Behavior Genetics, Vol. 12, No. 6, 1982

Dopamine-β-Hydroxylase Activity and Ethanol-Induced Sleep Time in Selectively Bred and Heterogeneous Stock Mice

Gary P. Horowitz,[1,5] Peter S. Dendel,[2,3] Andrea M. Allan,[1] and Leslie F. Major[2,4]

Received 9 Oct. 1981—Final 4 May 1982

Dopamine-β-hydroxylase (DBH), the enzyme responsible for the conversion of dopamine to norepinephrine, was measured in the plasma of two lines of mice selectively bred for differential sensitivity to hypnotic doses of ethanol. Plasma levels of DBH were measured by radioenzymatic assay in long sleep (LS) and short sleep (SS) male and female mice which had no prior exposure to ethanol. LS mice had significantly higher plasma DBH activity than did SS mice, although no significant sex difference was found. Fusaric acid, an inhibitor of both central and peripheral DBH, attenuated sleep time induced by either 3 or 4 g/kg ethanol in mice of a heterogeneous genetic stock. The results of these experiments may further implicate noradrenergic systems in mediating some of the depressed effects of ethanol.

KEY WORDS: mice; dopamine-β-hydroxylase; selective breeding; ethanol sensitivity; fusaric acid.

This work was supported by UAC Award 240-7395A from the Research Foundation of the State University of New York to the first author and by BRSG Grant S07RR07149-08 awarded by the Biomedical Research Support Grant Program, Division of Research Resources, National Institute of Health.

[1] Department of Psychology and Center for Neurobehavioral Sciences, State University of New York at Binghamton, Binghamton, New York 13901.
[2] Clinical Campus, University Center at Binghamton/Upstate Medical Center, Binghamton, New York 13901.
[3] Present address: University of Michigan Medical School, Ann Arbor, Michigan 48109.
[4] To whom all correspondence should be addressed.
[5] Present address: McNeil Pharmaceuticals, Spring House, Pennsylvania 19477.

0001-8244/82/1100-0549$03.00/0

INTRODUCTION

Using a selective breeding procedure, McClearn and Kakihana (1973) have produced two lines of mice which differ markedly in response to hypnotic doses of ethyl alcohol. The selective breeding methodologies for these two lines, designated long sleep (LS) and short sleep (SS), have been presented elsewhere (McClearn and Kakihana, 1973). Several studies have attempted to elucidate biochemical and neurochemical differences between these two lines which might underlie the differential response to ethanol treatment (e.g., Collins *et al.*, 1976; Collins and Deitrich, 1973; Heston *et al.*, 1974).

Given that previous research had demonstrated a significant negative correlation betweeen sleep time and alcohol dehydrogenase activity following alcohol challenge among mice of the heterogeneous founding population (Belknap *et al.*, 1972), the initial studies focused on factors which would affect alcohol metabolism. Heston *et al.* (1974) found no differences in the activity of either liver alcohol dehydrogenase or liver aldehyde dehydrogenase. In addition, blood alcohol levels appear to be similar in the LS and SS lines, making it unlikely that differences in absorption and distribution of alcohol account for the large differences in sleep time.

In another line of investigation, brain neurotransmitter systems were examined in the two lines. Whole-brain levels of dopamine and norepinephrine appear to be lower in LS mice than SS mice (Collins and Deitrich, 1973; Collins *et al.*, 1976). In addition, LS mice have been shown to have a lower cortical density of β-adrenergic receptors than SS mice (Dibner *et al.*, 1980).

Although the physiological consequences of bidirectional selective breeding for the phenotype of ethanol-induced narcosis among LS and SS mice are not fully understood, the results from nongenetic studies of neurotransmitter systems suggest a possible involvement of the noradrenergic system in this hypnotic response to ethanol. For example, Hayashida and Smith (1971) reported that sotalol, a β-adrenergic antagonist, significantly attenuated the duration of sleep time induced by 4 g/kg ethanol in Swiss–Webster mice. Additionally, these researchers (Smith *et al.*, 1970) have reported that low doses of propranolol, another β-adrenergic blocking agent and norepinephrine releaser, reduced ethanol sleep time in mice of the same line. Further support for the hypothesis of noradrenergic mediation of some of the depressant effects of ethanol in mice can be gleaned from a study by Matchett and Erickson (1977), who found that propranolol attenuated the initial hypoactivity produced by 2 g/kg ethanol. Interestingly, propranolol was without effect on the later hyperactivity produced by this subhypnotic dose of ethanol. How-

ever, the α-adrenergic blocking agent, phentolamine, antagonized the hyperactivity phase but was without effect on the earlier phase of hypoactivity. These investigators concluded that the depressant effect of alcohol on locomotor activity may be mediated by central β-adrenergic receptors, while the stimulant effect of ethanol may be mediated by central α-adrenergic receptors. In light of this hypothesis, it is interesting to note that, while LS mice are clearly more sensitive to the effects of hypnotic doses of ethanol, SS mice have been reported to show a greater increase in locomotor activity following lower doses of ethanol (Sanders, 1976).

If noradrenergic neurotransmitter systems are involved in the depressant effects of ethanol in general, or in the phenotypic difference between LS and SS mice in ethanol-induced narcosis in particular, then alterations in the enzymes responsible for norepinephrine metabolism might be evident as correlated responses to selection. However, differences between LS and SS mice were not found by Collins *et al.* (1976) in certain enzymes of catecholamine synthesis (tyrosine hydroxylase, dopa decarboxylase) or degradation (monoamine oxidase). These researchers were unable to measure dopamine-β-hydroxylase (DBH) from brain tissue, presumably because of high levels of endogenous inhibitors (Nagatsu *et al.*, 1967). DBH [3,4-dihydroxyphenylethylamine ascorbate: *O*-oxidoreductase (hydroxylating; EC 1.14.2.1)] is the enzyme responsible for the conversion of dopamine to norepinephrine. This enzyme is localized in noradrenergic storage vesicles, where it exists in both soluble and membrane-bound forms (Viveros *et al.*, 1968; Smith and Winkler, 1972). During exocytosis, a portion of the soluble fraction of DBH may be released into the extracellular fluid. Studies involving *in vitro* preparations of noradrenergic tissue show that norepinephrine and DBH are released proportionally in response to depolarizing agents (Geffen *et al.*, 1969; Viveros *et al.*, 1968; Weinshilboum *et al.*, 1971).

Numerous DBH inhibitors have been shown to reduce norepinephrine levels significantly, both centrally and peripherally, thereby altering noradrenergic activity. Hidaka *et al.* (1974) have shown that DBH inhibitors which do not cross the blood–brain barrier, such as 5-dimethyldithiocarbamylpicolinic acid (YP-279), have no effect on ethanol-induced sleeping time, while inhibitors such as 5-butylpicolinic acid (fusaric acid), which does pass the blood–brain barrier, significantly prolongs ethanol sleep time in mice.

Two experiments were conducted to elucidate further the possible role of noradrenergic systems in mediating the loss of righting reflex induced by hypnotic doses of ethanol. In the first experiment, plasma DBH activity was measured in LS and SS mice in the absence of alcohol. In the second experiment, the effect of fusaric acid, a DBH inhibitor, on

ethanol-induced narcosis was examined in mice of heterogeneous genetic origin.

EXPERIMENT 1

In the first study, plasma DBH activity was examined in LS and SS mice as a further investigation of potential differences in the noradrenergic nervous system between LS and SS mice. Brain DBH activity was not measured because, in previous attempts, we were unable to measure mouse brain DBH activity using a double radioenzymatic assay. This finding is in agreement with a previous report by Collins *et al.* (1976), who were also not able to measure brain tissue DBH levels. We found 0% recovery of exogenously added purified DBH in all brain homogenate samples, which would support the hypothesis that high levels of endogenous inhibitors are responsible for the apparent lack of DBH activity in mouse brain.

Materials and Methods

The subjects were descendents of the mice selectively bred by McClearn and Kakihana (1973) for differential sensitivity to hypnotic doses of ethanol. Mating pairs were obtained from the Institute for Behavioral Genetics (University of Colorado, Boulder) at generation 18 and have been maintained at the present laboratory with relaxed selection within each line. A minimum of 15 mating pairs has been maintained for each line, with the restriction that the progeny of each pair did not share common grandparents. In the present study, a total of 13 SS (females, $N = 6$; males, $N = 7$) and 14 LS (females, $N = 6$; males, $N = 8$) mice was tested. The animals were weaned at 21 days and housed as like-sexed littermates until the beginning of the experiment. During rearing, the animals were exposed to a 12-h light/12-h dark photoperiod, with the light period beginning at 0730 each morning, with food and water being available ad libitum. The ages of the mice at the time of testing were approximately equal within each line (mean ± SE—114.9 ± 8.5 and 114.2 ± 8.3 days for LS and SS mice, respectively).

Mice were sacrificed by decapitation and blood was collected in a 30-ml beaker containing 100 IU sodium heparin. Samples were kept on ice until centrifuged at 1500 *g* for 10 min at 4°C. Plasma samples were then stored at −40°C until assayed.

DBH was assayed by a modification of a double radioenzymatic method (Molinoff *et al.*, 1971). Each sample was analyzed in duplicate, using a 15-μl aliquot of plasma added to a tube containing the following:

sodium acetate buffer (2.5×10^{-5} mol), ascorbic acid (3×10^{-6} mol), pargyline hydrochloride (5×10^{-7} mol), *N*-ethylmaleimide (2.5×10^{-6} mol), sodium fumarate (5×10^{-6} mol), catalase (2250 U; Calbiochem, La Jolla, Calif.), tyramine hydrochloride (3×10^{-6} mol), and H_2O to bring the final volume to 500 μl. Tubes were incubated for 60 min at 37°C. Following this incubation, 205 μl of a mixture containing potassium phosphate buffer (1.5×10^{-4} mol), cysteine (5×10^{-6} mol), ^{14}C-*S*-adenosylmethionine (sp act, 50–60 Ci/mmol; 3.4×10^{-6} mol; ICN Radiochemicals, Irvine, Calif.), and 5 μl of a phenylethanolamine-*N*-methyltransferase (PNMT) solution (purified from bovine adrenyl glands) was added to all tubes before a second incubation at 37°C for 40 min. All reagents were purchased from the Sigma Chemical Co., St. Louis, Mo., unless otherwise noted.

Octopamine formed in the first reaction was converted to ^{14}C-synephrine in the second (PNMT) step. The second reaction was terminated by adding 0.5 ml of 0.5 *M* sodium borate buffer, pH 10, to all tubes and removing them from the water bath. ^{14}C-Synephrine was extracted away from radioactive precursors using a toluene isoamyl alcohol solvent system. Final activity of DBH was quantified using octopamine standards. Liquid scintillation spectrometry utilized a Packard Tri-Carb counter (Packard Instruments, Downers Grove, Ill.).

Recovery of DBH was measured by adding known amounts of purified DBH (Sigma) to plasma samples and testing assay recovery of exogenously added enzyme.

Results and Discussion

Mouse plasma samples were found to have measurable levels of DBH activity which were significantly different between LS and SS mice. The enzyme activity scores were subjected to a 2 (genotype) × 2 (sex) unweighted-means analysis of variance. As seen in Fig. 1, the main effect of genotype was significant, [$F(1,23) = 32.1$, $P \leq 0.001$], with LS mice displaying greater enzyme activity than SS mice regardless of sex. A main effect of sex was not detected [$F(1,23) = 1.98$, $P \geq 0.05$], nor was there a significant genotype × sex interaction, $F \leq 1$.

Assay recovery of DBH from plasma was estimated in each line and sex. Recovery was approximately 90%, with no apparent differences between genotype or sexes.

Several individual factors may affect plasma DBH activity and must be considered in the interpretation of the differences presently reported between LS and SS mice. No difference was observed in plasma levels

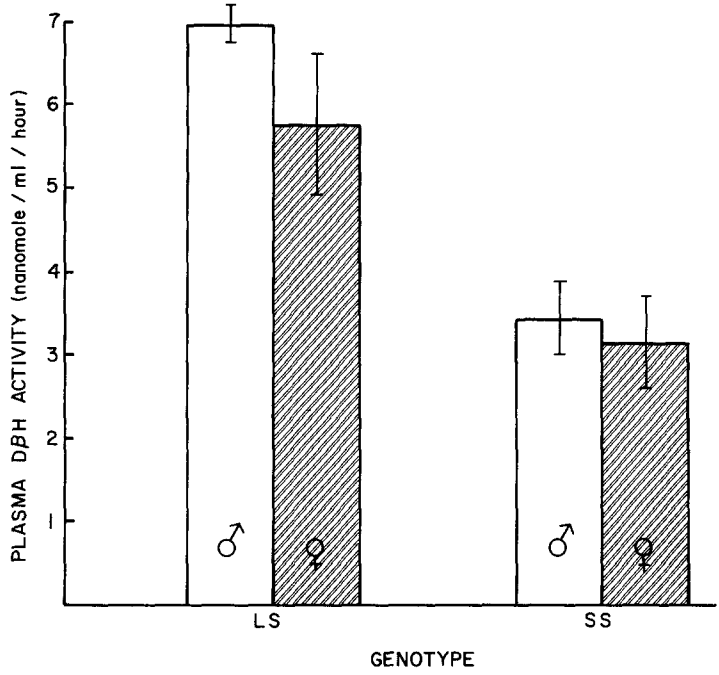

Fig. 1. Plasma dopamine-β-hydroxylase (DBH) activity, expressed as nanomoles of product per milliliter of plasma per hour, in long sleep (LS) and short sleep (SS) mice of each sex. Values shown are the means (±SE) for six to eight mice.

of endogenous inhibitors, as recovery of exogenous DBH was found to be the same in both lines. This finding rules out artifactual differences in DBH activity caused by differing inhibitor levels. However, factors other than sympathetic activity, especially metabolic clearance, may exert an overriding influence on the level of serum enzyme activity.

The previously reported studies showing higher levels of whole-brain norepinephrine in SS mice (Collins and Deitrich, 1973; Collins *et al.*, 1976) and higher levels of cortical β-adrenergic receptor density in SS animals (Dibner *et al.*, 1980) suggest that increased central noradrenergic activity is somehow responsible for the early recovery from hypnotic doses, and perhaps the greater stimulatory effects of lower doses, of ethanol in SS mice. Our present finding of lowered plasma DBH activity in SS mice would be consistent with these previous findings if, in fact, lowered plasma DBH activity in LS mice is a reflection of increased central noradrenergic inhibitory control of sympathetic outflow.

The hypothesis that differences in central noradrenergic systems are responsible for the seemingly paradoxical finding of greater peripheral DBH activity in LS mice can be tested by the use of relatively specific DBH inhibitors. Since the inhibition of DBH by fusaric acid has been shown to reduce central norephinephrine levels, (Hidaka *et al.*, 1974), the activity of both α- and β-noradrenergic systems should be reduced by this agent. If α-noradrenergic systems mediate the activating effects of ethanol, as suggested by Matchett and Erickson (1977), then DBH inhibition might be predicted to prolong ethanol-induced sleep time. In the second experiment, the effects of fusaric acid on ethanol-induced sleep time was assessed in mice of heterogeneous genetic origin.

EXPERIMENT 2

The results of the first experiment suggest that LS mice, which shows greater sensitivity to the loss of righting reflex induced by ethanol, also show higher activity levels of DBH. These findings suggest a relationship between the noradrenergic nervous system and the ethanol-induced sleep time. Other evidence for noradrenergic involvement comes from the reports that β-adrenergic antagonists, such as propranolol and sotalol, attenuate ethanol-induced sleep time in mice (Hayashida and Smith, 1971; Smith *et al.*, 1970) and that propranolol antagonizes the depressant effect of ethanol on locomotor activity (Matchett and Erickson, 1977). There is one report in the literature on the effects of a DBH inhibitor on ethanol-induced sleep. Hidaka *et al.* (1974) have reported that pretreatment with fusaric acid significantly prolonged ethanol-induced sleep time in mice of the DBA inbred strain. Since strain differences in response to a variety of pharmacologic agents have now been well documented, this finding may be specific to DBA mice. Thus, the second experiment was designed to evaluate the effects of DBH inhibition on ethanol-induced narcosis in mice of a heterogeneous genetic stock. It should be noted that the LS and SS lines were derived from a similar, but not identical, founding population.

Materials and Methods

A total of 44 mice of the Binghamton heterogeneous (HET) stock, 82–105 days of age, was used as subjects. These mice were derived from a systematic cross of eight inbred strains (see Fuller, 1979) and are maintained through breeding pairs which do not share common grandparents.

The subjects represented the progeny of 16 different mating pairs. Following weaning at postnatal day 21, they were housed as like-sexed littermates in a temperature (22 ± 1°C)- and photoperiod (12:12-h light:dark cycle; lights on at 0800 h)- controlled vivarium. Each subject was individually housed on the day prior to its first use in the experiment. Food and water were available continuously in the home cage throughout rearing and the experiment.

On the first morning of the experiment, all subjects were weighed and randomly assigned to be injected ip (0.02 ml/g body wt) with either 3 ($N = 24$) or 4 ($N = 20$) g/kg ethanol (from 15 or 20%, w/v, respectively). When each mouse lost the ability to maintain an upright position, it was placed on its side in a stainless-steel V-shaped trough, and the time was recorded. If the mouse turned to the prone position, it was placed on its back. An animal was judged to have regained the righting reflex if it could return to an upright position twice within 30 s. The duration of the loss of righting reflex was thus calculated as the difference in time between losing and regaining the ability to maintain an upright position.

Following this initial evaluation of ethanol sensitivity, all subjects were returned to their home cages in the vivarium at 6 days. For each dose of ethanol, two groups of equal numbers of animals were formed on the basis of prior sensitivity to ethanol-induced narcosis (see Table I). One week after the initial exposure to ethanol, mice were weighed and injected with either 25 mg/kg of fusaric acid (Sigma) or an equal volume of physiological saline, the vehicle for fusaric acid. Thirty minutes after this pretreatment, each subject received an injection of ethanol equal to the dose administered to it on the first day of the experiment. The duration of the loss of righting reflex was determined as above.

Results and Discussion

The duration of the loss of righting reflex, or the sleep time, served as the dependent measure of alcohol responsiveness. Sleep times were subjected to a 2 (treatments) × 2 (doses of alcohol) × 2 (trials) unweighted-means repeated-measure analysis of variance. The results of the experiment are summarized in Table I.

Analysis of the variance in sleep times revealed a main effect of dose [$F(1,40) = 124.01, P \leq 0.01$]. As seen in Table I, the sleep time was greater following the higher dose of ethanol, regardless of treatment with fusaric acid or saline. Similarly, there was a main effect of treatment [$F(1,40) = 4.96, P \leq 0.05$]. Since the two treatment groups were matched prior to treatment, this difference reflects the lower sleep time of animals re-

Table I. The Effects of Fusaric Acid or Saline on Ethanol-Induced Sleep Time in HET Mice

Dose of ethanol (g/kg)	Sleep time (min)			
	Saline		Fusaric acid	
	Pre[a]	Post	Pre[a]	Post
3				
$\bar{X}$	35.12	34.65	32.37	18.54
SE	5.24	2.94	4.81	2.43
4				
$\bar{X}$	95.47	77.79	94.82	56.48
SE	7.96	5.79	9.22	3.82

[a] Ethanol-induced sleep time 1 week prior to treatment with saline or fusaric acid (see text).

ceiving fusaric acid on the second trial. A main effect of trials [$F(1,40) = 33.71$, $P \leq 0.01$], is also due mainly to the lower sleep times of fusaric acid-treated animals on the second test. However, control mice receiving 4 g/kg of ethanol and given saline prior to the second trial also exhibited lower sleep times on the latter trial, contributing to a significant dose × trial interaction [$F(1,40) = 11.88$, $P \leq 0.01$]. The reason for this decrease is not apparent, but it was not as large as the decrease observed in mice receiving this dose of ethanol following fusaric acid pretreatment (see Table I). The treatement × trial interaction was also significant [$F(1,40) = 7.89$, $P \leq 0.01$]. Given the matching procedure based on the results of the first trial, this finding is again indicative that fusaric acid attenuated the sleep time induced by both doses of ethanol. Finally neither the treatment × dose nor the treatment × dose × trials interaction approached statistical significance ($F < 1$ for each case).

These results are not in agreement with the previous report by Hidaka *et al.* (1974) that fusaric acid lengthened ethanol-induced sleep time in DBA mice. It may be that procedural differences account for these discrepancies between the two studies. Hidaka *et al.* (1974) used DBA mice, an inbred strain, while we used mice of heterogeneous genetic origin. Perhaps DBA mice show a unique response to this combination of drug treatments. The DBA genotype is represented in the inbred strains from which our mice were derived. In fact, of the 22 mice tested in the present study showed a slight increase in ethanol-induced sleep time in the absence of pretreatment. However, the most extreme of these three mice showed only a 10-min increase, while the mean decrease in all mice

(including these three mice) receiving this dose of ethanol following fusaric acid was about 14 min.

A second procedural difference between the two studies is related to drug dosage and vehicle. While in both studies, 25 mg/kg fusaric acid was injected 30 min prior to ethanol administration, the doses of ethanol were different. Hidaka *et al.* (1974) gave subjects 4.8 g/kg ethanol. Since we were interested in the possible role of DBH as a response to selection, we injected doses of ethanol that are closer to those employed in the selection procedure. It is possible that the fusaric acid–ethanol combination has a biphasic dose–response curve. Additionally, the vehicle for fusaric acid in the previous study was carboxymethylcellulose (CMC), while we dissolved fusaric acid in physiologic saline. Hidaka *et al.* (1974) reported that the mean sleep time of DBA mice pretreated with CMC alone, followed by 4.8 g/kg ethanol, was approximately 82 min. Belknap *et al.* (1972) have previously reported that DBA mice slept for an average of 91 min following 3.55 g/kg ethanol, and Damjanovich and MacInnes (1973) similarly reported a sleep time of about 130 min for these mice following 4.1 g/kg ethanol. It is possible that CMC alone decreases sleep time but is antagonistic in its interaction with fusaric acid.

GENERAL DISCUSSION

The two experiments reported in this paper add further evidence to the role of the noradrenergic nervous system in the mediation of ethanol-induced loss of the righting reflex in mice. However, the exact nature of this involvement remains to be elucidated. We have mentioned a number of previous studies which have similarly demonstrated either differences in the noradrenergic nervous system between LS and SS mice or the ability to alter sleep time by manipulating the noradrenergic nervous system in various ways in other populations of mice.

These previous studies, as well as the results of the first experiment reported herein, led to the hypothesis that central noradrenergic systems might be inhibitory on peripheral sympathetic activation. Specifically, the greater plasma DBH activity of LS mice observed in Experiment 1 might reflect a lower central noradrenergic activity in mice of this line. A similar mechanism has been proposed to explain the finding that the central administration of norepinephrine or propranolol produces reductions in blood pressure in rats (Tackett *et al.*, 1981). This hypothesis would predict that DBH inhibition should potentiate ethanol-induced sleep time. However, the results of our second experiment are not consistent with this prediction; pretreatment with fusaric acid attenuated

sleep time in mice of heterogeneous genetic stock following 3- or 4-g/kg ethanol doses.

The collective results of the present experiments do not support the hypothesis that the great peripheral DBH activity in LS mice contributes to the greater sensitivity to the hypnotic effects of ethanol via the specific mechanism of deficient central noradrenergic inhibitory control of peripheral sympathetic activity. However, it should be noted that the results of the two present experiments are internally consistent. That is, if the increased basal plasma DBH activity of LS mice is correlated with the longer sleep time following hypnotic doses of ethanol observed in these mice, then inhibition of DBH should attenuate ethanol-induced sleep time in mice of a heterogeneous genetic stock. This prediction was realized, as indicated by the results of the second experiment.

Although many DBH inhibitors also inhibit other sulfhydryl-containing enzymes, fusaric acid has been shown to have no effect on other enzymes of monoamine metabolism, including aldehyde dehydrogenase, at concentrations of up to 10^{-4} *M* (Nagatsu *et al.*, 1970). Thus, it is unlikely that the effects of fusaric acid observed in our study are due to indirect effects on ethanol metabolism. Furthermore, inhibition of ALDH should prolong, not attenuate, ethanol-induced sleep time. Nonetheless, further experiments using specific α- and β-noradrenergic agonists and antagonists should prove useful in clarifying the role of noradrenergic systems as possible contributors to the observed phenotypic differences between LS and SS mice in the selection criterion of ethanol-induced sleep time. These experiments are currently being conducted in our laboratory.

It is also possible, of course, that any observed phenotype correlation between DBH activity and the previous selection index of ethanol-induced sleep time is fortuitous and the result of either a founder effect in establishing our colony or a stochastic association of separate genetic systems. It would be interesting to note whether the presently observed differences in plasma DBH activity are also found among the LS and SS mice maintained at the University of Colorado.

The use of selective breeding techniques in the area of pharmacogenetics, as exemplified by the Colorado sleep lines, is valuable in at least two respects (Crabbe and Belknap, 1980). First, the successful response to selection demonstrates a heritable component to the trait under investigation. Second, the products of such breedng studies are a valuable source of hypotheses of the relationship between the selected phenotype and other traits of interest. The present experiments failed to confirm one such hypothesis but generated further hypotheses regarding the role of

noradrenergic neurotransmitters systems in the differential response to ethanol by LS and SS mice. It is hoped that these results might be integrated with other research to contribute to our overall knowledge of the neurochemical mechanisms of the depressant effects of ethanol.

REFERENCES

Belknap, J. K., MacInnes, J. W., and McClearn, G. E. (1972). Ethanol sleep times and hepatic alcohol and aldehyde dehydrogenase activities in mice. *Physiol. Behav.* **9**:453–457.

Collins, A. C., and Deitrich, R. A. (1973). Alterations in catecholamine turnover by ethanol in lines of mice which differ in ethanol sleep time. Paper presented at meeting of Behavior Genetics Association, Chapel Hill, N.C.

Collins, A. C., Lebsack, M. E., and Yeager, T. N. (1976). Mechanisms that underlie sex-linked and genotypically determined differences in the depressant actions of alcohol. *Ann. N.Y. Acad. Sci.* **273**:303–316.

Crabbe, J. C., and Belknap, J. K. (1980). Pharmacogenetic tools in the study of drug tolerance and dependence. *Subst. Alc. Act./Misuse* **1**:385–413.

Damjanovich, R. P., and MacInnes, J. W. (1973). Factors involved in ethanol narcosis: Analysis in mice of three inbred strains. *Life Sci.* **13**:55–65.

Dibner, M. D., Zahniser, N. R., Wolfe, B. B., Rabin, R. A., and Molinoff, P. B. (1980). Brain neurotransmitter receptor systems in mice genetically selected for differences in sensitivity to ethanol. *Pharmacol. Biochem. Behav.* **12**:509–513.

Fuller, J. L. (1979). Fuller BWS lines: History and results. In Hahn, M. E., Jensen, C., and Dudek, B. C., (eds.), *Development and Evolution of Brain Size: Behavioral Implications*, Academic Press, New York, pp. 187–204.

Geffen, L. B., Livett, B. G., and Rush, R. A. (1969). Immunological localization of chromogranins in sheep sympathetic neurons and their release by nerve impulses. *J. Physiol.* **204**:58–59.

Hayashida, K., and Smith, A. (1971). Reversal of sotalol of the respiratory depression induced in mice by ethanol. *J. Pharm. Pharmacol.* **23**:718–719.

Heston, W. D. W., Erwin, V. G., Anderson, S. M., and Robbins, H. (1974). A comparison of the effects of alcohol on mice selectively bred for differences in ethanol sleep-time. *Life Sci.* **14**:365–370.

Hidaka, H., Fumiko, H., Harada, N., Hashizyme, Y., and Yano, M. (1974). Selective inhibition of dopamine-β-hydroxylase in the peripheral tissues by 5-dimethyldithiocarbamylpicolinic acid; Its effect on stress-induced ulcer, ethanol-induced sleep and blood pressure. *J. Pharmacol. Exp. Ther.* **191**:384–392.

Matchett, J. A., and Erickson, C. K., (1977). Alteration of ethanol-induced changes in locomotor activity by adrenergic blockers in mice. *Psychopharmacology* **52**:201–206.

McClearn, G. E., and Kakihana, R. (1973). Selective breeding for ethanol sensitivity in mice. *Behav. Genet.* **3**:409–410.

Molinoff, P. B., Weinshilboum, R., and Axelrod, J. (1971). A sensitive enzymatic assay for dopamine-β-hydroxylase. *J. Pharmacol. Exp. Ther.* **178**:425–431.

Nagatsu, T., Kuzuya, H., and Hidaka, H. (1967). Inhibition of dopamine-β-hydroxylase by sulfhydryl compounds and the nature of the natural inhibitors. *Biochim. Biophys. Acta* **139**:319-327.

Nagatsu, T., Hidaka, H., Kuzuya, H., Takeya, K., Umezawa, H., Takeuchi, T., and Suda, H. (1970). Inhibition of dopamine-β-hydroxylase by fusaric acid (5-butylpicolinic acid) *in vitro* and *in vivo*. *Biochem. Pharmacol.* **19**:35–44.

Sanders, B. (1976). Sensitivity to low doses of ethanol and pentobarbital in mice selected for sensitivity to hypnotic doses of ethanol. *J. Comp. Physiol. Psychol.* **90**:394–398.

Smith, A. D., and Winkler, H. (1972) Fundamental mechanisms in the release of catecholamines. In Blaschko, H., and Muscholl, E. (eds.), *Handbook of Experimental Pharmacology, Vol. 33. Catecholamines,* Springer, Berlin, pp. 538–617.

Smith, A., Hayashida, K., and Kim, Y. (1970). Inhibition by propranolol of ethanol-induced narcosis. *J. Pharm. Pharmacol.* **22**:644–645.

Tackett, R. L., Webb, J. G., and Privitera, P. J. (1981). Cerebroventricular propranolol elevates cerebrospinal fluid norepinephrine and lowers blood pressure. *Science* **213**:911–913.

Viveros, O. H., Argueros, L., and Kirshner, N. (1968). Release of catecholamines and dopamine-β-hydroxylase from the adrenal medulla. *Life Sci.* **7**:609–618.

Weinshilboum, R. M., Thoa, N. B., Johnson, D. G., Kopin, I. J., and Axelrod, J. (1971). Proportional release of norepinephrine and dopamine-β-hydroxylase from sympathetic nerves. *Science* **174**:1349–1351.

Edited by Glayde Whitney