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A Relationship between Alcohol Intoxication and the Disordering of Brain Membranes by a Series of Short-Chain Alcohols[1]

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ABSTRACT

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This study has established a correlation between the hypnotic potencies of aliphatic alcohols and their abilities to disrupt the structure of neuronal membranes *in vitro*. The hypnotic potency was determined in mice from the ED_{50} for loss of righting reflex. The alcohol-induced perturbation of mouse brain synaptosomal plasma membranes was measured by a sensitive electron paramagnetic resonance technique. The membrane disordering potency was determined from the slope of the concentration-dependent decrease in order parameter observed for each alcohol. Significant reductions in the order parameter were observed at nerve blocking concentrations. The following alcohols were investigated: ethanol, 1-propanol, 2-propanol, 1-butanol, 2-butanol, 2-methyl-1-propanol, 2-methyl-2-propanol, 1-pentanol, 2-pentanol, 3-methyl-1-butanol, 1-hexanol and 1-octanol. The disordering potency of each alcohol was closely related to its membrane solubility, based on published oil/water partition coefficients. Structural disorganization resulting from the incorporation of alcohols into neuronal membranes may be an integral step in the mechanism of alcohol intoxication. For a given degree of membrane disorder, intramembrane alcohol concentrations and intramembrane alcohol volumes were estimated from published partitioning and molecular volume data and compared for constancy. The data did not favor either the intramembrane drug concentration or the intramembrane drug volume as a more effectual determinant of disordering potency.

It is widely accepted that the anesthetic site of action of alcohols is located within neuronal membranes (Seeman, 1972; Hunt, 1975). Interactions between alcohols and hydrophobic sites (both lipid and protein) appear to be physical in nature, lacking chemical specificity. The relationship between the anesthetic potency of a compound and its lipid solubility has been clearly established (Meyer and Gottlieb, 1926; Seeman, 1972). The classical work of Meyer and Overton introduced the concept that anesthesia occurs when a compound located within the hydrophobic regions of a cell reaches a critical molar concentration (Meyer and Gottlieb, 1926). Considering perturbations due to molecular volume, Mullins (1954) proposed a modification of this rule, suggesting that narcosis occurred when a critical volume within the membrane was occupied by an anesthetic agent.

Alcohols at nerve-blocking concentrations expand biological membranes (Seeman *et al.*, 1969; Seeman, 1974), suggesting that the occupation of membrane space by an anesthetic results in physical disordering of the membrane structure. The structural disordering of natural membranes and lipid bilayers by alcohols has been demonstrated by biophysical techniques utilizing EPR (Paterson *et al.*, 1972; Chin and Goldstein, 1977), fluorescence (Lee, 1976; Zavoico and Kutchai, 1980), nuclear magnetic resonance (Metcalfe *et al.*, 1968; Turner and Oldfield, 1979) and differential scanning calorimetry (Jain *et al.*, 1975; Krishnan and Brandts, 1979). Appreciable disordering of membranes occurs at low aqueous concentrations (11–44 mM) of ethanol (Chin and Goldstein, 1977) and clinical gaseous concentrations (1.3% v/v) of halothane (Mastrangelo *et al.*, 1978), so this action may contribute significantly to the mechanism of anesthesia.

The present study is an application of an EPR technique to several alcohols. We have measured the membrane disordering potency of six straight-chain primary alcohols (C2–C6 and C8), two isoprimary alcohols (C4 and C5), three secondary alcohols (C3–C5) and one tertiary alcohol (C4). The reduction in order parameter of synaptosomal plasma membranes from mice was measured after the *in vitro* addition of each alcohol to membranes spin-labeled with 5-doxylstearic acid. This technique was sensitive to changes in order parameter at nerve blocking concentrations of these alcohols.

A good correlation between membrane disordering potency

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ABBREVIATION: EPR, electron paramagnetic resonance; SPM, synaptosomal plasma membranes; G, gauss.

and membrane partitioning is expected if the degree of membrane disordering depends on the lipid solubility of each alcohol. This relationship has been examined in a preliminary study (Lyon *et al.*, 1980). Since the magnitudes of the oil/water partition coefficients, as reported by Lindenberg (1951), depend on the number of carbon atoms and the molecular structure of each alcohol, it could be predicted that: 1) the disordering potency would increase with the addition of each methylene group, as shown for the disordering of lipid bilayers (Paterson *et al.*, 1972) and 2) the order of relative disordering potency for structural isomers would be straight-chain primary> isoprimary>secondary>tertiary.

Mullins (1954) suggested that, for equal degrees of narcosis produced by different agents, the intramembrane volume is, empirically, a better constant than the intramembrane concentration. A given degree of membrane disordering may require a critical concentration of alcohol in the membrane (Meyer and Overton) or a critical volume of alcohol in the membrane (Mullins). We checked the fit of our data to both hypotheses.

Linear relationships between the lipid solubilities of alcohols and their relative biological effects have been demonstrated for numerous systems (Hansch and Dunn, 1972). A few investigators have examined the comparative intoxicating effects of short-chain alcohols in rats (Wallgren, 1960; LeBlanc and Kalant, 1975), and McCreery and Hunt (1978) established a correlation between the ataxic effects on rats and the membrane/buffer partition coefficients for several alcohols. This correlation suggests that the ataxic potency of an alcohol is dependent on its ability to incorporate within the membrane bilayer. The purpose of this study was to examine the relationship between the hypnotic potency of an alcohol administered to Swiss-Webster mice and the ability of that alcohol to disorder brain membranes from mice of the same strain. The hypnotic potency was determined from the ED_{50} for loss of righting reflex, a well defined behavioral endpoint.

Methods

Materials. Ethanol was purchased from U.S. Industrial Chemicals Company (New York, NY); 1-propanol, 2-propanol, 1-butanol, i-butanol (2-methyl-1-propanol), *t*-butanol (2-methyl-2-propanol), 1-hexanol, 1-heptanol and 1-octanol were purchased from J. T. Baker Chemical Co. (Phillipsburg, NJ); 2-butanol, 2-pentanol and i-pentanol (3-methyl-1-butanol) were purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI); and 1-pentanol was purchased from Allied Chemical Co. (Morristown, NJ). All alcohols were of reagent grade and were used without further purification. The spin label was 5-doxylstearic acid (N-oxyl-4'-4'-dimethyloxazolidine derivative of 5-ketostearic acid; Syva, Palo Alto, CA).

Membrane preparation. SPM were prepared from whole brains of male Swiss Webster mice (8–10 weeks old; Charles River Breeding Laboratories, Inc., Wilmington, MA) by the combined flotation-sedimentation density gradient centrifugation technique of Jones and Matus (1974). After removal from the sucrose gradient, the SPM were washed with isotonic buffer and pelleted by centrifugation at 100,000 × *g* for 20 min. The pellet was stored in liquid nitrogen, thawed before the spin-labeling procedure and then diluted with isotonic buffer (Chin and Goldstein, 1977). Silanized conical tubes were coated with spin label during solvent (ethanol) evaporation and kept under vacuum overnight to remove all traces of solvent. The stock SPM were transferred into the coated tubes and incubated with spin label (12 μg of spin label per milligram of membrane protein) at 37°C for 30 min. Each EPR sample (20–25 μl) was prepared by adding spin-labeled stock membranes to buffer (control) or buffer containing alcohol, refrigerated overnight and transferred to a 50-μl heat-sealed capillary tube. A final protein concentration (Lowry *et al.*, 1951) of 20 mg/ml was maintained and the spin-label concentrations were calculated to be less than 1% by weight of membrane lipid. Each alcohol, with the exception of 1-octanol, was solubilized completely in buffer before the solution was mixed with membrane suspension. To achieve the highest 1-octanol concentration (16 mM) in membrane suspension, 1-octanol was diluted 398/1 (v/v) with membrane suspension (at 20 mg/ml of protein concentration). This produced a nominal aqueous 1-octanol concentration of approximately 1.2 mM (estimated from equation 3 below), which is less than the saturated aqueous concentration of 4.5 mM (Butler *et al.*, 1933).

Measurement of order parameters. Each sample was incubated at 37°C for 10 min and centered vertically to equilibrate in the microwave cavity thermostated at 37.0 ± 0.1°C. The temperature was controlled by a Varian E-257 variable temperature device and monitored by a Newport digital pyrometer. The first derivative spectra were collected by a Varian E-104A EPR spectrometer at 5 mW of microwave power and 1 G modulation amplitude over a 100-G range at a field of about 3200 G. The spectral data were digitized on line at intervals of 0.04 G by a PDP 11/03 computer. Segments of spectra were displayed and amplified on a VT-55 Decscope. The locations of the inflection points were estimated from a third-order curve fitting of the appropriate peaks and were used to calculate the inner and outer hyperfine splittings. The order parameter, an index of membrane fluidity, was calculated by the method of Hubbell and McConnell (1971). Gaffney's factor was used to correct the observed inner hyperfine splittings (Gaffney, 1976) and 5-doxylpalmitate was used as a crystalline standard. Each sample was scanned and analyzed three times to produce an average order parameter for that sample. Samples were prepared at each alcohol concentration from at least four different spin-labeled membrane preparations.

Intoxication studies. Male Swiss-Webster mice (5–8 weeks old; Simonsen Labortories, Gilroy, CA) were injected i.p. with various doses of each alcohol to determine the ED_{50} for loss of righting reflex. Ethanol, 1-propanol, 2-propanol, 1-butanol, i-butanol, 2-butanol and *t*-butanol were injected as solutions of 20% w/v in 0.9% saline. Due to the limited solubility in water, the longer chain alcohols were prepared in corn oil solutions (1-pentanol at 8.0%, 1-hexanol at 6.0% and 1-heptanol at 6.0% w/v); 1-octanol was injected as a pure alcohol. Control animals were injected with either 0.9% saline or corn oil (0.2–1.0 ml) and no loss of righting reflex was observed. The alcohols were administered at the same time each day to avoid circadian effects. The criterion for loss of righting reflex was that the mouse failed to right itself for at least 3 min after it had been placed on its back.

The ED_{50} values for loss of righting reflex for ethanol, 1-propanol, 2-propanol, 1-butanol, i-butanol, 2-butanol and *t*-butanol were calculated by the method of Litchfield and Wilcoxon (1949), using at least five doses for each drug, with six mice per group. The ED_{50} values for 1-pentanol, 1-hexanol, 1-heptanol and 1-octanol were estimated by the Dixon (1965) modified up-and-down method for small samples. Four to six mice were tested for each alcohol.

Drug concentration in the aqueous phase. When highly lipid-soluble alcohols were used, enough drug dissolved in the membrane to deplete the aqueous phase. The total added concentration was distributed as follows:

$$C_t = (C_a V_a + C_m V_m)/V_t \quad (1)$$

where C is concentration, V is volume and subscripts a, m and t represent aqueous, membrane and total, respectively. Defining the partition coefficient, $P_{memb} = C_m/C_a$ and solving for C_a,

$$C_a = (C_t V_t)/(V_a + P_{memb} V_m). \quad (2)$$

Our preparations contained 20 mg/ml of protein. Estimating equal amounts by weight of lipid and protein and overall membrane density of about 1, we have

$$C_a = C_t/(0.96 + 0.04\, P_{memb}). \quad (3)$$

Membrane disordering potency. The reduction in order parameter per 100 mM alcohol in the total membrane suspension was deter-

mined from the slope of each regression line in figure 1. Each value was converted to the reduction in order parameter per 100 mM alcohol in the aqueous phase, defined as the membrane disordering potency, by multiplying each value by the factor (0.96 + 0.04 P_{memb}) associated with each alcohol.

Intramembrane parameters. The intramembrane concentrations (millimoles per gram of membrane) were calculated by multiplying the aqueous concentration of each alcohol by its membrane/buffer partition coefficient, P_{memb} [(moles per kilogram of membrane)/(moles per liter of water)]. The oil/water partition coefficients, P_{oil}, for all of the alcohols were measured in a single study by Lindenberg (1951), with the exception of 1-hexanol and 1-octanol. We estimated the values for these latter two alcohols from the linear relationship between the measured log P_{oil} (from Lindenberg) and the number of alcohol carbon atoms (C1–C5). The P_{oil} values were converted to octanol/water partition coefficients, P_{oct}, by the use of the solvent regression equations described by Leo *et al.* (1971). The P_{memb} for each alcohol was calculated by dividing the P_{oct} by 5. Roth and Seeman (1972) demonstrated that the P_{memb} for alcohols in erythrocyte and neuronal membranes is equal to one-fifth of the P_{oct}. The P_{memb} values are similar to those derived from the P_{oct} values of Leo *et al.* (1971) which were assembled from four different sources. This review included measured P_{oct} values for 1-hexanol and 1-octanol. Both studies (Leo *et al.*, 1971; Lindenberg, 1951) show the same slope (0.55) for log P_{memb} *vs.* the number of carbon atoms, for *n*-alcohols up to 1-pentanol (Lindenberg, 1951) and up to 1-octanol (Leo *et al.*, 1971). We used Lindenberg's data because his is the most complete partitioning study of short-chain alcohols reported by a single investigator. The intramembrane volume (microliters per gram of membrane) of each alcohol was estimated by multiplying the intramembrane concentration by its molecular volume. The molecular volumes were calculated by the method of Bondi (1964).

Results

Membrane disordering. All of the alcohols tested reduced the order parameter in a linear, concentration-dependent fashion (fig. 1). The membrane disordering potency for each alcohol was determined from the slope of each least-squares regression line and expressed as the change in order parameter per 100 mM alcohol in the aqueous phase, as described under "Methods." The membrane disordering potency for each alcohol was as follows: ethanol, 0.0016; 1-propanol, 0.0057; 1-butanol, 0.019; 1-pentanol, 0.064; 1-hexanol, 0.28; 1-octanol, 4.0; i-butanol, 0.015; i-pentanol, 0.049; 2-propanol, 0.0026; 2-butanol, 0.0095; 2-pentanol, 0.035; *t*-butanol, 0.0035. A value of 1.0 was estimated for 1-heptanol from the regression line for log disordering potency *vs.* the number of carbons for *n*-alcohols (see fig. 2). Values are presented to reflect relative potencies with the understanding that changes in the order parameter cannot exceed 1.0. The potencies varied over a 2500-fold range from ethanol to 1-octanol.

Significant membrane disordering was observed for ethanol, 1-propanol, 2-propanol, 1-butanol, 1-pentanol and 1-hexanol at alcohol concentrations reported to be required for nerve block-

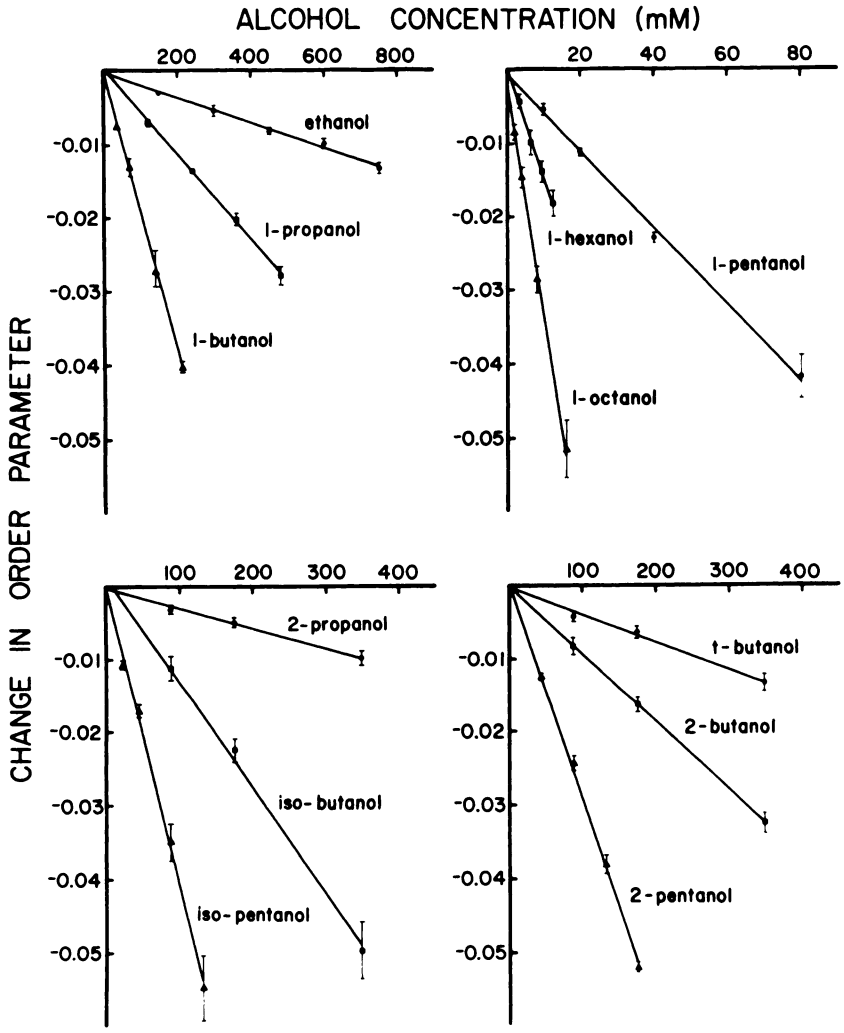

Fig. 1. Concentration-dependent disordering of spin-labeled (5-doxylstearic acid) synaptosomal plasma membranes at 37°C by short-chain alcohols. A base-line order parameter was measured for each membrane preparation in the absence of alcohol. The ordinate is the reduction in order parameter from the base line produced by the *in vitro* addition of an alcohol to a membrane preparation. The abscissa represents the total alcohol concentration in the membrane-buffer suspension. The mean base-line order parameter of 0.5902 (S.E. = 0.0007) was determined from 22 separate preparations. Lines are from least-squares analyses; points are means ($N \geq 4$); vertical bars represent S.E.

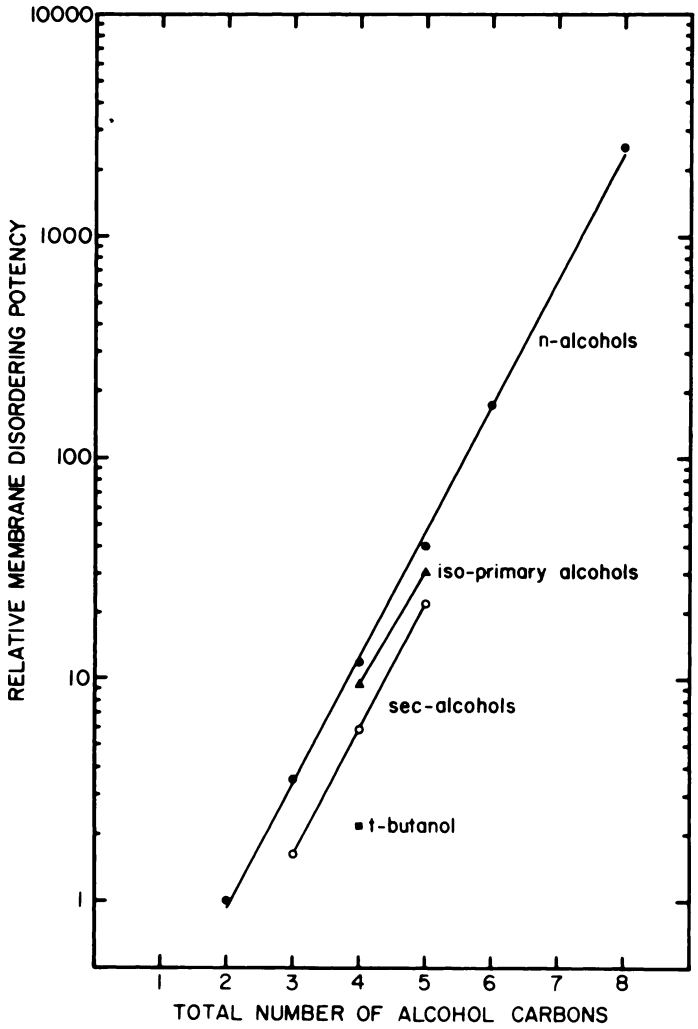

Fig. 2. Dependence of membrane disordering potency on the number of alcohol carbons and the effects of alcohol structure; straight-chain primary alcohols (●), isoprimary alcohols (▲), secondary alcohols (○) and tertiary alcohol (■). Disordering potencies, calculated as the reduction in order parameter per 100 mM alcohol in the aqueous phase, are expressed relative to the value for ethanol.

ing (Skou, 1958; Seeman, 1972). A constant reduction in order parameter of about 0.01, ranging from 0.0085 to 0.0168, was detected for these alcohols at nerve blocking concentration in the aqueous phase, suggesting that a specific degree of membrane disordering may be associated with a given anesthetic effect, such as peripheral nerve block.

As expected, the disordering potency increased logarithmically with the number of methylene groups as shown in figure 2. The structure of the alcohol was also important; the relative disordering potencies by groups of structural isomers were straight-chain primary>isoprimary>secondary>tertiary. The slopes of the regression lines (log potency *vs.* number of carbons) for *n*-alcohols (0.57), isoprimary alcohols (0.52) and *sec*-alcohols (0.57) did not differ significantly from each other. A slope of 0.57 means that the potency increased geometrically by a factor of $10^{0.57}$ or 3.7-fold for each additional methylene group. A plot (not shown) of the log membrane/buffer partition coefficients (see table 1) *vs.* the number of alcohol carbons produced a very similar pattern with slopes of 0.55 (*n*-alcohols), 0.53 (isoprimary alcohols) and 0.56 (*sec.*-alcohols).

These relationships suggest that the disordering potency is primarily a reflection of the ability of an alcohol to partition into the membrane bilayer. A correlation between the membrane disordering potency and the membrane/buffer partition coefficient is illustrated in figure 3. The slope (including the 95% confidence limits) of the regression line was 1.07 ± 0.09 with a correlation coefficient of 0.994. A slope of 1.0 would indicate, on a relative basis, that a given alcohol was equally effective in both systems.

Intoxication studies. The ED_{50} values (millimoles per kilogram) for loss of righting reflex were as follows: ethanol, 70.1 1-propanol, 24.6; 1-butanol, 14.2; 1-pentanol, 3.8; 1-hexanol, 2.1; 1-heptanol, 6.0; 1-octanol, 330; i-butanol, 14.9; 2-propanol, 27.4; 2-butanol, 12.8; and *t*-butanol, 14.6. The relative hypnotic potencies were determined by dividing the ethanol ED_{50} value by that for each alcohol. As demonstrated for disordering and partitioning, the hypnotic potency increased logarithmically with the number of methylene groups, but only slight differences between the ED_{50} values for structural isomers were noted. The relationship between the log of the hypnotic potency (*n*-alcohols, excluding 1-heptanol and 1-octanol) and the number of alcohol carbon atoms (plot not shown) produced a slope of 0.39 with a correlation coefficient of 0.987. This slope was significantly less than the slope of 0.57 observed for disordering by *n*-alcohols (fig. 2). The hypnotic potency increased geometrically by a factor of only 2.5-fold for each additional methylene group. A correlation between the log of the hypnotic potency and the log of the membrane disordering potency is shown in figure 4. The slope of the regression line (excluding 1-heptanol and 1-octanol) was 0.60 ± 0.19 with a correlation coefficient of 0.942. In general, the enhancement in potency produced by the addition of each methylene group was greater for disordering than for hypnotic effect.

Intramembrane parameters. Table 1 shows the aqueous alcohol concentration required to produce a reduction in order parameter of 0.01 for each alcohol, calculated from the regression lines shown in figure 1. This approximates the disordering produced at nerve-blocking concentrations. As described under "Methods," the intramembrane concentrations of these alcohols associated with a 0.01 reduction in order parameter were estimated (table 1) from the aqueous concentrations and partitioning data and compared for constancy. A 4-fold range in values from 0.054 (1-octanol) to 0.20 (*t*-butanol) mmoles/g of membrane was observed, with a mean of 0.12 mmoles/g of membrane. This is a small variability compared with the range of corresponding aqueous concentrations. The intramembrane volumes of alcohol responsible for a reduction in order parameter of 0.01 were estimated from the intramembrane concentrations and the molecular volumes (table 1). A 4-fold range in values from 2.9 (ethanol) to 11 (*t*-butanol) μl/g of membrane was observed, with a mean of 6.4 μl/g of membrane. The values for intramembrane concentration and intramembrane volume were normalized by expressing each as a fraction of their respective means. We found that the variance of intramembrane concentration did not differ significantly from that of intramembrane volume [$F(11,11) = 1.07$; $P > .05$].

Discussion

Membrane disordering may be an essential step in the progression of events leading to intoxication by alcohols (Lenaz *et al.*, 1978). A simplified mechanism of action at the membrane level would involve: 1) incorporation of anesthetic into target

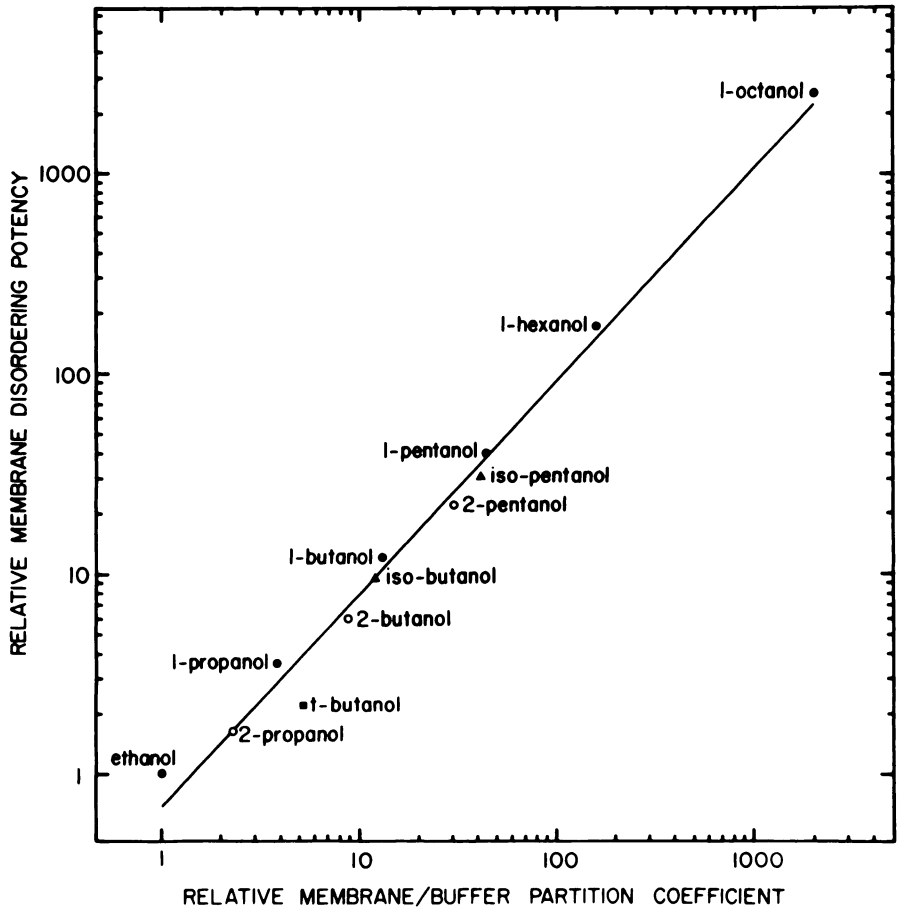

Fig. 3. Correlation between relative membrane disordering potency and relative membrane/buffer partition coefficients (from table 1). All values are relative to the value for ethanol (disordering potency as in fig. 2). The regression line (slope = 1.07) from least-squares analysis.

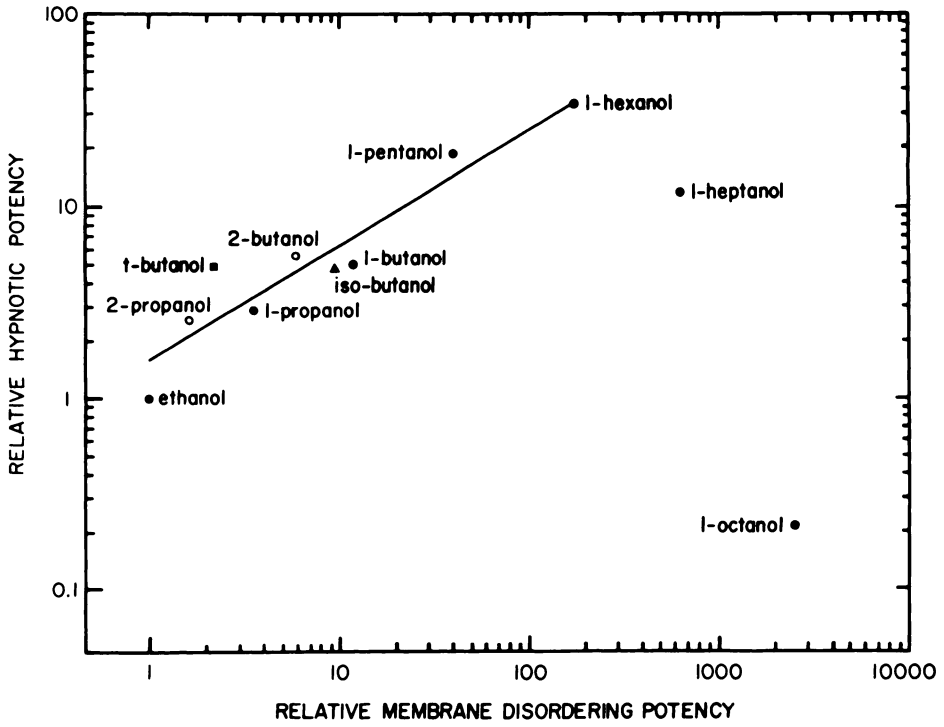

Fig. 4. Correlation between relative hypnotic potency and relative disordering potency. Relative hypnotic potencies were calculated from the ED_{50} values for loss of righting reflex by dividing the dose for each alcohol into the dose for ethanol. The disordering potency was calculated as in figure 2. The regression line (slope = 0.60) from least-squares analysis excludes 1-heptanol and 1-octanol.

membranes; 2) structural disorganization of membrane bilayers, including perturbations in lipid and protein conformations; and 3) disruption of membrane functions, including the blockage of action potentials. A direct correlation between the ability of an alcohol to produce intoxication in higher animals and its ability to disorder neuronal membranes from these animals has not previously been reported.

This study has established a linear relationship between the

TABLE 1

Aqueous concentrations, intramembrane concentrations and intramembrane volumes of alcohols required to produce 0.01 change in order parameter

Alcohol	Aqueous Conc.[a]	P_{memb}[b]	Intramembrane Conc.[c]	Molecular Volume[d]	Intramembrane Volume[e]
	M	*mol/kg membrane/mol/l water*	*mmol/g membrane*	*μl/mmol*	*μl/g membrane*
Ethanol	0.60	0.15	0.090	31.9	2.9
1-Propanol	0.18	0.57	0.10	42.2	4.3
1-Butanol	0.049	2.0	0.098	52.4	5.1
1-Pentanol	0.013	6.6	0.086	62.6	5.4
1-Hexanol	0.0035	24	0.084	72.9	6.1
1-Octanol	0.00018	302	0.054	93.3	5.1
i-Butanol	0.081	1.8	0.15	52.4	7.6
i-Pentanol	0.020	6.1	0.12	62.6	7.6
2-Propanol	0.37	0.35	0.13	42.2	5.5
2-Butanol	0.10	1.3	0.13	52.4	6.8
2-Pentanol	0.033	4.5	0.15	62.6	9.3
t-Butanol	0.26	0.78	0.20	52.4	11

[a] Calculated from the linear regression lines for concentration-dependent disordering at 0.01 change in order parameter.
[b] Converted from P_{oil} from Lindenberg (1951), except for values for 1-hexanol and 1-octanol as described under "Methods."
[c] Aqueous conc. × P_{memb}.
[d] Calculated by method of Bondi (1964).
[e] Intramembrane conc. × molecular volume.

log of the hypnotic potency and the log of the membrane disordering potency for several short-chain alcohols (C2-C6). The slope of this relationship was less than one (fig. 4). Thus, the progressive increase in membrane disordering potency (with increasing chain length and lipid solubility) was greater than the progressive increase in the potency for loss of righting reflex. Among these alcohols, the lipid solubility increases by a factor of about 3.5 with each additional carbon atom (Lindenberg, 1951). This factor of 3.5 is directly applicable to the membrane disordering potency (fig. 2), but not to the *in vivo* data. We find a factor of 2.5 for hypnotic potency, in agreement with Wallgren (1960) and McCreery and Hunt (1978), who studied ataxic effects. The *in vivo* potency is still proportional to chain length, but the slope is less than for *in vitro* potency. This may reflect the difference between *in vivo* and *in vitro* exposure of neuronal membranes to alcohol. The *in vivo* potency may be the result of an increase in disordering with chain length partially offset by diversion of the more lipid-soluble compounds into tissues en route from the peritoneal cavity to the brain. McCreery and Hunt (1978) attempted to evaluate this latter factor numerically, but their equations would apply only at equilibrium. Even so, conversion of dose to units of aqueous concentration by their method brings our *in vivo* and *in vitro* potencies into line. The log of the aqueous concentration (converted from the ED_{50} values) *vs.* the log of the membrane disordering potency produced a slope of 1.1.

The log of the membrane disordering potency was linearly related to the log of the membrane/buffer partition coefficient with a slope of about one. This suggests that the membrane disordering is sensitive to the same hydrophobic forces that regulate the alcohol partitioning into the membrane (Hansch and Dunn, 1972). The logarithmic increase in disordering potency with the number of methylene groups (fig. 2) was a reflection of the relationship between potency and alcohol carbon number observed for partitioning. Disordering and partitioning data were both sensitive to the effects of structural isomers. Each relationship was characterized by a slope of about 0.55. Alcohol partitioning is dependent upon the change in free energy associated with the transfer of each methylene group from the aqueous to the membrane phase. This change in free energy can be calculated from the slope of the log P_{memb} *vs.* the number of alcohol carbon atoms (Cratin, 1968). For example, a slope of 0.55 yields a value of −780 cal/mole at 37°C. The membrane disordering potency appears to be dependent upon this change in free energy associated with the partitioning of alcohol into the membrane.

The maximal hypnotic potency was observed for 1-hexanol. The hypnotic potency observed for 1-heptanol and 1-octanol were 8-fold and 1000-fold less than the values predicted from the linear relationship between log hypnotic potency for *n*-alcohols (C2-C6) and carbon number (plot not shown). This "cutoff" effect, beyond which anesthetic potency no longer correlates with lipid solubility, has been observed in several studies (Seeman, 1972). Within a homologous series of *n*-alcohols up to 1-decanol (McCreery and Hunt, 1978), 1-hexanol also was the most potent for intoxication in rats. The low water solubility of alcohols of longer chain length than hexanol may severely restrict their diffusion from the peripheral tissues to the site of action within the central nervous system.

For an equivalent degree of membrane disordering, intramembrane parameters (concentration and volume) were estimated and compared for constancy. The disordering data did not preferentially support either the Meyer-Overton concept of constant concentration or the Mullins hypothesis of constant volume. For comparison, we estimated intramembrane concentrations and volumes of the alcohols required for nerve block (Skou, 1958; Seeman, 1972) for ethanol, 1-propanol, 2-propanol, 1-butanol, 1-pentanol and 1-hexanol. The intramembrane concentrations exhibited a 2-fold range (0.08–0.14 mmol of alcohol per gram of membrane) compared with the 5-fold range (2.3–11 μl of alcohol per gram of membrane) of intramembrane volumes. In this case, the intramembrane volumes were significantly more variable [$F(6,6) = 6.99$; $P < .05$] than the intramembrane concentrations; this conforms to the Meyer-Overton concept.

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References

Bondi, A.: van der Waals volumes and radii. J. Phys. Chem. **68:** 441–451, 1964.

Butler, J. A. V., Thomson, D. W, and Maclennan, W. H.: The free energy of the normal aliphatic alcohols in aqueous solution. J. Chem. Soc. (Lond.) 674–686, 1933.

Chin, J. H. and Goldstein, D. B.: Effects of low concentrations of ethanol on the fluidity of spin-labeled erythrocyte and brain membranes. Mol. Pharmacol. **13:** 435–441, 1977.

Cratin, P. D.: Partitioning at the liquid-liquid interface. Ind. Eng. Chem. **60:** 14–19, 1968.

Dixon, W. J.: The up-and-down method for small samples. J. Am. Stat. Assoc. **60:** 967–978, 1965.

Gaffney, B. J.: Practical considerations for the calculation of order parameters for fatty acid or phospholipid spin labels in membranes. *In* Spin Labeling, Theory and Applications, ed. by L. J. Berliner, pp. 567–571, Academic Press, New York, 1976.

Hansch, C. and Dunn, W. J., III: Linear relationships between lipophilic character and biological activity of drugs. J. Pharm. Sci. **61:** 1–19, 1972.

Hubbell, W. L. and McConnell, H. M.: Molecular motion in spin-labeled phospholipids and membranes. J. Am. Chem. Soc. **93:** 314–326, 1971.

Hunt, W. A.: The effects of aliphatic alcohols on the biophysical and biochemical correlates of membrane function. Adv. Exp. Med. Biol. **56:** 195–210, 1975.

Jain, M. K., Wu, N. Y.-M and Wray, L. V.: Drug-induced phase change in bilayer as possible mode of action of membrane expanding drugs. Nature (Lond.) **255:** 494–496, 1975.

Jones, D. H. and Matus, A. I.: Isolation of synaptic plasma membrane from brain by combined flotation-sedimentation density gradient centrifugation. Biochim. Biophys. Acta **356:** 276–287, 1974.

KRISHNAN, K. S. AND BRANDTS, J. F.: Interaction of phenothiazines and lower aliphatic alcohols with erythrocyte membranes: A scanning calorimetric study. Mol. Pharmacol. **16:** 181–188, 1979.

LEBLANC, A. E. AND KALANT, H.: Ethanol-induced cross tolerance to several homologous alcohols in the rat. Toxicol. Appl. Pharmacol. **32:** 123–128, 1975.

LEE, A. G.: Interactions between anesthetics and lipid mixtures. Normal alcohols. Biochemistry **15:** 2448–2454, 1976.

LENAZ, G., CURATOLA, G., MAZZANTI, L., BIGI, A. AND BERTOLI, E.: A conformational model for the action of general anesthetics at the membrane level. I. Theoretical considerations. Ital. J. Biochem. (Engl. Ed.) **27:** 378–400, 1978.

LEO, A., HANSCH, C. AND ELKINS, D.: Partition coefficients and their uses. Chem. Rev. **71:** 525–616, 1971.

LINDENBERG, B. A.: Sur la solubilité des substances organiques amphipatiques dans les glycerides neutres et hydroxyles. J. Chim. Phys. **48:** 350–355, 1951.

LITCHFIELD, J. T., JR. AND WILCOXON, F.: A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. **96:** 99–113, 1949.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. AND RANDALL, R. J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:** 265–275, 1951.

LYON, R. C., SCHREURS, J. AND GOLDSTEIN, D. B.: Disordering of spin-labeled synaptosomal plasma membranes by several short-chain alcohols. Drug Alcohol Depend. **6:** 69–70, 1980.

MASTRANGELO, C. J., TRUDELL, J. R., EDMUNDS, H. N. AND COHEN, E. N.: Effect of clinical concentrations of halothane on phospholipid-cholesterol membrane fluidity. Mol. Pharmacol. **14:** 463–467, 1978.

MCCREERY, M. J. AND HUNT, W. A.: Physico-chemical correlates of alcohol intoxication. Neuropharmacology **17:** 451–461, 1978.

METCALFE, J. C., SEEMAN, P. AND BURGEN, A. S. V.: The proton relaxation of benzyl alcohol in erythrocyte membranes. Mol. Pharmacol. **4:** 87–95, 1968.

MEYER, H. H. AND GOTTLIEB, R.: Theory of narcosis. *In* Experimental Pharmacology as a Basis for Therapeutics, 2nd ed., transl. by V. E. Henderson, pp. 116–129, Lippincott, Philadelphia, 1926.

MULLINS, L. J.: Some physical mechanisms in narcosis. Chem. Rev. **54:** 289–323, 1954.

PATERSON, S. J., BUTLER, K. W., HUANG, P., LABELLE, J., SMITH, I. C. P. AND SCHNEIDER, H.: The effects of alcohols on lipid bilayers: A spin label study. Biochim. Biophys. Acta **266:** 597–602, 1972.

ROTH, S. AND SEEMAN, P.: The membrane concentrations of neutral and positive anesthetics (alcohols, chlorpromazine, morphine) fit the Meyer-Overton rule of anesthesia; negative narcotics do not. Biochim. Biophys. Acta **255:** 207–219, 1972.

SEEMAN, P.: The membrane actions of anesthetics and tranquilizers. Pharmacol. Rev. **24:** 583–655, 1972.

Seeman, P.: The membrane expansion theory of anesthesia: Direct evidence using ethanol and a high-precision density meter. Experientia (Basel) **30:** 759–760, 1974.

SEEMAN, P., KWANT, W. O., SAUKS, T. AND ARGENT, W.: Membrane expansion of intact erythrocytes by anesthetics. Biochim. Biophys. Acta **183:** 490–498, 1969.

SKOU, J. C.: Relation between the ability of various compounds to block nervous conduction and their penetration into a monomolecular layer of nerve-tissue lipoids. Biochim. Biophys. Acta **30:** 625–629, 1958.

TURNER, G. L. AND OLDFIELD, E.: Effect of a local anaesthetic on hydrocarbon chain order in membranes. Nature (Lond.) **277:** 669–670, 1979.

WALLGREN, H.: Relative intoxicating effects on rats of ethyl, propyl and butyl alcohols. Acta Pharmacol. Toxicol. **16:** 217–222, 1960.

ZAVOICO, G. B. AND KUTCHAI, H.: Effects of n-alkanols on the membrane fluidity of chick embryo heart microsomes. Biochim. Biophys. Acta **600:** 263–269, 1980.

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