Biochemical Pharmacology, Vol. 28, pp. 3075–3080.

0006-2952/79/1015-3043 $02.00/0

EFFECTS OF PENTOBARBITAL, ETHANOL AND MORPHINE ON SUBCELLULAR LOCALIZATION OF CALCIUM AND MAGNESIUM IN BRAIN

WILLIAM F. HOOD and R. ADRON HARRIS

Department of Pharmacology, University of Missouri School of Medicine, Columbia, MO, 65212, and Veterans Administration Medical Center, Columbia, MO 65201, U.S.A.

(*Received* 24 *February* 1979; *accepted* 27 *April* 1979)

Abstract—The effects of acute and chronic administration of pentobarbital and ethanol and of acute administration of moprphine on the subcellular localization of calcium and magnesium were determined in brain. Acute administration of pentobarbital to mice significantly decreased the calcium content of synaptic plasma membranes, (SPM-1), while increasing the calcium content of extrasynaptosomal mitochondria. After chronic administration of pentobarbital, the calcium content of SPM-1 remained depressed but no other alterations were detected. Neither acute nor chronic treatment with pentobarbital altered the subcellular localization of magnesium in brain. Acute injection of morphine decreased the synaptosomal calcium concentration in rat brain. In contrast to these effects of pentobarbital and morphine, acute administration of ethanol to mice or rats failed to alter the calcium content of any subcellular fraction studied. In addition, acute treatment with either ethanol or morphine failed to alter the calcium or magnesium content of tissue samples from rat cortex. Chronic ethanol treatment did not alter the concentration of calcium in any subcellular fraction, but did decrease the concentration of magnesium in both myelin and serum. This finding is discussed in terms of the role of magnesium deficiency in chronic alcoholism. Taken together, these results indicate that pentobarbital, ethanol and morphine each produce distinct alterations in the subcellular localization of calcium and magnesium in brain.

Recent investigations have linked ethanol, pentobarbital and morphine intoxication and dependence to alterations in calcium metabolism. Elevation of brain calcium, apparently at intracellular sites, has been shown to increase ethanol- and pentobarbital-induced sleeping time [1, 2] while antagonizing the anti-nociceptive effects of morphine [3]. At the same time, the calcium chelator ethyleneglycolbis(aminoethylether)tetra-acetate (EGTA) decreased ethanol sleeping time, while potentiating the analgesic effect of morphine. In addition, Ross *et al.* [4] and Ross [5] reported that the calcium content of rat brain was decreased significantly following the acute administration of ethanol, morphine, or high doses of pentobarbital. Moreover, the depletion of brain calcium produced by ethanol and morphine was prevented by pretreatment with the opiate antagonist naloxone [4, 6], whereas the depletion produced by pentobarbital was not [5]. Subsequently, the decrease in brain calcium seen after acute ethanol or morphine was localized in the synaptosomal fraction [6–8]. In contrast, chronic treatment with morphine or ethanol produced an increase in the calcium content of the synaptosomal fraction [7, 8].

Magnesium, on the other hand, has not yet been shown to be as important as calcium in acute ethanol or morphine intoxication. Intracerebroventricularly administered $MgCl_2$ does not alter ethanol sleeping time [1, 2], and acute morphine fails to change the magnesium content of cortical tissue [6]. However, Belknap *et al.* [9] have recently reported that mice chronically exposed to ethanol have brain magnesium concentrations during withdrawal lower than control animals. Based on the similarities between the signs of alcohol withdrawal and the signs of magnesium deficiency, these workers suggested that the decrease in brain magnesium produced by alcohol exposure could contribute to the alcohol withdrawal syndrome.

Thus, several studies have demonstrated that acute and chronic treatments with opiates, alcohol or barbiturates may alter the calcium or magnesium content of brain tissue. In an attempt to confirm and extend these important findings, we determined the calcium and magnesium content of brain tissue and brain subcellular fractions after acute treatment with morphine, ethanol or pentobarbital, and after chronic treatment with ethanol or pentobarbital.

MATERIALS AND METHODS

Materials. The chemicals and their suppliers are as follows: morphine sulfate, S. B. Penick & Co. (Lyndhurst, NJ); sodium pentobarbital, *N*-2-hyrdoxy-ethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), *N*-tris(hydropymethyl)-methylglycine (tricine), Ficoll, and lanthanum oxide, Sigma Chemical Co. (St. Louis, MO); ethanol (95 and 99.5%, v/v), Commercial Solvents Corp. (Terre Haute, IN); Sustacal, Mead Johnson Laboratories (Evansville, IN); ethylenediamine tetra-acetic acid (EDTA) and NaOH, Fluka-Tridom Chemical Inc. (Hauppauge, NY); sucrose, $CaCl_2$ and $MgCl_2$, Fisher Scientific Co. (St. Louis, MO); and hydrochloric acid and hydrogen peroxide, J. T. Baker Chemical Co. (Phillipsburg, NJ).

Animal treatments. Male Sprague–Dawley rats (Charles River Breeding Lab, Wilmington, MA) and male Swiss–Webster mice (Charles River Breeding Lab, Portage, MI), used in various experiments, were maintained on a standard laboratory diet and tap water

except for the chronic ethanol studies, as described below. To test the acute effects of pentobarbital, rats were given 60 mg/kg and mice 75 mg/kg intraperitoneally (i.p.) 20 min prior to decapitation. For acute administration of morphine, rats were given 30 mg/kg subcutaneously (s.c.) 30 min before being killed. For acute studies with ethanol, rats were given either 2.5 g/kg (i.p.) 30 min before killing or 2 g/kg 10 min prior to decapitation. In mice, ethanol was given at a dose of either 4 g/kg (i.p.) 20 min before the animals were killed or 3.3 g/kg or 3.5 g/kg 10 min before decapitation. In all cases, ethanol was administered as a 20% (w/v) solution in 0.9% NaCl and equal volumes of saline were given to animals in the control groups. Morphine sulfate and sodium pentobarbital were dissolved in 0.9% NaCl; dosages are expressed as the salts. The injection volumes were 1 ml/kg for rats and 10 ml/kg for mice. Barbiturate tolerance and dependence were induced in mice by subcutaneous implantation of a pentobarbital pellet for 72 hr [10], concomitant with injections of pentobarbital (75 mg/kg) given 24, 48 and 56 hr after pellet implantation. The pellets contained $MgSO_4$, rather than the $CaSO_4$ used in the original formulation [11]. Control groups of mice were implanted with placebo pellets and injected with saline. In one experiment, the pentobarbital pellet was implanted for only 9 hr and no injections of pentobarbital were given. In each study, placebo and pentobarbital pellets were not removed prior to death.

For chronic ethanol treatment, mice were kept on a liquid diet containing Sustacal and 7% (v/v) ethanol for 7 days. The control group was pair-fed a diet containing Sustacal with sucrose substituted isocalorically for ethanol. Mice which were fed the liquid diet, consumed an average of 18.1 g/kg/day of ethanol. The diets were withdrawn 7 hr before death. Immediately prior to decapitation, the withdrawal signs were rated by the method of Goldstein [12]. Only those mice showing a withdrawal score of one or more were used.

Serum and erythrocyte preparation. After decapitation, trunk blood from two mice was collected with 0.2 ml of a heparin solution (1000 units/ml of saline). This was then spun for 20 min at 4000 *g* to separate the serum and erythrocytes for calcium and magnesium analysis.

Subcellular fractionation. Whole brain homogenates [10 vol. of 0.32 M sucrose, 3 mM Hepes (pH 7.5), and 4–6 mouse brains or 1–2 rat brains/tube] were fractionated by a modification of the methods described by Cotman and Matthews [13] and Gurd *et al.* [14]. All procedures were carried out at 0–5°. A crude nuclear fraction was prepared by centrifugation of the homogenate at 1,100 *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) containing microsomes and soluble material. The microsomes were separated from the soluble fraction by centrifugation at 68,000 *g* for 60 min. The crude mitochondrial pellet was washed once and layered over a gradient of 7.5 and 13% (w/v in 0.32 M sucrose and 3 mM HEPES, p*H* 7.5) Ficoll. After centrifugation at 65,000 *g* for 60 min the material between 0 and 7.5% Ficoll was taken as the myelin fraction, while the pellet in the bottom of the tube was taken as extrasynaptosomal mitochondria. Once separated, the myelin, extrasynaptosomal mitochondria, and a portion of the nerve ending (synaptosomal) fraction, isolated between the 7.5% and 13% regions of the Ficoll gradient, were diluted with double distilled water and pelleted at 100,000 *g* for 30 min. The pellets were then homogenized with either 1 N NaOH or double distilled water and frozen until analysis. The remainder of the synaptosomal fraction was hypotonically lysed with 6 mM tricine, pH 8.1, for 90 min. This was then layered over a discontinuous gradient of 10, 20, 25, 32.5 and 38% (w/w) sucrose. After centrifugation at 65,000 *g* for 90 min, the fractions were obtained from the following interfaces: SPM-1 (20 to 25%), SPM-2 (25 to 32.5%), and intrasynaptosomal mitochondria (bottom of tube).* In contrast to the procedures of Cotman and Matthews [13] and Gurd *et al.* [14], none of these solutions contained calcium chelators such as EDTA or EGTA. The nomenclature used for the various fractions indicates enrichment in these components. This is based on analysis of the enzyme activities and membrane composition of these fractions as determined in this laboratory [15] and others [16–18].

Calcium and magnesium content. Aliquots of the various subcellular fractions were prepared for atomic absorption analysis of calcium and magnesium by the method of McDonald *et al.* [19]. Briefly, this method involves digesting the samples in 1 N NaOH and then adding appropriate aliquots of this digestate to 1 ml of a La^{3+}–EDTA solution.

For whole tissue analysis, pieces of cortex (3–15 mg dry weight) were removed, rinsed with double distilled water, blotted on low ash filter paper, and put in preweighed polyethylene mini vials. Then, based on the method of Jones and Shain [20], samples were dried at 110° for 20 hr, weighed and ashed with 1–2 ml of 30% H_2O_2 at 70–75° overnight. Next, the ash was dissolved in 1–3 ml of a solution of 2 g/l of La_2O_3 in 0.6 N HCl for atomic absorption spectroscopy. Samples were run in duplicate and concentrations of calcium and magnesium were determined using a Perkin–Elmer 372 atomic absorption spectrophotometer.

Protein concentrations were run in duplicate and determined through a modification of the phenol method [21]. Delipidated bovine serum albumin (BSA) was used as the standard. Statistical analysis for significance was performed using Student's two-tailed *t*-test, except where noted, and P values are shown in the illustrations.

RESULTS

Effects of acute injection of pentobarbital, ethanol or morphine on the subcellular localization of calcium and magnesium. Acute injection of mice with pentobarbital (75 mg/kg) significantly decreased the calcium content of brain myelin and synaptosomal plasma membranes (SPM-1), while increasing the calcium content of extrasynaptosomal mitochondria (Fig. 1, upper panel). Although not statistically significant, acute pentobarbital also appeared to decrease the total calcium content of brain synaptosomes by 14% and the calcium content of SPM-2 by 18%, without changing the calcium content of microsomes or the microsomal supernatant fraction

* SPM = synaptic plasma membranes.

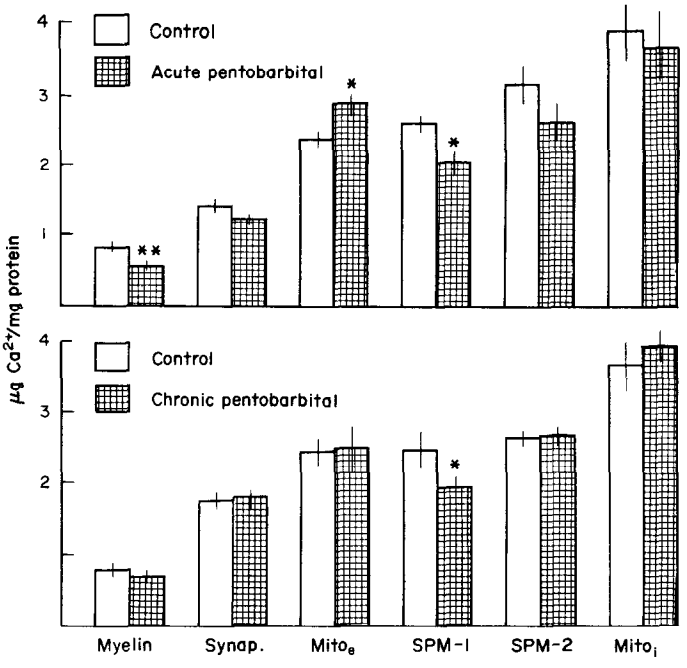

Fig. 1. Effects of acute and chronic pentobarbital on the subcellular localization of calcium in mouse brain. For acute studies mice were given pentobarbital (75 mg/kg i.p.) 20 min before death, while for chronic studies mice were implanted with pentobarbital pellets for 72 hr and given injections of pentobarbital (75 mg/kg) 24, 48 and 56 hr after pellet implantation. Abbreviations: (SPM) synaptic plasma membranes; (synap.) intact synaptosomes; ($mito_e$) extrasynaptosomal mitochondria; and ($mito_i$) intrasynaptosomal mitochondria. Vertical bars represent ± S.E.M.; n = 6–9. Significantly different from control: the single asterisk(*) indicates $P < 0.01$; The double asterisk (**) indicates $P < 0.005$

(data not shown). In contrast to the effects of pentobarbital, injection of ethanol (4.0 g/kg) 20 min before death did not alter the calcium content of any fraction studied (Fig. 2, upper panel). Moreover, injection of ethanol in mice (3.5 g/kg and 3.3 g/kg) or rats (2 g/kg) 10 min before death failed to alter the calcium levels in any of the brain fractions studied (data not shown).

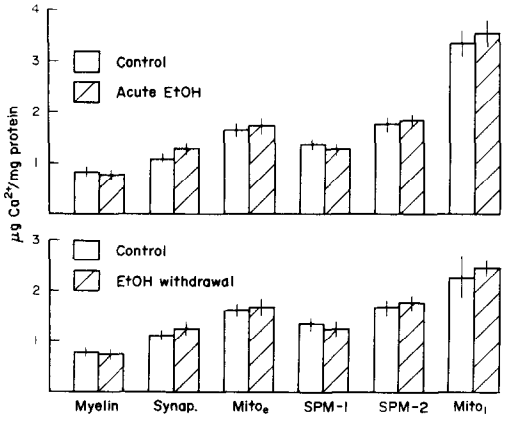

Fig. 2. Effects of acute ethanol and ethanol withdrawal on the subcellular localization of calcium in mouse brain. For acute studies mice were given ethanol (4 g/kg, i.p.) 20 min before death while for chronic studies mice were pair-fed a liquid diet of Sustacal with 7% (v/v) ethanol substituted isocalorically for sucrose for 7 days. Mice were killed 7 hr after withdrawing the diet. Abbreviations: (SPM) synaptic plasma membranes; (synap.) intact synaptosomes; ($mito_e$) extrasynaptosomal mitochondria; and ($mito_i$) intrasynaptosomal mitochondria. Vertical bars represent ± S.E.M.; n = 6–9.

With regard to magnesium localization, acute injection of ethanol produced a small but significant increase in the magnesium content of synaptosomes (Table 1). However, acute treatment with pentobarbital had no effect on the magnesium content of the various subcellular fractions from either mice or rats.

Acute administration of morphine significantly reduced the calcium content of synaptosomes (μg calcium/mg of protein ± S.E.M.: 1.33 ± 10 vs 1.15 ± 0.02; $P < 0.05$) but did not significantly alter their magnesium content (μg magnesium/mg of protein ± S.E.M.: 1.59 ± 0.02 vs 1.52 ± 0.03). Furthermore, neither the calcium nor the magnesium content of pieces of cortical tissue was changed after acute injection of ethanol or morphine (μg calcium/mg dry wt ± S.E.M.: 196 ± 7, saline; 184 ± 4, ethanol; and 186 ± 5, morphine; μg magnesium/mg dry wt ± S.E.M.: 573 ± 33, saline; 526 ± 32, ethanol; and 542 ± 35, morphine). The lack of effect of the drugs on the total calcium and magnesium content of brain tissue is in agreement with the modest and discrete effects of these treatments on the subcellular localization of these ions.

Effects of chronic administration of pentobarbital or ethanol on the subcellular localization of calcium and magnesium. In mice tolerant to and dependent on pentobarbital, the calcium levels of SPM-1 were decreased significantly from those of controls, while the calcium content of the other fractions was unaffected by

Table 1. Effects of acute pentobarbital or acute ethanol on the subcellular localization of magnesium in mouse brain*

Subcellular fraction	Control	Pentobarbital†	Control	Ethanol†
Myelin	1.78 ± 0.07	1.70 ± 0.04	1.64 ± 0.04	1.70 ± 0.06
Synaptosomes	1.49 ± 0.10	1.48 ± 0.10	1.52 ± 0.04	1.62 ± 0.02 ‡
Extrasynaptosomal mitochondria	1.12 ± 0.04	1.13 ± 0.03	1.11 ± 0.06	1.14 ± 0.08
SPM-1	3.42 ± 0.15	3.52 ± 0.14	2.48 ± 0.05	2.56 ± 0.09
SPM-2	2.64 ± 0.16	2.65 ± 0.12	2.17 ± 0.05	2.16 ± 0.10
Intrasynaptosomal mitochondria			0.98 ± 0.09	1.15 ± 0.09
Microsomes	2.03 ± 0.11	2.09 ± 0.09	1.77 ± 0.09	1.76 ± 0.05
Microsomal supernatant fraction	0.98 ± 0.04	0.97 ± 0.03	1.00 ± 0.08	0.98 ± 0.08

* All values represent $\mu g\ Mg^{2+}$/mg of protein and are presented as the means ± S.E.M.; n = 6–9.
† Mice were given either pentobarbital (75 mg/kg) or ethanol (4 g/kg), i.p. 20 min before death. Controls were given an equal volume of saline.
‡ Significantly different from control, $P < 0.05$.

Table 2. Effects of chronic pentobarbital or ethanol withdrawal on the subcellular localization of magnesium in mouse brain*

Subcellular fraction	Placebo	Pentobarbital†	Control diet	Chronic ethanol‡
Myelin	2.03 ± 0.15	1.99 ± 0.13	1.60 ± 0.03	1.44 ± 0.04§
Synaptosomes	1.77 ± 0.08	1.71 ± 0.09	1.63 ± 0.08	1.69 ± 0.08
Extrasynaptosomal mitochondria	1.12 ± 0.04	1.18 ± 0.09	1.01 ± 0.01	1.03 ± 0.02
SPM-1	3.39 ± 0.18	3.30 ± 0.20	2.37 ± 0.03	2.22 ± 0.07
SPM-2	2.81 ± 0.11	2.76 ± 0.13	1.91 ± 0.04	1.88 ± 0.04
Intrasynaptosomal mitochondria	1.08 ± 0.06	1.06 ± 0.02	1.01 ± 0.06	1.06 ± 0.05
Microsomes			1.97 ± 0.09	1.89 ± 0.05

* All values represent $\mu g\ Mg^{2+}$/mg of protein and are presented as the means ± S.E.M.; n = 6–9.
† Mice were implanted with a pentobarbital pellet for 72 hr and given pentobarbital (75 mg/kg) injections 24, 48 and 56 hr after pellet implantation.
‡ Mice were fed a liquid diet of Sustacal and 7% (v/v) ethanol for 7 days. The control group was pair-fed a diet containing Sustacal with sucrose substituted isocalorically for the ethanol. Mice were killed 7 hr after withdrawal from the diet.
§ Significantly different from control, $P < 0.005$.

Table 3. Effects of acute and chronic ethanol treatments on the calcium and magnesium concentrations of serum and erythrocytes

	Serum*		Erythrocyte*	
	Ca^{2+}	Mg^{2+}	Ca^{2+}	Mg^{2+}
Saline control	105 ± 4	22.4 ± 0.6	0.055 ± 0.008	0.190 ± 0.008
Acute ethanol†	88 ± 2‡	29.3 ± 1.0§	0.043 ± 0.001‖	0.172 ± 0.006
Control diet	122 ± 3	23.1 ± 1.8	0.060 ± 0.003	0.174 ± 0.007
Ethanol withdrawal¶	125 ± 3	19.0 ± 0.5**	0.065 ± 0.003	0.187 ± 0.008

* All values represent μg/mg of protein and are presented as the means ± S.E.M.; n = 12.
† Mice were given ethanol (4 g/kg) or saline, i.p., 20 min before death.
‡ Significantly different from saline control, $P < 0.01$.
§ Significantly different from saline control, $P < 0.001$.
‖ Significantly different from saline control, $P < 0.005$.
¶ Mice were fed a liquid diet of Sustacal and 7% (v/v) ethanol for 7 days. The control group was pair-fed a diet containing Sustacal with sucrose substituted isocalorically for the ethanol. Mice were killed 7 hr after withdrawal from the diet.
** Significantly different from control diet, $P < 0.05$.

chronic pentobarbital exposure (Fig. 1, lower panel). Moreover, when mice were killed after 9 hr of pentobarbital pellet implantation, the only change from controls was a decrease in the calcium content of the SPM-1 fraction (data not shown). The SPM-1 fraction demonstrated a decrease of approximately 20 per cent after 9 hr and 72 hr of pellet implantation. After chronic ethanol treatment we observed no change from control in the calcium content of any of the fractions studied (Fig. 2). However, the magnesium content of myelin decreased significantly (10 per cent, $P < 0.005$) after chronic ethanol exposure. In contrast to this effect of ethanol, we observed no change in magnesium content in any of the fractions from mice tolerant to pentobarbital (Table 2). The implantation of a placebo or pentobarbital pellet appeared to increase the magnesium content of the fractions (Tables 1 and 2). This may be due to the presence of $MgSO_4$ in the pellets.

Effects of acute and chronic ethanol on the calcium and magnesium content of erythrocytes and serum. Acute administration of ethanol significantly decreased the calcium content of erythrocytes and serum, while increasing the concentration of magnesium in serum. Serum from mice chronically treated with ethanol had magnesium levels lower than those of controls, although the calcium content of serum and erythrocytes was not affected during ethanol withdrawal (Table 3).

DISCUSSION

Reports of marked effects of ethanol on the calcium content of brain tissue have led to considerable interest in the role of calcium in ethanol intoxication [22]. However, the present results demonstrate clearly that neither acute ethanol (4 g/kg) nor ethanol withdrawal produced alterations in the subcellular localization of calcium in mouse brain. Moreover, in similar experiments with rats, we could not detect any change in the calcium content of cortical tissue or synaptosomes following acute ethanol (2 g/kg) treatment. These findings are in direct contrast to those of Ross [5, 7] who reported that injection of ethanol (2 g/kg) 30 min prior to decapitation decreased the calcium content of all brain regions, as well as of synaptosomal fractions prepared from brain. We also injected doses of 3.3 and 3.5 g/kg ethanol in mice and 2 g/kg ethanol in rats 10 min before death. Yet, none of these regimes of ethanol administration was capable of decreasing the calcium content of synaptosomes in either species. In contrast to these negative results with brain tissue we did detect a decrease in the calcium content of serum and erythrocytes after acute ethanol treatment, which is in agreement with Peng and Gitelman [23]. It is unclear why we were not able to confirm the decrease of brain calcium following acute ethanol administration which was noted by Ross' group. Even after morphine treatment we were unable to obtain decreases of similar magnitude in the calcium content. For example, we found a 20 per cent decrease in the calcium content of synaptosomes which was similar to the 29 per cent decrease obtained by Yamamoto *et al.* [8] but was considerably less than the 72 per cent decrease in the calcium content of synaptosomes reported by Cardenas and Ross [6].

Ethanol, however, did affect the localization of brain magnesium. Following acute ethanol administration there was a significant increase in the magnesium content of synaptosomes, and during ethanol withdrawal there was a decrease in the magnesium content of serum, which is consistent with a number of clinical studies [22]. Chronic alcohol ingestion also decreased the magnesium content of myelin. This confirms and extends the findings of Belknap *et al.* [9] that chronic alcohol exposure produced about a 6 per cent decrease in the total magnesium content of mouse brain. It is important to note that a 5 per cent decrease in brain magnesium, produced by a magnesium-deficient diet, results in some of the signs of the alcohol withdrawal syndrome, such as convulsions, tremor, straub tail, hyper-reactivity, and increased seizure susceptability. Furthermore, in magnesium-deficient animals these signs were suppressed after administration of ethanol [9]. Magnesium is also essential for normal myelin growth [24], and chronic alcohol consumption results in an abnormal myelination in both neonates [25] and adults [26]. Hence, the reduction of myelin magnesium produced by chronic ethanol exposure might be related to some of the signs of the alcohol withdrawal syndrome or to some of the neuropathology associated with chronic alcohol ingestion.

With regard to the effects of acute pentobarbital treatment on calcium localization, we noted significant decreases in myelin and SPM-1 fractions, while the synaptosomes, SPM-2, and intrasynaptosomal mitochondria also showed slight decreases. Some of the calcium removed by acute pentobarbital may have been sequestered in the extrasynaptosomal mitochondria since we were also able to detect a significant increase in the calcium content of this fraction. However, when calcium levels were measured in those mice implanted with pentobarbital pellets, we discovered that, when the fractions were isolated after 9 or 72 hr of pellet implantation, the calcium content of all the fractions had returned to control levels except the SPM-1 which still showed a decrease in the calcium content similar to that seen after acute pentobarbital. This suggests that tolerance develops to several of the effects of pentobarbital on brain calcium localization; the failure of the calcium levels of the SPM-1 fraction to return toward those found in control animals following chronic pentobarbital exposure may possibly be related to the inability of the central nervous system to develop tolerance to some effect of the barbiturates. Recently Belknap *et al.* [27] reported that consumption of phenobarbital for 6 days decreased the magnesium content of brain about 9 per cent. However, after implantation of a pentobarbital pellet for only 3 days we were unable to detect any changes in brain magnesium. This may be related to the lower level of physical dependence produced by this pellet implantation procedure [10, 27].

In summary, while acute and chronic pentobarbital decreased the calcium content of synaptic plasma membranes, neither acute nor chronic ethanol treatments altered the calcium content of brain subcellular fractions. Chronic ethanol treatment consistently produced a slight, but highly significant, decrease in the magnesium content of myelin and this decrease may be related to the alcohol withdrawal syndrome. Finally, although we could not detect a decrease in the calcium content of cortical tissue with acute morphine, our results from

subcellular fractionation confirm those of others, indicating that acute morphine treatment decreases the calcium content of rat synaptosomes.

Acknowledgements—This work was supported in part by Research Starter Grants from the Pharmaceutical Manufacturers Association Foundation and the National Council on Alcoholism, and by funds from the Veterans Administration.

REFERENCES

1. R. A. Harris, *Pharmac. Biochem. Behav.* **10**, 527 (1979).
2. C. K. Erickson, T. D. Tyler and R. A. Harris, *Science* **199**, 1219 (1978).
3. R. A. Harris, H. H. Loh and E. L. Way, *J. Pharmac. exp. Ther.* **195**, 488 (1975).
4. D. H. Ross, M. A. Medina and H. L. Cardenas, *Science* **186**, 63 (1974).
5. D. H. Ross, *Ann. N. Y. Acad. Sci.* **273**, 280 (1976).
6. H. L. Cardenas and D. H. Ross, *Br. J. Pharmac.* **57**, 521 (1976).
7. D. H. Ross, in *Alcohol and Intoxication and Withdrawal* (Ed. M. Gross) Vol. IIIa p. 459. Plenum Press, New York (1977).
8. H. Yamamoto, R. A. Harris, H. H. Loh and E. L. Way, *J. Pharmac. exp. Ther.* **205**, 255 (1978).
9. J. K. Belknap, J. H. Berg and R. R. Coleman, *Pharmac. Biochem. Behav.* **9**, 1 (1978).
10. I. K. Ho, I. Yamamoto and H. H. Loh, *Eur. J. Pharmac.* **30**, 164 (1975).
11. R. O. Gibson and J. E. Tingstad, *J. pharm. Sci.* **59**, 426 (1970).
12. D. B. Goldstein, *J. Pharmac. exp. Ther.* **180**, 203 (1972).
13. C. W. Cotman and D. A. Matthews, *Biochim. biophys. Acta* **249**, 380 (1971).
14. J. W. Gurd, L. R. Jones, H. L. Mahler and W. J. Moore, *J. Neurochem.* **22**, 281 (1974).
15. R. N. Fontaine, R. A. Harris and F. Schroeder, *J. Neurochem.* in press.
16. I. G. Morgan, L. S. Wolfe, R. Mandel and G. Gombos, *Biochim. biophys. Acta* **241**, 737 (1971).
17. A. A. Abdel-Latif, *Biochim. biophys. Acta*, **121**, 403 (1966).
18. A. P. Smith and H. H. Loh, *J. Neurochem.* **28**, 887 (1977).
19. J. M. McDonald, D. E. Bruns, L. Jarett and J. E. Davis, *Analyt. Biochem.* **82**, 485 (1977).
20. A. W. Jones and M. L. Shain, *Am. J. Physiol.* **223**, 105 (1972).
21. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
22. R. A. Harris, in *Pharmacology of Ethanol* Vol. 2 (Eds. E. Majchrowicz and E. P. Noble), Plenum Publishing Corp. New York (1979).
23. T. C. Peng and H. J. Gitelman, *Endocrinology* **94**, 608 (1974).
24. W. Craelis, N. A. Newlay and F. C. Thomas, *Soc. Neurosci. Abstr.* **4**, 313 (1978).
25. M. J. Druse and J. H. Hofteig, *Drug Alcohol Depend.* **2**, 421 (1977).
26. S. W. French, in *The Biology of Alcoholism* (Eds. B. Kissin and H. Begleiter), p. 437. Plenum Press, New York (1971).
27. J. K. Belknap, J. H. Berg, G. Ondrusek and S. Waddingham, *Psychopharmacology* **59**, 299 (1978).