Pharmacology Biochemistry & Behavior, Vol. 12, pp. 509–513. Printed in the U.S.A.

Brain Neurotransmitter Receptor Systems in Mice Genetically Selected for Differences in Sensitivity to Ethanol[1]

M. D. DIBNER,[2,3] N. R. ZAHNISER,[3] B. B. WOLFE, R. A. RABIN[3] AND P. B. MOLINOFF

University of Colorado Health Sciences Center, Department of Pharmacology, 4200 East Ninth Avenue, Denver, CO 80262

Received 6 August 1979

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.* PHARMAC. BIOCHEM. BEHAV. 12(4) 509–513, 1980.—The density of receptors for neurotransmitters and receptor-mediated cellular responsiveness were determined in tissues from two lines of mice selectively bred for differences in sleep time after ethanol administration. The studies were undertaken to ascertain if differences in neurotransmitter receptors between the lines might explain any of the differences seen in initial sensitivity to ethanol. The results were compared with those obtained in studies using the parent heterogeneous stock (HS). The animals used in the current experiments did not receive ethanol. β-Adrenergic receptor density, as measured by the binding of (^{125}I)-iodohydroxybenzylpindolol, was lower in cortices of alcohol-sensitive long-sleep mice (LS) as compared to the short-sleep (SS) animals or to HS controls. The densities of β_1 and β_2-adrenergic receptors were lower by approximately the same amount. The results were tissue specific in that there was no difference in the density of β-adrenergic receptors in the cerebellum. Cyclic AMP accumulation in response to isoproterenol was not significantly different in the cortex of LS mice despite the decrease in β-adrenergic receptors. No differences were found in ^{3}H-spiroperidol binding to dopamine receptors in the striatum or in ^{3}H-quinuclidinylbenzilate binding to muscarinic cholinergic receptors in the cortex, striatum or hippocampus. Dopamine-stimulated adenylate cyclase activity was, however, lower in striata of SS mice. The affinities of the receptors for the various ligands studied were the same in the three lines of mice.

Genetics Ethanol β-Adrenergic receptor Muscarinic cholinergic receptor Dopaminergic receptor Adenylate cyclase

TWO lines of mice which differ in their initial response to alcohol have been derived from a genetically heterogeneous (HS) stock [5, 11, 15, 16]. The HS line, created by intercrossing 8 inbred strains of mice (A, AKR, BALB/c, C3H/2, C57/BL, DBA/2, Is/Bi and RIII), has been subsequently maintained by a random mating procedure as previously described by McClearn *et al.* [23]. Sleep time was originally measured in HS animals following injection of 3.4 g of ethanol/kg body weight. Those animals which exhibited the shortest sleep times were mated to produce the first generation of the short sleep (SS) line. Similarly, the first generation of long sleep (LS) mice was produced by mating those animals with the longest sleep times. In subsequent generations selection pressure has been maintained so that the differences in sensitivity to ethanol have been enhanced.

In addition to an altered sleep time the SS mice have an increased ED_{50} for righting response following alcohol administration [11]. Alcohol metabolism is, however, similar in SS and LS mice [15] and sensitivity to other (non-alcohol) sedative hypnotics is similar in the two lines of mice [11].

The chronic administration of ethanol has been shown to alter the properties of several central nervous system neurotransmitter receptor/effector systems. Decreases in the density of β-adrenergic receptors have been observed following alcohol treatment in rat [1] and mouse cerebral cortex [26]. Alterations in the turnover of brain norepinephrine and in the sensitivity to norepinephrine have also been reported following ethanol administration [13, 18, 36]. Effects of ethanol on acetylcholine release have also been reported [9]. Several groups of investigators have presented data which implicates dopaminergic systems in the central actions of ethanol. Thus, the administration of l-DOPA augments ethanol induced narcosis in mice [3] and both l-DOPA and intracranially injected dopamine results in attenuation of the convulsions induced following ethanol withdrawal [2]. Carlsson *et al.* [4] have shown that dopamine receptor agonists inhibit ethanol induced stimulation of locomotor activity and dopamine synthesis. Finally, changes in

[1]Supported by the USPHS (AA 03527)
[2]Present Address: University of California, San Diego, Department of Medicine, Division of Pharmacology, Box M-013, La Jolla, CA 92093
[3]Supported by the USPHS (MD: NS 05714, NZ: NS 05970 and RR: AA 03527)

TABLE 1

β-ADRENERGIC RECEPTORS IN CORTEX AND CEREBELLUM OF HS, LS AND SS MICE

	HS	LS	SS
Cortex: (% HS)	100 ± 2.4	86 ± 2.1*	95 ± 3.8
Cerebellum: (% HS)	100 ± 3.0	94 ± 4.4	94 ± 3.6

*Differs from both HS and SS $p<0.01$.

Homogenates were incubated with 40–250 pM IHYP and data were analyzed by Scatchard analysis. Results are expressed as the mean percent of HS (Bmax ± SEM) for analyses carried out on 7–24 animals. The average mean Bmax for HS animals was approximately 90 fmol/mg protein for cortex and 21 fmol/mg protein for cerebellum.

TABLE 2

β-ADRENERGIC RECEPTOR SUBTYPES IN HS, LS AND SS MICE

	% β_1	% β_2	Bmax
HS	82.0 ± 3.50	18.0 ± 3.50	79.8 ± 7.03
LS	81.7 ± 4.37	18.3 ± 4.39	59.9 ± 5.07*
SS	79.5 ± 3.95	20.5 ± 3.95	77.1 ± 6.23

*Differs from both HS and SS $p<0.05$.

The percentage of β_1 and β_2-adrenergic receptor were calculated as described in METHOD. The percentage of receptors of either subtype did not vary between the groups. Data represent mean percentage of total ± SEM for determinations from 8–10 animals taken from two separate experiments. The total number of β receptors in cortices from these animals (Bmax) were determined by Scatchard analysis of IHYP binding. The Bmax data are expressed as fmols/mg protein.

dopamine synthesis [33] and in dopamine stimulated adenylate cyclase activity have been described [17,34].

The LS and SS lines provide a useful tool with which to study the regulation of the responsiveness to ethanol [7]. The present studies were undertaken to determine whether there are differences in various central neurotransmitter/effector systems between LS, SS and HS mice.

METHOD

Male LS, SS and HS mice were obtained from the Institute for Behavioral Genetics (University of Colorado, Boulder, CO). Animals weighing from 21–26 g (50–90 days of age) were housed in plastic cages and given water and Purina Lab Chow ad lib. The animals used in these studies were from the 25th generation. The 25th generation is the 17th generation of selection since selection pressure was not applied for generations 6, 7 and 19–24.

Animals were killed by cervical dislocation and brains were removed. The cerebral cortex, caudate, hippocampus and cerebellum were dissected on ice and homogenized (Brinkmann Polytron) in cold isotonic saline containing 20 mM Tris, pH 7.5. Homogenates were centrifuged at 20,000×g for 10 min and the pellets were resuspended in fresh buffer.

To measure the density of β-adrenergic receptors, aliquots (50–75 μg protein) were incubated with 35–250 pM ^{125}I-iodohydroxybenzylpindolol (IHYP) for 30 min at 37°C as described by Sporn and Molinoff [31]. Specific binding, defined as the amount of IHYP bound in the absence of a competing ligand minus that bound in the presence of 20 μM l-isoproterenol, comprised 70% of total binding. The affinity of IHYP for the receptor (K_D) and receptor density (Bmax) were calculated by the method of Scatchard [28]. A computer aided graphic analysis of the inhibition of specific IHYP binding by the β_2-selective agonist zinterol provided a measure of the densities of β_1 and β_2 receptors [25]

To assay dopamine receptors homogenates of striatum (70–150 μg protein) were incubated with 50–600 pM ^{3}H-spiroperidol [12, 20, 38] for 10 min at 37°C. Specific binding (80% of total binding) was defined as total binding minus binding in the presence of 2 μM (+)-butaclamol.

Muscarinic cholinergic receptors were assayed with 100–1000 pM ^{3}H-quinuclidinylbenzilate (QNB) [37]. Samples of cortex (100 μg protein), striatum (40 μg protein) and hippocampus (35 μg protein) were incubated for 20 min at 37°C. Specific binding was defined in the absence and presence of 0.3 μM atropine (95% of total binding). Data from assays for dopaminergic and muscarinic cholinergic receptors were also analyzed by the method of Scatchard [28].

To measure β-adrenergic receptor stimulated cyclic AMP accumulation cerebral cortices were sliced with a McIlwain Tissue Chopper (0.26×0.26×1.0 mm). Slices were prelabeled with ^{3}H-adenine and reincubated in the presence of a maximally effective concentration of l-isoproterenol (30 μM). The percent of ^{3}H-ATP converted to ^{3}H-cyclic AMP was determined by a modification [14] of the method of Shimizu *et al.* [29].

Adenylate cyclase activity was determined in striatal homogenates by measuring the conversion of (α-^{32}P)-ATP to ^{32}P-cyclic AMP [27]. Dopamine stimulated adenylate cyclase activity was determined in the presence of 50 μM dopamine.

Protein was determined by the method of Lowry *et al.* [21] using fraction V bovine serum albumin as a standard.

Data were subjected to an analysis of variance and Student's t-test for examination of statistical significance. Statistical computations, quantitation of β-adrenergic receptor subtypes and Scatchard [28] analyses were performed on a Wang 2200T computer system.

RESULTS

The possibility that the density of β-adrenergic receptors is different in brains of mice with increased or decreased sensitivity to ethanol was investigated. Homogenates of cerebral cortex and cerebellum were employed. The density of receptors was approximately 15% lower in cerebral cortices of LS mice as compared to SS mice or HS controls (Table 1). In contrast, IHYP binding in cerebellum was similar in the 3 lines of mice (Table 1). The affinity of the receptor for IHYP was not significantly different in SS, LS or HS mice in either brain region.

It has recently been shown that β_1 and β_2 adrenergic receptor subtypes are regulated independently [24]. It was therefore of interest to determine whether the difference in the density of β-adrenergic receptors observed in the cerebral cortex of LS animals was specific for either receptor subtype. The ratio of β_1 to β_2 receptors was, however, approximately 4:1 in all 3 lines of mice (Table 2).

The densities and properties of dopaminergic and mus-

TABLE 3

DOPAMINE RECEPTOR BINDING IN STRIATUM OF HS, LS AND SS MICE

	HS	LS	SS
Bmax (pmol/mg)	0.98 ± 0.074	0.97 ± 0.07	0.98 ± 0.081
K_D (pM)	82.5 ± 8.0	94.5 ± 7.5	90.5 ± 6.5

Homogenates of caudate from HS, LS and SS animals were incubated with 50–600 pM (^{3}H)-spiroperidol and binding data were analyzed by the method of Scatchard to give the maximal number of binding sites (Bmax) and the affinity (K_D) of the receptor for spiroperidol. The results are expressed as mean ± SEM for groups of 16 animals and represent pooled data from 2 separate experiments.

TABLE 4

MUSCARINIC CHOLINERGIC RECEPTORS IN HS, LS AND SS MICE

	HS	LS	SS
Cortex	2.0 ± 0.10 (412 ± 21)	2.1 ± 0.10 (456 ± 27)	2.1 ± 0.12 (421 ± 15)
Striatum	4.4 ± 0.19 (327 ± 19)	4.2 ± 0.20 (326 ± 13)	4.1 ± 0.19 (315 ± 18)
Hippocampus	2.8 ± 0.11 (250 ± 20)	2.6 ± 0.13 (251 ± 10)	2.6 ± 0.10 (235 ± 14)

Homogenates were incubated with 100–1000 pM ^{3}H-QNB and binding data were analyzed by the method of Scatchard. The density of binding sites (pmol/mg) and the K_D value (pM, in parentheses) are shown. The results are expressed as mean ± SEM for groups of 12 animals and represent pooled data from 2 separate experiments.

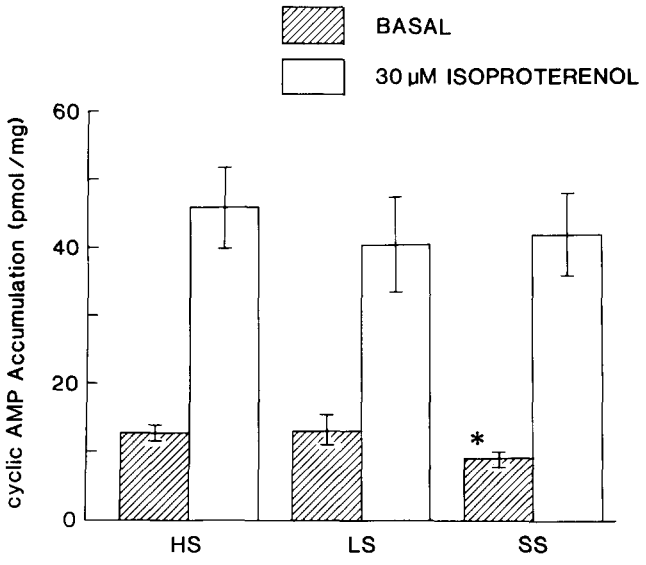

FIG. 1. Catecholamine-stimulated cyclic AMP accumulation in cortices for HS, LS and SS animals. Basal and isoproterenol-stimulated (30 μM) cyclic AMP accumulation were measured in slices of cerebral cortex. Values are mean ± SEM for determinations from 8 animals. *Differs from basal levels in HS and LS mice ($p<0.05$).

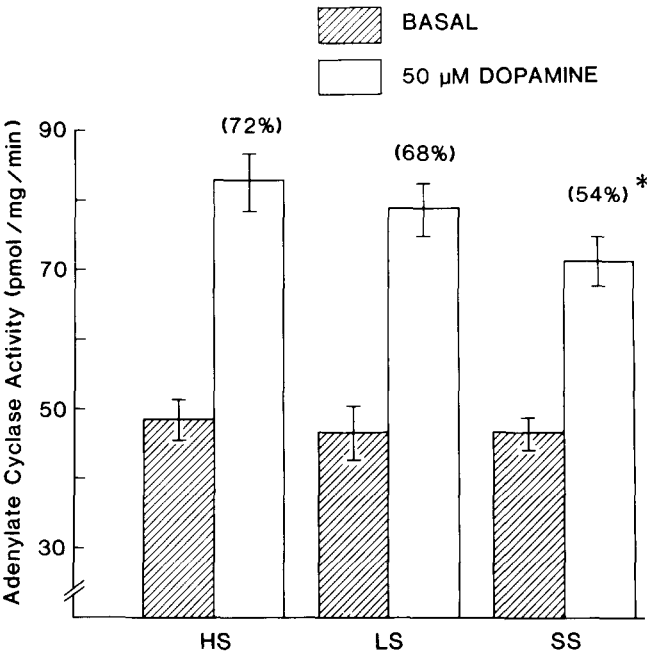

FIG. 2. Dopamine stimulated adenylate cyclase activity in caudate of HS, LS and SS mice. Homogenates of caudate were assayed for basal and dopamine-stimulated (50 μM) adenylate cyclase activity. Values represent the mean ± SEM for groups of 13 animals pooled from two separate experiments. Average percent stimulation above basal is in parentheses above each bar. *Significantly less than the % stimulation in HS and LS mice ($p<0.01$).

carinic cholinergic receptors were also determined. The density of striatal binding sites for ^{3}H-spiroperidol was the same in the three lines of mice (Table 3). The K_D of the receptor for ^{3}H-spiroperidol was also identical in HS, LS and SS mice (Table 3). Muscarinic cholinergic receptors were examined in cerebral cortex, hippocampus and caudate by studying the binding of ^{3}H-QNB. The density of receptors and K_D values in these three brain areas were not different in the three lines (Table 4).

Cortical tissue was further examined to determine whether an alteration in β-adrenergic receptor-mediated cyclic AMP accumulation paralleled the decrease in β-adrenergic receptor density in LS mice. Basal levels of cyclic AMP accumulation were slightly lower in slices of cortex from SS mice as compared to those in HS and LS mice (Fig. 1). However, stimulation of cyclic AMP accumulation by a maximally effective concentration of isoproterenol resulted in similar levels of cyclic AMP in the three groups (Fig. 1).

Homogenates of caudate nucleus were assayed for basal and dopamine-stimulated adenylate cyclase activity. Basal levels were similar in the three groups (Fig. 2). In contrast, adenylate cyclase activity stimulated by 50 μM dopamine was diminished in SS mice (Fig. 2) as compared to the LS and SS animals.

DISCUSSION

The lines of mice used in this study provide important evidence that the initial sensitivity to ethanol is under genetic control [22]. Previous investigations of the action of ethanol in the mammalian central nervous system have suggested that specific neurotransmitter/effector systems may be affected [1, 26, 35]. Thus, alterations observed in the HS, LS and SS lines could provide useful information with regard to the effects of chronic ethanol ingestion. It is important to note that the selection pressure is applied only for the single behavioral trait of ethanol induced "sleep/time." Since the animals are outbred they should be heterogenous for characteristics that do not influence initial sensitivity to ethanol. The use of the HS line of animals as a comparison group, in which no selection pressure has been applied, provides a positive control for environmental effects as well as nonspecific genetic effects.

The density of β-adrenergic receptors was 15–20% lower in the cerebral cortex of the alcohol-sensitive LS mice as compared to the SS animals or to the HS controls (Tables 1–2). Alterations in adrenergic receptors have been reported in the central nervous system of mice given ethanol. Chronic administration of ethanol to mice for 60 days resulted in a decreased density of β-adrenergic receptors as measured by the binding of ^{3}H-dihydroalprenolol [1] and IHYP [26]. Withdrawal for 48–72 hr following chronic ethanol administration led to an increased density of β-adrenergic receptors [1]. The alcohol insensitive (SS) mice might correspond to chronically treated animals with a high tolerance for alcohol. This does not appear to be the case as far as β-adrenergic receptors are concerned. Thus, the density of β-adrenergic receptors was lower in LS mice than in the less sensitive SS mice.

Chronic ethanol intake has been reported to affect the ability of norepinephrine to increase cyclic AMP accumulation in rat cerebral cortex [13, 19, 30, 36] and to cause changes in norepinephrine turnover in the brain [18]. Basal and β-adrenergic receptor mediated cyclic AMP accumulation was measured in slices of cerebral cortex from HS, LS and SS mice. Except for a slightly lower basal activity in SS mice, the results were comparable in the three lines of mice (Fig. 1).

Cortical acetylcholine release is depressed in rabbits and rats by ethanol infusion [9]. A cholinergic mechanism has also been implicated in the reduction of ethanol-induced sleep time by thyrotropin-releasing hormone [6]. Additionally, cholinergic neurons may be involved in the regulation of the effects of ethanol on rat brain wave synchrony [10]. Nonetheless, there was no difference in the density of muscarinic cholinergic receptors in the cortex, striatum or hippocampus of HS, LS or SS mice (Table 4).

The dopaminergic system has been widely studied in relation to central effects of ethanol. Tabakoff and coworkers [17,34] have reported a decrease in dopamine-sensitive adenylate cyclase activity in striatal tissue of mice withdrawn from ethanol. However, attempts to reproduce this finding in our laboratory have thus far been unsuccessful [26]. Other investigators have reported an increased dopamine sensitivity in rat nucleus accumbens following chronic ethanol treatment [8]. In the present experiments dopamine-stimulated adenylate cyclase activity was lower in the SS mice as compared to the HS controls (Fig. 2). This change was not associated with a change in the density of dopamine receptors (Table 3).

The changes observed in the current study are different from those observed in animals with decreased alcohol sensitivity due to chronic ethanol administration [32]. It should be noted that changes in ethanol sensitivity following chronic ethanol administration may involve different molecular mechanisms than are responsible for the differences between SS and LS mice which have an altered initial sensitivity to ethanol. It is interesting that these genetically selected animals do not exhibit an altered metabolism of ethanol [16] as is seen following chronic ethanol administration. Thus, the mechanisms underlying differing sensitivity to alcohol remain to be elucidated.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the technical assistance of Ms. Beth Goens, and Mr. Jeffrey Kleiner. Ms. Candy Plesha provided expert secretarial assistance.

REFERENCES

1. Banerjee, S. P., V. K. Sharma and J. M. Khanna. Alterations in β-adrenergic receptor binding during ethanol withdrawal. *Nature* **276:** 407–408, 1978.
2. Blum, K., J. D. Eubank, J. E. Wallace and H. A. Schwertner. Suppression of ethanol withdrawal by dopamine. *Experientia* **32:** 493–495, 1973.
3. Blum, K., W. Calhoun, J. Merritt and J. E. Wallace. l-DOPA: Effect of ethanol narcosis and brain biogenic amines in mice. *Nature* **242:** 407–409, 1973.
4. Carlsson, A., J. Engel, V. Strömbom, T. H. Svensson and B. Waldeck. Suppression by dopamine agonists of the ethanol-induced stimulation of locomotor activity and brain dopamine synthesis. *Naunyn-Schmied. Arch. Pharmac.* **283:** 117–128, 1974.
5. Collins, A. C., T. N. Yeager, M. E. Lebsack and S. Panter. Variations in alcohol metabolism: Influence of sex and age. *Pharmac. Biochem. Behav.* **3:** 973–978, 1975.
6. Cott, J. M., G. R. Breese, B. R. Cooper, T. S. Barlow and A. J. Prangle. Investigations in to the mechanism of reduction of ethanol sleep by thyrotropin-releasing hormone (TRH). *J. Pharmac. exp. Ther.* **196:** 594–604, 1976.
7. Deitrich, R. A. and A. C. Collins. Pharmacogenetics of alcoholism. In: *Alcohol and Opiates: Neurochemical and Behavioral Mechanisms,* edited by K. Blum. New York: Academic Press, 1977, pp. 109–139.
8. Engel, J. and S. Liljequist. The effect of longterm ethanol treatment on the sensitivity of the dopamine receptors in the nucleus accumbens. *Psychopharmacology* **49:** 253–257, 1976.
9. Erickson, C. K. and D. T. Graham. Alteration of cortical and reticular acetylcholine release by ethanol *in vivo*. *J. Pharmac. exp. Ther.* **185:** 583–593, 1973.
10. Erickson, C. K. and K. J. Chai. Cholinergic modification of ethanol-induced electroencephalographic synchrony in the rat. *Neuropharmacology* **15:** 39–43, 1976.

11. Erwin, V. G., W. D. W. Heston, G. E. McClearn and R. A. Deitrich. Effect of hypnotics on mice genetically selected for sensitivity to ethanol. *Pharmac. Biochem. Behav.* **4:** 679–683, 1976.
12. Fields, J. Z., R. D. Reisine and H. I. Yamamura. Biochemical demonstration of dopaminergic receptors in rat and human brain using ^{3}H-spiroperidol. *Brain Res.* **136:** 576–584, 1977.
13. French, S. W., D. S. Palmer, M. E. Narod, P. E. Reid and C. W. Ramey. Noradrenergic sensitivity of the cerebral cortex after ethanol ingestion and withdrawal. *J. Pharmac. exp. Ther.* **194:** 319–326, 1975.
14. Harden, T. K., B. B. Wolfe, J. R. Sporn, B. K. Poulos and P. B. Molinoff. Effects of 6-hydroxydopamine on the β-adrenergic receptor/adenylate cyclase system in the rat cerebral cortex. *J. Pharmac. exp. Ther.* **203:** 132–147, 1977.
15. Heston, W. D. W., S. M. Anderson, V. G. Erwin and G. E. McClearn. A comparison of the actions of various hypnotics on mice selectively bred for sensitivity to ethanol. *Behav. Genet.* **3:** 402–403, 1973.
16. 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.
17. Hoffman, P. L. and B. Tabakoff. Alterations in dopamine receptor sensitivity by chronic ethanol treatment. *Nature* **268:** 551–553, 1977.
18. Hunt, W. A. and E. Majchrowicz. Alterations in the turnover of brain norepinephrine and dopamine in alcohol-dependent rats. *J. Neurochem.* **23:** 549–552, 1974.
19. Kuriyama, K. and M. A. Israel. Effect of ethanol administration on cyclic 3′,5′-adenosine monophosphate metabolism in brain. *Biochem. Pharmac.* **22:** 2919–2922, 1973.
20. Leysen, J. E., W. Gommeren and P. M. Laduron. Spiperone: A ligand of choice for neuroleptic receptors. I. Kinetics and characteristics of *in vitro* binding. *Biochem. Pharmac.* **27:** 307–316, 1978.
21. Lowry, O. H., M. J. Rosebrough, A. L. Farr and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193:** 265–275, 1951.
22. McClearn, G. E. and R. Kakihana. Selective breeding for ethanol sensitivity in mice. *Behav. Genet.* **3:** 409–410, 1973.
23. McClearn, G. E., J. R. Wilson and W. Meredith. In: *Contribution to Behavior-Genetic Analysis: The Mouse as a Prototype*, edited by G. G. Lindsey and D. D. Thressen. New York: Appleton-Century Crafts, 1970, pp. 3–22.
24. Minneman, K. P., M. D. Dibner, B. B. Wolfe and P. B. Molinoff. β_1 and β_2-Adrenergic receptors in rat cerebral cortex are independently regulated. *Science* **204:** 866–868, 1979.
25. Minneman, K. P., L. R. Hegstrand and P. B. Molinoff: Simultaneous determination of β_1 and β_2-adrenergic receptors in tissues containing both receptor subtypes. *Molec. Pharmac.* **16:** 34–46, 1979.
26. Rabin, R. A., B. B. Wolfe, M. D. Dibner, N. R. Zahniser, C. Melchior and P. B. Molinoff. Effects of ethanol administration and withdrawal on neurotransmitter receptor systems in C57 mice. *J. Pharmac. exp. Ther.*, in press.
27. Salomon, Y., C. Landos and M. Rodbell. A highly sensitive adenylate cyclase assay. *Analyt. Biochem.* **58:** 541–548, 1974.
28. Scatchard, G. The attractions of proteins for small molecules and ions. *Ann. N.Y. Acad. Sci.* **51:** 660–672, 1949.
29. Shimizu, H., J. W. Daly and C. R. Creveling. A radioisotopic method for measuring the formation of adenosine 3′-5′-cyclic monophosphate in incubated slices of brain. *J. Neurochem.* **16:** 1609–1619, 1969.
30. Smith, T., A. Shen, A. Jacobyansky and R. G. Thurman: Rapid adaptation of norepinephrine-stimulated adenylate cyclase of rat brain cerebral cortex during ethanol withdrawal reaction. *Fedn. Proc.* **36:** 331, 1977.
31. Sporn, J. R. and P. B. Molinoff. β-Adrenergic receptors in rat brain. *J. Cyc. Nuc. Res.* **2:** 149–161, 1976.
32. Tabakoff, B. Neurochemical aspects of ethanol dependence. In: *Alcohol and Opiates: Neurochemical and Behavioral Mechanisms*, edited by K. Blum. New York: Academic Press, 1977, pp. 21–39.
33. Tabakoff, B. and P. L. Hoffman. Alterations in receptors controlling dopamine synthesis after chronic ethanol ingestion. *J. Neurochem.* **31:** 1223–1229, 1978.
34. Tabakoff, B., P. L. Hoffman and R. F. Ritzmann. Dopamine receptor function after chronic ingestion of ethanol. *Life Sci.* **23:** 643–648, 1978.
35. Tabakoff, B. and R. F. Ritzmann. The effects of 6-hydroxydopamine on tolerance to and dependence on ethanol. *J. Pharmac. exp. Ther.* **203:** 319–321, 1977.
36. Volicer, L., R. Mirin and B. I. Gold. Effect of ethanol on the cyclic AMP system in rat brain. *J. Stud. Alcohol* **38:** 11–24, 1977.
37. Yamamura, H. I. and S. H. Snyder. Muscarinic cholinergic receptor binding in the longitudinal muscle of the guinea pig ileum. *Molec. Pharmac.* **10:** 861–867, 1974.
38. Zahniser, N. R. and P. B. Molinoff. Effect of guanine nucleotides on striatal dopamine receptors. *Nature* **275:** 453–455, 1978.