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BRIEF COMMUNICATION

The Ethanol Sensitivity of Calcium Taken up by a Depolarization-Dependent Process in Mouse Strains DBA and C57BL[1]

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LACE, J W., C W SCHNEIDER AND R. A HARTLINE. *The ethanol sensitivity of calcium taken up by a depolarization-dependent process in mouse strains DBA and C57BL* PHARMACOL BIOCHEM BEHAV 24(4) 1137–1139, 1986.—*In vitro* effects of ethanol on calcium taken up by synaptosomes were examined in two strains of mice, C57BL and DBA, that exhibit marked differences in alcohol sensitivity and preference. There were no significant strain differences in basal or depolarization-dependent synaptosomal calcium levels. Ethanol did not reduce the basal calcium level but instead reduced depolarization-dependent calcium levels with the same potency in both strains. These results do not support a role for changes of calcium levels as the basis for differences in ethanol sensitivity in these mouse strains

Ethanol sensitivity Depolarization-dependent calcium levels Strain differences

ALCOHOLISM is the most common form of drug addiction in the United States, and has become a serious public health problem. The etiology of this disorder involves a multidimensional array of factors that include genetic, psychological, social and cultural factors interacting in a very complex manner. While genetic factors may play a role [16], it is not clear how they might be expressed. One possibility may be an increased risk because of an inherent difference in central nervous system (CNS) tolerance to the effects of the drug. However, despite years of research, the mechanism(s) of action of alcohol on the CNS remains unclear.

Mice from the C57BL and the DBA strains possess a genetically determined high and low preference for alcohol, respectively [10]. While these strains cannot logically represent alcoholic and non-alcoholic humans, they apparently possess differential CNS sensitivity to ethanol and other alcohols [7, 12, 13], and, therefore, may serve as a model to investigate CNS mediators of alcohol tolerance. There are many avenues an investigator may pursue in studying this problem. For example, ethanol has been shown, *in vitro*, to inhibit calcium uptake into synaptosomes isolated from rat and mouse brain [6]. Concentrations of ethanol which do not alter synaptosomal membrane potentials show this inhibitory effect [15]. Depolarization-dependent calcium uptake has been shown to be similar to the process in intact nerve endings, and is due neither to nonspecific membrane binding nor uptake by mitochondria in the synaptosomal preparations [2, 3, 5]. Ethanol inhibits the release of a variety of neurotransmitters [4]. Since the influx of calcium into the synaptic terminal is essential for the release of neurotransmitter after neuronal depolarization [11], changes in the terminal calcium levels could be the mechanism of the depressive effects of ethanol on neuronal activity [6] and may explain differences in the two strains. This study tested these mouse strains for differences in the sensitivities to ethanol of synaptosomal calcium levels resulting from calcium uptake.

METHOD

A total of 80 animals, half from the C57BL/6J strain and half from the DBA/2J strain, were obtained from the Jackson Laboratory, Bar Harbor, ME. They were 10–12 weeks of age

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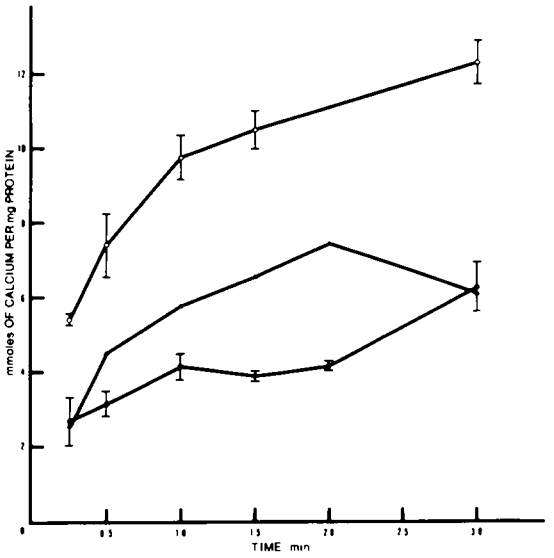

FIG 1 Time course of calcium uptake by synaptosomes Synaptosomes from DBA mice were incubated with $^{45}CaCl_2$ in either Na-5K or Depolarizing solution for different times from 0 25 to 3 minutes (●) Basal calcium uptake, (○) Stimulated calcium uptake, (△) Depolarization-dependent calcium uptake The bars represent the standard errors of triplicate determinations

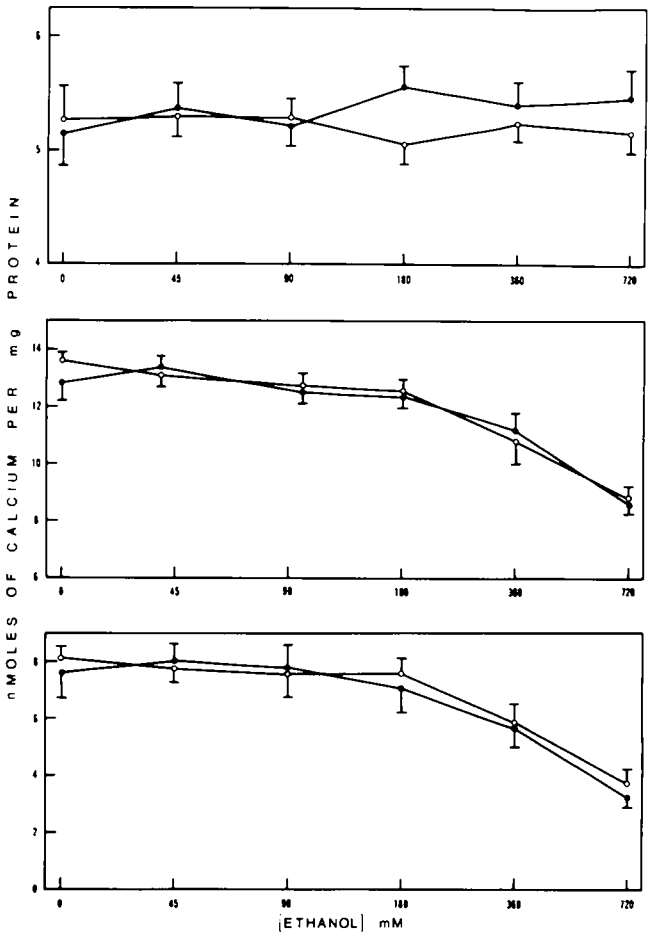

FIG 2 Effect of ethanol on synaptosomal calcium uptake, comparison of mouse strains DBA (○) and C57BL (●) Synaptosomes from each strain were preincubated with different concentrations of ethanol for 12 minutes before assaying calcium uptake (Top) Basal Uptake The bars represent the standard errors of 16–18 determinations (Middle) Stimulated Uptake The bars represent the standard errors of 15–18 determinations (Bottom) Depolarization-Dependent Uptake The bars represent the standard errors of 6 determinations

at the time of sacrifice by cervical dislocation All were housed under standard and similar conditions, 6 per cage, with free access to water and standard mouse chow.

Ficoll Type 400, Trizma Base, HEPES and EGTA were obtained from Sigma Chemical Company. $^{45}CaCl_2$ was obtained from New England Nuclear. All other reagents were obtained from Fisher Scientific

Synaptosomes were prepared by the method of Smith and Loh [14] Five brains were pooled for each synaptosomal preparation. Synaptosomes were suspended at 1 mg protein per ml in Na-5K which was 1.2 mM in $CaCl_2$ and incubated at 37° for 15 minutes Total protein was 0.3 mg. The Na-5K solution contains 132 mM NaCl, 5 mM KCl, 1 3 mM $MgCl_2$, 1 2 mM NaH_2PO_4, 10 mM Glucose and 20 mM Tris at pH 7 4. A 0.1 ml aliquot of either double distilled H_2O (control) or ethanol to the final assay concentration was added to six tubes containing the synaptosomes and the incubation continued for 12 minutes. To initiate calcium uptake, 0 5 ml of either Na-5K ^{45}Ca (specific activity, 0 2 microcuries/micromole in the final solution) or depolarizing solution with ^{45}Ca (in which KCl at 137 mM is substituted for NaCl) were added to each of three tubes and incubated for 90 seconds with the exception of time course studies. Uptake was terminated by addition of 0.5 ml of ice-cold EGTA-halting solution (132 mM NaCl, 5 mM KCl, 1 3 mM $MgCl_2$, 1 2 mM NaH_2PO_4, 30 mM EGTA, and 30 mM Tris at pH 7.4) plus 5 ml of ice-cold, Ca-free Na-5K Samples were immediately filtered under vacuum over Ca-free Na-5K premoistened Whatman GF/C filters and washed twice with 10 ml of ice-cold Ca-free Na-5K The termination procedure took less than 20 seconds Isotope on the filters was determined by liquid scintillation spectrometry The final synaptosomal suspensions were assayed for protein [9]. Basal calcium uptake is that measured in the presence of high external sodium Stimulated calcium uptake is that measured in the presence of high external potassium Depolarization-dependent calcium uptake is the difference between the means of triplicate assays of basal and stimulated uptake performed on a particular synaptosomal preparation

RESULTS

Basal, stimulated, and depolarization-dependent calcium levels increased linearly with tissue protein from 0 1 to 0 3 mg (data not shown) In all subsequent experiments the quantity of protein used was 0 3 mg

Figure 1 shows the time course of calcium uptake Depolarized synaptosomes approach equilibrium within 2 minutes of incubation An incubation time of 90 seconds was chosen to measure the calcium levels for subsequent experiments The synaptosomal calcium levels of 90 seconds reflects the uptake process(es) in effect during the first 90 seconds Figure 2 compares the two mouse strains, C57BL and DBA, with regard to the sensitivity of synaptosomal calcium

levels to reduction by ethanol. Figure 2 (top) shows that the basal level of calcium is not reduced by ethanol, and is not different between strains. However, calcium levels in synaptosomes depolarized in the presence of high external K^+ concentrations is lowered by high concentrations of ethanol in the assay, in agreement with published results [6]. This primarily reflects reduction of depolarization-dependent calcium levels as seen in Fig 2 (bottom) No difference was seen between DBA and C57BL in the sensitivity of depolarization-dependent synaptosomal calcium levels to ethanol as seen in Fig 2 (middle and bottom)

DISCUSSION

The values obtained for basal and depolarization-dependent synaptosomal calcium levels are similar to values previously reported [6]. No differences were seen in the basal level of calcium between the strains (Fig. 2, top) We verified that ethanol does not reduce the basal level and that ethanol reduces the depolarization-dependent level in a dose-dependent manner (data not shown) No differences were found in the reduction of calcium levels by ethanol between strain C57BL, the ethanol tolerant strain, and DBA, the low tolerant strain (Fig 2, bottom). These data suggest that reduction of calcium levels in presynaptic terminals fails to account for the differences in neural sensitivity to ethanol observed in these mouse strains. It has been shown that differences in neural function may be apparent only after modification of the internal composition of the synaptosome [1,8] and Harris and Hood [6] showed that synaptosomes isolated from mice chronically treated with ethanol exhibited significantly decreased uptake of calcium that could be reversed by the *in vitro* addition of low concentrations of ethanol. Therefore, the data does not rule out that chronic alcohol ingestion, compared with animal not ingesting alcohol, could conceivably reveal phenotypic differences in the development of tolerance in the depolarization dependent calcium channel.

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