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Calcium Influence on Neuronal Sensitivity to Ethanol in Selectively Bred Mouse Lines[1]

ELIZABETH L. MORROW AND V. GENE ERWIN[2]

Center for Alcohol Research, School of Pharmacy
University of Colorado, Boulder, CO 80309

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MORROW, E L AND V G ERWIN *Calcium influence on neuronal sensitivity to ethanol in selectively bred mouse lines* PHARMACOL BIOCHEM BEHAV 24(4) 949–954, 1986 —Sensitivity to ethanol, as measured by blood ethanol concentration at loss of righting reflex, was increased significantly in SS but not LS mice following intracerebroventricular (ICV) administration of calcium chloride or A23187, a calcium ionophore Magnesium chloride or lanthanum chloride, ICV, did not alter sensitivity to ethanol in either SS or LS mice, further indicating a specificity for calcium cation Calcium was without effect on sensitivity to halothane narcosis in LS or SS mice Endogenous brain calcium content was similar in these mouse lines, and ethanol administration either *in vivo* or *in vitro* did not alter brain calcium concentration These results indicate that differences in brain sensitivity to ethanol are mediated, in part, by genetic differences in calcium-related processes and support the hypothesis that ethanol-induced narcosis may be due to alterations in calcium metabolism in the CNS

Calcium Magnesium Ethanol Halothane Sensitivity Selective breeding

WHILE the neurochemical processes through which ethanol elicits its hypnotic action in the central nervous system (CNS) have not been defined, elucidation of these mechanisms has been facilitated by the establishment of mouse lines which differ in CNS sensitivity to the acute narcotic actions of ethanol [7, 14, 19] Since these lines, long sleep (LS) and short sleep (SS), have been selected for their sensitivity (LS) or resistance (SS) to acute ethanol intoxication, any measured physiological or biochemical differences which exist should be related to their differential behavioral response to ethanol Brain sensitivity differences to ethanol-induced narcosis in these mouse lines have been highly correlated with the sensitivity of cerebellar purkinje cells to ethanol [28,29] To date no comparable biochemical correlates of the behavioral and electrophysiological effects of ethanol have been observed Studies suggest differences exist in central neurotransmission, including receptor density [3], distinct ethanol-induced alteration in neurotransmitter concentration, and receptor-coupled response [2, 3, 9, 18, 22]

Several aspects of neurotransmission are calcium dependent, including membrane permeability, impulse propagation, stimulus-secretion coupling, and receptor-mediated response [26]. Early investigations reported the acute administration of ethanol decreased calcium levels in the brain [23,25] Although these investigations remain controversial [8,15], ethanol exerts an effect on many biochemical processes which modulate the functional concentration of intracellular calcium [10, 12, 13, 16, 24, 27, 30] Other investigations demonstrated that by increasing brain calcium levels through intracerebroventricular (ICV) administration of this cation, ethanol-induced narcosis could be enhanced in genetically heterogeneous mice and rats [4, 5, 11]. These results suggest that alterations in intracellular calcium metabolism in the CNS may be involved in the behavioral manifestation of acute ethanol intoxication

The purpose of this investigation was to examine the influence of exogenous administration of calcium as well as other cations on the sensitivity of LS and SS mice to ethanol. These studies are especially relevant in light of the evidence suggesting a possible relationship of the genetically determined differential sensitivity with modest differences in neurotransmission, since these discrete differences may be magnified by ethanol-induced alterations in intracellular calcium metabolism

METHOD

Animals

Male LS and SS mice as well as the genetically heteroge-

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[2]Requests for reprints should be addressed to V Gene Erwin, School of Pharmacy, Campus Box 297, University of Colorado, Boulder, CO 80309

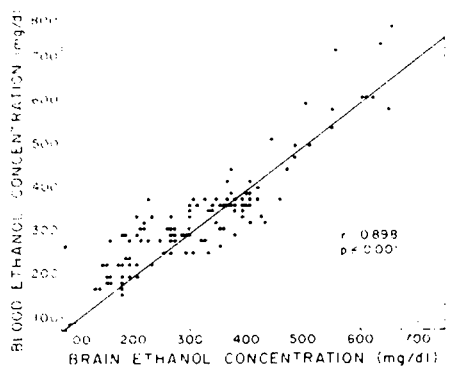

FIG 1 Correlation of blood and brain ethanol concentrations in LS and SS mice at LRR following administration of ethanol (7 g/kg, LS, or 10 g/kg, SS)

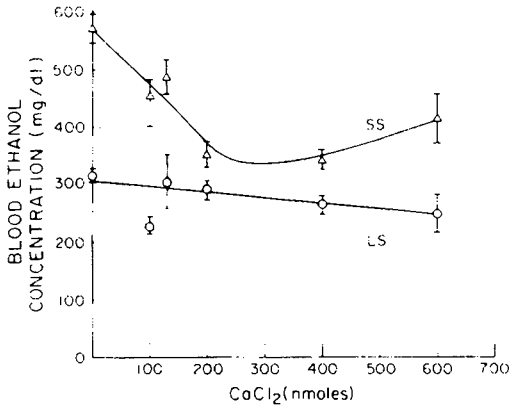

FIG 2 Effect of varying calcium concentration on sensitivity of LS and SS mice to ethanol-induced narcosis Ethanol was administered IG at 7 g/kg (LS) or 10 g/kg (SS) 15 min following ICV injection of $CaCl_2$ or 0 9% NaCl (0 $CaCl_2$) Values are mean±S E M Significantly different from 0 $CaCl_2$ at $p<0$ 05

neous stock (HS) from which the lines were derived were obtained from the Institute for Behavioral Genetics, University of Colorado, Boulder, CO The animals were of the 35th generation and were tested at 50 to 80 days of age

Materials

The chemicals and supplies were as follows Absolute ethanol, Aapen Alcohol and Chemical Co (Shelbyville, KY), halothane, 2-bromo-2-chloro-1,1,1-trifluoroethane, containing 0 01% thymol, Ayerst (New York, NY), chloride salts of calcium and magnesium, Mallinkrodt (St Louis, MO), lanthanum chloride, A D MacKay (Darien, CT), A23187, alcohol dehydrogenase, β-nicotinamide adenine dinucleotide, Sigma Chemical Co (St Louis, MO), Ready-Solv, Beckman (Fullerton, CA), ^{45}Ca, New England Nuclear (Boston, MS), lanthanum oxide, Research Organic/Inorganic Chemical Corp (Sun Valley, CA), [ethylenebis (oxyethylene-nitrilo)] tetraacetic acid (EGTA), Eastman Kodak Co (Rochester, NY)

Intracerebroventricular Injections

Cations and the calcium ionophore A23187 were administered, ICV, to halothane-anesthetized mice using gross anatomical structures for needle placement such that an equilateral triangle was visualized between the eye, the ear, and the apex, located at intersection of bregma with the coronal suture Injection was made 1 to 2 mm lateral to the apex on bregma through the scalp using a 26-gauge needle ensheathed with polyethylene tubing to alter the depth of needle penetration Slight differences exist between LS and SS mouse lines in depth at which the lateral ventricle occurs To adjust for this difference, the polyethylene sleeve length was cut so that the needle penetrated 2 5 mm in the LS and 3 mm in SS line Injection volume was 5 μl and in the case of multiple injections, total injection volume did not exceed 10 μl Placement of the solutions into the lateral ventricle was verified routinely by post-mortem examination $CaCl_2$, $MgCl_2$, and $LaCl_3$ were dissolved in 0 9% NaCl A23187 was solubilized in absolute ethanol and brought to the desired concentration by dropwise addition of deionized water so that the final solvent concentration was 70% (v/v) Doses of cations and the calcium ionophore A23187 were based on previous investigations [4, 5, 11]

Drug Administration

Mice were administered ethanol intragastrically (IG) using a stainless steel feeding tube (Poppen and Sons, Inc , New Hyde Park, NY) attached to a 1-cc syringe 15 min following the initial ICV injection of cations, A23187, 0 9% NaCl or 70% ethanol LS mice received 7 g/kg and SS mice received 10 g/kg ethanol (60% v/v in saline) The time schedules for calcium and ethanol administration were based on a preliminary examination of the time course of distribution of ICV administered $CaCl_2$ (200 nmol) containing ^{45}Ca (specific activity 0 075 μCi/μmole $CaCl_2$ in final solution) These studies were carried out in both LS and SS mouse lines and revealed a similar biphasic disappearance of the cation An initial rapid disappearance of calcium was observed following ICV administration with only 32% of cation remaining at 15 min This rapid elimination was followed by a slower elimination rate over the next 60 min To insure that a significant concentration of calcium was present during the assessment of sensitivity a 15 min interval between ICV calcium administration and IG ethanol administration was chosen This time was maintained in the experimental protocols with other cations and with the calcium ionophore being administered ICV prior to calcium Concurrent cation administrations were avoided in an effort to minimize drastic alterations in osmolarity

In one experiment, mice were administered halothane by inhalation 15 min following ICV calcium administration Mice were placed in a 2-liter jar into which halothane was introduced as a 1 5% solution in O_2 using an anesthesia pump (Ohio Medical Products, Madison, WI)

Assessment of Sensitivity, Rectal Temperature

Sensitivity to ethanol or halothane was measured behaviorally as that time to onset of the loss of righting reflex (LRR) following drug administration LRR was measured by the inability of an animal to right itself three times in 30 sec

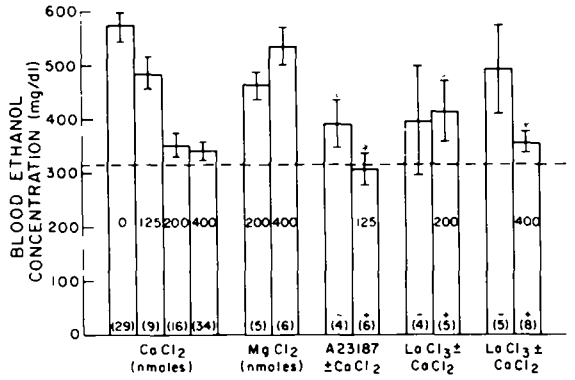

FIG 3 Effect of cations and A23187 on sensitivity of SS mice to ethanol-induced narcosis Ethanol was administered (IG, 10 g/kg) 15 min following the ICV administration of $CaCl_2$ (125, 200, 400 nmol), $MgCl_2$ (200, 400 nmol) or 0 9% NaCl, A23187 (25 nmol) or 70% (v/v) ethanol (data not shown) followed 2 min later by ICV $CaCl_2$ (125 nmol) or 0 9% NaCl, $LaCl_3$ (100 nmol) followed 5 min later by $CaCl_2$ (200 nmol) or 0 9% NaCl, or $LaCl_3$ (200 nmol) followed 5 min later by $CaCl_2$ (400 nmol) or 0 9% NaCl (----BEC at LRR in LS mice, 0 $CaCl_2$) Values are mean S E M Number of animals indicated in parentheses *Significantly different from 0 $CaCl_2$ at $p<0$ 05

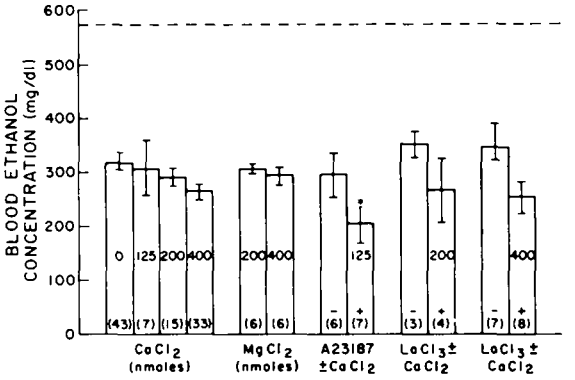

FIG 4 Effect of cations and A23187 on sensitivity of LS mice to ethanol-induced narcosis Ethanol was administered (IG, 7 g/kg) 15 min following the ICV administration of cations or A23187 as described in legend to Fig 3 *Significantly different from 0 $CaCl_2$ at $p<0$ 05

when placed on its back in a Plexiglas trough Time to LRR was approximately 4 min in LS mice with ethanol (7 g/kg), whereas SS mice did not lose righting reflex following IG administration of 7 g/kg ethanol An ethanol dose of 10 g/kg IG was required to produce a loss of righting response in SS mice Halothane-induced LRR occurred in SS and LS mice within 4 min At this time blood samples were obtained by retro-orbital sinus puncture, and the animal was immediately sacrificed and the brain removed for analysis of ethanol concentration.

Body temperature was determined using a telethermometer (Bailey Instruments, Inc , Saddle Brook, NJ) The rectal probe was lubricated and inserted 2.5 cm into the rectum. To determine the effect of ICV calcium administration on body temperature, measurements were made immediately prior to the ICV injection and at 15 min thereafter. Animals were maintained at an ambient temperature of 23° to 24°C

Analysis of Blood and Brian Ethanol and Halothane Concentration

Ethanol concentration in blood and brain samples obtained at LRR was analyzed spectrophotometrically (Beckman Instruments, Inc , Model 25, Irvine, CA) [17] Blood halothane concentration at LRR was determined by gas chromatography (Hewlett Packard, Model 5710A, Palo Alto, CA) using a head-space technique [6]

Analysis of Brain Calcium Concentration

For whole brain calcium analysis, aliquots of 10% homogenates (w/v in 0.9% saline, 0.9% saline containing 10^{-3} M EGTA, or 0.4% ethanol) were diluted with one volume of HNO_3 and La_2O_3 so that the final concentration was 10% HNO_3 and 2% La_2O_3. The remaining homogenate was centrifuged at 150,000 × g for 1 hr. The pellet obtained was resuspended and the supernatant diluted with 1 volume HNO_3 and La_2O_3 to a final concentration of 10% HNO_3 and 2% La_2O_3 Standards were prepared in the appropriate tissue preparations using calcium carbonate. Samples were run in duplicate and concentrations of calcium were determined using a Perkin-Elmer 423 atomic absorption spectrophotometer (Danbury, CT) In one experiment animals received 4 1 g/kg ethanol intraperitoneally and were sacrificed 5 min after LRR Brains were removed and prepared as described

Statistical Methods

The effect of ions and A23187 on ethanol sensitivity was evaluated with a two-way analysis of variance Significance was determined using the Scheffé test. Body temperature and brain calcium concentration were evaluated by Student's t-test for unpaired data

RESULTS

Correlation of Blood and Brain Ethanol Concentration at LRR Following Intragastric Ethanol Administration

As demonstrated by regression analysis, blood and brain ethanol concentrations (mg/dl) were highly correlated ($r=0.898$, $p<0.001$) at LRR with this mode of drug administration (Fig 1). Based on this analysis, brain sensitivity of LS and SS mice was defined as blood ethanol concentration (BEC) at LRR.

Effect of Varying Calcium Concentration on Sensitivity of LS and SS Mice to Ethanol-Induced Narcosis, Rectal Temperature

In the absence of calcium, the differential sensitivity of LS and SS mice to ethanol-induced narcosis is demonstrated by BEC at which each line lost its righting reflex, 316.0±10.7 mg/dl and 517.0±25.1 mg/dl, respectively (Fig 2). In LS mice, pretreatment with calcium was without effect on sensitivity to ethanol at any concentration examined However, administration of 200, 400, and 600 nmol calcium, ICV, 15 min prior to ethanol, significantly increased the sensitivity of

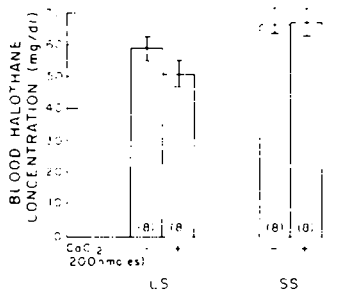

FIG 5 Effect of calcium on sensitivity of LS and SS mice to halothane-induced narcosis Halothane was administered as 1 5% halothane 98 5% O_2 at a flow rate of 3 L/min, 15 min following the ICV administration of $CaCl_2$ (200 nmol) or 0 9% NaCl Values are mean+S E M Number of animals indicated in parenthesis

SS mice Although a reversal of calcium-induced enhancement of sensitivity appeared to occur at 600 nmol calcium, this was probably a consequence of calcium toxicity Mice exhibited tremors at this calcium concentration The increase in sensitivity at 200 to 600 nmol calcium was manifested by a significant decrease in BEC at LRR At these concentrations of calcium SS mice exhibit an ethanol sensitivity comparable to that seen the LS line, approximately 350 mg/dl A significant degree of hypothermia was seen in the absence of ethanol 15 min following calcium (200 nmol) administration, in both LS and SS mice However, there was no difference between mouse lines in the extent of hypothermia Both lines exhibited a 2 7±0 4°C decrease in temperature

Effect of Cations and A23187 on Sensitivity of LS and SS mice to Ethanol-Induced Narcosis

The results presented above demonstrate clearly that ethanol sensitivity of SS mice is significantly enhanced by pretreatment with calcium It was of interest to determine if this effect was specific to calcium This was accomplished by examining the effect of other cations, as well as the calcium ionophore A23187 Magnesium is a divalent cation which mimics the membrane-stabilizing properties of calcium This ion at equimolar doses was ineffective in altering ethanol sensitivity in SS mice (Fig 3) Lanthanum which antagonizes calcium by blocking calcium channels [20] was also without effect on ethanol sensitivity in SS mice in the absence of calcium Furthermore, concurrent administration of lanthanum in a 1 2 molar ratio with calcium did not reverse the calcium enhancement of ethanol sensitivity (Fig 3) Administration of A23187 (25 nmol), ICV, in the absence of added calcium resulted in a significant increase in sensitivity of SS mice to ethanol Concomitant administration of A23187 (25 nmol) and calcium (125 nmol) also resulted in increased ethanol sensitivity of SS mice The actions of A23187 and calcium appear to be additive since administration of 125 nmol calcium in the absence of ionophore did not alter sensitivity (Fig 3) It should be noted that A23187 was dissolved in 70% (v/v) ethanol as described in methods and ICV administration of vehicle (70% ethanol v/v) was used as a control Although this concentration of ethanol is extremely high and some tissue damage is likely, it was the only appropriate vehicle for the calcium ionophore LS and SS mice receiving 5 μl ICV, ethanol (70% v/v) recovered from halothane anesthesia in a time equivalent to that of

TABLE 1

TOTAL LEVELS AND DISTRIBUTION OF CALCIUM IN LS, AND SS MICE

Mouse Line	Homogenizing Medium*	Total†	Calcium‡ Supernatant	Pellet
LS	Saline	1 10	0 22	0 80
		1 20	0 36	0 88
LS	EGTA	1 09	0 70	0 62
		1 15	0 72	0 59
SS	Saline	1 07	0 25	0 80
		1 10	0 35	0 84
SS	EGTA	1 05	0 20	0 79
		1 09	0 49	0 69

*Either saline (0 9% NaCl) or 10^{-3} M Disodium EGTA in saline was used as the brain homogenizing medium

†Aliquots of homogenate were taken for total calcium assays, and remaining homogenate was centrifuged at 150,000 × g for 1 hr Calcium content of the resulting supernatant and resuspended pellet was then analyzed

‡Values represent μmoles calcium ion per g brain wet weight measured by atomic-absorption spectrometry Values represent 2 separate experiments using brains from 8 mice (LS or SS) pooled for each experiment

TABLE 2

EFFECTS OF ETHANOL *IN VIVO* AND *IN VITRO* CALCIUM LEVELS IN HS MOUSE BRAINS

Homogenizing Medium	Ethanol IP†	Total	Calcium‡ Supernatant	Pellet
Saline	–	1 15	0 26	0 78
		1 10	0 22	0 89
Saline	+	1 25	0 26	0 93
		1 30	0 27	1 03
Ethanol	–	1 20	0 17	1 05
		1 24	0 19	0 98
Ethanol	+	0 96	0 16	0 83
		1 05	0 20	0 81

*Brain homogenizing medium was either saline (0 9% NaCl) or 0 4% ethanol in saline

†Ethanol (4 1 g/kg) was administered, IP, and animals were sacrificed and brains homogenized 5 min after loss of righting response

‡Values are expressed as μmoles calcium ion per g brain wet weight and represent 2 experiments with brains from 8 animals pooled for each experiment

animals receiving ICV saline BEC at LRR in both mouse lines administered ethanol (IG) subsequent to ICV ethanol administration did not differ from that obtained in animals administered ICV saline prior to ethanol (data not shown), indicating this concentration of ethanol ICV was not of functional consequence in producing LRR

Administration of magnesium or lanthanum as well as calcium was without effect on the sensitivity of LS mice to ethanol (Fig 4) However, A23187 increased ethanol sen-

sitivity when administered in combination with calcium (125 nmol), but had no significant effect on sensitivity of LS mice when administered alone (Fig. 4)

Effect of Calcium on Sensitivity of LS and SS Mice to Halothane-Induced Narcosis

The generalizability of the effect of calcium to potentiate anesthesia in SS mice was examined by determining the interaction of this ion with halothane-induced narcosis Previous studies have demonstrated that LS and SS mice do not differ in sensitivity to halothane-induced anesthesia [1] This finding was confirmed in the present study by observing similar blood halothane concentrations at LRR in LS and SS mice (Fig 5) Administration of calcium 15 min prior to halothane exposure had no effect on sensitivity to halothane anesthesia in either mouse line (Fig 5)

Brain Calcium Concentration in LS, SS, and HS mice

Total brain calcium concentration was identical in LS and SS mice (Table 1) Furthermore, distribution of brain calcium between bound and free form appeared to be similar

As measured in HS mice, ethanol *in vivo* or *in vitro* did not appear to alter total endogenous calcium levels in brain or the distribution of calcium between free and bound forms (Table 2)

DISCUSSION

Administration of calcium ICV enhanced the sensitivity of SS but not LS mice to ethanol-induced narcosis The ability of calcium to potentiate the response of SS mice to ethanol was indicated by a dramatic decrease in BEC measured at LRR, SS mice become virtually LS-like with respect to BEC at loss of righting response. Increasing calcium concentration did not result in a further enhancement of sensitivity, indicating maximum sensitivity had been attained The resistance of LS mice, in ability of calcium to augment sensitivity, also seems to indicate a maximum level of sensitivity.

The potentiation of ethanol sensitivity was unique to calcium and appeared due to a specific rather than non-specific mechanism Magnesium, which, as calcium, has membrane-stabilizing properties [26], did not alter ethanol sensitivity The enhancement appears to occur through a channel independent, intracellular process, inasmuch as the calcium ionophore A23187 in the presence or absence of calcium augmented ethanol sensitivity in SS mice and lanthanum was unable to antagonize it. These results are comparable to those of other investigations which have demonstrated an increase in ethanol-induced sleep time in response to calcium administration [4, 5, 11]. However, these studies found no significant difference between sex, strain, or animal species In contrast, we have demonstrated a differential response to calcium pretreatment in LS and SS mouse lines.

Calcium elicits hypothermia in rodents when administered centrally, and the hypothermia acts in synergy with anesthetic agents to enhance anesthesia [21] In this investigation, no difference in degree of hypothermia evoked by calcium was observed between LS and SS mice and therefore does not appear responsible for differential enhancement of ethanol sensitivity Another mechanism which might be involved in the differential response is distinctions in endogenous brain calcium levels between LS and SS lines However, calcium concentration or distribution of calcium between free and bound forms was similar in LS and SS brains Furthermore, ethanol, *in vivo* or *in vitro*, did not alter endogenous calcium levels It should be noted that small changes (μM) in calcium concentrations or in its microenvironment would not have been detected by this method of analysis

Calcium administration was without effect on sensitivity to halothane-induced narcosis. Since LS and SS mice do not exhibit differential sensitivity to halothane [1], this lack of response to calcium suggests that the differential effect of calcium on ethanol-induced narcosis is in some way related to the genetic determination of differential sensitivity of LS and SS mice to ethanol

Demonstration of modulation of ethanol sensitivity by calcium suggests that the mechanism by which ethanol causes intoxication is through an alteration in the mobilization of intraneuronal calcium pools, especially those integral to neurotransmission or membrane permeability The differential effect of calcium in modulating ethanol sensitivity in LS and SS mice suggests that brain calcium metabolism in these lines is distinctly susceptible to interaction with ethanol Investigation continues in the laboratory to elucidate potential sites of interaction of ethanol with calcium-related processes in the CNS

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REFERENCES

1 Baker, R , C Melchior and R Deitrich The effect of halothane on mice selectively bred for differential sensitivity to alcohol *Pharmacol Biochem Behav* **12:** 691–695, 1980

2 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

3 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

4 Erickson, C K and T Tyler Ethanol Modification of acute intoxication by divalent cations *Science* **199:** 1219–1221, 1978

5 Erickson, C K , T D Tyler, L K Beck and K L Duensing Calcium enhancement of alcohol and drug-induced sleeping time in mice and rats *Pharmacol Biochem Behav* **12:** 651–656, 1980

6 Eriksson, C J P , H W Sippel and O A Forsander The determination of acetaldehyde in biological samples by headspace gas chromatography *Anal Biochem* **80:** 116–124, 1977

7 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 *Pharmacol Biochem Behav* **4:** 679–683, 1976

8 Ferko, A P and E Bobyock A study on regional brain calcium concentrations following acute and prolonged administration of ethanol in rats *Toxicol Appl Pharmacol* **55:** 179–187, 1980
9 French, T A , J M Masserano and N Weiner Ethanol-induced changes in tyrosine hydroxylase activity in adrenal glands of mice selectively bred for differences in sensitivity to ethanol *J Pharmacol Exp Ther* **232:** 315–321, 1985
10 Garrett, K M and D H Ross Effects of *in vivo* ethanol administration on Ca^{+2}/Mg^{+2} ATPase and ATP-dependent Ca^{2} uptake activity in synaptosomal membranes *Neurochem Res* **8:** 1013–1028, 1983
11 Harris, R A Alteration of alcohol effects by calcium and other inorganic cations *Pharmacol Biochem Behav* **10:** 527–534, 1979
12 Harris, R A and D Fenner Ethanol and synaptosomal calcium binding *Biochem Pharmacol* **31:** 1790–1792, 1982
13 Harris, R A and W F Hood Inhibition of synaptosomal calcium uptake by ethanol *J Pharmacol Exp Ther* **213:** 562–568, 1980
14 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
15 Hood, W F and R A Harris Effects of pentobarbital, ethanol and morphine on subcellular localization of calcium and magnesium *Biochem Pharmacol* **28:** 3075–3080, 1979
16 Hood, W F and R A Harris Effects of depressant drugs and sulfhydryl reagents on the transport of calcium by isolated nerve endings *Biochem Pharmacol* **29:** 957–959, 1980
17 Lundquist, F The determination of ethyl alcohol in blood and tissue *Methods Biochem Anal* **7.** 217–251, 1959
18 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
19 McClearn, G E and R Kakihana Selective breeding for ethanol sensitivity in mice *Behav Genet* **3:** 409–410, 1973
20 Miledi, R Lanthanum ions abolish the "calcium response" of nerve terminal *Nature* **229:** 410–411, 1971
21 Meyers, R D and P D Brody Temperature changes in the rat produced by altering the sodium-calcium ratio in the cerebral ventricles *Neuropharmacology* **11:** 351–361, 1972
22 Proctor, W R and T V Dunwiddie Behavioral sensitivity to purinergic drugs parallels ethanol sensitivity in selectively bred mice *Science* **224.** 519–521, 1984
23 Ross, D H Selective actions of alcohols on cerebral calcium levels *Ann NY Acad Sci* **273:** 280–294, 1976
24 Ross, D H Adaptive changes in Ca^{++}-membrane interactions following chronic ethanol exposure In *Alcohol Intoxication and Withdrawal*, edited by M Gross New York Plenum Press, 1977, pp 459–471
25 Ross, D H , M A Medina and H L Cardenas Morphine and ethanol Selective depletion of regional brain calcium *Science* **186:** 63–65, 1974
26 Rubin, R P *Calcium and the Secretory Process* New York Plenum Press, 1974
27 Seeman, P , M Chau, M Goldberg, T Sauks and L Sax The binding of Ca^{+2} to the cell membrane increased by volatile anesthetics (alcohols, acetone, ether) which induce sensitization of nerve or muscle *Biochim Biophys Acta* **225:** 185–193, 1971
28 Sorensen, S , T Dunwiddie, G McClearn, R Freedman and B Hoffer Ethanol-induced depressions in cerebellar and hippocampal neurons of mice selectively bred for differences in ethanol sensitivity An electrophysiological study *Pharmacol Biochem Behav* **14.** 227–234, 1981
29 Sorensen, S , M Palmer, T Dunwiddie and B Hoffer Electrophysiological correlates of ethanol-induced sedation in differentially sensitive lines of mice *Science* **210.** 1143–1145, 1980
30 Stokes, J A and R A Harris Alcohols and synaptosomal calcium transport *Mol Pharmacol* **22** 99–104, 1982