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# Alteration of Alcohol Effects by Calcium and Other Inorganic Cations[1]

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HARRIS, R A *Alteration of alcohol effects by calcium and other inorganic cations* PHARMAC. BIOCHEM BEHAV 10(4) 527–534, 1979 —The duration of loss of righting reflex (sleeping-time) produced by ethanol in mice was increased by intracerebroventricular (IVT) administration of $CaCl_2$ or $MnCl_2$ but was not altered by injection of $MgCl_2$, $LaCl_3$ or verapamil Calcium also increased *t*-butanol, chloral hydrate and pentobarbital sleeping-time and decreased the $ED_{50}$ for loss of righting reflex for ethanol, *t*-butanol, chloral hydrate and pentobarbital In contrast to the enhancement of sleeping-time, calcium did not potentiate the hypothermic effects of these four drugs However, lanthanum, a calcium antagonist, was found to antagonize the hypothermic effect of ethanol In other experiments, mice were made dependent upon ethanol by a liquid diet technique After withdrawal of the diet, convulsions on handling and body temperature were evaluated hourly to estimate withdrawal severity Injection of calcium during withdrawal was found to markedly suppress the convulsions on handling and to completely reverse the hypothermia produced by withdrawal of ethanol Injection of magnesium also suppressed signs of withdrawal, but was less effective than calcium Further evidence for the involvement of calcium in the actions of alcohols was obtained in experiments where acute administration of ethanol reduced the toxicity produced by IVT injection of EGTA, a specific calcium chelator In addition, the CNS toxicity of EGTA was increased during ethanol withdrawal These results demonstrate that signs of both alcohol and sedative intoxication and alcohol withdrawal can be modulated by the availability of brain calcium, and suggest that acute and chronic ethanol treatments may alter the availability of calcium in brain

Calcium Magnesium Ethanol Pentobarbital *t*-Butanol Chloral hydrate Ethanol dependence

A NUMBER of reports have suggested that ingestion of ethanol alters the metabolism of calcium and magnesium in brain and other tissues (reviewed in refs [6,11]) For example, acute administration of ethanol has been reported to decrease levels of calcium in the brain while chronic ethanol ingestion increased the calcium content of synaptic membranes [23–25]. Other studies have shown that ethanol exposure alters the binding of calcium to membranes prepared from brain, heart and erythrocytes [20, 24, 27]. In addition, hypocalcemia and hypomagnesemia have been noted in alcoholic patients during ethanol withdrawal [5,19] and administration of calcium or magnesium has been reported to relieve some of the symptoms of alcohol withdrawal [5,28] These results raised the possibility that alterations in calcium and magnesium metabolism may be involved in the production of ethanol intoxication as well as in the development of ethanol dependence This in turn suggested that alterations in brain calcium or magnesium availability might alter the intensity of both ethanol intoxication and dependence The effects of calcium and magnesium administration during alcohol withdrawal are of particular interest as the importance of hypocalcemia and hypomagnesemia in the alcohol withdrawal syndrome as well as the therapeutic value of calcium and magnesium supplementation during withdrawal remain controversial [6,11] The present study evaluates the effects of cations, cation antagonists and a cation chelator on alcohol and sedative intoxication and on signs of ethanol withdrawal in laboratory animals. A similar investigation has been carried out independently by Tyler and Erickson [3, 4, 29]

## METHOD

### *Materials*

The chemicals and their suppliers were as follows: ethanol, 95 and 99 5% v/v, Commercial Solvents Corp (Terre Haute, IN), vitamin diet fortification mixture and salt mixture XIV, ICN Pharmaceuticals (Cleveland, OH), chloral hydrate, sodium pentobarbital, ethyleneglycol-bis-(β-aminoethyl ether) N,N′-tetraacetic acid (EGTA), magnesium gluconate and calcium gluconate, Sigma Chemical Co (St Louis, MO), verapamil hydrochloride, a gift from

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Knoll Pharmaceutical Co (Whippany, NJ), *t*-butanol, chloride salts of calcium, magnesium, manganese and lanthanum, Fisher Scientific Co. (St. Louis, MO)

*Animals*

Male Swiss-Webster mice were obtained from National Laboratories (St. Louis, MO) and Charles River Laboratories (Portage, MI) The effectiveness of the drugs used in this study was found to vary between mice from these two suppliers and to vary with the body weight of the mice Thus each experiment was conducted with mice from a single supplier of about the same weight ( ± 2 g) although the weight range for different experiments was 22–32 g.

*Intracerebroventricular Injections*

Intracerebroventricular (IVT) injections were performed as described by Harris *et al* [13] using an injection volume of 5 μl/mouse, except for Ca-EGTA which was injected in a volume of 10 μl due to solubility. $LaCl_3$ was dissolved in a bicarbonate-free Krebs-Ringer buffer at pH 6 7 [12], EGTA was dissolved in saline at pH 7.8, $CaCl_2$, $MgCl_2$ and $MnCl_2$ were dissolved in artificial cerebrospinal fluid (ACSF) [21] at a pH of 7.2, and verapamil was dissolved in ACSF at pH 6 2 The ACSF had the following composition (g/l). NaCl, 8.1; KCl, 0 25; $CaCl_2$, 0 11, $MgCl_2$, 0.11; $NaHCO_3$, 1.76, $NaH_2PO_4$, 0 07; urea, 0.13, glucose, 0.61

*Effects of Cations on Loss of Righting Reflex Induced by Ethanol, t-Butanol, Chloral Hydrate and Pentobarbital*

Chloride salts of various cations ($Ca^{++}$, $Mg^{++}$, $Mn^{++}$, $La^{+++}$ and verapamil) or ACSF was injected IVT in mice and 30 minutes later either ethanol, *t*-butanol, chloral hydrate or sodium pentobarbital was injected IP Selection of ions, their dosages and times of administration were based on the studies by Harris *et al* [12,14] Shortly after alcohol or drug injection, each animal was placed on its back and the righting reflex was taken as the ability to right itself onto all four paws twice within one minute. As a graded analysis, the duration of loss of righting reflex (sleeping-time) was determined as the length of time after alcohol or drug injection which was necessary for restoration of the righting reflex No attempt was made to quantitate the onset of loss of righting reflex The loss of righting reflex was also used as a quantal measure to determine a $ED_{50}$ for each drug and alteration of the $ED_{50}$ by calcium administration. These $ED_{50}$'s were determined by the method of Litchfield and Wilcoxon [17] using at least three doses of each drug and testing six to eight mice at each dosage

*Effects of Cations, Ethanol, t-Butanol, Chloral Hydrate and Pentobarbital on Body Temperature*

Temperature was determined using a Yellow Spring Instruments telethermometer (model 42SC) and probe (model 402) The probe was lubricated and inserted 2 5 cm past the rectal sphincter. Measurements were carried out at an ambient temperature maintained between 24.5 and 25.5°C To determine the effects of IVT calcium or lanthanum administration on body temperature, ions were injected as described above and rectal temperature was determined before ion injection (control temperature) and 30, 60, 90 and 180 min after injection To determine the effects of calcium administration on the hypothermic effects of various drugs, calcium or ACSF was injected IVT and 30 min later various doses of ethanol, *t*-butanol, pentobarbital or chloral hydrate were injected IP. Temperature was determined before calcium injection (control temperature) and 30, 60, 90 and 180 min after ion injection Each treatment was evaluated using groups of seven to ten mice. Each group of mice was tested at all four time points and then discarded.

*Chronic Administration of Ethanol*

The liquid diet method of Walker and Freund [30] was used for chronic ethanol administration The alcohol diets contained 7% v/v ethanol, 10% v/v tap water and 83% v/v Sego liquid diet food The diets were fortified with 3 g/l vitamin diet fortification mixture and 5 g/l salt mixture XIV In the control diet, sucrose was substituted equicalorically for ethanol by adding 7% v/v of a solution of 1.43 g/ml sucrose in tap water. Diets were administered in calibrated bottles which were cleaned and filled daily with fresh diet Mice were housed five per cage and were food deprived overnight before being given access to the liquid diets To assure that the control group received a diet which was equicaloric to that consumed by the alcohol group, the average consumption of each cage of alcohol animals during the previous 24 hr was determined and this volume of the sucrose diet was then given to each cage of control mice Ethanol intake averaged about 20 g/kg/day Seven days exposure to the diets produced a mean weight loss of about 4 g for both alcohol and sucrose groups

*Effects of Calcium and Magnesium on Signs of Ethanol Withdrawal*

After mice had been given access to ethanol-containing diet for seven days, the diet was removed. Beginning three hours later, each animal was scored hourly for convulsions on handling as described by Goldstein [7] This scoring system was modified slightly to include a score of 0 5 if the mouse vocalized when lifted by the tail as this sign was prevalent in mice withdrawn from alcohol but not in control animals After each animal was scored, body temperature was determined as described previously. Ethanol withdrawal has been shown to alter body temperature in mice [22]. Six hours after withdrawal, mice were injected IVT with either ACSF, calcium (0 2 or 0.4 μmol/mouse) or magnesium (0 2 or 0 4 μmol/mouse) or were injected SC with either saline or calcium gluconate (1 g/kg) or IP with saline or $MgCl_2$ (300 mg/kg) Thirty minutes after ion administration, withdrawal scores and body temperatures were again determined This evaluation was repeated 30 min later (7 hours after withdrawal) and at hourly intervals until 9 hours had elapsed since withdrawal We observed, as have Goldstein [8] and Goldstein and Arnold [9], that the severity of withdrawal among individual mice, especially those of the Swiss-Webster strain, varies greatly even though they have been exposed to the liquid diet for the same length of time. In order to select animals with a high degree of physical dependence, any animal with a withdrawal score of less than 1 at the time of ion injection (six hours after withdrawal) was eliminated from the study. This resulted in rejection of about 30% of the mice tested

*Determination of EGTA $LD_{50}$*

To determine the effects of acute ethanol treatment on the toxicity of EGTA, mice were injected IVT with various

doses of EGTA and were then immediately injected IP with 4 g/kg ethanol. One hour later, the number of dead animals in each group was recorded. To determine EGTA toxicity during ethanol withdrawal, mice were given an ethanol containing diet or an equicaloric diet (described above) for 5 days In an attempt to equalize the time of withdrawal, ethanol consuming animals were given ethanol (1 2 g/kg, IP) at the time of withdrawal (the morning of the sixth day of ethanol consumption). This protocol produced a low level of dependence Withdrawal signs were rated hourly as described above Seven hours after removal of the diets, both groups of mice were injected IVT with various doses of EGTA and the number of deaths was recorded one hour later To determine the toxicity of the Ca-EGTA complex, a solution containing equimolar concentrations of $CaCl_2$ and EGTA (acid form) was prepared in saline by adding NaOH to adjust the pH to 7 8 For this solution, the injection volume was increased to 10 $\mu$l due to solubility limitations.

*Statistical Methods*

$ED_{50}$'s and $LD_{50}$'s and their 95% confidence limits were determined from a probit vs log dose analysis using the method of Litchfield and Wilcoxon [17]. Between group differences in sleeping-time, body temperature and withdrawal scores were evaluated by the Student's *t*-test for unpaired data, while within-group changes in body temperature (after ion or drug treatment) were evaluated by a Student's *t*-test for paired observations When more than one treatment group was compared with a control group the level of significance was determined using Dunnett's tables for multiple comparisons with a control. The effects of ions on the time course of ethanol withdrawal (Figs. 5 and 6) were evaluated by an analysis of variance for repeated measures Individual points on the time course were compared by a Student's *t*-test

## RESULTS

*Effects of Cations on the Duration of the Loss of Righting Reflex Produced by Ethanol*

Pretreatment of mice with calcium increased, in a dose dependent manner, the duration of the loss of righting reflex (sleeping time) produced by ethanol. As can be seen in Fig 1, IVT injection of calcium at a dose of 0 2 $\mu$mol/mouse increased ethanol sleeping time by two- to three-fold, while a dose of 0.4 $\mu$mole increased sleeping time by three- to four-fold (Fig 2). A dose of 0.1 $\mu$mole increased sleeping-time by 35%, which was not statistically significant (data not shown) This effect of calcium was mimicked by manganese, but ethanol sleeping-time was not altered by magnesium or by the calcium antagonists lanthanum and verapamil when the agents were injected IVT (Fig 1). Magnesium also failed to alter the effects of calcium when the two ions were administered simultaneously at equimolar concentrations (Fig 1).

*Effects of Calcium on the Duration of the Loss of Righting Reflex Produced by Other Drugs*

Pretreatment with calcium lengthened the duration of the loss of righting reflex produced by *t*-butanol, chloral hydrate and pentobarbital. As shown in Fig. 2, a calcium dose which increased ethanol sleeping time by three- to four-fold also greatly increased *t*-butanol sleeping time. However, the calcium treatment was less effective in prolonging the actions of

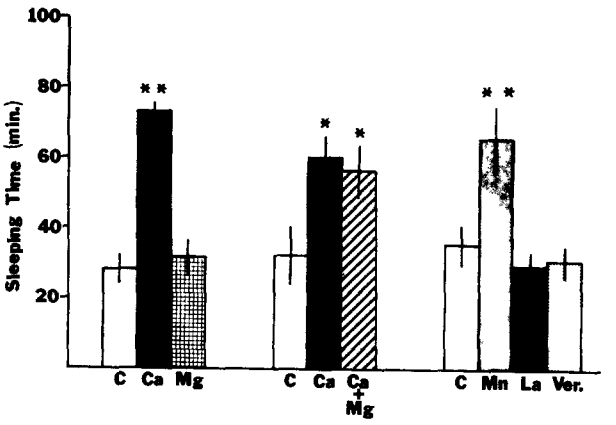


FIG 1 Effects of cations on the duration of loss of righting reflex (sleeping time) produced by ethanol (4 g/kg, IP) Ions were injected IVT 30 min before ethanol administration Dosages were $Ca^{++}$, $Mg^{++}$, $Mn^{++}$ and $La^{+++}$, 0 2 $\mu$mol/mouse, verapamil (Ver.) 0 1 $\mu$mol/mouse C indicates control animals pretreated with appropriate vehicle Vertical bars represent ± SEM * Significantly different from control, $p<0$ 05,**, $p<0$ 01

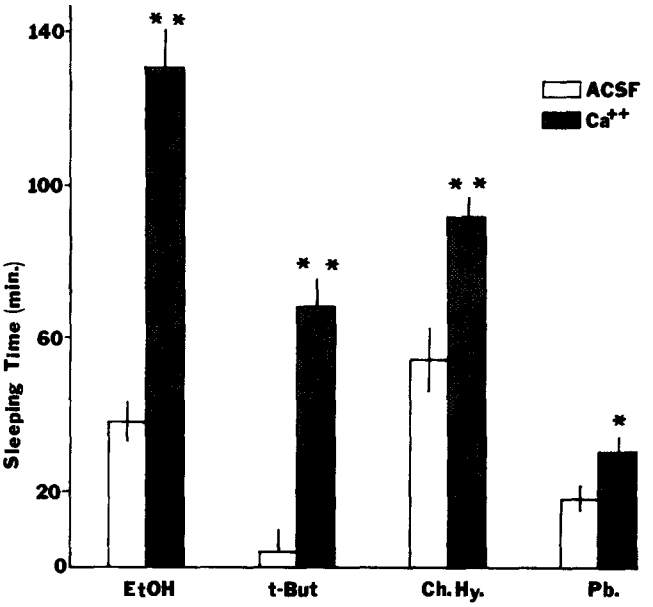


FIG 2 Effects of calcium on the duration of loss of righting reflex (sleeping time) produced by IP administration of ethanol (4 g/kg), *t*-butanol (1 g/kg), chloral hydrate (400 mg/kg) and pentobarbital (50 mg/kg) ACSF or $Ca^{++}$ (0 4 $\mu$mol/mouse) were injected IVT 30 min before drug injection Vertical bars represent ± SEM *Significantly different from ACSF, $p<0$ 05,**, $p<0$ 01

chloral hydrate or pentobarbital. Chloral hydrate sleeping time was increased by about 70%, while pentobarbital sleeping time was increased by only 50% Thus, calcium potentiates the effects of several sedative-hypnotic drugs, but it was most effective with the two alcohols tested.

*Effects of Calcium on the $ED_{50}$ for Loss of Righting Reflex*

The results presented above clearly demonstrate that

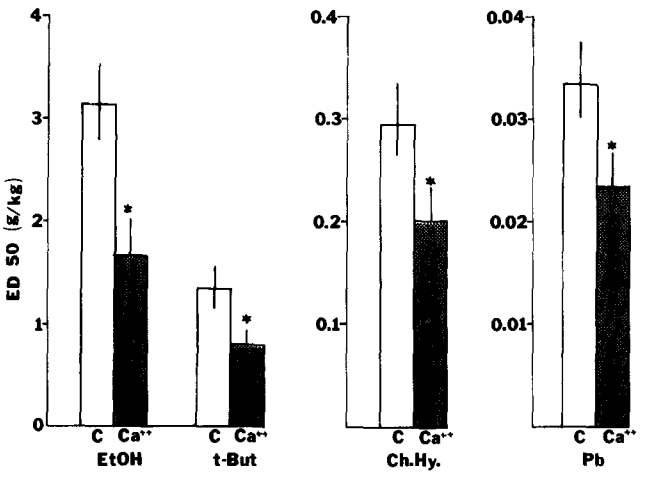


FIG 3 Effects of calcium on the $ED_{50}$ for loss of righting reflex for ethanol, *t*-butanol, chloral hydrate and pentobarbital $Ca^{++}$ (0 4 μmol/mouse) or ACSF (denoted C) was injected IVT 30 min before the other drugs were injected IP Vertical bars represent 95% confidence limits *Significantly different from control, $p<0$ 05

calcium increases the duration of the narcosis induced by relatively high doses of ethanol and other sedatives It was also of interest to determine if calcium could potentiate the effects of lower doses of these drugs This was accomplished by determining the effect of calcium on the $ED_{50}$ for loss of righting reflex for several drugs From Fig. 3 it can be seen that calcium pretreatment decreased the $ED_{50}$ of ethanol, *t*-butanol, chloral hydrate and pentobarbital This potentiation of the drug effect was most pronounced with ethanol and less marked for *t*-butanol, chloral hydrate and pentobarbital. With each of the four drugs tested, a dose of the drug which produced no loss of righting reflex in animals pretreated with ACSF produced a loss of righting reflex in 75 to 100% of the animals pretreated with calcium These results clearly indicate that pretreatment with calcium decreases the threshold dose required to produce a loss of the righting reflex It should be noted that the dosages of calcium used in these studies did not themselves produce loss of righting reflex or any signs of sedation

*Effects of Cations on Ethanol-Induced Hypothermia*

Acute administration of ethanol produced a marked hypothermia, and injection of calcium alone also produced a significant, though less pronounced, decrease in body temperature However, as is shown in Fig 4 (upper panel) the hypothermic effect of ethanol was not additive with that of calcium Alone, or in conjunction with calcium, ethanol hypothermia was maximal at about 30 min after injection and persisted for at least 120 min (Fig 4) Similar results were obtained using various doses of pentobarbital, chloral hydrate or *t*-butanol, as well as other doses of ethanol and calcium (not shown) The effects of lanthanum on body temperature and ethanol hypothermia are also shown in Fig 4 Lanthanum, like calcium, produced a significant hypothermia when administered alone When ethanol was given to mice pretreated with lanthanum, body temperature followed a time course similar to that of animals given only lanthanum

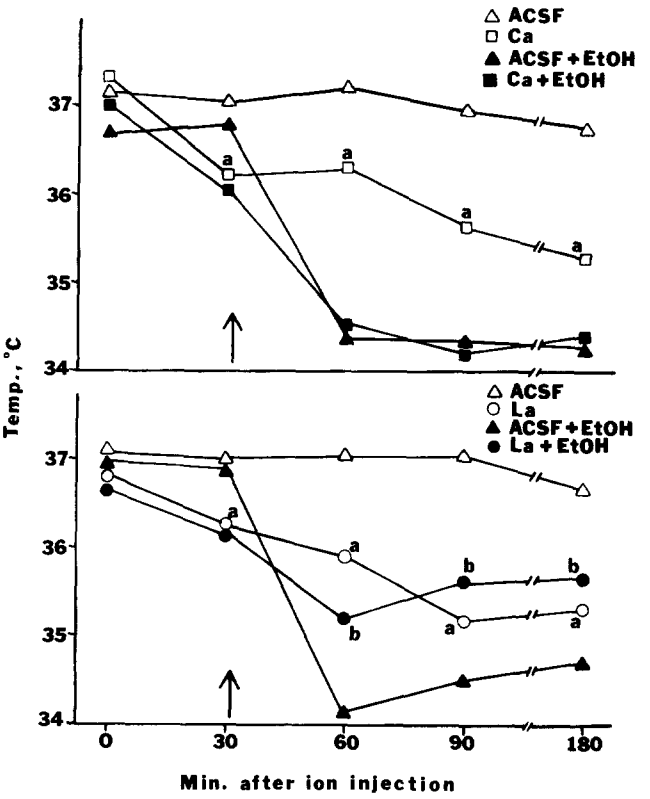


FIG 4 Effects of calcium, lanthanum and ethanol on body temperature Rectal temperature was determined immediately before IVT injection of ACSF, $Ca^{++}$ or $La^{+++}$ (time 0) and 30, 60, 90 and 180 minutes after ion injection Immediately after the 30 min temperature measurement, half of each of the three groups (ACSF, $La^{+++}$, $Ca^{++}$) were injected IP with 4 g/kg ethanol (denoted by vertical arrow) "a" Indicates significantly different from ACSF, $p<0$ 05, "b" is significantly different from ACSF plus ethanol, $p<0$ 05 The SEM of the values ranged from 0 1 to 0 3°C

(Fig 4, bottom panel) Thus, lanthanum antagonized the hypothermic effects of ethanol

*Effects of Ethanol Treatments on the Toxicity of EGTA*

In view of the data presented above suggesting an involvement of calcium in the actions of ethanol, it was of interest to determine if ethanol treatment would alter the effects of the specific calcium chelator, EGTA To investigate this possibility, the effects of acute ethanol injection and the effects of ethanol withdrawal on the IVT toxicity of EGTA were studied As is shown in Table 1, acute ethanol intoxication significantly increased the EGTA $LD_{50}$ by almost 2-fold while ethanol withdrawal decreased the EGTA $LD_{50}$ by almost 3-fold Thus, acute ethanol treatment protected the animals against the toxic effects of EGTA while ethanol withdrawal increased the CNS toxicity of this calcium chelator The toxicity of EGTA may be attributed to chelation of brain calcium as EGTA is at least 15 times more toxic than the Ca-EGTA complex (Table 1) The increased toxicity of EGTA appears to be a sensitive indicator of ethanol withdrawal as these animals had consumed alcohol for only five days and showed only slight signs of withdrawal

TABLE 1

EFFECTS OF ETHANOL TREATMENTS ON THE TOXICITY OF EGTA IN MICE

| Treatments | EGTA $LD_{50}$ μmol/mouse, IVT (95% C L) |
|---|---|
| Saline* | 0 28 (0 20–0 39) |
| Ethanol, 4 g/kg | 0 49 (0 41–0 59)‡ |
| Withdrawal from control diet† | 0 37 (0 24–0 57) |
| Withdrawal from ethanol diet | 0 14 (0 10–0 20)§ |
| Untreated | Ca-EGTA $LD_{50}$ >5 0¶ |

*Saline or ethanol was injected IP immediately after IVT injection of various doses of EGTA The EGTA $LD_{50}$ was determined after one hr

†Mice were given either control or ethanol containing liquid diets (see Methods for details) for 5 days Both diets were removed and seven hr later EGTA was injected IVT The EGTA $LD_{50}$ was determined after one hr

‡Significantly different from saline, $p<0$ 05

§Significantly different from control diet, $p<0$ 05

¶Of 10 mice given 5 μmol/mouse Calcium EGTA, one of the mice died within one hr

(average score of 0.45 for convulsions on handling while control mice had a mean score of 0.11) immediately before injection of EGTA

*Alteration of Ethanol Withdrawal by Calcium and Magnesium Administration*

After consuming a liquid diet containing 7% v/v ethanol for seven days, mice showed clear signs of withdrawal (tremor, irritability, seizure susceptibility and hypothermia) when deprived of ethanol The severity of these withdrawal signs and their time course were quantified by scoring the response of the animals to handling as described by Goldstein [7] and by measurement of body temperature as described by Ritzman and Tabakoff [22] To test the effects of calcium and magnesium on withdrawal, mice were rated hourly from 3 to 6 hours after withdrawal. At 6 hours after withdrawal, those mice having a withdrawal score of 1 0 or greater were injected with either ACSF, calcium or magnesium Withdrawal signs were rated 30 minutes later and at hourly intervals thereafter Analysis of the time courses presented in Fig. 5 indicated a significant $F(2/32)=4$ 4, $p<0$ 02, effect of the ion injections The effects of IVT calcium injection (0.4 μmol/mouse) on withdrawal scores are presented in the bottom panel of Fig. 5 Within 30 min after calcium injection, the withdrawal signs dramatically decreased to a score of 0 2 (Fig 5, bottom panel). At an hour after injection, the withdrawal signs had increased somewhat but remained well below their pre-injection levels A lower dose of calcium (0 2 μmol/mouse IVT) produced a slight suppression of withdrawal, while SC administration of calcium gluconate (1000 mg/kg) did not significantly alter the withdrawal score (not shown) Magnesium, like calcium, also suppressed withdrawal signs but was less effective at the same dosage As can be seen in Fig 5 (upper panel), for the magnesium group the withdrawal score reached a maximum of about 2.0 at six hours after withdrawal of magnesium administration at

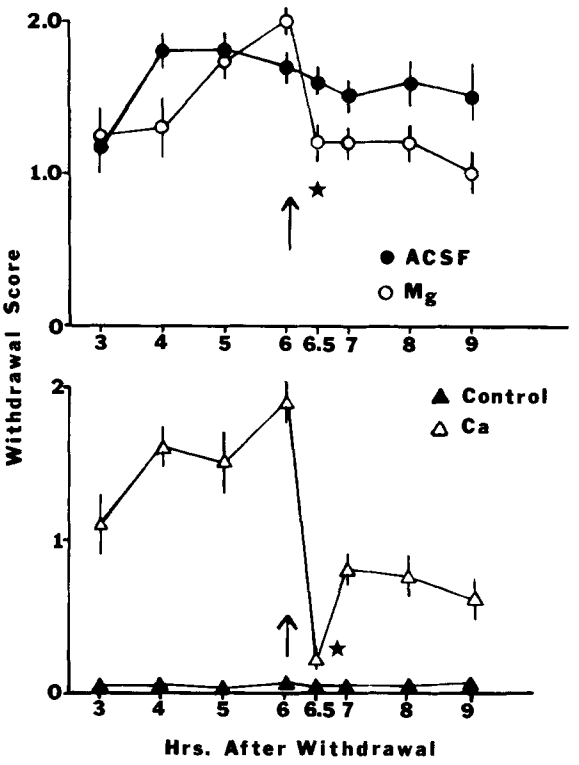


FIG 5 Effects of calcium and magnesium on ethanol withdrawal signs Mice ingesting ethanol or sucrose containing diets for seven days were withdrawn from the diet and scored for withdrawal signs [7] from three to nine hours after removal of the diets After being scored at the sixth hour of withdrawal, mice consuming the ethanol diet were injected IVT with ACSF (n=10), $Ca^{++}$ (0 4 μmol/mouse, n=11) or $Mg^{++}$ (0 4 μmol/mouse, n=14) Control mice consuming the sucrose diet (n=6) were not injected Vertical bars represent ± SEM Stars signify significantly different values ($p<0$ 01) from preceding time point

this time produced a partial suppression of withdrawal signs. A lower dose of magnesium (0.2 μmol/mouse) or IP administration of $MgCl_2$ (300 mg/kg) failed to suppress withdrawal (not shown). In contrast to the reduction of withdrawal by calcium and magnesium, injection of ACSF did not alter the time course of withdrawal (Fig 5, top panel) In addition, mice consuming a control diet did not show any signs of withdrawal after removal of this diet (Fig 5, bottom panel).

Body temperature was also measured in the animals which were evaluated for withdrawal signs and these data are shown in Fig 6. Injection of ions significantly, $F(2/32)=14.0$, $p<0$ 001, altered the time course of the withdrawal hypothermia The bottom panel of Fig. 6 shows a progressive decrease in temperature during withdrawal reaching about 35.2° by 6 hours after withdrawal Injection of calcium at this time resulted in a dramatic reversal of this hypothermia with body temperature increasing to 37 7° by 1 hour after calcium injection The increase in body temperature produced by calcium in ethanol dependent mice is in sharp contrast to hypothermic effects of calcium noted in non-dependent animals (Fig 4) In contrast to the effects of calcium, magnesium injection did not produce a clear reversal of the withdrawal hypothermia At one hour after mag-

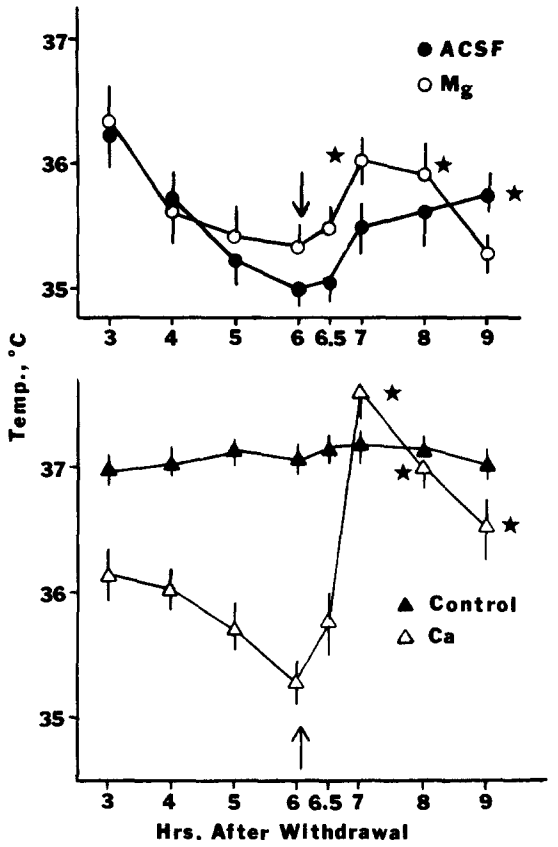


FIG 6 Effects of calcium and magnesium on body temperature during ethanol withdrawal Mice ingesting ethanol or sucrose containing diets for seven days were withdrawn from the diet and rectal temperature was measured at intervals from three to nine hours after removal of the diets After temperature was determined at the sixth hour of withdrawal, mice consuming the ethanol diet were injected IVT with ACSF (n=10), $Ca^{++}$ (0 4 μmol/mouse, n=11) or $Mg^{++}$ (0 4 μmol/mouse, n=14). Control animals consuming the sucrose diet (n=6) were not injected Vertical bars represent ± SEM Stars signify significantly different ($p<0$ 01) from values at six hours after withdrawal

nesium injection, body temperature was increased by 0.7°, but one hour after injection of ACSF body temperature increased by 0.4°, indicating that magnesium was less effective than calcium in reversing the hypothermia produced by ethanol withdrawal (Fig. 6, upper panel). Mice withdrawn from a control diet maintained their body temperature at about 37° (Fig 6, bottom panel).

## DISCUSSION

Calcium administration was found to potentiate the narcosis produced by ethanol, *t*-butanol, chloral hydrate and pentobarbital in mice This potentiation by calcium was seen as an increased duration of the loss of righting reflex as well as a reduced $ED_{50}$ for narcosis Calcium was most effective in potentiating the effects of ethanol and *t*-butanol and was less effective in increasing the effects of chloral hydrate and pentobarbital, suggesting quantitative, if not qualitative, differences between these drugs. This effect of calcium was mimicked by manganese, but neither magnesium, nor the calcium antagonists, lanthanum and verapamil, affected ethanol narcosis In addition, administration of magnesium in conjunction with calcium did not reduce the effect of calcium An important function of calcium in the nervous system involves the regulation of neurotransmitter release, however, the effects of magnesium and manganese suggest that calcium was not acting by directly increasing neurotransmitter release as this effect of calcium is not mimicked by manganese and is blocked by magnesium [26] The ability of calcium and manganese, but not other ions, to potentiate ethanol narcosis is in agreement with the results of Tyler and Erickson [29] and Erickson *et al* [4] In view of the pronounced effect of calcium on ethanol narcosis, it is surprising that lanthanum, a calcium antagonist, was without effect Tyler and Erickson [29] have reported that the calcium chelator, EGTA, reduced the sleeping-time of ethanol when no exogenous calcium was given, suggesting that there may be a pool of brain calcium critical for ethanol narcosis which is affected by EGTA but not by lanthanum The ineffectiveness of verapamil may be related to recent observations suggesting that it is relatively ineffective in blocking calcium channels in the central nervous system [18] In the present experiments, EGTA toxicity was reduced by acute administration of ethanol This observation is consistent with the hypothesis that ethanol affects a fraction of the brain calcium which is available for chelation by EGTA, although a physiological antagonism between the sedative effects of ethanol and the excitatory effects of EGTA has not been ruled out

The hypothermic effect of ethanol was affected quite differently by ions than was the soporific effect as central administration of calcium did not alter ethanol hypothermia The effects of lanthanum and ethanol on thermoregulation were of particular interest as lanthanum antagonized the hypothermic effects of ethanol although it did not reduce ethanol narcosis. As was seen with ethanol, calcium failed also to potentiate the hypothermic effects of pentobarbital, chloral hydrate and *t*-butanol although it did potentiate their soporific effect The differential effects of ions on the soporific effect and hypothermic effect of these drugs implies that the effects may be independently manipulated and suggests that different mechanisms are responsible for these two effects of alcohols and sedatives. Since calcium administration did not enhance all of the effects of ethanol or other sedatives, it is unlikely that the mechanism underlying its potentiation of the soporific effects involved an increase in the brain levels of these drugs. Indeed, Erickson *et al* [3,4] noted that calcium administration does not alter uptake of ethanol into the brain

In addition to its effect on ethanol narcosis, calcium injection also dramatically altered the signs of ethanol withdrawl When calcium was injected at the time of peak withdrawal, the convulsions on handling, a useful estimator of intensity of withdrawal [7,9], were promptly suppressed. In addition, the hypothermia which is another index of severity of withdrawal [22], was effectively reversed by calcium administration This reversal of withdrawal hypothermia was particularly striking since calcium itself produced hypothermia in untreated animals, yet calcium was able to elevate body temperature to normal levels in animals undergoing alcohol withdrawal. The importance of calcium availability during ethanol withdrawal was also studied by comparing the CNS toxicity of EGTA in mice withdrawn from either an ethanol diet or a control diet During ethanol with-

drawal the toxicity of EGTA was increased. This increased toxicity was demonstrated with a regimen of ethanol administration which produced only minimal signs of dependence, indicating that EGTA toxicity may represent a sensitive indicator of ethanol withdrawal. The data obtained with EGTA, taken together with the effects of calcium on withdrawal, suggest that a relative deficiency of brain calcium exists during alcohol withdrawal. When this deficit is corrected by administration of exogenous calcium, withdrawal signs are suppressed, while chelation of brain calcium by EGTA during withdrawal produces enhanced toxicity Withdrawal signs were also suppressed by central injection of magnesium, but at an equimolar dosage magnesium was considerably less effective than calcium. Systemic administration of calcium gluconate or $MgCl_2$ did not alter the signs of alcohol withdrawal, although serum and brain magnesium are known to be reduced in mice during ethanol withdrawal [1,15] Although chronic administration of magnesium has been found to reduce symptoms of ethanol withdrawal in humans [5, 28], the ineffectiveness of acute treatments is not surprising since a single peripheral injection of these agents produces only a small increase in the level of magnesium and no increase in the level of calcium in brain tissue [2,31]. Thus, the failure of peripheral administration of magnesium to reduce signs of withdrawal in the present study may be due to the use of a single injection of magnesium rather than chronic infusion

The interactions between inorganic ions and opiates have been the subject of several investigations and it is of interest to compare the results to those obtained with alcohols and sedatives in the present study. Calcium, manganese and magnesium reduced the analgesic effects of morphine [12,16] while calcium and manganese, but not magnesium, increased the soporific effect of alcohols and sedatives Both lanthanum and EGTA potentiated the effects of morphine [12–14] while lanthanum antagonized the hypothermic effects of ethanol and EGTA antagonized the narcotic effect [29]. Thus, the influence of ions on the actions of opiates was generally opposite to their influence on the actions of alcohols and sedatives. This is somewhat surprising since both opiates and alcohols have been reported to reduce the calcium content of brain tissue [23,25]. However, other studies indicate that morphine, but not ethanol, decreases the calcium content of brain synaptosomes [15]. The ability of EGTA to reduce ethanol narcosis and of ethanol to reduce EGTA toxicity suggest that acute ethanol administration may allow normal brain functions despite the chelation of a portion of the brain calcium. This would suggest either a decreased requirement for calcium or an increased calcium availability during ethanol intoxication. In contrast to these effects of acute ethanol intoxication, ethanol withdrawal appears to produce a deficit of brain calcium. This could be due to a depletion of brain calcium during withdrawal or to an increased requirement for calcium after chronic ethanol treatment

These results demonstrate that the intoxication produced by alcohols and sedatives may be modulated by the availability of inorganic cations, particularly calcium in brain tissue. In addition, signs of ethanol withdrawal may also be modified by altering the availability of brain calcium and magnesium The possibility that alterations in brain calcium and magnesium localization are related to the mechanism underlying the actions of alcohols and sedatives is currently under investigation in this laboratory [15]

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