Pharmacology Biochemistry & Behavior, Vol. 20, pp. 91–94, 1984. © Ankho International Inc. Printed in the U.S.A.

Apomorphine Effects on Behavioral Response to Ethanol in Mice Selectively Bred for Differential Sensitivity to Ethanol

BRUCE C. DUDEK, MICHAEL E. ABBOTT, AJAY GARG AND TAMARA J. PHILLIPS

Department of Psychology and The Neurobiology Research Center
State University of New York at Albany, 1400 Washington Ave., Albany, NY 12222

Received 6 March 1982

DUDEK, B. C., M. E. ABBOTT, A. GARG AND T. J. PHILLIPS. *Apomorphine effects on behavioral response to ethanol in mice selectively bred for differential sensitivity to ethanol.* PHARMACOL BIOCHEM BEHAV **20**(1) 91–94, 1984.—Two lines of mice selectively bred for differences in response to a hypnotic dose of ethanol were administered apomorphine alone or in combination with ethanol. When administered by itself, apomorphine produced similar dose-dependent depression of locomotor activity and increases in stereotypy in the two lines. Doses of apomorphine (0.5 μM/kg and 2 μM/kg) thought to bind only presynaptic dopamine receptors blocked the slight locomotor activation to 1.5 g/kg ethanol in the ethanol-sensitive Long-Sleep (LS) mice; in the ethanol-insensitive Short-Sleep (SS) mice which show marked activation to all subhypnotic doses of ethanol, these doses of apomorphine only attenuated the activation. A higher apomorphine dose (8 μM/kg) antagonized the locomotor depressant effects of 2.0 and 2.5 g/kg of ethanol in LS mice but did not alter the shape of the SS ethanol dose response curve for locomotor activity. Apomorphine (2 and 8 μM/kg) potentiated ethanol-induced loss of the righting reflex in LS mice in a dose dependent fashion, but did not alter this soporific effect of ethanol in SS mice. These findings extend the data base suggesting a role for dopamine both in the mechanism(s) differentiating the LS and SS mice and the stimulant and intoxicating properties of ethanol.

Ethanol Apomorphine Genetics Dopamine Locomotor activity Stereotypy

IT has been argued that the stimulant properties of ethanol (ETOH), its "euphoric" actions, may be a source of its reinforcing properties [19,20]. Neuropharmacological work has implicated the function of dopamine (DA) neurotransmitter systems in the mechanisms underlying the stimulant effect. Treatment with alpha-methyl tyrosine antagonizes the locomotor stimulation effects of ETOH in mice and rats [4], and blocks the euphoric properties of the drug in man [1]. This antagonism in rodents is partially reversed by treatment with *l*-dopa [11]. The stimulant action of ETOH in mice was also antagonized by treatment with the DA receptor agonist apomorphine [22]. Other work has shown apomorphine to antagonize the discoordinating effects of a sub-hypnotic dose of ETOH in rats [2].

Genetically based variation in the magnitude of the stimulant ETOH effect has been demonstrated in inbred and selected lines of mice [16,21]. Long-Sleep (LS) mice selectively bred for extended soporific response to ETOH [17] show locomotor depression to most sub-hypnotic doses. The relatively ETOH-insensitive Short-Sleep (SS) mice show marked locomotor stimulation in response to sub-hypnotic doses. Part of the effectiveness of the selection for these lines may result from differences in DA systems. Biochemical studies demonstrated a two-fold greater decrease in whole brain DA turnover in LS mice than in SS mice following a soporific dose of ETOH [6]. DA stimulation of striatal adenylate cylcase was about 25% greater in LS than in SS mice [7]. Pharmacological studies have shown the two lines to respond differently to a variety of agents which act on central DA systems (e.g., salsolinol, amphetamine, gamma-butyrolactone, haloperidol; [5,9]).

The present studies examined the effects of apomorphine on locomotor activity and stereotypy in these mice as well as its effects on behavioral response to hypnotic and subhypnotic doses of ETOH. In order to assess the ability of apomorphine to modify the locomotor and soporific responses to ETOH, it was first necessary to study the effects of apomorphine given by itself to LS and SS mice. Apomorphine has previously been shown to shorten the latency to fluorothyl-induced seizures in LS mice while it had little effect on SS latencies [12]. The slightly greater effectiveness of haloperidol to induce catalepsy in SS mice [9] is consistent with the finding that DA turnover rate is higher in SS mice [6]. Experiment 1 examined the dose response curve of apomorphine effects on locomotor activity and stereotyped behavior in the two lines. Experiment 2 examined the effects

of several apomorphine doses on stimulant effects assessed by the shape of the sub-hypnotic ETOH dose response curve; apomorphine effects on duration of the loss of the righting reflex following hypnotic doses were also examined.

METHOD

Experiment 1

Mice of the Albany colony of LS and SS mice are descended from breeding stock obtained at generation 18. Male and female mice of the present study came from generations 27 and 29 and ranged in age from 55 to 100 days. Apomorphine-HCl (Merck) was prepared each day in 0.9% NaCl with 0.1% Na metabisulfite, and injected at 3–5°C. Mice were injected IP in a volume of 10 ml/kg; each mouse was tested only once.

Locomotor activity testing was done in a circular open field (LVE model PAC-001, 61 cm diameter, modified to have a Plexiglas floor which could be cleaned between tests). No illumination was present. Mice were placed singly in a holding cage for ten min following injection prior to testing. Activity was monitored for 15 min.

Stereotypy was assessed in a cage similar to the home cage, with fresh bedding (hardwood chips) present, by noting the presence or absence of four behaviors: sniffing/gnawing, straub tail, stiff gait, exaggerated posture (extended limbs). Rating was done for 30 sec every five min for 35 min following drug administration. The total number of behaviors present was summed across the seven observation periods and analysis of variance was performed on the square root of this score, taken to diminish proportionality of means and variances.

Experiment 2

Locomotor activity was assessed in the same manner as in Experiment 1. A total of 516 mice of both sexes came from generation 30 and ranged in age from 50 to 118 days. ETOH was prepared in 0.9% NaCl and injected IP in a 20 ml/kg volume so that dose was varied by concentration. The range of ETOH doses was chosen so that apomorphine effects could be described in terms of the shape of the ETOH dose response curve. Apomorphine doses were chosen on the basis of Experiment 1 data to represent (1) the descending portion of the "presynaptic" component—0.5 μM/kg, (2) the maximal depression of the "presynaptic" component—2 μM/kg, and (3) a high dose—8 μM/kg which surely involves postsynaptic receptor effects. Apomorphine was prepared and injected as in Experiment 1. Mice were treated with apomorphine (or saline vehicle) ten min prior to ETOH administration of one of three doses of ETOH or its saline vehicle, and then immediately placed in the circular activity monitor for fifteen min. Each mouse was tested only once.

Study of the effects of apomorphine on ETOH-induced loss of the righting reflex (LRR) was made possible by the choice of two ETOH doses that would produce approximately the same durations. This procedure was indicated since assessment of effects of pretreatment would be confounded by the temporal difference in LRR durations when the same dose is administered to the two lines. Such an approach is warranted because the rate of ETOH metabolism is similar in the two lines [13]. The doses were 4.0 (SS) and 2.4 (LS) g/kg, administered IP in 0.9% saline in a 20 ml/kg volume. Mice were pretreated with 0.0, 2.0 or 8.0 μM/kg apomorphine, prepared as above, and injected IP five min prior to ETOH treatment. LRR duration was assessed by measurement of time from loss of the righting reflex to spontaneous righting three times within 30 sec.

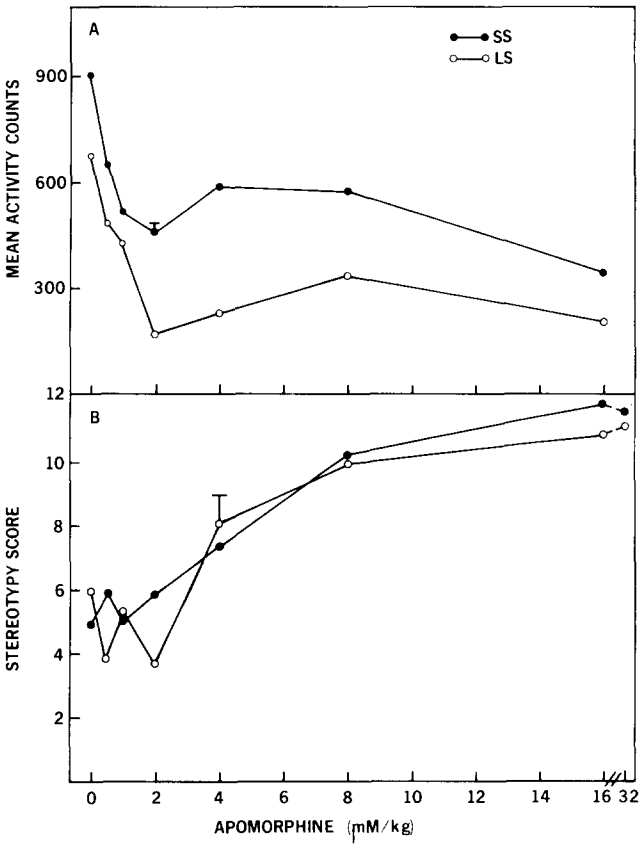

FIG. 1. Panel A: Dose response curves for apomorphine effects on locomotor activity counts for the 15 min test session. Each data point represents 6 or 7 mice of each sex (12–14 total). The vertical bar represents a generalized standard error of the mean derived from the analysis of variance error term. Note that 16 μM/kg is equivalent to 5 mg/kg of apomorphine-HCl. Panel B: Dose response curves for apomorphine-induced stereotypy. The maximum possible score was 14. Each data point represents 7 or 8 mice.

RESULTS

Experiment 1

The dose response curve for effects of apomorphine on locomotor activity (Fig. 1, Panel A) was the complex triphasic curve previously reported in the literature [8,18]. Since no sex differences were apparent, both the figures and analyses are for the sexes combined. The drug decreased activity in both lines, $F(6,141)=19.46$, $p<0.001$; the interaction of dose and genotype was non-significant; SS mice were generally more active at all doses including the saline control, $F(1,141)=50.99$, $p<0.001$. The triphasic shape of the dose response curve, an initial depression followed by relative activation, and then decreases at high doses due to stereotypy is statistically verifiable by significance of the cubic component of trend analysis on the dose variable in both lines, $F(1,141)=29.94$ and 16.47 for LS and SS respec-

tively; $p<0.001$. This complex nature of the dose response curve is consistent with reports in the literature which attribute the depression of locomotor activity in rodents at low doses to preferential apomorphine binding to presynaptic DA receptors, and the subsequent activation at higher doses to postsynaptic receptor binding. The present findings are consistent with the report that spiroperidol binding is similar in the LS and SS mice [7].

The dose response curve for apomorphine-induced stereotypy was also similar for the two lines (Fig. 1, Panel B). The doses of apomorphine could be divided into two classes on the basis of the post-hoc comparisons (Duncan's test $\alpha=0.05$). The first class was made up of the three lower doses that did not differ among themselves or from vehicle controls. The second class was made up of the three highest doses which were not different from each other, but were different from the three lower doses. The intermediate 4 μM/kg dose was not significantly different from the first or second class. The fact that only higher doses produced significant stereotypy permits conclusion that the locomotor depression induced by low doses is not due to competing stereotyped behaviors, but the secondary depression at 16 μM/kg probably is. Taken together, these two studies indicate that LS and SS mice respond similarly to apomorphine; the study of interactions of ethanol, apomorphine, and genotype in the next experiment could proceed without the complications of initial sensitivity differences to apomorphine in addition to the marked differences in sensitivity to ETOH.

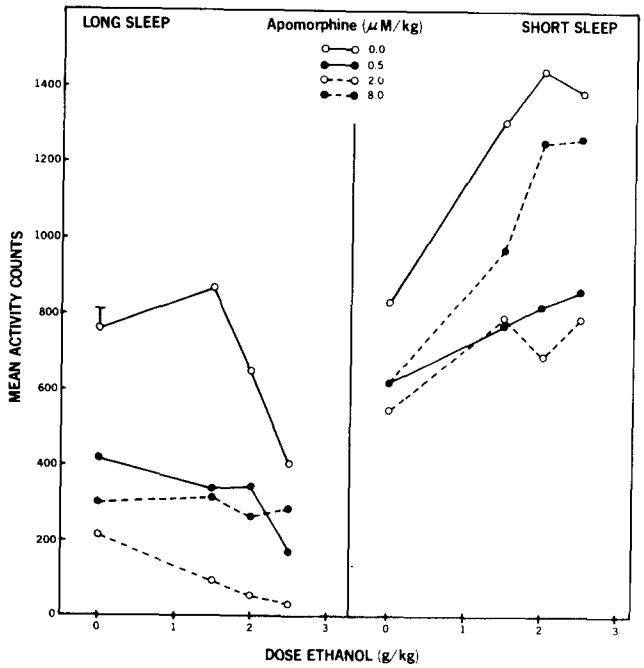

FIG. 2. Mean locomotor activity counts of mice treated with various combinations of doses of ETOH and apomorphine and tested for 15 min. Each point represents 7–10 mice of each sex (14–18 total). The vertical bar represents the generalized standard error of the mean derived from the error term of the analysis of variance.

Experiment 2

Female mice were significantly more active than males across all treatment conditions, but since sex did not interact with apomorphine or ETOH dose in the analysis of variance, both the figure (Fig. 2) and this discussion refer to data of the sexes combined. Mice not treated with apomorphine showed the usual genotype difference as a function of ETOH treatment. SS mice were activated at all ETOH doses (ETOH-linear component: $F(1,452)=65.37$, $p<0.001$). LS mice showed some activation at 1.5 g/kg but depression at the higher doses (ETOH-quadratic component: $F(1,452)=21.98$, $p<0.001$). LS and SS mice receiving neither drug were not significantly different (Duncan's test).

Apomorphine treatment markedly changed the dose response curves in both lines, but the specific changes depended on genotype. All doses of apomorphine antagonized the small activation to 1.5 g/kg ETOH in LS mice. The two higher apomorphine doses produced a flattened ETOH curve in LS mice, indicating a partial antagonism of the depressant effects of larger ETOH doses. In SS mice both 0.5 and 2.0 μM/kg of apomorphine antagonized the stimulant effects of ETOH, but did not eliminate it as was the case for LS mice. The linear component of ETOH dose effects was significant for SS mice at both these apomorphine doses, $F(1,452)=21.49$ and 8.89 for 0.5 and 2.0 μM/kg respectively, $p<0.001$ and 0.01. The slopes of these curves were clearly less than for SS mice untreated with apomorphine or mice treated with the higher dose, 8 μM/kg. Interestingly, no apomorphine dose was able to produce much antagonism of the stimulation at 1.5 g/kg ETOH in SS mice. The 8 μM/kg apomorphine dose which did not alter the shape of the ETOH curve in SS mice was the dose with the most dramatic effects in LS mice, eliminating both stimulant and depressant effects. ETOH by apomorphine interactions were significant in both lines, $F(9,452)=2.51$ and 4.09, $p<0.025$, for LS and SS mice respectively. The genotype-dependent nature of the apomorphine-ETOH interactions described above is reflected in the significance of the three-way interaction of genotype, ETOH dose and apomorphine dose, $F(9,452)=3.96$, $p<0.001$.

Since the largest dose of apomorphine appeared to antagonize the depressant effects of ETOH in LS mice, we attempted to directly assess this possibility by examination of apomorphine effects on ETOH-induced narcosis, the original selection phenotype. While apomorphine antagonism of ETOH-induced impairment of coordination has been reported [2], we thought it possible that in the locomotor activity study reported here, the apparent antagonism of depression could be due to a "floor" effect since LS mice treated with both drugs were generally very inactive. LS and SS mice untreated with apomorphine lost the righting reflex for 33.14 ± 6.51 and 36.73 ± 4.69 min respectively, indicating that the dose adjustment procedure was successful. LS mice treated with apomorphine lost the righting reflex for 73.22 ± 6.74 (Mean±S.E.M.) and 90.12 ± 9.52 min at 2 and 8 μM/kg respectively. This potentiation of ETOH-induced narcosis was not seen for SS mice where the apomorphine pretreated animals lost the righting reflex for 36.16 ± 3.84 and 37.32 ± 4.80 min at 2 and 8 μM/kg. This qualitatively different effect of apomorphine in the two lines was reflected in a genotype by apomorphine dose interaction, $F(2,52)=10.64$, $p<0.001$. Therefore, it seems unlikely that the antagonism of ETOH-induced depression of locomotor ambulation seen above actually reflects an antagonism of intoxication.

DISCUSSION

The fact that no differential response of the LS and SS mice occurred when apomorphine was administered alone is somewhat surprising given earlier reports of differences in response to other agents with DA actions, including the DA receptor antagonist haloperidol [9]. These data are consistent with the report of no LS/SS differences in DA receptors as measured by spiroperidol binding [7]; this occurs even though DA stimulated increases in adenyl cyclase are greater in LS mice [7].

In the study of ETOH effects on activity in Experiment 2, it is possible to interpret the change in the ETOH dose response curve for SS mice treated with the two lower doses of apomorphine as a simple shift of the curve to the right. This type of interpretation was not possible in earlier literature of apomorphine effects on ETOH-induced stimulation [4,22] since ETOH dose response curves were not examined. Our data from the Short-Sleep mice, which are a good model for the stimulant effects of ETOH, clearly indicate that complete antagonism of ETOH-induced stimulation by apomorphine does not occur. The facts that no dose of apomorphine completely antagonized the stimulant effects in SS mice and the highest dose of apomorphine (which should have both pre- and postsynaptic receptor activity) had no effect imply that neural systems other than DA are involved in the stimulation. Recent work suggests that cholinergic systems may be important for genotype-dependent ETOH effects [10]. Since cholinergic function may differ between the lines [15], an hypothesis of joint cholinergic/DA involvement is reasonable.

While definitive evidence of presynaptic DA receptor involvement in ETOH-induced stimulation awaits biochemical study, the present study supports this conclusion as drawn from previous work [4, 11, 22]. As is often seen in pharmacology work though, the nature and magnitude of drug effects depended on a genetic variable [14,16]. The genetic tool provided by the LS and SS mice has repeatedly pointed to DA systems involvement in the effects of hypnotic doses of ETOH [3, 6, 7, 9, 12]. The potentiation of ETOH-induced narcosis by apomorphine in LS mice but not in SS mice as reported in Experiment 2 reemphasizes this point. The data from apomorphine effects on locomotor response to ETOH suggest a similar conclusion about effects of sub-hypnotic doses of ETOH.

ACKNOWLEDGEMENTS

We thank Susan Hrvatin for assistance in data collection. Generous provision of breeding stock of LS and SS mice by G. E. McClearn is appreciated. Supported in part by a grant from the SUNY Research Foundation.

REFERENCES

1. Ahlenius, S., A. Carlsson, T. H. Svensson and P. Sodersten. Antagonism by alpha-methyltyrosine of the ethanol-induced stimulation and euphoria in man. *Clin Pharmacol Ther* **14:** 586–591, 1973.
2. Bacopoulos, N. G., I. Bize, J. Levine and L. S. van Orden, III. Modification of ethanol intoxication by dopamine agonists and antagonists. *Psychopharmacology (Berlin)* **60:** 195–201, 1979.
3. Baizer, L., J. M. Masserano and N. Weiner. Ethanol-induced changes in tyrosine hydroxylase activity in mouse brains of mice selectively bred for differences in sensitivity to ethanol. *Pharmacol Biochem Behav* **15:** 945–949, 1981.
4. Carlsson, A., J. Engel, U. Strombom, T. Svensson and B. Waldeck. Suppression by dopamine agonists of the ethanol induced stimulation of locomotor activity and brain dopamine synthesis. *Naunyn Schmiedebergs Arch Pharmacol* **283:** 117–128, 1974.
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. Collins, A. C., M. E. Lebsack and T. N. Yeager. Mechanisms that underlie sex-linked and genotypically determined differences in the depressant actions of alcohol. *Ann NY Acad Sci* **273:** 303–316, 1976.
7. Dibner, M. D., N. R. Zahniser, B. B. Wolfe, R. A. Rabin and P. B. Molinoff. Brain neurotransmitter receptor systems in mice genetically selected for differences in sensitivity to ethanol. *Pharmacol Biochem Behav* **12:** 509–513, 1980.
8. DiChiara, G., G. V. Corsini, G. P. Mereu, A. Tissari and G. L. Gessa. Self-inhibitory dopamine receptors: Their role in the biochemical and behavioural effects of low doses of apomorphine. In: *Dopamine*, edited by L. L. Iverson. *Advances in Biochemical Psychopharmacology*, vol 19. New York: Raven Press, 1978, pp. 275–292.
9. Dudek, B. C. and R. J. Fanelli. Effects of gamma-butyrolactone, amphetamine and haloperidol in mice differing in sensitivity to alcohol. *Psychopharmacology (Berlin)* **68:** 89–97, 1980.
10. Durkin, T. P., H. Hashem-Zadeh, P. Mandel and A. Ebel. A comparative study of the acute effects of ethanol on the cholinergic system in hippocampus and striatum of inbred mouse strains. *J Pharmacol Exp Ther* **220:** 203–208, 1982.
11. Engel, J., U. Strombom, T. Svensson and B. Waldeck. Suppression by α-methyltyrosine of ethanol-induced locomotor stimulation: Partial reversal by *l*-dopa. *Psychopharmacologia* **37:** 275–279, 1974.
12. Greer, C. and H. Alpern. Mediation of myoclonic seizures by dopamine and clonic seizures by acetylcholine and GABA. *Life Sci* **21:** 385–392, 1977.
13. Heston, W. D. W., V. G. Erwin, S. M. Anderson and M. Robbins. A comparison of the effects of alcohol on mice selectively bred for differences in ethanol sleep-time. *Life Sci* **14:** 365–370, 1976.
14. Horowitz, G. P. and B. C. Dudek. Behavioral pharmacogenetics. In: *Behavior Genetics: Principles and Applications*, edited by J. L. Fuller and E. Simmel. Hillsdale, NJ: L. Erlbaum, 1983, pp. 117–154.
15. Masserano, J. M. and N. Weiner. Investigations into the neurochemical mechanisms mediating differences in ethanol sensitivity in two lines of mice. *J Pharmacol Exp Ther* **221:** 404–409, 1982.
16. McClearn, G. E. and S. M. Anderson. Genetics and ethanol tolerance. *Drug Alcohol Depend* **4:** 61–76, 1979.
17. 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*, edited by G. E. McClearn, R. A. Deitrich and V. G. Erwin. Washington, DC: NIAAA Research Monograph No. 6, U.S. Govt. Printing Office, 1982, pp. 147–159.
18. Nagy, J. I., T. Lee, P. Seeman and H. C. Fibiger. Direct evidence for presynaptic and postsynaptic dopamine receptors in brain. *Nature* **274:** 278–281, 1978.
19. Parker, E. S., J. M. Moribisa, R. J. Wyatt, B. L. Schwartz, H. Weingartner and R. C. Stillman. The alcohol facilitation effect on memory: A dose-response study. *Psychopharmacology (Berlin)* **74:** 88–92, 1981.
20. Pohorecky, L. A. Biphasic action of ethanol. *Biobehav Rev* **1:** 231–240, 1977.
21. Sanders, B. Sensitivity to low doses of ethanol and pentobarbital in mice selected for sensitivity to ethanol. *J Comp Physiol Psychol* **90:** 394–398, 1976.
22. Strombom, U., T. Svensson and A. Carlsson. Antagonism of ethanol's central stimulation in mice by small doses of catecholamine-receptor agonists. *Psychopharmacology (Berlin)* **51:** 293–299, 1977.