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ETHANOL AND PENTOBARBITAL INHIBITION OF INTRASYNAPTOSOMAL SEQUESTRATION OF CALCIUM

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Abstract—Synaptic membranes sequester calcium by a nonmitochondrial, ATP-dependent mechanism. This process appears to be important in maintaining low intrasynaptosomal levels of ionized calcium. *In vitro* addition of ethanol or pentobarbital to synaptic membranes isolated from mouse brain inhibited this ATP-dependent calcium uptake. Ethanol inhibited the uptake in a concentration-dependent manner over the range of 50–1600 mM. The inhibitory effects of low concentrations of ethanol were enhanced by a reduction in the ionized calcium concentration. The effects of even the highest concentration of ethanol were completely reversed when the drug was removed from the membranes. The effects of ethanol and pentobarbital were temperature dependent. The drugs produced a significant inhibition of uptake at assay temperatures of 23–37°, but not at assay temperatures of 1–18°. A single injection of ethanol inhibited the ATP-dependent sequestration of calcium and reduced the sensitivity of the isolated membranes to *in vitro* ethanol exposure, which suggests the development of an acute tolerance. Compared with membranes from control mice, membranes from mice that were chronically ingesting ethanol accumulated less calcium and were tolerant to the *in vitro* effects of ethanol and pentobarbital. These results suggest that the known stimulatory effects of ethanol and pentobarbital on the resting release of neurotransmitters, and the development of tolerance to these effects, may be due to alterations in the intrasynaptosomal sequestration of calcium.

There is considerable evidence that ethanol and pentobarbital alter the transport of calcium in brain tissue [1–4]. These studies have shown that the depolarization-stimulated uptake of calcium by intact synaptosomes is inhibited by ethanol. The intrasynaptosomal availability of calcium is also influenced by the storage of calcium within the nerve. Recent studies have demonstrated intrasynaptosomal [5–10] and intraxonal [11, 12] sequestration of calcium. These storage sites are nonmitochondrial and accumulate calcium by a high-affinity, ATP-dependent uptake system. Little attention has been given to the effects of neuropharmacological agents on this uptake system. These considerations led us to study the effects of *in vitro* exposure to ethanol or pentobarbital and of *in vivo* administration of ethanol on the intrasynaptosomal calcium uptake. This ATP-dependent calcium uptake was assayed in lysed synaptosomes following the inhibition of mitochondrial uptake. This procedure allows the analysis of intrasynaptosomal mechanisms involved in the storage of calcium.

The results presented here demonstrate that *in vitro* exposure to ethanol and pentobarbital inhibited the ATP-dependent uptake of calcium by lysed synaptosomes. These results are discussed in terms of the stimulatory effects of these drugs on the resting release of neurotransmitters.

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MATERIALS AND METHODS

Materials. The chemicals used in this study were: sodium pentobarbital, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), tris(hydroxymethyl)aminomethane (Tris), Na_2ATP (low calcium, vanadium free), 2,4-dinitrophenol, NaN_3, oligomycin, Ficoll, and ethylene-glycolbis-(aminoethylether)tetra-acetate (EGTA) (Sigma Chemical Co., St. Louis, MO); $^{45}CaCl_2$ (sp. act. 21 mCi/mg) (New England Nuclear Corp., Boston, MA); NaCl, KCl, $MgCl_2$, NaH_2PO_4, glucose, sucrose, and $CaCl_2$ (Fisher Scientific Co., St. Louis, MO). Other materials used were Whatman GF/C filters; Sustacal Liquid Diet (Mead Johnson & Co., Evansville, IN), and Budget Solve scintillation counting mixture (Research Products International Corp., Elk Grove Village, IL).

Experimental procedures. Male CD-1 mice (Charles River Breeding Laboratories, Portage, MI), 20–30 g, were housed five or six per cage and were given free access to water and standard laboratory chow before being used for the experiments.

Acute administration of ethanol. To study the effects of acute alcohol intoxication in mice, 4 g/kg ethanol [20%(w/v) in saline], or an equal volume of saline, was adminstered intraperitoneally. The ethanol-treated mice were divided into two groups. One group was killed upon losing righting reflex (3–5 min after injection); the other group was killed upon regaining righting reflex (about 80 min after injection). The saline-injected group was killed about 20 min after injection.

Chronic administration of ethanol. For studies involving chronic administration of ethanol, mice

were given a liquid diet, which contained Sustacal with 5% (v/v) water and 7% (v/v) ethanol, for 7 days. Mice were killed between 8:00 and 9:00 a.m. The control group was pair-fed a diet containing Sustacal with 12% (v/v) water and 10% (w/v) sucrose substituted isocalorically for ethanol. The control group was pair-fed an amount of the sucrose diet equal to that consumed on the previous day by the ethanol diet group. Mice fed the liquid ethanol diet consumed an average of 15.4 ethanol·kg^{-1}·day^{-1}. All mice were given free access to water.

Preparation of synaptosomes. Mice were decapitated and whole brain homogenates (10 vol. of 0.32 M sucrose, 3 mM HEPES, pH 7.5, at room temperature, two to four mouse brains per tube) were fractionated by a modification of the methods described by Cotman and Matthews [13]. The purity of this preparation has been verified in our laboratory [14]. All procedures were carried out at 0–5°. The crude nuclear fraction was removed by centrifugation of the homogenate at 1100 *g* for 5 min. The resulting supernatant fraction was then centrifuged at 17,300 *g* for 12 min to yield a crude mitochondrial pellet (P_2) and a supernatant fraction (S_2). The supernatant fraction was discarded and the pellet was washed once and layered over a gradient of 7.5 and 13% (w/v in 0.32 M sucrose, 3 mM HEPES, pH 7.5, Ficoll) in 1 in. × 3 in. cellulose-nitrate tubes. After centrifugation at 65,000 *g* for 60 min in an SW27 rotor (Beckman Instruments, Palo Alto, CA), the synaptosomal fraction was removed from the 7.5–13% interface and prepared for ^{45}Ca uptake experiments, as described below.

ATP-stimulated uptake of ^{45}Ca. ATP-dependent uptakes were assayed as described by Blaustein *et al.* [5, 6]. The synaptosomal band was removed from the Ficoll gradient, slowly diluted 5-fold with ice-cold calcium-free Na^+–$5K^+$ (132 mM NaCl, 5 mM KCl, 1.3 mM $MgCl_2$, 1.2 mM NaH_2PO_4, 10 mM glucose, and 20 mM Tris; pH 7.4), and pelleted at 15,000 *g* for 6 min. The pellet was then resuspended in hypotonic lysis solution (1.3 mM $MgCl_2$, 2.4 mM NaH_2PO_4 and 20 mM HEPES; pH 7.4) and incubated at 37° for 3 min to disrupt the synaptosomes. This suspension was placed on ice and diluted with an equal volume of a solution containing 362 mM KCl, 1.95 mM $MgCl_2$, 3.6 mM NaH_2PO_4, 30 mM HEPES, 0.5 mM NaN_3, 0.5 mM dinitrophenol and 5 μg/ml oligomycin; pH 7.4. Aliquots (0.8 ml; 0.4–0.8 mg protein) were added to tubes containing 0.2 ml of various drug solutions or distilled water (control) and incubated at room temperature for 15 min, followed by incubation at 37° for 1 min. At this point, 1 ml of the EGTA-buffered ^{45}Ca solution (80 μM $CaCl_2$, 0.5 μCi/ml $^{45}CaCl_2$, 145 mM KCl, 1.3 mM $MgCl_2$, 0 or 2 mM MgATP, and various concentrations of EGTA) was added and incubation was continued for 5 min at 37°. In agreement with Blaustein *et al.* [5, 6], we found that ATP-dependent uptake was linear for at least 10 min. Ethanol produced similar inhibitory effects, with uptake periods of 2, 5, and 8 min. Based on these observations, a 5-min uptake period was used in all of the experiments reported here. Unless otherwise noted, the final concentration of free calcium was calculated to be 0.5 μM [15]. In some experiments, the ionized calcium concentration was varied by changing the EGTA concentration. The uptake was terminated by rapid filtration through GF/C glass fiber filters which were washed three times with 5 ml of a solution containing 145 mM KCl, 1.2 mM $CaCl_2$ and 1.4 mM $MgCl_2$. The amount of radioactivity on the discs was determined by liquid scintillation spectrometry. The ATP-dependent uptake (ΔATP) was considered to

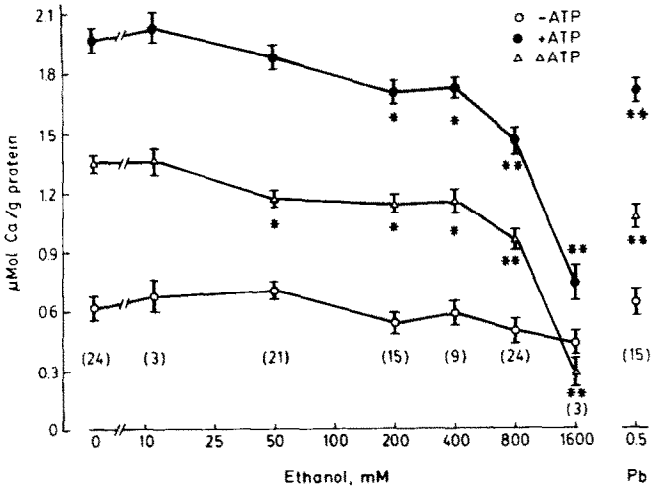

Fig. 1. Effects of *in vitro* exposure to ethanol or pentobarbital (Pb) on the uptake of calcium by synaptic membranes. Key: (○) uptake in the absence of ATP (−ATP), (●) uptake in the presence of ATP (+ATP), and (△) ATP-dependent uptake. Ethanol concentrations were varied between 10 and 1600 mM, while pentobarbital was tested at 0.5 mM. Vertical bars represent ± S.E.M. Numbers in parentheses denote the number of different membrane preparations tested under each condition. Each membrane preparation was assayed in duplicate. * Significantly different from H_2O control, $P < 0.05$. **Significantly different from control, $P < 0.01$.

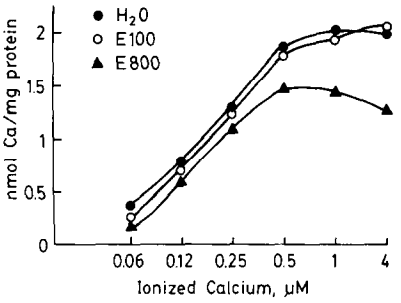

Fig. 2. Effects of ethanol on the ATP-dependent uptake of calcium as a function of ionized calcium concentrations. Key: (●) H_2O control, (○) 100 mM ethanol, and (▲) 800 mM ethanol. Each point is the mean from four to six membrane preparations.

be the difference between the uptake in the absence of ATP (−ATP) and in the presence of ATP (+ATP).

Other methods. Protein concentrations were determined by using a modification of the phenol method [16]. Delipidated bovine serum albumen (BSA) was used as the standard. Statistical analysis was performed using Student's *t*-test for unpaired samples (for comparisons between different membrane preparations) and for paired samples (for comparison of drug effects on the same membrane preparations). For multiple comparisons with a control, significance levels were obtained from Dunnett's tables [17]. Dose–response curves were compared by analysis of variance of bioassay data [18].

RESULTS

Effects of in vitro *ethanol on ATP-dependent uptake of calcium.* Figure 1 shows the effects of *in vitro* addition of various concentrations of ethanol on the uptake of calcium by lysed synaptosomes. Control values show that addition of ATP increased calcium uptake by about 3-fold (+ATP compared to −ATP). These values are similar to those obtained by Blaustein *et al.* [5, 6]. The difference between the amount of calcium accumulated in the presence of ATP and that accumulated in the absence of ATP (basal uptake) is the ATP-dependent (ΔATP) uptake. *In vitro* addition of ethanol inhibited the ATP-dependent uptake, without affecting the basal uptake (Fig. 1). Pentobarbital also inhibited the ATP-dependent uptake, without affecting basal uptake. Inhibition was obtained with ethanol concentrations as low as 50 mM; however, very high concentrations were required for marked suppression of the ATP-dependent uptake, resulting in a biphasic dose–response curve.

The effects of low (100 mM) and high (800 mM) concentration of ethanol were evaluated as a function of the concentration of ionized calcium in the uptake medium. The inhibitory effects of the low ethanol concentration were enhanced by a reduction in ionized calcium and were abolished by increasing the free calcium to 4 μM (Fig. 2). In contrast, the inhibition produced by the high ethanol concentration was similar at all concentrations of calcium.

Influence of the duration of ethanol exposure and of temperature on calcium uptake. Before measurement of calcium uptake, lysed synaptosomes were exposed to ethanol at room temperature for periods ranging from 30 sec to 120 min before the beginning of the 5-min uptake period. Calcium uptake was measured at either 37° or 18°. Ethanol inhibited the ATP-dependent uptake to about the same extent, regardless of the duration of preincubation with the drug (Fig. 3). The effects of ethanol may be decreased after 120 min at room temperature; this long incubation period, however, may produce membrane degradation. However, when uptake was measured at 37°, the inhibitory effects of ethanol were greater than when uptake was measured at 18° (except at the 120 min time point) (Fig. 3). To further

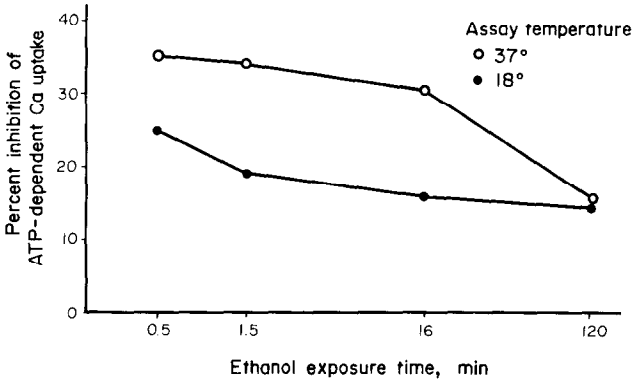

Fig. 3. Effects of duration of ethanol exposure and incubation temperature on ATP-dependent uptake of calcium by lysed synaptosomes. Synaptic membranes were exposed to 800 mM ethanol for 0.5–120 min prior to the measurement of calcium uptake. Uptake of ^{45}Ca was measured for 5 min at either 37° (○) or 18° (●). The results represent the percent inhibition of ATP-dependent uptake (ΔATP) produced by the drug. All points represent a significant ($P < 0.05$) inhibition of ΔATP, as compared to membranes tested without drug under the same conditions. Each point is the mean of three different membrane preparations which were assayed in duplicate. The S.E.M. varied between 12 and 18 percent of the mean.

Table 1. Effects of temperature on the inhibition of the ATP-dependent uptake of calcium by ethanol and pentobarbital

Assay temperature (°)	ΔATP*			% Inhibition	
	H_2O	Ethanol	Pentobarbital	Ethanol	Pentobarbital
37	1.32 ± 0.10	0.82 ± 0.11†	1.02 ± 0.09†	38	23
28	1.10 ± 0.10	0.81 ± 0.09†	0.90 ± 0.09	26	18
23	0.91 ± 0.06	0.74 ± 0.05†	0.73 ± 0.05†	19	20
18	0.68 ± 0.08	0.60 ± 0.06	0.59 ± 0.07	12	13
12	0.47 ± 0.05	0.43 ± 0.06	ND‡	9	ND‡
1	0.09 ± 0.01	0.12 ± 0.02	0.09 ± 0.02	−33	0

* Values are nmoles calcium/mg protein and are given as the mean ± S.E.M. (N = 3–6). The final concentration of ethanol was 800 mM and the final concentration of pentobarbital was 0.5 mM.
† Significantly different from H_2O control, $P < 0.05$.
‡ Not determined.

explore this temperature dependence, the effects of ethanol and pentobarbital were evaluated at six different assay temperatures (Table 1). It can be seen that at higher temperatures ethanol and pentobarbital inhibited calcium uptake, but these effects were not seen at lower temperatures. Similar results were obtained when a lower concentration of ethanol was tested in conjunction with a lower concentration of ionized calcium (Table 2).

Reversibility of the effects of in vitro *exposure to ethanol.* This experiment evaluated the hypothesis that high concentrations of ethanol inhibit calcium uptake by irreversibly damaging the ATP-dependent uptake system. To test this, synaptic membranes were incubated with either water or 1600 mM ethanol and were then washed by centrifugation. The membranes were then resuspended and their ATP-dependent calcium uptake was assayed in the presence of either ethanol (800 mM) or water (control). Preincubation with ethanol followed by washing (ethanol–H_2O) did not affect uptake, as compared to membranes preincubated with water and then washed (H_2O–H_2O) (Table 3). Thus, the marked inhibitory effects of a very high concentration of

Table 2. Time and temperature dependence of the effects of ethanol on the ATP-dependent uptake of calcium at low calcium concentrations

Addition	Ethanol exposure time (min)	Uptake					
		37°		20°		0°	
		ΔATP	% Change	ΔATP	% Change	ΔATP	% Change
H_2O (control)	30	0.60 ± 0.04*	−22	0.45 ± 0.01	−11	0.07 ± 0.01	+43
Ethanol, 100 mM		0.47 ± 0.02		0.40 ± 0.01		0.10 ± 0.01	
H_2O (control)	0.5	0.65 ± 0.02	−18	0.45 ± 0.02	−13	0.08 ± 0.01	+25
Ethanol, 100 mM		0.53 ± 0.02		0.39 ± 0.03		0.10 ± 0.02	

* Values are nmoles calcium/mg protein, mean ± S.E.M. for N = 6. The concentration of ionized calcium was 0.1 μM. Under each condition, ethanol produced a significant ($P < 0.05$) change in uptake.

Table 3. Reversibility of the *in vitro* effects of ethanol on calcium uptake

Treatments*		Calcium uptake†		
First incubation	Second incubation	−ATP	+ATP	ΔATP
H_2O	H_2O	0.38 ± 0.04	1.28 ± 0.06	0.88 ± 0.06
Ethanol	H_2O	0.32 ± 0.04	1.22 ± 0.06	0.90 ± 0.08
H_2O	Ethanol	0.34 ± 0.02	1.01 ± 0.06‡	0.67 ± 0.04‡
Ethanol	Ethanol	0.30 ± 0.02	0.80 ± 0.04§	0.50 ± 0.06§

* Synaptosomes were prepared from rat brain and were lysed. During the first incubation, the membranes were exposed to H_2O or ethanol (1600 mM) for 15 min at room temperature. The membranes were then washed once by centrifugation (15,000 g × 6 min), resuspended, and incubated (second incubation) with H_2O or ethanol (800 mM) for 15 min; the ATP-dependent uptake (ΔATP) was evaluated as described under Materials and Methods.
† Values are mean nmoles calcium/mg protein ± S.E.M., N = 6.
‡ Significantly different from H_2O–H_2O group, $P < 0.01$.
§ Significantly different from H_2O–ethanol group, $P < 0.05$.

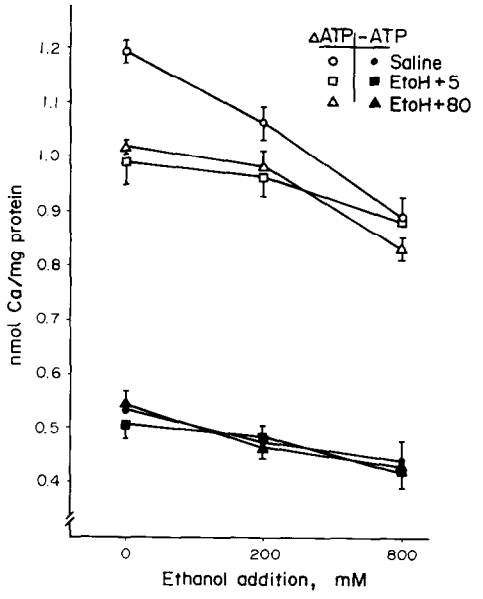

Fig. 4. Effects of acute administration of ethanol on the uptake of calcium by synaptic membranes. Mice were injected with 4 g/kg ethanol either 5 min (□, ■) or 80 min (△, ▲) before death. Another group was given saline (○, ●). The ATP-dependent (ΔATP, open symbols) and the basal (−ATP, filled symbols) uptake of calcium was assayed under control conditions and in the presence of 200 and 800 mM ethanol. Ethanol injection inhibited ($P < 0.01$) uptake (ΔATP) assayed in the absence of ethanol. *In vitro* addition of 200 or 800 mM ethanol inhibited the uptake (ΔATP) only in membranes from saline-treated mice. Each point is the mean ± S.E.M. of nine membrane preparations assayed in duplicate.

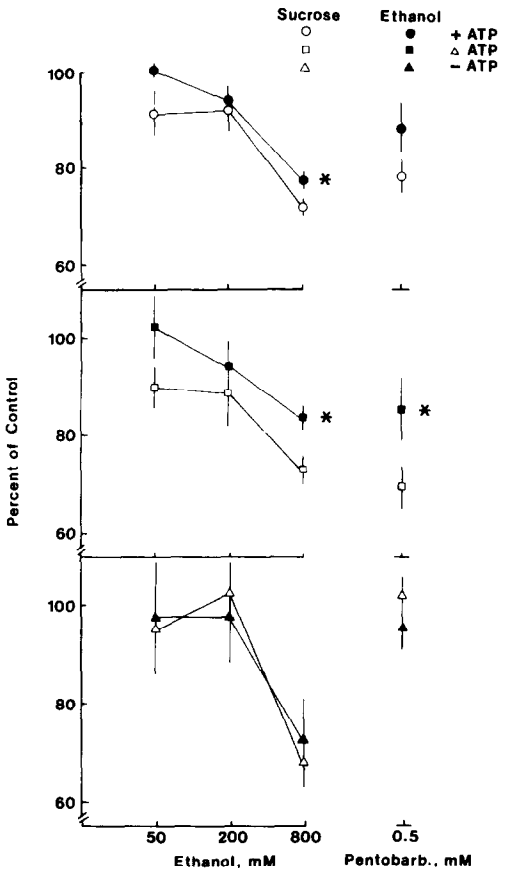

Fig. 5. Effects of chronic ingestion of ethanol on the inhibition of calcium uptake by ethanol and pentobarbital. The effects of ethanol (50, 200, and 800 mM) and pentobarbital (0.5 mM) on the uptake of calcium in the absence of ATP (−ATP, lower panel), in the presence of ATP (+ATP, upper panel), and the ATP-dependent uptake (ΔATP, middle panel) are presented as a percentage of the control values (no drug addition). Filled symbols represent the chronic ethanol group, while open symbols represent the sucrose control group. For the ATP-dependent uptake, the ethanol dose–response curves are significantly different ($P < 0.02$) and the effects of pentobarbital are different ($P < 0.05$) for the sucrose and ethanol groups. Each point is the mean ± S.E.M. for nine separate membrane preparations assayed in duplicate.

ethanol (Fig. 1) were reversible. The effects of a lower concentration of ethanol (100 mM) were also reversible (data not shown). Preincubation with ethanol, however, did make the membranes more sensitive to a subsequent exposure to ethanol. This is indicated by the greater inhibition of uptake in the ethanol–ethanol group, as compared to the H_2O–ethanol group (Table 3). Preincubation with water followed by washing appeared to reduce calcium uptake compared to untreated membranes (Table 3 vs Figs. 1 and 4).

Acute administration of ethanol and the uptake of calcium. Mice were injected with ethanol either 5 or 80 min before death. These times correspond to the time of loss of righting reflex and time of regaining the righting reflex. Synaptic membranes from mice injected with ethanol at either time displayed less ATP-dependent calcium uptake than did synaptic membranes from mice injected with saline (Fig. 4). Basal (−ATP) uptake of calcium was not affected by ethanol treatments. When ethanol was added *in vitro*, the membranes from ethanol-treated mice were resistant to the inhibitory effects of ethanol on the ATP-dependent uptake. Although the membranes from saline-treated mice demonstrated that 200 and 800 mM ethanol inhibited ATP-dependent uptake, membranes from mice treated with ethanol 5 or 80 min before death were not affected by either of these ethanol concentrations (Fig. 4). These changes cannot be attributed to the residual presence of the drug in the isolated membranes, since ethanol in the brain tissue at the time of death would be removed by the extensive washing involved in the preparation of membranes.

Chronic ingestion of ethanol and the uptake of calcium. Mice drinking an ethanol-containing liquid diet for 7 days have been shown to become tolerant to, and physically dependent upon, ethanol [19]. Data in Fig. 5 indicate that synaptic membranes from mice chronically exposed to ethanol in this manner were tolerant to the inhibitory effects produced by *in vitro* addition of ethanol. This is demonstrated by a shift to the right of the dose–response curve for the inhibition of the ATP-dependent uptake by ethanol (Fig. 5, middle panel). Membranes from ethanol-tolerant mice were also tolerant to the effects

of pentobarbital. Pentobarbital (0.5 mM) inhibited ATP-dependent uptake by 31 percent ($P < 0.01$) in control mice, but by only 14 percent (not significantly) in ethanol-tolerant mice (Fig. 5). The magnitude of this tolerance is consistent with behavioral studies [2] demonstrating that the anesthetic effects of ethanol and pentobarbital are reduced by 30–40 percent after this chronic treatment regimen.

Not only did the membranes from the sucrose and ethanol groups respond differently to *in vitro* challenges of ethanol and pentobarbital, but differences in their ability to accumulate calcium also were apparent without an *in vitro* drug addition. Membranes isolated from mice chronically exposed to ethanol displayed a decrease in ATP-dependent calcium uptake. Synaptic membranes from mice drinking the sucrose-containing diet were found to have an ATP-dependent calcium uptake of 1.65 ± 0.06, whereas the group drinking the ethanol-containing diet had an uptake of 1.43 ± 0.07 (mean nmoles calcium/mg protein $\pm$ S.E.M, $N = 9$) ($P < 0.05$).

DISCUSSION

These studies demonstrated that ethanol and pentobarbital inhibited ATP-dependent uptake of calcium by brain synaptic membranes. This inhibition of calcium storage would be expected to increase intracellular levels of free calcium and thus increase the resting release of neurotransmitters. Indeed, ethanol and barbiturates have been shown to increase the non-stimulated release of neurotransmitters from several neural preparations. Ethanol has been reported to increase the resting release of acetylcholine from the mouse phrenic nerve [20] and the rat phrenic nerve [21]. In addition, ethanol has been shown to increase the resting release of dopamine from rat caudate synaptosomes [22] and to increase the non-stimulated release of serotonin, dopamine, and γ-aminobutyric acid (GABA) from rat brain cortical slices [23]. Pentobarbital has been shown to increase the release of acetylcholine from rat cerebral cortex slices [24], whereas methohexital has been reported to increase the frequency of resting miniature end-plate potentials at the rat neuromuscular junction, suggesting an increase in the release of acetylcholine [25]. These findings have been taken as evidence that these drugs directly affect the membrane processes involved in vesicle fusion and neurotransmitter release [21]; however, the results presented here raise the possibility that ethanol and pentobarbital may also increase the release of neurotransmitters by altering the intrasynaptosomal storage of calcium.

In vitro exposure to ethanol concentrations as low as 50–100 mM produced inhibition of the ATP-dependent uptake of calcium, and this effect was enhanced by a reduction in the ionized calcium concentration. An ethanol concentration of 50 mM is associated with a moderate degree of intoxication in rodents [26]. In the present studies, however, high concentrations of ethanol were needed for marked inhibition of the ATP-dependent calcium uptake. In this regard, it is important to note that concentrations of ethanol ranging from 200 to 1600 mM have been required to increase the resting release of various neurotransmitters [20, 23]. The present results demonstrate that these same ethanol concentrations inhibit intrasynaptosomal storage of calcium. Curran and Seeman [21] reported that phrenic nerves from rats chronically treated with ethanol were tolerant to the stimulatory effects of ethanol on acetylcholine release. In the present study, chronic ethanol ingestion produced tolerance to its effects on ATP-dependent calcium storage. Thus, the effects of ethanol, pentobarbital, and other agents [3] are consistent with the hypothesis that stimulation of release of neurotransmitters by these drugs may be due, at least in part, to inhibition of calcium sequestration by intrasynaptic membranes.

A single injection of ethanol inhibited the ATP-dependent calcium uptake and reduced the sensitivity of the uptake system to subsequent *in vitro* addition of ethanol. This was surprising because ethanol would be removed from the tissue during the preparation of synaptosomes and the inhibitory effects of *in vitro* exposure to ethanol were readily reversed when the membranes were washed. Exposure to ethanol *in vivo* apparently produces intrasynaptosomal alterations, which are initiated very shortly after the injection of ethanol and which persist after the ethanol is removed from the membranes. Acute administration of ethanol also reduced the sensitivity of the uptake system to subsequent *in vitro* challenges with ethanol. This would be analogous to the acute tolerance observed *in vivo* after a single injection of ethanol [26, 27]. An inhibition of calcium uptake and decreased sensitivity to ethanol was also produced by consumption of ethanol for 7 days. Thus, similar effects were obtained with an exposure to ethanol for 5 min, 80 min, or 7 days.

It is likely that the effects of ethanol and pentobarbital on ATP-dependent calcium uptake involve an inhibition of Ca^{2+}-ATPase. This enzyme appears to be responsible for ATP-dependent transport of calcium by synaptic membranes [7, 8], and there is preliminary evidence that the enzyme is inhibited by ethanol [28]. The activity of this enzyme is sensitive to its lipid environment [29], and the inhibitory effects of ethanol and pentobarbital may be due to their ability to increase the fluidity of the lipid phase of brain membranes [30–32]. If this is the case, decreasing the temperature of the membrane should offset the fluidizing effects of ethanol and pentobarbital and abolish their inhibitory action. Indeed, at low temperatures, neither ethanol nor pentobarbital significantly inhibited the ATP-dependent calcium uptake. It could also be argued that at all temperatures ethanol and pentobarbital should produce the same effect as an increase in temperature, i.e. an increase in calcium uptake. However, an increase in temperature produces complex effects due to changes in protein conformation and rates of chemical reactions in addition to effects on membrane fluidity. The present results suggest that intrasynaptosomal storage of calcium may prove useful for studying the effects of drugs on the coupling between the physical and the functional properties of brain membranes.

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