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Tolerance and Cross-Tolerance in Chronic Alcoholics: Reduced Membrane Binding of Ethanol and Other Drugs

Abstract. *Membrane binding of ethanol, anesthetics, and hydrophobic molecules in brain synaptosomes and liver mitochondria from rats is conspicuously reduced after long-term consumption of ethanol. The membranes are resistant to structural disordering by both ethanol and halothane. Tolerance, cross-tolerance, and dependence in chronic alcoholics may in part result from membrane alterations that inhibit the binding of ethanol and other drugs.*

In chronic alcoholics ethanol tolerance and dependence probably arise from alterations in the membrane properties of the nervous system (*1*). This is consistent with the fact that the acute effects of ethanol, similar to other anesthetics, result from membrane interactions (*2*). We recently reported that liver mitochondrial membranes from rats chronically fed ethanol are resistant to the uncoupling effect of ethanol, which is associated with a resistance to the disordering of the membrane lipid structure by ethanol (*3*). The resistance is caused by altered lipid composition of the mitochondrial membranes. We now report that this resistance is associated with a drastic reduction in the partitioning of ethanol into liver mitochondrial membranes. We also report that brain synaptosomal plasma membranes in ethanol-fed rats are resistant to disordering by ethanol, a finding similar to previous observations in ethanol-fed mice (*1*). While the cause of the latter resistance may arise, in part, from increased cholesterol content (*4*), the end result of ethanol feeding is the same for both mitochondrial and synaptosomal membranes, namely, a reduction in the binding of ethanol. Synaptosomal and mitochondrial membranes from ethanol-fed rats are also resistant to structural disordering by the inhalation anesthetic halothane, and this resistance is also associated with reduced binding of halothane by these membranes. The binding of the sedative phenobarbital is also reduced in membranes from ethanol-fed rats.

To study the effect of ethanol and halothane on membrane structure, we employed two spin-labeled membrane probes. We calculate the order parameter S from the hyperfine splitting of the electron paramagnetic resonance (EPR) spectra of 5-doxyl stearate, a fatty acid analog incorporated into the lipid domain in cell membranes. The order parameter provides a measure of the freedom of motion of the probe in the membrane and is decreased when the membrane becomes more fluid (*5*, *6*). The nitroxide-labeled decane 4-butyl-2,2-dimethyl-1, 4-pentyloxazolidine -*N*- oxyloxazolidine (5N10) is a partition probe. The partition coefficient of the spin probe between the hydrophobic membrane and the medium is calculated from the contribution of the bound and free species to the composite EPR spectrum (*7*). The partitioning into the membrane increases as the membrane becomes more fluid.

Figure 1 shows the results of these measurements in liver mitochondria and brain synaptosomes from ethanol-fed rats and their pair-fed controls. Each curve represents membranes from a different rat. Both the order parameters and the probe partitioning show that membranes from ethanol-fed rats are more rigid than those from the controls. When titrated with ethanol (25 mM to 1.0M), control membranes become much more fluid, as indicated by a decrease in the order parameter and an increase in the partition coefficient. Membranes from ethanol-fed rats are much less affected by the addition of ethanol and remain relatively rigid even at high concentrations of ethanol. In the presence of moderate concentrations of ethanol, membranes from ethanol-fed animals are as fluid as membranes from the control animals in the absence of ethanol. The latter presumably are in a state that is optimal for membrane function.

There are similar differences in the

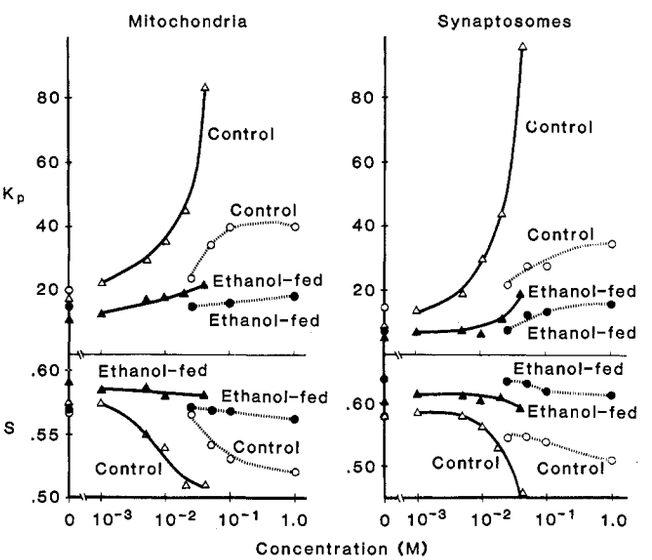

Fig. 1. Effect of ethanol (○, ●) and halothane (△, ▲) on the 5N10 partition coefficient K_p (upper panels) and on the 5-doxyl stearate order parameter S (lower panels) in liver mitochondrial membranes and brain synaptosomal membranes from ethanol-fed rats and their pair-fed controls. EPR spectra were obtained at 35° ± 0.2°C with a Varian E-109 spectrophotometer. The nitroxide-decane derivative 5N10 was added to membrane suspensions (10 mg of protein per milliliter) to give an apparent concentration of 0.1 mM. Partition coefficients were calculated by estimating the amount of bound and free probes from the EPR spectra (*7*). Sufficient 5-doxyl stearic acid was added to the membrane suspension to obtain a probe-to-lipid ratio of 1:200. The order parameter was estimated by the method described by Gaffney (*6*). Rats were given ethanol (14 g per kilogram of body weight per day) for 35 days; pair-fed controls received isocaloric carbohydrate instead of ethanol (*3*). Liver mitochondria (*3*) and brain synaptosomal (*9*) membranes were prepared by conventinal procedures. For EPR measurements the membranes were suspended in 0.25M sucrose, 20 mM tris-Cl (*p*H 7.4), 2 μM rotenone, and 10 μM ferrocyanide. At least three pairs were tested for each of the effects shown. The results are typical examples of these experiments.

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Table 1. Partition coefficients of ethanol and anesthetics in brain synaptosomal and liver mitochondrial membranes from ethanol-fed and control rats. The coefficients were determined by incubating the membranes with a ^{14}C-labeled compound and ^3H_2O. The $^{14}C/^3H$ ratio in the supernatant and the pellet was determined after sedimentation of the membranes. Partition coefficients were calculated on the assumption that the amount of ^{14}C-labeled compound in the pellet in excess of the amount dissolved in the pellet water is dissolved in the membrane lipids (*10*). The number of pairs of rats used for each determination is given in parentheses. Values are means ± standard deviations. All the experimental values are significantly different from the corresponding control values at $P < .01$ (paired t-test).

Group	Ethanol	Halothane	Phenobarbital
	Mitochondrial membranes		
Ethanol-fed	1.17 ± .634 (8)	21.4 ± 2.71 (5)	22.1 ± 3.3 (5)
Control	3.60 ± .740	28.6 ± 5.58	33.0 ± 1.5
Control/ethanol-fed	4.24	1.35	1.54
	Synaptosomal membranes		
Ethanol-fed	0.33 ± 0.11 (5)	21.7 ± 10.2 (7)	
Control	1.00 ± 0.42	27.5 ± 11.3	
Control/ethanol-fed	3.07	1.38	

response to halothane. Halothane is about 50 times more potent than ethanol in disordering membranes. In a titration from 1 to 40 m*M* there is strong disordering in control membranes, as indicated by the lowering of the order parameter and by the increase in probe partioning. However, in this case, too, membranes from ethanol-fed rats are much less affected, even at high concentrations of halothane. While the response of synaptosomes and mitochondria is quite similar, in general, synaptosomal membranes are considerably more rigid than mitochondrial membranes.

These results suggest that the altered membranes are the origin of physical dependence, tolerance, and cross-tolerance. Dependence may arise if the membranes of ethanol-fed rats are too rigid for optimal function in the absence of ethanol, since in the presence of ethanol their rigidity is similar to that of normal membranes. Tolerance may be due to resistance of the membranes to disordering by high concentrations of ethanol, whereas cross-tolerance to anesthetics is indicated by membrane resistance to disordering by halothane. While the partition probe is used here to monitor membrane structure, the results also suggest the mechanism for the acquired resistance. If the increased membrane rigidity results in decreased binding of ethanol, anesthetics, and other drugs, then resistance to these compounds is expected. Table 1 shows the results of experiments in which the partition coefficients of ethanol, halothane, and phenobarbital (an anesthetic, commonly used as sedative) were determined in liver mitochondrial and brain synaptosomal membranes. The partition coefficient of ethanol is much lower in both kinds of membranes from ethanol-fed rats. In mitochondrial membranes from controls the partition coefficient of ethanol is actually more than four times higher than in mitochondrial membranes from ethanol-fed rats. Synaptosomal membranes are more rigid; hence, the ethanol partition coefficient is much lower and the determination is more difficult. Nevertheless, here too the partition coefficient of ethanol for controls is at least three times higher than that for ethanol-fed animals. Similarly, the partition coefficients of halothane and phenobarbital are lower in mitochondrial membranes from ethanol-fed rats than in those from the controls. The partition coefficients of halothane are also lower in synaptosomal membranes from ethanol-fed rats. The partition coefficients of phenobarbital in synaptosomal membranes show a similar trend; however, the difference is small, thus excluding a definite conclusion. Preliminary experiments suggest that the binding of this and possibly other drugs to membranes cannot be fully characterized by a partition coefficient. A more detailed study of the binding of these drugs to the membranes should clarify this issue.

We have observed changes comparable to those shown in Fig. 1 in red blood cell membranes. This finding suggests that comparable changes occur in most membranes and may play a role in many diseases related to alcoholism. The differences in the partition coefficients of ethanol, halothane, and phenobarbital between control and ethanol-fed rats are similar in magnitude to the differences in the partition coefficients of the decane spin probe (1.4- to 4-fold difference). Since the latter are strongly correlated with the membrane order parameter, it is apparent that the changes in membrane rigidity are related to the binding of alcohol and anesthetics. Whether the reduced membrane binding is sufficient to fully explain the acquired resistance to the disordering effects of these compounds can only be answered by further refinement of the measurements.

In conclusion, we suggest a simple, unified explanation for physical dependence, tolerance, and cross-tolerance: chronic consumption of ethanol induces an adaptation of the membrance composition causing increased membrane rigidity (decreased fluidity), which leads to a reduction in the binding of alcohol to the membrane and hence to the acquisition of tolerance. The increased rigidity impairs normal membrane function in the absence of ethanol, but in the presence of moderate concentrations of ethanol the membrane becomes sufficiently fluid to resemble normal membranes (dependence). Since the membrane is more rigid it also binds fewer molecules of anesthetics (cross-tolerance). Other drugs whose activity depends on membrane binding should also be cross-tolerated by the alcoholic. Similarly, the effect of alcohol on membrane fluidity may increase drug binding or permeability in nerve and other membranes and thus may be related to many alcohol-drug interactions (*8*).

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10. Mitochondria (6 mg of protein per milliliter) were suspended in 0.2*M* sucrose containing ^3H_2O (0.25 μC/ml) and ^{14}C-labled drug (0.1 μC/ml). The mitochondrial pellet was sedimented by centrifugation (Beckman Microfuge). A 50-μl sample from the supernatant and the pellet were mixed with 0.5 ml of 1.0*M* $HClO_4$; after extraction and sedimentation of the precipitate, a 0.3-

ml sample was mixed with 5 ml of scintillation liquid for counting. Synaptosomes were treated similarly except that they were suspended in 0.32*M* sucrose and sedimented in a Beckman Airfuge ultracentrifuge. In the case of halothane and ethanol, which are volatile, the pellet-to-supernatant ratio was extrapolated to the time of pellet separation by measuring the ratio at several time intervals after separation of the pellet. To calculate the partition coefficient from the pellet-to-supernatant ratio, we determined the fractions of pellet volume occupied by lipids and water. Under our centrifugation conditions, the fraction of pellet volume occupied by lipid, f, was 0.12 for mitochondria and 0.07 for synaptosomes. It is easy to show (H. Rottenberg, unpublished manuscript) that if $^{14}Cp/^{3}Hp$ is the pellet ratio and $^{14}Cs/^{3}Hs$ is the supernatant cation, then $K_p = (^{14}Cp^{3}Hs)/(^{14}Cs^{3}Hpf) - 1/f$.

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Radiation Doses from Mount St. Helens 18 May 1980 Eruption

Recently published work by Fruchter *et al.* (*1*) characterizes the ash from the Mount St. Helens eruption of 18 May 1980 and provides a data base that can be used to estimate the radiation dose equivalent to individuals in the fallout area. Fruchter *et al.* reported relatively high concentrations of radon daughters (Table 1) in newly fallen ash in the vicinity of Richland, Washington, approximately 225 km due east of the volcano, with significantly smaller quantities of ^{226}Ra, ^{232}Th, and ^{40}K and a respirable dust fraction (< 3.5 μm) ranging from 1 to 3 percent by weight of the ash. Using these data as a starting point, it is possible to calculate the dose equivalent to the critical organs (lung and bronchial epithelium) from the 18 May eruption.

Inspection of the data in (*1*) indicates that about one-tenth of the activity was associated with particles of respirable size. An air sample collected at the Hanford Meteorological Station on 18 May showed a 24-hour (midnight to midnight) mean dust concentration of 10,600 μg/m^3 (*2*). The meteorologist reported the passage time for the Mount St. Helens cloud as ~ 5 hours (1100 to 1600). Assuming that essentially all the dust collected on the filter was from the Mount St. Helens plume, the mean dust concentration over the 5-hour cloud passage period is 5×10^4 μg/m^3 (0.05 g/m^3). If the dust collected at the Hanford Meteorological Station was similar in radioactivity concentration and particle size distribution to that reported by Fruchter *et al.*, then the activity reaching the bronchi and lungs from inhalation at the rate of 1.2 m^3/hour (*3*) over the 5-hour period is

$$270 \text{ pCi/g} \times 0.05 \text{ g/m}^3 \times 1.2 \text{ m}^3\text{/hour} \times 5 \text{ hours} \times 0.1 = 8 \text{ pCi}$$

Using dose conversion factors calculated by McPherson (*4*), the combined 50-year radiation dose equivalent from inhalation of the three radon daughters would be 4×10^{-6} rem to the bronchial epithelium and 1.5×10^{-6} rem to the pulmonary lung. These can be compared to a dose equivalent of ~ 3×10^{-4} rem received during a 5-hour period from inhalation of natural radon-thoron daughters normally present in the air, reported as an average for the continental United States (*5*).

Eventually, all of the radon daughters will decay to ^{210}Pb, which, because of its relatively low specific activity, would have an initial activity concentration of only about 1.7×10^{-6} that of the grandparent ^{214}Bi. However, because of its long half-life (22.3 years), this nuclide will remain in the environment for a long period of time. Estimates of potential dose equivalent commitments from lifetime inhalation of resuspended ^{210}Pb must take into account initial ground deposition, particle size, gradual agglomeration into larger size particles, weathering of the deposited ash, and wind profiles.

The depth of ash in the vicinity of the Hanford Meteorological Station was approximately 3 mm on 18 May. Taking the density of the ash as 2 g/cm^3, the total ^{210}Pb activity deposited is estimated as

$$3 \times 10^{-3} \text{ m} \times 2 \times 10^6 \text{ g/m}^3 \times 270 \text{ pCi/g} \times 1.7 \times 10^{-6} = 2.8 \text{ pCi/m}^2$$

where 270 pCi/g represents ^{214}Bi and 1.7×10^{-6} is the ratio of the activity of ^{210}Pb to that of ^{214}Bi. McPherson and Watson (*6*) integrated the resuspension equation of Anspaugh *et al.* (*7*) for a 50-year period and obtained a value of 8.9×10^{-3} day per meter. Applying this factor, an inhalation rate of 23 m^3/day (*3*), and the value of 2.8 pCi/m^3 for ^{210}Pb as the initial deposition yields a total of 0.57 pCi of ^{210}Pb inhaled, mostly in the first few years. Assuming this to be insoluble dust, the 50-year integrated dose equivalent to the lung is ~ 2×10^{-4} rem, with a similar dose equivalent delivered to bone from ^{210}Pb deposited there. These are insignificant in comparison to the dose equivalent of 5 to 10 rem received from normal levels of natural radioactivity over the same time period. Further, the estimated ^{210}Pb concentration in the ash, 4×10^{-4} pCi/g, is about 0.1 percent of the naturally occurring concentration of 0.3 pCi/g for soil in the vicinity of the Hanford Meteorological Station.

Table 1. Radionuclides in Mount St. Helens ash at Richland, Washington, 18 May 1980 (*1*).

Nuclide	Concentration (pCi/g)	Comment
^{214}Pb	212	Fresh ash fallout collected 3 hours 43 minutes after eruption
^{214}Bi	274	
^{214}Po	274	
^{226}Ra	0.376	
^{232}Th	0.088	
^{40}K	7.73	

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