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Alcohols and Synaptosomal Calcium Transport

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SUMMARY

Synaptosomes were isolated from rat and mouse brain and the effects of alcohol addition on calcium uptake were studied *in vitro*. Ethanol inhibited potassium-, glutamate-, and veratridine-stimulated calcium uptake. The inhibitory effects were obtained with concentrations of ethanol (≤100 mM) which did not alter synaptosomal membrane potentials. Elevation of extrasynaptosomal calcium reduced the inhibitory effect of ethanol, resulting in apparently competitive kinetics. The inhibitory potencies of a series of alkanols were correlated with their membrane/buffer partition coefficients, implicating hydrophobic regions of the membrane as the site of alcohol action. However, not all nerve endings were equally sensitive to ethanol. Inhibition of potassium-stimulated calcium uptake was greater with synaptosomes prepared from cerebellum and striatum than with preparations from cortex or brain stem. In contrast, veratridine-stimulated calcium uptake (which is dependent upon sodium influx) was inhibited more strongly in cortex than in cerebellum. These results suggest that the calcium channels in cerebellum are more sensitive to ethanol than the calcium channels in cortex, whereas sodium channels in cortex are more sensitive than those in cerebellum.

INTRODUCTION

Neuronal calcium fluxes play a critical role in the regulation of synaptic activity and neurotransmission. An influx of calcium at the nerve ending initiates neurotransmitter release, alters the activity of intracellular enzymes, and may affect the physical properties of the presynaptic membrane (1, 2). Neuronal depolarization produced by elevated potassium or veratridine stimulates calcium entry through voltage-dependent channels, whereas neurotransmitters such as acetylcholine and glutamate appear to stimulate calcium entry through voltage-independent channels (1, 3, 4). Phosphatidate, a membrane phospholipid, has been suggested as a neuronal calcium ionophore (5). It is apparent that calcium channels are potential sites of action for psychoactive drugs. Indeed, barbiturates, anticonvulsants, cannabinoids, and ethanol alter some aspects of neuronal calcium transport (6, 7). Ethanol exposure *in vitro* and *in vivo* has been shown to inhibit depolarization-induced calcium uptake by isolated brain synaptosomes (7). However, electrophysiological and behavioral observations indicate that different brain regions or synaptic pathways must vary in their sensitivity to ethanol. Because calcium is a ubiquitous link in stimulus-secretion coupling, it is not clear whether the effects of ethanol on calcium transport display sufficient selectivity to be useful in understanding the neuropharmacological actions of the drug. The purpose of the present study was to determine which, if any, factor might alter the sensitivity of synaptosomal calcium transport to the effects of ethanol. Experiments were designed to answer the following questions about the inhibitory effect of ethanol: (a) Is it dependent on the degree of depolarization? (b) Is it dependent on the extrasynaptosomal concentration of calcium? (c) Is it equal synaptosomes from different brain regions? (d) Is it equal for calcium uptake induced by potassium, veratridine, glutamate, and phosphatidate? (e) Is it the result of changes in synaptosomal membrane potentials? (f) Is it related to the ability to partition into the membrane? Results from these experiments indicate that the sensitivity of synaptosomal calcium transport to ethanol depends on several of these factors.

METHODS

Animals. Male CD-1 mice, 20–30 g (Charles River Breeding Laboratory, Inc., Portage, Mich.), were housed six per cage, and male Sprague-Dawley rats, 150–300 g (Charles River Breeding Laboratories, Wilmington, Mass.), were housed three per cage. All animals had free access to water and standard laboratory chow for at least 5 days prior to experimentation.

Materials. Drugs and suppliers were as follows: veratridine, L-glutamate, and sodium phosphatidate (egg yolk) (Sigma Chemical Company, St. Louis, Mo.); tritiated

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TPP[1] (kindly provided by Dr. Ronald Kaback, Roche Institute, Nutley, N. J.); nonradioactive TPP chloride (Tridom [Fluka] Chemical Company, Hauppauge, N. Y.). Other materials were obtained from sources indicated in a previous report (7).

Preparation of synaptosomes and measurement of calcium accumulation. Ficoll density gradient centrifugation was used to prepare synaptosomes from mouse and rat brain regions (7). The procedure was identical for all tissues. Calcium accumulation by depolarized and nondepolarized synaptosomes was determined as in a previous study (7). Unless otherwise indicated, the uptake period was 1 min.

In one experiment, 20 mM Hepes was substituted for the 20 mM Tris normally used to buffer the solutions. In another experiment, the calcium concentration was varied from 1.1 to 5.5 mM. Glutamate-stimulated uptake was produced by adding 0.3, 1.0, or 3.0 mM L-glutamate. Veratridine concentrations of 1.5–75 μM were used for veratridine-stimulated calcium uptake. The uptake period for both glutamate- and veratridine-induced calcium accumulation was 5 min.

Phosphatidate-induced calcium accumulation was done by allowing 0.05 ml of 2 mM phosphatidate (suspected in water) to incubate with the synaptosomes for 2 min before a nondepolarizing potassium-^{45}Ca solution was added (5). The uptake period was 4 min.

Measurement of TPP accumulation. Synaptosomal membrane potential was estimated by the accumulation of [^{3}H]TPP, as described by Lichtschtein *et al.* (8). A 0.1-ml aliquot of synaptosomes (5 mg of protein per milliliter) was incubated for 4 min at 30°, followed by a 1-min incubation with 20 μl of water (control) or ethanol-water solutions, and a 5-min incubation with 0.1 ml of [^{3}H]TPP in Na-5K (5 μM final concentration, specific activity 400 Ci/mole of TPP). The synaptosomes were then exposed for 5 min to 0.2 ml of either a depolarizing solution (65 mM K^+ final concentration) or to a nondepolarizing solution (5 mM K^+ final concentration). The solutions were the same as used for the calcium uptake experiments and have been described in detail previously (7). The solution was transferred to two 400-μl microcentrifuge tubes and the synaptosomes were pelleted. The supernatant was aspirated, the tips of the tubes were cut off, and the pellets were digested overnight (0.5 ml of 0.1 N NaOH). A scintillation cocktail was added, and liquid scintillation spectrometry was used to determine radioactivity.

Determination of intrasynaptosomal volume. Synaptosomes were incubated for 10 min at 30° in depolarizing (65 mM K^+) or nondepolarizing (5 mM K^+) solutions containing ^3H_2O and [^{14}C]sorbitol (New England Nuclear Corporation, Boston, Mass.) and then pelleted in 400-μl microcentrifuge tubes. After the supernatant was aspirated, the pellet was solubilized (0.1 N NaOH), and radioactivity was determined using a Beckman LS9000 instrument programmed for dual isotope quantitation. The total volume of the pellet was determined from the ^3H_2O content, the extracellular space by the [^{14}C]sorbitol content, and the intracellular space by the difference between the two (8). An average value for intrasynaptosomal volume was 3.6 μl/mg of protein. Neither depolarization nor addition of ethanol altered the synaptosomal volume. Concentration of gradients were calculated as $[TPP]_{in}/[TPP]_{out}$ and can be used to determine ψ_m by the method of Lichtschtein *et al.* (8).

Other methods. Protein was determined by the method of Lowry *et al.* (9). A *t*-test for paired observations was used for statistical evaluation of the effects of ethanol.

RESULTS

Ethanol effects on depolarization-dependent calcium uptake. The *in vitro* effect of ethanol on calcium uptake in mouse brain synaptosomes stimulated by increasing concentrations of potassium is shown in Fig. 1. A greater than 2-fold increase in calcium uptake was seen when the potassium concentration was raised from 5 mM to 35 mM; increasing the potassium concentration beyond this level had a smaller effect on calcium uptake. Ethanol (100 m M and 400 mM) inhibited uptake in a concentration-dependent manner in synaptosomes exposed to more than 5 mM potassium. The effects of ehtanol were greater on depolarization-induced uptake than on resting uptake, but the degree of depolarization was not an important determinant of the inhibitory potency of ethanol.

Calcium dependence of ethanol inhibition of depolarization-dependent calcium uptake. Figure 2 shows the effects of extracellular calcium on the depolarization-dependent calcium uptake of mouse brain synaptosomes. Increasing the calcium concentration increased the calcium uptake, although not in a proportional manner; calcium increases of 270% and 490% caused only a 30% and 40% rise, respectively, in uptake.

Ethanol effects were measured at each calcium level and, as before, ethanol produced a concentration-dependent inhibition of calcium uptake. A double-reciprocal plot (Fig. 2) yielded a straight line for the control and for each of the two ethanol doses. The close grouping of the *y*-intercepts for the three lines suggest that the apparent V_{max} for calcium transport was similar, but the apparent calcium binding coefficient (K_m) was reduced by ethanol. A 100 mM concentration of ethanol reduced the apparent K_m by almost 50% ($p < 0.05$).

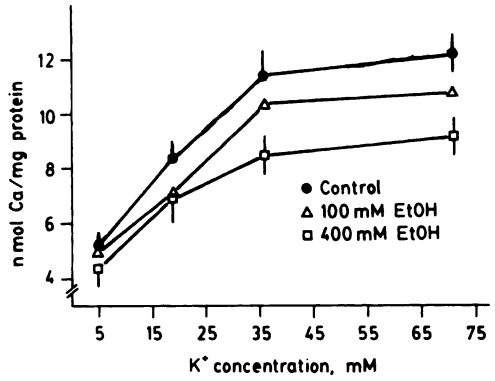

FIG. 1. *Effects of ethanol on potassium-stimulated synaptosomal calcium uptake*

Mouse brain synaptosomes were preincubated *in vitro* with no ethanol (●), 100 mM ethanol (△), or 400 mM ethanol (□) and exposed to the concentrations of potassium shown on the abscissa. The amount of calcium accumulated within a 1-min period is shown on the ordinate. Vertical bars represent ± standard error of the mean, $N = 4$–6.

[1] The abbreviations used are: TPP, tetraphenylphosphonium, Hepes, 4-(2-hydroxyethyl)-1piperazineethanesulfonic acid.

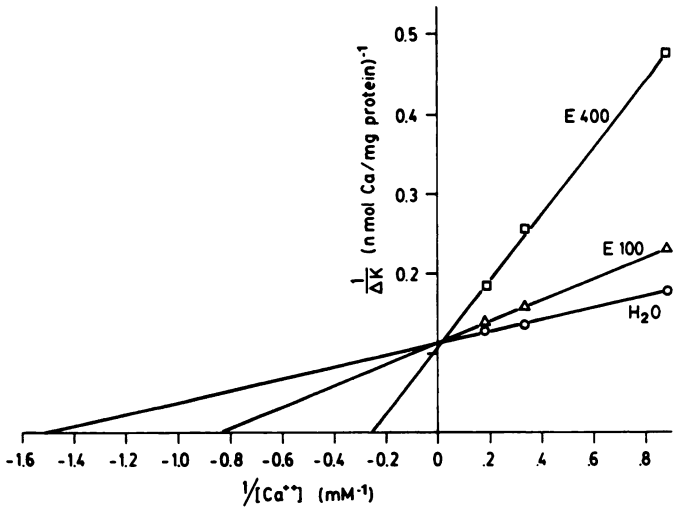

FIG. 2. *Effects of ethanol synaptosomal calcium uptake as a function of calcium concentration*

Mouse brain synaptosomes were preincubated with no ethanol (○), 100 mM ethanol (△), or 400 mM ethanol (□), and potassium-dependent uptake was determined with three concentrations of calcium. The potassium concentration was 68 mM. Each point represents the mean of four determinations.

Membrane partitioning and alkanol effects on depolarization-dependent calcium uptake. Parameters of dose-response curves for a series of alkanols and their effects on potassium-stimulated calcium uptake over a range of concentrations are listed in Table 1. The series includes the straight-chain alkanols through hexanol and one side-chain derivative, *t*-butanol. Membrane-buffer partitioning coefficients ($P_{m/b}$) were used to determine the dose of each alkanol that would result in similar molar concentrations in the membrane (10). A dose-response curve was plotted for each alkanol as "percentage of control ΔK value" versus "log (alkanol concentration)", and the slopes and correlation coefficients of the linear regressions for those lines are listed in Table 1. The alkanol concentration necessary to produce a 50% inhibition of calcium uptake (IC_{50}) was also determined from the regression lines. As noted in a previous report (7), the effects of ethanol are biphasic—low concentrations (50–200 mM) produce some inhibition, and high concentrations (>400 mM) produce a marked inhibition. This was reflected in the relatively low correlation coefficient (0.67) for ethanol (Table 1). In contrast, the dose dependent for the other alkanols was linear and their slopes were similar (Table 1).

The IC_{50} and $P_{m/b}$ for each alkanol were plotted on a log-log scale (Fig. 3). The two parameters were found to be linearly proportional ($r^2 = 0.98$), indicating that the ability of the alkanols to penetrate into hydrophobic membrane regions is the major determinant of their potency.

Ethanol effects on calcium uptake by synaptosomes from brain areas. The *in vitro* effects of ethanol on calcium uptake by synaptosomes from various rat brain areas are shown in Fig. 4. Ethanol caused concentration-dependent inhibition of calcium uptake in each brain area, but the areas were not equisensitive to its effects. A low concentration of ethanol (50 mM) selectively inhibited calcium uptake in cerebellum and striatum, without affecting brain stem or cortex; a high concentration (400 mM) inhibited calcium uptake in all brain regions. Using synaptosomes from pig brain, we also found cerebellum to be more sensitive than cortex to ethanol (data not shown).

Control values for calcium uptake varied among brain regions, with uptake in cortex and striatum being greater than uptake in cerebellum and brain stem (Fig. 4). This may reflect the higher density of nerve endings in cortex and striatum, resulting in a more pure synaptosomal preparation from these regions. Alternatively, nerve endings from different brain regions may differ in their calcium transport properties. There was no apparent

TABLE 1

Effects of in vitro addition of n-alcohols on potassium-stimulated calcium accumulation by brain synaptosomes

Curves were determined from three or four different alcohol concentrations; $N = 4$–14 for each concentration.

Alcohol	IC_{50}[a]	Slope	r^2
	mM		
Ethanol	1331.0	−44	0.67
Propanol	133.0	−47	0.89
Butanol	37.0	−36	0.96
t-Butanol	76.0	−36	0.97
Pentanol	8.7	−40	0.96
Hexanol	1.8	−32	0.97

[a] IC_{50}, the concentration of each alcohol required to reduce uptake by 50%, was derived from the regression line of percentage of control calcium uptake versus log[alcohol concentration]. The percentage of control uptake represents (uptake in the presence of drug)/(uptake in the absence of drug) × 100. "Uptake" refers to the depolarization-dependent accumulation of ^{45}Ca.

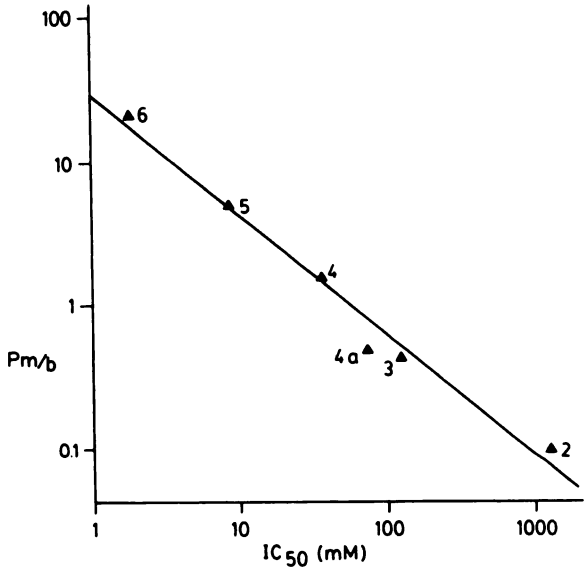

FIG. 3. *Correlation of the effect of alcohols on synaptosomal calcium transport with their membrane/buffer partition coefficients*

The abscissa presents the concentration of each alcohol required to inhibit synaptosomal calcium uptake by 50% (IC_{50}), and the ordinate presents the membrane/buffer partition coefficients (10). The number by each symbol signifies the chain length: *2*, ethanol; *3*, *n*-propanol; *4*, *n*-butanol; *4a*, *t*-butanol; *5*, *n*-pentanol; *6*, *n*-hexanol. The line was fit by linear regression analysis, $r^2 = 0.98$. (See Table 1 for details).

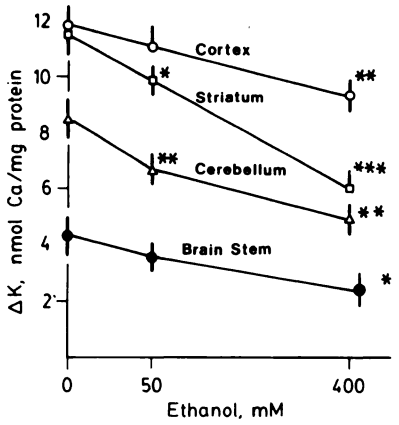

FIG. 4. *Effects of ethanol on calcium uptake by synaptosomes from regions of rat brain*

The abscissa represents the concentration of ethanol added *in vitro*; the ordinate represents the potassium-dependent uptake of calcium (ΔK, the difference between uptake in the presence of 5 mM and 68 mM KCl) during a 1-min period. ○, cerebral cortex; □, corpus striatum; △, cerebellum; ●, brain stem. Vertical bars represent ± standard error of the mean, $N = 8$. *Significant effect of ethanol, $P < 0.05$; **$p < 0.01$; ***$p < 0.001$.

relationship between control calcium uptake and the inhibitory effects of ethanol, and it is unlikely that the purity of the synaptosomes influenced the actions of ethanol.

Effects of ethanol on glutamate-stimulated calcium uptake. Electrophysiological studies indicate that glutamate enhances neuronal calcium current by a mechanism that does not involve depolarization (4). In our study, *in vitro* addition of glutamate increased synaptosomal calcium uptake (Fig. 5). The maximal stimulation (1.5- to 2-fold) occurred within 5 min after addition 1 μM glutamate and was not changed for at least 5 more min. Thus, compared with K^+-induced uptake, glutamate-induced uptake was slower in onset and smaller in magnitude.

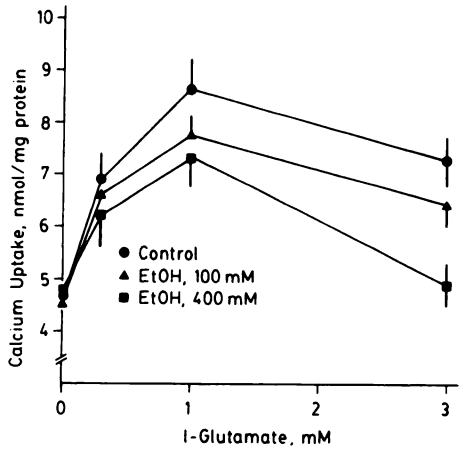

FIG. 5. *Effects of ethanol on glutamate-stimulated calcium uptake*

Mouse brain synaptosomes were preincubated with no ethanol (●), 100 mM ethanol (▲), or 400 mM ethanol (■) and exposed to the concentrations of glutamate shown on the abscissa. The uptake of calcium over a 5-min period is given on the ordinate. Vertical bars represent ± standard error of the mean, $N = 6$–8. Both concentrations of ethanol are significantly different from control ($p < 0.05$, Wilcoxon's sign-rank test).

Under conditions that stimulate calcium uptake, glutamate does not appear to depolarize synaptosomes (11), which allows a further distinction between glutamate- and K^+-stimulated calcium uptake. Despite these distinctions, *in vitro* addition of ethanol inhibited glutamate-stimulated calcium uptake in a concentration-dependent manner (Fig. 5), and the sensitivity to ethanol of glutamate-stimulated uptake was similar to that of K^+-stimulated uptake (Fig. 1). The glutamate analogue, kainate, produced similar effects (data not shown).

Effects of ethanol on veratridine-stimulated calcium uptake. Veratridine prevents the inactivation of neuronal sodium channels, leading to sodium influx and membrane depolarization (12). This sodium-dependent depolarization stimulates synaptosomal calcium uptake (13). We found that concentrations of veratridine as low as 1.5 μM enhanced calcium uptake by synaptosomes from rat cortex, whereas higher concentrations were required to stimulate uptake by cerebellar synaptosomes (Fig. 6). Ethanol inhibited veratridine-stimulated calcium uptake by cortical synaptosomes. This effect was most pronounced with the lower concentrations of veratridine, where 100 mM ethanol almost completely abolished the veratridine-dependent uptake. In contrast to cortex, ethanol produced only a modest, but significant ($p < 0.05$), inhibition of veratridine-stimulated uptake in cerebellum (Fig. 6).

Comparison of Tris and Hepes buffers. Tris has been reported to decrease calcium uptake in smooth muscle and alter the effects of ethanol in these tissues (14). To determine whether Tris exerts similar effects on brain synaptosomes, we studied potassium-dependent calcium uptake and ethanol effects in solutions buffered by equimolar amounts of Tris or Hepes. Hepes reduced both resting and stimulated calcium accumulation by 25–40%, but the inhibitory effect of ethanol was similar with the two buffers (Table 2).

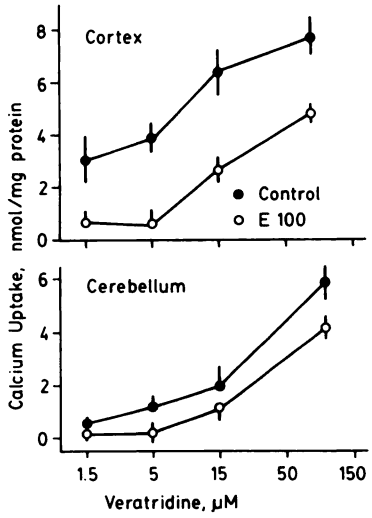

FIG. 6. *Effects of ethanol on veratridine-stimulated calcium uptake*

Synaptosomes from rat brain cortex (*upper panel*) or cerebellum (*lower panel*) were incubated with no ethanol (●) or 100 mM ethanol (○) and the concentration of veratridine shown on the abscissa on a log scale. The veratridine-dependent uptake of calcium (uptake in the presence of veratridine minus uptake in the absence of veratridine) over a 5-min period is given on the ordinate. Vertical bars represent ± standard error of the mean, $N = 4$.

TABLE 2

Comparison of Tris and Hepes buffers on potassium-stimulated calcium accumulation by mouse brain synaptosomes

[K]	Tris (20 mM)	Hepes (20 mM)[a]
mM	*nmoles calcium/mg protein*	
5	4.4 ± 0.3	2.7 ± 0.2[b]
19	8.6 ± 0.4	5.9 ± 0.3[b]
36	11.4 ± 0.4	8.3 ± 0.4[b]
71	12.6 ± 1.1	9.2 ± 0.5[b]
71 + 200 mM ethanol	9.1 ± 0.9[c]	7.1 ± 0.6[b, c]

[a] Values are means ± standard error of the mean, $N = 3$.
[b] Significantly different from Tris ($p < 0.05$).
[c] Significantly different from 71 mM K^+ control ($p < 0.05$).

Effects of ethanol on phosphatidate-stimulated calcium uptake. We have recently shown that phosphatidate stimulates synaptosomal calcium uptake (5). This membrane lipid acts as a calcium ionophore and may be involved in stimulus-secretion coupling; therefore, we were interested in determining whether ethanol inhibits phosphatidate-stimulated calcium uptake. Although phosphatidate produced a marked enhancement of synaptosomal calcium accumulation, this effect was insensitive to *in vitro* addition of ethanol (Table 3).

Effects of ethanol on synaptosomal accumulation of TPP. We determined [^{3}H]TPP accumulation in mouse brain synaptosomes in order to explore the possibility that ethanol inhibits calcium uptake by altering membrane potentials. TPP is a lipophilic cation, and its accumulation is proportional to membrane potential (8). The accumulation difference between depolarizing and nondepolarizing conditions (ΔK) reflects the plasma membrane potential, whereas the residual accumulation under depolarizing conditions ($+K$) reflects primarily the mitochondrial membrane potential (8). In our study, a 100 mM concentration of ethanol did not affect accumulation of [^{3}H]TPP, but an 800 mM concentration reduced accumulation by depolarized synaptosomes, without affecting the accumulation by nondepolarized synaptosomes (Table 4). This suggests that a moderate concentration of ethanol did not affect membrane potentials whereas a high concentration decreased the mitochondrial membrane potential and increased the plasma membrane potential.

DISCUSSION

In vitro addition of ethanol inhibited synaptosomal calcium uptake produced by exposure to glutamate, potassium, or veratridine. Studies of potassium-stimulated calcium uptake demonstrated that the inhibitory effects of ethanol were independent of the degree of depolarization and reflected an apparent decrease in the affinity of calcium for the uptake system. Phosphatidate has been suggested as a neuronal calcium carrier (5), and ethanol could produce its effects by altering the calcium-phosphatidate interaction. However, this was not the case, as even very high concentrations of ethanol did not alter phosphatidate-mediated calcium uptake. These results do not rule out the possibility that ethanol inhibits calcium transport by altering the synthesis or degradation of endogenous phosphatidate. The failure of ethanol to affect phosphatidate-stimulated calcium transport supports our theory that ethanol directly inhibits calcium influx and does not affect calcium efflux or binding (6, 7). Recent electrophysiological evidence indicates that glutamate enhancement of neuronal calcium flux does not require depolarization (4). Likewise, our experiments using TPP to measure synaptosomal membrane potentials demonstrate that glutamate does not depolarize synaptosomes under the conditions used in the present study to stimulate calcium uptake (11). The similar inhibition of glutamate-stimulated and potassium-stimulated calcium uptake by ethanol indicates that the drug acts by directly reducing calcium transport rather than by affecting the membrane potential. This conclusion is supported by lack of effect of 100 mM ethanol on synaptosomal accumulation of TPP.

The ability of a series of alcohols to inhibit calcium uptake was correlated with their membrane partitioning. This indicates a hydrophobic site of action for these compounds; this site is most likely membrane lipid, although a hydrophobic region of a protein cannot be eliminated. The potency of the alcohols in fluidizing synaptic membranes is directly related to their potency in inhibiting calcium uptake,[2] but further studies are required to establish the role of membrane fluidity in calcium transport.

Although ethanol concentrations of 100 mM or less consistently inhibited synaptosomal calcium uptake, the degree of inhibition observed in this study and others (7) was modest. These results were obtained with synaptosomes prepared from whole brain homogenates and represent many different types of nerve endings. Several studies have shown that the inhibitory effects of barbiturates and cannabinoids on synaptosomal calcium trans-

TABLE 3

Effects of ethanol on synaptosomal calcium accumulation induced by phosphatidate

	Calcium accumulation	
	Resting[a]	Phosphatidate-stimulated[a]
	nmoles calcium/mg protein	
Control	4.1 ± 0.1	10.8 ± 0.5
Ethanol		
100 mM	3.7 ± 0.1	11.3 ± 0.9
400 mM	3.6 ± 0.1	10.8 ± 0.5
800 mM	4.3 ± 0.1	10.0 ± 0.14

[a] Values are means ± standard error of the mean, $N = 4$.

TABLE 4

Effects of in vitro addition of ethanol on the accumulation of [^{3}H] TPP by mouse brain synaptosomes

	Nondepolarized ($-K^+$)[a]	Depolarized ($+K^+$)[a]	ΔK^+ [a]
Control	37 ± 2	18 ± 2	19 ± 2
Ethanol			
100 mM	37 ± 2	15 ± 1	22 ± 2
800 mM	38 ± 3	10 ± 1[b]	28 ± 3[b]

[a] Values represent the intrasynaptosomal concentration of TPP divided by the extrasynaptosomal TPP concentration. Values are means ± standard error of the mean, $N = 3$.
[b] Significantly different from control ($p < 0.05$).

[2] J. A. Stokes and R. A. Harris, unpublished observations.

port are more pronounced in certain brain regions (15, 16). These observations suggest that different nerve endings vary in their sensitivity to these drugs. Likewise, we found that potassium-stimulated calcium uptake by cerebellar and striatal synaptosomes was inhibited by 50 mM ethanol, whereas synaptosomes from cortex and brain stem were less sensitive to ethanol. In contrast to potassium-stimulated uptake, veratridine-stimulated calcium uptake by cortical synaptosomes was quite sensitive to ethanol; in fact, cortical synaptosomes were more sensitive than cerebellar preparations. These results suggest important differences in the effects of ethanol on potassium- and veratridine-stimulated calcium uptake. Potassium produces a direct depolarization which activates voltage-sensitive calcium channels, whereas veratridine prevents inactivation of sodium conductance which leads to membrane depolarization and activation of voltage-sensitive calcium channels (12, 13). In light of this information, our results suggest that in the cortex ethanol has more pronounced effects on sodium channels than on calcium channels, whereas in the cerebellum the calcium channels are more sensitive than the sodium channels. A difference between cortical and cerebellar sodium channels is also indicated by the lower sensitive to veratridine displayed by cerebellar synaptosomes. The greater sensitivity of cortical sodium channels as compared with calcium channels in excellent agreement with the results of Sunahara and Kalant (17). They reported that ethanol (110 mM) inhibited the electrically stimulated release of acetylcholine from rat cortical slices by 50–60% but did not alter potassium-stimulated release or resting release. These investigators concluded that "ethanol acts primarily on sodium influx during the action potential." In addition, ethanol (110 mM) has been reported to inhibit sodium influx with electrically stimulated cortical slices (18). Ethanol alters the physical properties of the acyl groups of synaptic membrane lipids (19, 20), and this represents a mechanism by which it could inhibit sodium influx. The sodium channel appears to be a lipoprotein, and its conformation is influenced by the surrounding lipids (12, 21). We have found that fluidization of synaptosomal membranes by insertion of unsaturated fatty acid methyl esters (22) inhibits veratridine but not potassium-stimulated calcium uptake.[2] These results suggest that ethanol-induced perturbation of membrane lipids reduces sodium conductance while ethanol may act by other ways to reduce calcium influx. However, this indirect evidence for an effect of ethanol on sodium transport must be interpreted with caution, as high concentrations of ethanol are required to inhibit sodium movement in squid axon (23). Synaptosomes have recently been reported to have "fast" and "slow" calcium channels (24). It is possible that these channels differ in their sensitivity to veratridine- and potassium-induced depolarization and to ethanol. An understanding of the sensitivity of veratridine-stimulated calcium uptake to ethanol requires an analysis of the effects of ethanol on synaptosomal sodium transport and "fast" and "slow" calcium channels. Such techniques have recently become available (24, 25).

In summary, it appears that ethanol can alter synaptic function by inhibiting sodium influx as well as glutamate-dependent and voltage-dependent calcium influx. The magnitude of inhibition of calcium influx was shown to depend on the extracellular calcium concentration and on the brain region assayed. These observations suggest that not all synapses will be affected equally by ethanol, and provide a mechanism for selective action.

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