Salsolinol Differentially Affects Mice Selected for Sensitivity to Alcohol

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Abstract. Salsolinol, a compound putatively formed following alcohol ingestion, differentially decreased the activity of lines of mice after 18 generations of genetic selection for alcohol sensitivity. Low doses of salsolinol produced significantly lower activity levels in the alcohol-sensitive long-sleep (LS) line than in the alcohol-insensitive short-sleep (SS) line. A hypnotic dose of salsolinol induced significantly longer sleep-times in the LS line than in the SS line. Results are interpreted as supporting the hypothesis that salsolinol-like substances may mediate some of the effects of alcohol on the central nervous system.

Key words: Salsolinol - Ethanol narcosis - Locomotor activity - Selected lines - Intracisternal - Behavior genetics.

INTRODUCTION

A number of authors have suggested that compounds formed from the condensation reaction of catecholamines and acetaldehyde (an ethanol metabolite) may be involved in mediating some of the pharmacological actions of ethanol (Davis, 1971, 1973; Davis and Walsh, 1970; Cohen, 1973; Cohen and Collins, 1970; Collins, 1973; Walsh, 1973). The group of compounds consistently mentioned as such condensation products is the tetrahydroisoquinoline (TIQ) group. Recently, the in vivo formation of salsolinol (the TIQ formed from the reaction of dopamine with acetaldehyde) has been detected in the rat brain under certain conditions during acute ethanol exposure (Collins and Bigdeli, 1975). A potential physiological role of TIQ is suggested by its uptake into sympathetic nerve terminals and subsequent binding to norepinephrine (NE) vesicles, and its release following neuron stimulation (Tennyson et al., 1973; Heikkila et al., 1971; Greenberg and Cohen, 1973; Locke et al., 1973). Evaluation of the sympathomimetic properties of TIQ indicated a weak direct adrenergic activity and an indirect effect based upon the release of NE (Simpson, 1975).

The behavioral effects of TIQ's have not been extensively examined. In the present study, the animals chosen to investigate the behavioral actions of a TIQ were two lines of mice selectively bred for long (LS) or short (SS) sleep-time response to hypnotic doses of ethanol. The mice are the product of 18 generations of selection (McClearn and Kakihana, 1973) and thus represent a valuable genetic tool for studying the actions of alcohol on the central nervous system. The value lies in the fact that the genetic treatment separates mice into two distinct physiological types by a process which avoids the acute trauma associated with many other procedures such as chemical lesions or surgery. The two lines are practically non-overlapping in duration of sleep-time following a standard intraperitoneal injection of ethanol. Since these mice show little difference in their rates of ethanol metabolism, their difference in sleep-time probably reflects genetic differences in their central nervous system sensitivity to ethanol (Heston et al., 1974).

METHODS

The subjects were males and females, 45–70 days old. They were group-housed in transparent plastic cages containing pine shavings and were exposed to a 12 h/12 h light-dark cycle. A total of 155 animals were used with most groups consisting of 9 animals.

In the first study, activity of the animals was monitored using an hour-glass shuttle chamber described by Fuller (1966). The number of crossings (determined by a photocell) during a 10-min period in the apparatus was the index of activity. All animals received only one test. In the second study, sleep-time was defined as the duration of loss of the righting reflex following injection of the drug.
Prior to measuring activity, the experimental subjects were administered 10, 20, or 40 µg of salsolinol (Aldrich Chemical Co.) intracisternally (Schanberg et al., 1967) in 5 µl of Krebs-Ringer solution containing 0.01% ascorbic acid. The mice were etherized and then injected using a 0.64 cm, 27 gauge hypodermic needle attached to a graduated 50 µl Hamilton syringe. Test injections of hemotoxylin dye confirmed the site of injection to be the cisterna cerebellomedullaris. Subjects were tested 25 min following injection in order to minimize effects of etherization. If, following ether recovery, asymmetrical motor behavior (resulting from misdirected injection) was observed, the subject was immediately discarded. Three control groups were employed: NN—non-etherized, non-injected; EN—etherized, non-injected; EI—etherized, saline injected. Number of subjects in each group is nine with the exceptions of LS-20 (N = 10) and LS-40 (N = 8).

RESULTS

With respect to shuttle activity, the control groups were compared both individually using t-tests, and also together using a 2 × 3 analysis of variance.

Table 1. Percentage of animals receiving salsolinol with less than 50% of the mean crossings of control groups

<table>
<thead>
<tr>
<th>Salsolinol Dose (µg)</th>
<th>% &lt; ED50b</th>
<th>SS Control</th>
<th>LS Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>11</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>22</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>56</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

a SS and LS control group means were 81.6 and 73.7 crossings respectively.
b ED50, calculated by extrapolating the dosage producing a 50% decrease in crossings, was 32.5 µg for SS and 18.8 µg for LS.

Results indicated no significant differences among the control groups. However, when the groups receiving the different drug dosages were added to the analysis, the LS line was significantly lower in crossings than the SS line. Using a 2 × 6 analysis of variance, a main effect of genotype was found [F(1,96) = 7.35, P < 0.01]; the main effect of drug dosage was also significant [F(5,96) = 14.48, P < 0.01]. The simple main effect of genotype was significant at the 20 µg dose [F(1,96) = 9.13, P < 0.01]. Notably, the 20 µg dose also depressed crossing activity (relative to controls) to a greater extent in LS than in SS subjects. As shown in Figure 1, the 20 µg dose produced about a 70% drop in mean LS crossings relative to their control group, while decreasing SS crossings only 23%. The presence of this interaction was determined by comparing “change scores” (control mean minus experimental scores) between the mice lines using the Wilcoxon Rank Sum Test [P < 0.027 (Hollander and Wolfe, 1973)]. The ED50 for each line is presented in Table 1 along with an indication of how SS and LS groups were distributed across dosages relative to their ED50.

Further indication of higher LS sensitivity to salsolinol was determined by analysis of the hypnotic effects of a 240 µg dose. The LS line had significantly longer sleep-times than the SS line. As shown in Table 2, LS animals were not only more sensitive to intracisternal salsolinol, but they also slept significantly longer than the SS subjects following i.p. administration of either ethanol or acetaldehyde.

DISCUSSION

The results indicate that salsolinol produces a reduction in activity that is dose related. More significantly, there is a strong indication of differential effects dependent on genotype. Thus a genetic factor rendered the alcohol-sensitive LS mice more susceptible to the activity-reducing influences of salsolinol. Similarly, the LS line was significantly more sensitive to the