be 300 μM for LS mice and 1000 μM for SS mice. Although this represents a greater than three-fold difference between the lines, these concentrations are outside the physiological range. It is possible that salsolinol may be a more effective uptake inhibitor *in vivo* compared to *in vitro*.

DISCUSSION

The LS and SS mice used in this study were derived from a genetically heterogeneous stock (HS) of mice by means of selective breeding for differential response to the hypnotic effects of ethanol [32]. These lines of mice should respond in a dissimilar fashion only to those parameters which are related to their acute response to ethanol. Several studies have shown that the LS and SS mice differ in their behavioral and physiological responses to both ethanol and salsolinol: Shuttle chamber and open-field activity are depressed at a lower dose of salsolinol in LS mice compared to SS mice and LS mice lose the righting response for a longer period of time than do SS mice following injection of either ethanol or salsolinol. Salsolinol has also been found to prolong ethanol-induced sleep time and induce hypothermia to a greater degree in LS mice than in SS mice ([7,8], Smolen and Collins, submitted). The results of these studies suggested the possibility that some of the response to ethanol might be due to either differential formation of salsolinol (or some other similar compound) or to differential elimination of salsolinol. Technical difficulties [21, 35, 39, 40] have prevented us from measuring salsolinol formation in mouse brain following acute ethanol administration. Thus, the issue of differential formation of salsolinol by LS and SS mice remains unsettled. Nevertheless, we have shown that the elimination of salsolinol following a challenge dose yielded virtually identical half-lives for the two lines of mice (12.8 and 12.3 min). These data argue that the difference in response to intracerebrally injected salsolinol that we have seen in the LS and SS mice is not due to differences in elimination of salsolinol.

In the absence of differential rates of elimination it seems likely that the differences in response to injected salsolinol seen between LS and SS mice are due to differences in brain sensitivity to this agent. Numerous studies have suggested that salsolinol and other TIQs may act as false transmitters (see [18] for a review). In the present study salsolinol did not affect whole brain DA levels in either line of mice. Salsolinol treatment may have an effect on whole brain NE content: A modest reduction in NE was observed in the LS mice 20 min after salsolinol treatment. Further studies will be necessary to assess the potential effect of salsolinol on brain NE content. Nonetheless, the mouse line which is most responsive to salsolinol behaviorally is the line in which a reduction of brain norepinephrine levels was obtained. It should be remembered, however, that whole brain catecholamine measurements are a crude measure of catecholaminergic function. Therefore, further analysis of salsolinol-catecholamine interactions should assess effects on turnover, release and other measures of the dynamics of catecholamine action.

A number of studies have shown that salsolinol inhibits catecholamine uptake [1,25]. Alpers *et al.* [1] reported that salsolinol inhibited DA uptake by striatal synaptosomes with an inhibition constant, K_i, of 125 μM. This value is in general agreement with the present *in vitro* study in which significant inhibition of DA uptake by salsolinol occurred in the striatum at 300 μM for LS mice and 1000 μM for SS mice. A three-fold difference between the lines in IC_{50} values was found, but inhibition of uptake was seen only at high concentrations. At lower concentrations (1–100 μM) LS and SS mice did not significantly differ from each other. However, we can not rule out the possibility that *in vivo* the difference between the lines may be expressed at physiological concentrations of salsolinol. It has also been suggested that a metabolite of salsolinol, such as O-methylsalsolinol, may be the more active endogenous substrate [14,24]. Alternatively, salsolinol may interact with other neurochemical systems to influence the uptake of DA.

The effect of ethanol on dopamine uptake into striatal synaptosomes was nearly identical in these lines of mice (Fig. 2). High concentrations of ethanol were required to inhibit DA uptake. This finding is in agreement with a previous report on the effect of *in vitro* ethanol on the uptake of NE, choline and gamma-aminobutyric acid into synaptosomes of LS and SS mice [27]. Thus, our results suggest that it is unlikely that the acute physiological and behavioral effects of ethanol are mediated by direct inhibition of DA uptake.

In summary, we have demonstrated that the hypothesis of differential salsolinol elimination rates in LS and SS mice is inadequate to explain the behavioral and physiological differences in sensitivity to this drug. Several studies have found the concentration of the salsolinol precursor, DA, to be greater in the SS mouse line which is least sensitive to both ethanol and salsolinol [11, 12, 19] and the present study has shown that salsolinol treatment decreases whole brain NE content of the LS mouse line which is most sensitive to salsolinol's behavioral effects. The differential response of the LS and SS mice to salsolinol may be due to a differential effect of salsolinol on DA uptake. However, very high concentrations of salsolinol were required to inhibit uptake *in vitro*. Therefore, unless salsolinol is more effective as an uptake inhibitor *in vivo* than it is *in vitro*, the hypothesis that salsolinol elicits its behavioral and physiological actions via an effect on DA uptake remains open to question.

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