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Barbiturate Tolerance and Dependence: Effects on Synaptosomal Sodium Transport and Membrane Fluidity[1]

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MITCHELL, M. A., J. PERIS AND R. A. HARRIS. *Barbiturate tolerance and dependence: Effects on synaptosomal sodium transport and membrane fluidity.* PHARMACOL BIOCHEM BEHAV 22(6) 955–960, 1985.—DBA mice were fed lab chow containing phenobarbital for seven or eight days. Upon withdrawal of the phenobarbital diet, dependence was evidenced by appearance of hypothermia, handling-induced convulsions and lethal seizures. Functional tolerance was determined by injecting phenobarbital into mice treated with the phenobarbital diet or a pair-fed control diet and measuring the brain concentration of phenobarbital at the time of loss of righting reflex and the time of regaining righting reflex. Both measures demonstrated that chronic consumption of phenobarbital resulted in functional tolerance. When the diet was withdrawn for two days, tolerance was no longer present, indicating a rapid reversal of the adaptive changes. The veratridine-stimulated uptake of ^{24}Na by isolated brain synaptosomes was used as a measure of membrane function. Sodium uptake was inhibited *in vitro* by pentobarbital and ethanol, and the inhibitory effects of these drugs were attenuated by chronic *in vivo* phenobarbital treatment. The fluidity of brain synaptic plasma membranes was estimated by the fluorescence polarization of the fluorescent probe molecules 1-(4-trimethylammonium phenyl)-6-phenyl-1,3,5-hexatriene and 1,6-diphenyl-1,3,5-hexatriene. Synaptic membranes from mice treated chronically with phenobarbital did not differ from those of control mice with regard to either the baseline fluorescence polarization of the probes or the decrease in fluorescence polarization produced by *in vitro* exposure to phenobarbital or ethanol. Taken together, these results indicate that although chronic phenobarbital ingestion resulted in tolerance and dependence (studied *in vivo*), and adaptation of sodium channels (studied *in vitro*), there was no evidence that these changes were due to alterations in the membrane physical properties.

Membrane fluidity Barbiturate tolerance Barbiturate dependence DBA/2 mice Synaptosomes Sodium transport Ethanol

ACUTE exposure to general anesthetics is suggested to increase membrane fluidity thereby disrupting cellular processes and producing sedation [23,25]. In support of a common mechanism of anesthetic action, concentration-dependent increases in brain synaptosomal membrane (SPM) fluidity have been shown to occur following *in vitro* exposure to ethanol, barbiturates, and gaseous anesthetics [4, 11, 13, 14, 24].

Repeated exposure to ethanol causes a homeoviscous adapation of brain membranes that lessens the *in vitro* fluidizing drug effect (tolerance) [4, 15, 20, 27]. In contrast to these extensive studies of ethanol effects, investigations of the membrane effects of chronic barbiturate intoxication are scant. Johnson *et al.* [17] reported resistance to the fluidizing effects of ethanol in membranes from mice treated chronically with pentobarbital by a pellet implantation method. Although these results are suggestive of cross-tolerance to ethanol, the effects of barbiturates were not assessed, and membrane tolerance to barbiturates was therefore inferred but not proven.

We investigated the effects of chronic phenobarbital intoxication on brain membrane fluidity and function to determine the following: First, does chronic phenobarbital exposure produce changes in brain membranes similar to those treated chronically with ethanol (increased initial membrane rigidity and decreased *in vitro* drug response)? To answer this question, mice were made tolerant to and dependent on phenobarbital by the adulterated chow method of Belknap *et al.* [1]. The physical properties of brain membranes from control and tolerant-dependent mice were compared *in vitro* using fluorescence polarization of probes specific for different membrane regions. Secondly, we determined whether chronic ingestion of phenobarbital altered a specific membrane function: synaptic sodium transport. The voltage-

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dependent movement of sodium into isolated synaptosomes was chosen for study because it is inhibited by barbiturates and alcohols and appears to be altered by changes in membrane fluidity [11,22].

METHOD

Animals

DBA/2 (Charles River Breeding Lab, Portage, MI; Simonsen Labs, Gilroy, CA; Harlan Sprague-Dawley Inc., Indianapolis, IN; or Goodwin Cancer Institute, Plantation, FL), male mice (19–25 g) received from the Office of Mammalian Genetics, National Cancer Institute, were used in these experiments. Mice were housed 6 per cage in temperature regulated (22–23°C) animal facilities at least one week prior to use and maintained on a 12-hour light/dark cycle. Standard laboratory chow and water available ad lib.

Chronic Phenobarbital Administration

Mice, housed singly, were fed 2.2 g phenobarbital (free acid, Sigma Chemical Co., St. Louis, MO) per kg of ground standard laboratory chow and given water ad lib [1]. The control group was fed standard laboratory chow with availability group matched daily to that of the chow consumed by the phenobarbital group. After 7 or 8 days of phenobarbital consumption, mice were used immediately (non-withdrawn) or withdrawn 48 hr. Standard laboratory chow, given ad lib, replaced the phenobarbital diet during the withdrawal period.

Measurement of Withdrawal Signs

To assure that our animals were developing intoxication and physical dependence equivalent to that reported by others [1,2], a group of mice was evaluated for a 30 hr period following withdrawal of the phenobarbital diet. Animals were monitored for the presence of seizure on handling by the method of Goldstein [8], for withdrawal hypothermia [10], and for the incidence of lethal seizures during withdrawal.

Evaluation of Tolerance Development

To quantitate the development of cellular tolerance, brain phenobarbital content was determined at the time of loss or regaining of righting reflex in control and chronic phenobarbital-treated mice given an acute injection of phenobarbital. Phenobarbital was dissolved in saline (pH 9) and injected IP in a volume of 10 ml/kg. Animals were required to right themselves in a v-shaped trough (angle=120°). Loss of righting reflex was designated as the inability of the mouse to right itself within 30 sec following placement in a supine position. Regaining righting reflex was defined as the ability to immediately right when placed in the trough. Initial experiments with mice injected with a dose of 200 mg/kg phenobarbital yielded large differences in sleeptimes between controls and phenobarbital-exposed animals. To assure results were independent of factors related to sleeptime, additional data were obtained with phenobarbital dosages adjusted to yield similar sleeptimes for control and chronic phenobarbital mice. The dosages varied for different experiments, but were 180–200 mg/kg for control mice and 140–180 mg/kg for chronic phenobarbital groups.

Tolerance was also evaluated by determining brain phenobarbital concentrations at an ataxic threshold (end of barholding) [21]. Mice were given a 5 min pretest for barholding ability using a 9 mm diameter wooden dowel. Animals remaining on the bar for 5 min without falling were used for further testing. Following the pretest, each mouse was injected IP with phenobarbital (200 mg/kg) and returned to the bar. When the animal fell from the bar, it was immediately placed on the bar again. When the mouse fell a second time, it was quickly decapitated, and brain phenobarbital content was determined as described below.

Tolerance to the hypothermic effects of phenobarbital was also investigated. Control or chronic phenobarbital-treated mice were injected IP with 200 mg/kg phenobarbital, and body temperature was determined at 0, 20, 40, 60, and 80 min after injection [10].

All *in vivo* measurements were made between 8:00 a.m. and 12:00 noon. The light period was 6:00 a.m. to 6:00 p.m.

Phenobarbital Assay

Mice were decapitated and brains were removed immediately, divided along the mid-line, placed in vials and frozen at −10°C until used. At the time of assay, brains were weighed, hexobarbital was added as an internal standard, and the tissues were homogenized in 2 ml H_2O. Following acidification with HCl (4 N, 2 drops), the homogenate was extracted twice with chloroform. The chloroform phase was then made basic with NaOH (0.4 N, 0.2 ml) and extracted twice with water. The water fractions were saved, re-acidified (1 N HCl, 0.25 ml) and re-extracted with chloroform. The final chloroform phase was saved and dried under nitrogen, and the butylated derivatives of barbiturates were made using N,N-dimethylacitimide, saturated tetramethylammonium/methanol and 1-iodobutane in a 10:1:2 ratio [9]. Samples were dried under nitrogen and dissolved in methanol (50 μl). The butyl-derivatized phenobarbital was determined by gas chromatography (flame ionization, OV-17 glass column at 260° isothermal). Phenobarbital was quantified by the internal standard ratio method. The validity of chromatographic determination was verified by comparison with ^{14}C-labelled phenobarbital (New England Nuclear, Boston, MA) injected into mice at a 200 mg/kg dose, IP (100 μCi/kg body weight). Values determined by gas chromatography and ^{14}C-phenobarbital were equivalent.

Membrane Preparation and measurement of Fluorescence Polarization

At the time of withdrawal or 48 hr after withdrawal mice were decapitated and brain membranes prepared. Brains (2 to 3 per tube) were placed immediately in ice-cold 0.32 M sucrose containing 3 mM HEPES (pH 7.4) and 1 mM potassium ethylenediaminetetracetic acid (EDTA; Sigma Chemical Co., St. Louis, MO). Brain membranes were prepared using the Ficoll-sucrose density gradient centrifugation method [5,7]. Microsomal, mitochondrial, and synaptosomal plasma membrane (SPM 1+2) fractions were collected, washed, pelleted, and resuspended in 4-(2-hydroxyethyl)-1-piperazincethanesulfonic acid phosphate buffered saline (HEPES-PBS) [15] at a final concentration of 3–4 mg/ml. Removal of phenobarbital from brain membranes during SPM preparation was verified by IP injection of ^{14}C-labelled phenobarbital minutes before decapitation. Control animals were injected IP with 200 mg/kg phenobarbital containing [ring-2-^{14}C]-phenobarbital (0.2 μCi/mouse). One hour following the loss of righting reflex, animals were decapitated, and brain membranes were prepared as described below. Aliquots of each fraction were collected for determina-

tion of radioactivity by liquid scintillation spectrometry. Most of the phenobarbital was found in the soluble fraction with less than 0.5% of the total radioactivity (235,000 cpm per 3 brains) in the P_2 fraction. There was no detectable radioactivity in the SPM fraction.

Fluorescence polarization was determined using an HH-1 T format polarization spectrofluorimeter (BHL Associates, Burlingame, CA) with fixed excitation and emission polarization filters. Fluorescence intensity parallel ($I_{||}$) and perpendicular ($I_{\perp}$) to the polarization phase of the exciting light were monitored [15]. Polarization of fluorescence $[(I_{||}-I_{\perp})/I_{||}+I_{\perp})]$ and intensity of fluorescence $(I_{||}+2I_{\perp})$ were calculated by an on-line microprocessor. The excitation wavelength was 362 nm, a 03FCG001 filter (Melles Griot, Irvine, CA) was used in the excitation beam, and 003FIR045 filters were used for the emitted light. Cuvette temperature was maintained by a circulating water bath and monitored continuously by a thermocouple inserted into the curvette to a level just above the light beam.

The fluroescence probes, 1,6-diphenyl-1,3,5-hexatriene (DPH) and 1-(4-trimethylammonium phenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH) (Molecular Probes, Inc., Junction City, OR) were dissolved in THF (tetrahydrofuran) and THF-H_2O (1:1), respectively, and 0.05 μg of probe was added to 50 μg of membrane protein suspended in 1 ml of HEPES-PBS. The amount of THF added was less than 0.5 μl per sample. Probe incorporation was accomplished by incubating samples at 35°C for 10–15 min with occasional vortexing. Fluorescence polarization was measured at 37°C. Aliquots of drug (1–100 μl) were added to the samples, stirred and allowed to equilibrate a minimum of two min prior to determining polarization [15]. To minimize any systematic artifacts, membranes from control and phenobarbital-treated animals were tested in alternating order.

Brain Synaptosomal Sodium Uptake

As another means of investigating membrane tolerance, synaptosomal ^{24}Na uptake was determined. Synaptosomes were prepared from whole mouse brains using the Ficoll gradient centrifugation method [12]. The uptake of ^{24}Na was measured by a slight modification [11] of the procedure of Krueger and Blaustein [18]. Synaptosomes were removed from the Ficoll gradients, diluted slowly with low calcium buffer (145 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 10 mM glucose, 0.02 mM $CaCl_2$, 10 mM HEPES, pH adjusted to 7.5 at 25°C with Tris base), and pelleted at 17,369 g for 6 min. The pellet was resuspended in incubation buffer (140 mM choline Cl, 5 mM NaCl, 5 mM KCl, 1.4 mM $MgCl_2$, 1.0 mM $CaCl_2$, 20 mM NaH_2PO_4, 10 mM glucose, 20 mM Tris base, 20 mM HEPES, pH 7.4 at 25°C), and 180 μl aliquots (0.7 to 0.9 mg of protein) were incubated for 10 min at 30°C. Veratridine (10 μl to give a final concentration of 60 μM) was added followed immediately by addition of 10 μl aliquots of ethanol or pentobarbital solutions. All concentrations of drugs are expressed as the amount in the initial incubation volume. Ten min later, 50 μl of incubation buffer containing about 150 μCi ^{24}Na, kept at 30°C, was added while vortexing. Uptake of ^{24}Na was stopped 2 sec later (timed by metronome) by addition of 5 ml of ice-cold incubation solution and rapid filtration (Whatman GF/C). Filters were washed twice with 5 ml wash buffer, and radioactivity was determined by liquid scintillation counting (Beckman LS9000 with decay correction program). The veratridine-dependent ^{24}Na uptake (ΔV) was calculated as the difference between the uptake in the presence of veratridine and uptake in the absence of veratridine.

TABLE 1

DEPENDENCE DEVELOPMENT IN CHRONIC PHENOBARBITAL-TREATED MICE

Parameter	Control	Chronic Phenobarbital
Consumption (mg/kg/day)	—	368 ± 11* (145)
Lethal withdrawal seizure	0% (25)	18% (27/142)
Seizure on handling†‡	0% (25)	40% (49)
Withdrawal hypothermia‡	—	1.9°C (10)
Brain phenobarbital§	—	132 ± 10 (5)

*Mean ± S.E.M. Numbers in parentheses indicate number of animals tested.

†Values represent seizure on handling as determined by Goldstein [8] using a score of one or greater as a positive response.

‡Values determined at 21 hr of withdrawal.

§Phenobarbital concentration (μg/g brain wet wt.) at the time of withdrawal.

Statistics

Concentration-response curves were compared by analysis of variance for repeated measures. Effects of *in vitro* drug additions on ^{24}Na uptake were determined by a Student's *t*-test for paired samples. In all assays, the effects of chronic phenobarbital treatment were evaluated by a Student's *t*-test for unpaired samples.

Other Procedures

Protein was determined by the method of Lowry *et al.* [19].

RESULTS

Phenobarbital Tolerance and Dependence

The chronic phenobarbital intoxication method used in these studies resulted in a degree of intoxication and dependence equivalent to that reported in the literature [1] (Table 1). Mean consumption of phenobarbital was slightly higher than the value (272 mg/kg) reported by Belknap *et al.* [1] for DBA/2 mice. Brain phenobarbital concentrations at the end of intoxication were also higher than those of Belknap *et al.* [2] who found 81 μg/g and 111 μg/g after six and nine days, respectively, of phenobarbital consumption. Moreover, lethal seizure occurrence, handling-induced seizures and severity of withdrawal hypothermia were equivalent to or slightly greater than reported values for this strain of mice [1,2].

Evaluation of functional tolerance development in phenobarbital-exposed mice was determined by brain phenobarbital levels at the time of loss or regaining of righting reflex. These results indicated significant tolerance development in non-withdrawn mice (Table 2). In contrast, mice withdrawn from the phenobarbital diet for 48 hr prior to testing displayed no tolerance for the loss of regaining of the righting reflex (Table 2). The latter finding was further investigated by determination of brain phenobarbital levels at the time of loss of barholding ability ("ataxia") and by investigation of phenobarbital-induced hypothermia. In mice

TABLE 2

DETERMINATION OF PHENOBARBITAL TOLERANCE IN MICE TREATED CHRONICALLY WITH PHENOBARBITAL

Parameter	Brain Phenobarbital Concentration	
	Control*	Chronic Phenobarbital*
Non-Withdrawn		
Loss of righting reflex	100 ± 2†	163 ± 2§
Regain righting reflex	132 ± 1	155 ± 5‡
48 hr Withdrawn		
Loss of righting reflex	108 ± 4	92 ± 3
Regain righting reflex	104 ± 4	110 ± 10

*DBA/2 mice were fed a diet containing phenobarbital or pair-fed a control diet for 7–8 days. At the end of this time (non-withdrawn) or after removal of the phenobarbital diet for 48 hr (withdrawn), mice were injected IP with phenobarbital and killed at the time of loss of righting reflex or regaining of righting reflex for determination of brain phenobarbital concentrations.

†Values represent μg phenobarbital/g brain wet wt., mean± S.E.M., n=5–10.

‡Significantly different from control, $p<0.02$.

§Significantly different from control, $p<0.001$.

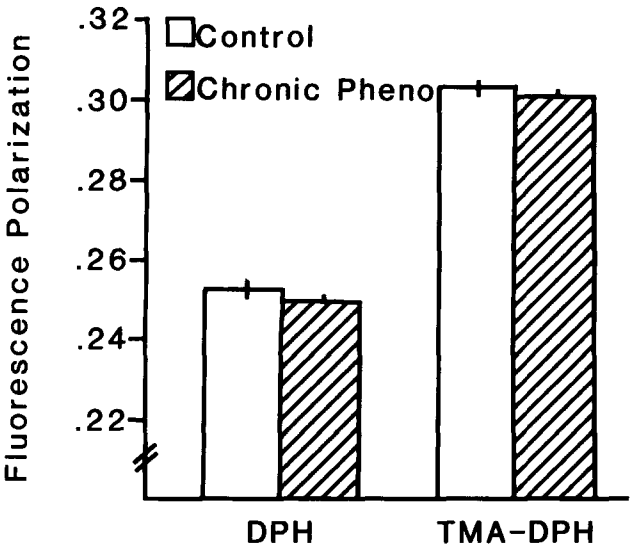

FIG. 1. Baseline fluorescence polarization of probe molecules incorporated into SPM from mice treated chronically with phenobarbital (hatched bars) or pair-fed controls (open bars). Diphenylhexatriene (DPH) or trimethylammonium-DPH (TMA-DPH) was incorporated into the membranes and fluorescence polarization was determined at 37°C. Vertical bars represent S.E.M.; DPH, n=9; TMA-DPH, n=3.

withdrawn 48 hr, no difference existed in brain phenobarbital levels between phenobarbital (38±9 μg/g brain, n=9) and control (41±17 μg/g brain, n=12) mice at the onset of ataxia. In addition, no difference in maximal phenobarbital-induced hypothermia was noted between groups (phenobarbital, 5.1°C vs. control, 5.7°C). These data indicate that phenobarbital tolerance decays completely within 48 hr following removal of phenobarbital adulterated diet.

Membrane Physical Properties

Fluorescent probe molecules were incorporated into SPM, and fluorescence polarization was used to estimate membrane fluidity. Alteration in fluidity of the central portions of the membranes was monitored by DPH, and the surface portions were monitored by TMA-DPH. SPM from tolerant-dependent mice showed no significant change in baseline rigidity of either membrane region compared to controls (Fig. 1). Furthermore, SPM from phenobarbital-tolerant mice showed no resistance to the *in vitro* fluidizing effects of phenobarbital, pentobarbital, or ethanol (Fig. 2). Indeed, the degree of fluidization produced by pentobarbital was significantly greater in SPM from phenobarbital tolerant-dependent mice than in pair-fed controls. The effects of phenobarbital and ethanol were also numerically greater for SPM from tolerant-dependent mice than from controls, but this difference was not statistically significant (Fig. 2). Investigation of mitochondrial and microsomal brain membrane fractions revealed no differences between phenobarbital and control mice in either baseline rigidity or response to *in vitro* exposure to pentobarbital or ethanol (data not shown).

Similar results were also obtained with repeated injections of phenobarbital. SPM from mice injected IP with phenobarbital at 12 hr intervals for 7 days (90 mg/kg/day for 5 days, then 120 mg/kg/day for 2 days) did not differ in baseline

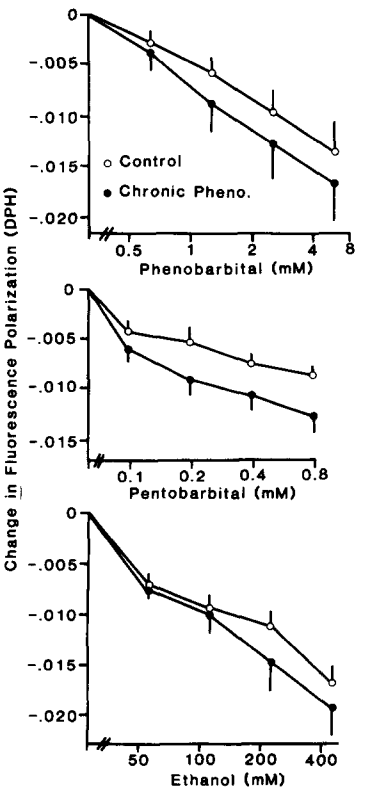

FIG. 2. Effect of *in vitro* exposure to phenobarbital (upper panel), pentobarbital (middle panel) or ethanol (lower panel) on the fluorescence polarization of DPH in SPM from control (open circles) and chronic phenobarbital (filled circles) mice. Vertical bars represent S.E.M., n=6–9. Significance values determined by ANOVA were phenobarbital, $F(1,10)=0.52$, $p=0.49$; pentobarbital, $F(1,14)=5.66$, $p=0.03$; and ethanol, $F(1,14)=0.65$, $p=0.43$.

rigidity (DPH or TMA-DPH) from SPM of mice injected with saline. The membrane disordering action of phenobarbital (as measured *in vitro* by DPH) was greater, $F(1,10)=9.9$, $p<0.05$, for SPM from the mice injected chronically with phenobarbital than for those receiving saline (data not shown). A single injection of phenobarbital (60 mg/kg, IP) 12 hr before death did not alter either the baseline rigidity or the phenobarbital sensitivity (both measured with DPH and TMA-DPH) of SPM.

Synaptosomal Sodium Transport

The veratridine-stimulated uptake of $^{24}Na^+$ by isolated synaptosomes was used as a measure of a functional property of the neuronal membrane. The use of a 2-sec uptake period and low (5 mM) extracellular sodium assured that the uptake of $^{24}Na^+$ represents a unidirectional flux through the voltage-sensitive channel and does not reflect processes such as Ca^{++}-Na^+ exchange or Na^+,K^+-ATPase [11,18]. *In vitro* exposure to ethanol (400 mM) or pentobarbital (1 mM) inhibited sodium uptake by 56% in synaptosomes from control mice but only by 36% in synaptosomes from phenobarbital-tolerant mice (Table 3). Large concentrations of ethanol and pentobarbital were used to produce a marked decrease of sodium influx. Concentrations of the drugs associated with intoxication *in vivo* produce a small, but significant, inhibition of sodium flux [11,22]. The uptake of sodium in the absence of drugs was not affected by phenobarbital ingestion. Thus, chronic treatment with phenobarbital produced adaptive changes in the sodium channel complex that reduced the effects of pentobarbital and ethanol.

TABLE 3

BRAIN SYNAPTOSOMAL ^{24}Na UPTAKE: TOLERANCE DEVELOPMENT TO THE *IN VITRO* EFFECTS OF ETHANOL AND PENOBARBITAL

	In Vivo Treatment	
In Vitro Addition	Pair-Fed Control Diet	Chronic Phenobarbital Diet
Water	1.76 ± 0.12*	1.70 ± 0.10
Pentobarbital (1 mM)	0.76 ± 0.05	1.08 ± 0.10†
Ethanol (400 mM)	0.78 ± 0.01	1.08 ± 0.13‡

*Values represent the veratridine-dependent sodium uptake as nmol/mg protein/2 sec, mean ± S.E.M., n=5.

†Significantly different from pair-fed control exposed to pentobarbital, $p<0.02$, two-tailed t-test.

‡Significantly different from pair-fed control exposed to ethanol, $p<0.05$, one-tailed t-test.

DISCUSSION

Administration of phenobarbital in ground lab chow to mice resulted in physical dependence and tolerance development similar to that reported by others using this technique [1,2]. Physical dependence was demonstrated in our animals by seizures on handling, lethal seizures and hypothermia during withdrawal. For determination of tolerance development, we chose two readily identifiable endpoints: the loss and regaining of the righting reflex following injection of phenobarbital. Our data indicate the presence of significant tolerance at the time of loss and regaining of righting reflex in non-withdrawn mice compared to controls. These results are in agreement with reports of functional tolerance in chronic phenobarbital-treated rats [6] and mice [2,26]. Although we demonstrated tolerance in non-withdrawn animals, 48 hr following the withdrawal of diet we were no longer able to detect tolerance. These results indicate a rapid decay of tolerance with a time course similar to that reported for the decay of physical dependence [3].

If disruption of membrane lipids represents the physical mechanism leading to the anesthetic action of barbiturates, then chronic exposure to these drugs might cause a homeoviscous adaptation of brain membranes analogous to that documented for chronic ethanol treatment [4, 15, 24]. Our results, however, indicate no increase in initial rigidity of the core or surface portions of SPM from phenobarbital tolerant-dependent mice. Moreover, no decrease in drug sensitivity was noted in SPM from tolerant-dependent mice when exposed *in vitro* to phenobarbital, pentobarbital, or ethanol; in fact, these membranes tended to be more sensitive than control membranes to the fluidizing action of the drugs. Thus, despite clear evidence of tolerance and dependence *in vivo*, we were unable to detect any differences in the physical properties of the isolated brain membranes. In contrast, Johnson *et al.* [17] reported both increased baseline rigidity and decreased ethanol sensitivity in vesicles prepared from lipid extracts of crude synaptosomal membranes from mice made tolerant to pentobarbital using the three-day pellet implantation method of Ho *et al.* [16]. The numerous methodological differences between the present study and that of Johnson *et al.* may account for the discrepancies in the results.

Acute exposure to barbiturates and ethanol is known to cause fluidization of SPM by disrupting the core of the membrane [13,14]. Differences exist in the effects of ethanol and barbiturates, however, in that pentobarbital reduces intrinsic tryptophan fluorescence while ethanol does not [14]. Such results suggest that chronic barbiturate treatment might affect membrane proteins. This speculation is supported by the observation that chronic ingestion of phenobarbital produced adaptation of synaptosomal sodium channels to the effects of barbiturates and ethanol. Similar evidence for membrane tolerance was presented by Elrod and Leslie [6] who found that chronic phenobarbital treatment attenuated the *in vitro* effects of barbiturates on synaptosomal calcium uptake. These results demonstrate that cellular tolerance to barbiturates is accompanied by changes in the functional properties of brain membranes that are consistent with barbiturate tolerance and ethanol cross-tolerance. These changes in membrane function cannot, however, be ascribed to changes in the bulk physical properties of brain membranes.

In conclusion, while similar signs of physical dependence occur in animals treated chronically with alcohols or barbiturates, the mode of development of dependence and tolerance may be different for these two classes of anesthetics. The increased rigidity and decreased sensitivity of brain membranes produced by chronic ethanol treatment was not found after chronic phenobarbital administration. Adaptation of the synaptosomal sodium transport system during chronic phenobarbital intoxication demonstrates the presence of membrane tolerance but suggests a restricted site of adaptation to barbiturates. Such a site may involve alterations in protein configuration or protein interaction with annular lipids in direct contact with the protein. This suggests that

the effects of barbiturates may be more discrete than those of ethanol. This is consistent with the idea that at least some of the actions of barbiturates are mediated by specific receptors [28], whereas there is no evidence for specific receptors for ethanol.

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