MOLECULAR PHARMACOLOGY, 26:83–89

Membrane-Disordering Potency and Anticonvulsant Action of Valproic Acid and Other Short-Chain Fatty Acids

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Received December 12, 1983; Accepted February 29, 1984

SUMMARY

The cell membrane-disordering potencies of sodium valproate, the sodium salts of other short-chain fatty acids, and ethanol were compared using fluorescence polarization with the probe 1,6-diphenyl-1,3,5-hexatriene. Valproate was about 7 times more potent in fluidizing synaptosomal plasma membranes than ethanol, a prototypic disordering agent. The disordering potency of the straight-chain fatty acids pentanoate through octanoate increased by a factor of 2.2 with each additional methylene group. The sedative potencies of the drugs were assessed by determining the brain concentration at which Swiss Webster mice lost the ability to balance on a stationary wooden dowel. Relative anticonvulsant potency was measured by determining the ED_{50} for protection against pentylenetetrazol-induced seizures and then determining the brain levels of drug that were actually achieved at the time of seizure protection. The ability of the fatty acids and ethanol to disorder membranes *in vitro* correlated closely with their ability to cause sedation and protect mice against pentylenetetrazol-induced seizures. These data suggest that valproic acid might exert some of its effects by disordering brain cell membranes—the proposed mechanism of action of ethanol and the general anesthetics.

INTRODUCTION

Sodium valproate, approved by the Food and Drug Administration in 1978 for the treatment of absence seizures, is a small, branched-chain fatty acid (di-*n*-propylacetate). Most studies of valproate's mechanism of action have focused on its effects on brain GABA[1] levels (1). *In vitro*, valproate can inhibit three enzymes in the GABA degradation pathway: GABA transaminase, succinic semialdehyde dehydrogenase, and aldehyde reductase. More important, it also can increase the activity of the GABA-synthesizing enzyme, glutamic acid decarboxylase (2). High doses of valproate can increase GABA brain levels, but valproate (3, 4) and some of its analogues (5) have anticonvulsant effects at doses that do not significantly increase GABA levels. Even high doses of valproate raise brain GABA levels less than 2-fold, whereas equivalent anticonvulsant doses of ethanolamine-*O*-sulfate (an irreversible inhibitor of GABA transaminase) can cause 10-fold increases in brain GABA (4). Thus it is not yet clear whether the GABA system is the primary site of action of valproate. Several other mechanisms of action have been suggested (6), but none has gained wide acceptance.

This work was supported in part by United States Public Health Service Grants DA 02584 and GM 07149.

[1] The abbreviations used are: GABA, γ-aminobutyric acid; PTZ, pentylenetetrazol; DPH, 1,6-diphenyl-1,3,5-hexatriene; PBS, phosphate-buffered saline; SPM, synaptosomal plasma membranes.

Valproate's simple amphiphilic structure, relatively low potency, and lack of a known specific binding site suggest that valproate might exert some of its effects via a cell membrane-perturbing action, the proposed mechanism of action of ethanol and the general anesthetics. These agents probably do not exert their effects by binding to a specific receptor but are thought to partition nonspecifically into cell membranes and alter membrane structure and function (7). Many studies using a variety of physical chemical techniques have shown that anesthetic agents can increase the fluidity of biological membranes and liposomes (8).

Short-chain fatty acids have been shown to have sedative (9) and anticonvulsant (5, 10) actions. Their potencies increase in general with lipid solubility, but adequate systematic studies are lacking. Short-chain fatty acids are also known to reduce the phase transition temperature of model membranes (11), indicating a membrane effect resembling that of anesthetic agents. Using EPR spectroscopy, Lyon and Goldstein (12) found that valproate and octanoate are potent membrane-disordering agents. The relative membrane-disordering potencies of valproate (12) and ethanol (13) agree roughly with their oral anticonvulsant potencies (14, 15). Valproate is 10–20 times more potent than ethanol *in vitro* and *in vivo*.

We measured the abilities of the straight-chain fatty acids C_5 through C_8, the two branched-chain acids valproate and 2-ethylbutyrate, and ethanol to cause sedation and protect mice against PTZ-induced seizures. The

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cell membrane-disordering potency of these agents was measured using fluorescence polarization with the fluorescent probe DPH. The ability of these drugs to disorder membranes correlated well with their anticonvulsant potency, suggesting that disruption of membrane structure might be a primary mechanism of anticonvulsant action.

MATERIALS AND METHODS

Swiss Webster male mice (Charles River Breeding Laboratories, Inc., Wilmington, Mass.), 7–8 weeks old, were housed with a 12-hr light/dark cycle for 1 week before use. Water and Purina rat chow were available ad libitum.

Fatty acid salt solutions. Pentanoic, hexanoic, octanoic, and 2-ethylbutyric acids (Aldrich Chemical Company, Milwaukee, Wisc.), valproic acid (gift of Abbott Laboratories, North Chicago, Ill.), and heptanoic acid (Aldrich Chemical Company or Eastman Kodak Company, Rochester, N.Y.) were used without further purification. Fatty acid salt solutions were made by dissolving the acids in 95% ethanol and adding a slight excess of aqueous NaOH. After shaking for several hours, the solutions were lyophilized to remove the solvent. For administration to mice, the salts were resuspended in glass-distilled water and adjusted to pH 7.4. For fluorescence studies, the salts were dissolved in a 10 mM phosphate buffer containing 2.7 mM KCl, and the solutions were brought to pH 7.4 and to a sodium concentration of 153 mM.

Fluorescence polarization. Synaptosomal plasma membrane fractions were prepared from whole brains (two to four brains per tube) by the sucrose density centrifugation technique of Jones and Matus (16). The fraction was pelleted by centrifugation at 27,000 × *g* and resuspended in PBS without Mg^{2+} or Ca^{2+} (17) at pH 7.4 at a concentration of about 20 mg of protein per milliliter. The suspensions were stored in 60-μl aliquots under N_2 at −80° before use. Protein concentration was determined by the method of Lowry *et al.* (18).

Membrane aliquots were thawed and diluted with PBS. Then 250 μM DPH (Sigma Chemical Company, St. Louis, Mo.) in tetrahydrofuran (Aldrich Chemical Company) was added while shaking to a concentration of about 1 mole of dye per 500 moles of membrane lipid. The labeled membrane suspension was dispensed into silanized tubes, and PBS or drug solution was added. The final membrane concentration was 30 μg of protein per milliliter. The samples were incubated in the dark at 25° in a shaking water bath for at least 30 min before measurements were taken.

Fluorescence intensity and steady-state anisotropy values were determined at 25° using an SLM 4048 polarization spectrofluorometer (SLM Instruments Inc., Urbana, Ill.). The excitation wavelength (360 nm) was isolated using a single grating monochromator (2-nm slit width) and a Corning 7-54 visible absorbing filter (Corning Glass Works, Corning, N. Y.). Emission above 420 nm was selected using Schott 418 cutoff filters (Jenaer Glaswerk Schott and Gen., Mainz, West Germany) and Kodak 47 B bandpass filters (Eastman Kodak Company). With this filter arrangement, light-scattering effects were in most cases negligible. Glan-Thompson polarizers were used for both excitation and emission. The SLM fluorometer contains two photomultiplier tubes and can measure the vertical and horizontal components of the fluorescence emission simultaneously.

Steady-state anisotropy, *r*, is defined as

$$\frac{I_\parallel - I_\perp G}{I_\parallel + 2I_\perp G}$$

where $I_\parallel$ and $I_\perp$ are the vertical and horizontal components of the fluorescence emission when the sample is excited with vertically polarized light. The denominator is equal to the total fluorescence intensity. *G* is an instrument factor that corrects for artifactual influences on the polarization (19). This is determined by measuring the ratio of the vertical and horizontal components of the emission when the sample is excited with horizontally polarized light. In the few instances where light scattering could not be neglected, anisotropy values were corrected for scattering (20) using identical membrane preparations that had not been labeled with fluorescent dye.

The temperature of the sample was maintained at 25.0 ± 0.1° using a Lauda circulating water bath (Brinkmann Instruments, Inc., Westbury, N. Y.) and a thermojacketted cuvette holder equipped with a magnetic stirrer. Samples were allowed to equilibrate in the cuvette for 10 min before readings were taken. Five determinations of the anisotropy were averaged for each sample. Four to eight different membrane preparations (each prepared from the pooled brains of two to four mice) were measured in duplicate at each drug concentration.

Fluorescence lifetime. To show that the drugs were affecting the membrane itself, rather than interacting with the fluorescent probe directly, the fluorescence lifetime of DPH in SPM was determined in the presence and absence of added drug. Lifetimes were determined at the Stanford Synchrotron Radiation Laboratory using the light pulses emitted from the electrons stored in the Stanford positron electron asymmetric ring (SPEAR) (21). These light pulses have a duration (width at half-height) of about 700 psec and are thus well suited for measuring lifetimes of the order of several nanoseconds. Samples were prepared as described for steady-state fluorescence work, except that the final protein concentration was 90 μg/ml. Samples were excited at 360 nm (slit width 10 nm). Polarizers were set at vertical for excitation and 55° for emission to minimize the effect of probe rotation on the measured lifetime. Fluorescence emission above 410 nm was isolated using a Schott 408 cutoff filter. Each signal from the photomultiplier tube was converted to a voltage proportional to the length of time between excitation and emission and then counted in the corresponding channel of a multichannel analyzer. Data were accumulated for 10 min, and monoexponential curve fits were determined by computer.

Sedative potency. The sedative potency of the drugs was assessed by determining the brain concentration at which the mice lost their ability to balance on a stationary dowel. Mice received i.p. injections of drug solution, 20 ml/kg (30 ml/kg in the case of pentanoate and 2-ethylbutyrate). After 20 sec, the mice were placed on a stationary wooden dowel clamped in a horizontal position 45 cm above a bed of shavings. The doses used caused the mice to fall from the dowel within 5 min, presumably when the drugs reached an effective concentration in the brain. Immediately after falling off the dowel, the mice were killed by cervical dislocation, and the brains were removed and homogenized in 9 volumes of ice-cold 0.9% NaCl solution. Aliquots (1 ml) were stored at −20° for later determination of drug concentrations.

Anticonvulsant potency. The drugs were tested for the ability to prevent PTZ-induced clonic convulsions 10 min after injection. Our protocol allowed us to determine the anticonvulsant ED_{50} values of the test drugs and then estimate their actual anticonvulsant brain concentrations. First, to determine the timing for subsequent anticonvulsant testing, the average latency to convulsion after PTZ injection was measured. A group of 12 mice received s.c. injections of PTZ, 85 mg/kg (Aldrich Chemical Company). This is the CD_{97} dose for PTZ in mice, i.e., the dose that causes clonic convulsions in at least 97% of a large population of control animals (15). The mean latency to convulsion was 7.5 min (± 2 min SD). The ED_{50} for protection against PTZ-induced seizures was then determined for each drug using the Dixon up-and-down method (22). Mice received i.p. injections of test drug, 20 ml/kg, and then at time 2.5 min received s.c. injections of PTZ, 85 mg/kg. With this protocol, unprotected mice would be expected on average to have a convulsion 10 min after the injection of test drug (7.5 min after the PTZ injection). A mouse was considered protected if a convulsion had not occurred by 2 SD (4 min) past the mean unprotected convulsion time, i.e., by 14 min after the injection of test drug. Each drug was tested with at least four groups of mice to provide independent estimates of the ED_{50}.

The brain levels of each drug that resulted from the ED_{50} dose were determined by administering to a new group of 6–12 mice i.p. injections of the calculated ED_{50} dose and killing them 10 min after injection. The brains were homogenized in 9 volumes of ice-cold 0.9% NaCl

solution, and 1-ml aliquots were frozen for later analysis of drug concentrations.

Assay of brain drug levels. Brain levels of the test fatty acids were determined by gas chromatography. The brain homogenates were thawed and centrifuged for 1 min in an Eppendorf centrifuge. An aliquot (0.5 ml) of the supernatant was acidified with 50 μl of 1 N HCl, and 10–15 μl of an internal standard (an aqueous solution of a straight-chain fatty acid close to the length of the test drug) and 100–200 μl of chloroform were added. The tube was vortexed and centrifuged, and 2 μl of the lower chloroform phase was injected into a Hewlett-Packard 5710 gas chromatograph equipped with a flame ionization detector. The 6-foot glass column was packed with 10% SP 216 PS (Supelco, Inc., Bellefonte, Pa.). The nitrogen flow rate was 20 ml/min. Injection port and detector temperatures were 200°. The column temperature was set between 140° and 165° depending upon the test drug. Peak areas were calculated by an HP 3380A integrator. Standard curves were prepared by adding known amounts of drug to brain homogenates and analyzing as described. Standard curves were linear in the range of 0.02–6.4 μmoles of drug per gram of brain (wet weight).

When ethanol was the test drug, brains were homogenized in 3.4% perchloric acid and brain levels were determined enzymatically (23).

RESULTS

Fluorescence polarization. The steady-state anisotropy of a fluorescent probe reflects the ability of the molecule to change orientation during the time it is in the excited state. This fluorescence lifetime can be affected by changes in the probe's environment. If the lifetime of the dye in the membrane is changed by the drug being tested, then the probe will have either more or less time to rotate before emission, and misleading anisotropy changes will be obtained. We observed that the fluorescence lifetime of DPH in SPM was not significantly changed by either 200 mM ethanol or 30 mM sodium valproate at 25° or 37° (Table 1). Thus, the drug effects on the measured anisotropy values are not due to changes in the fluorescence lifetime of DPH.

Ethanol and the fatty acids caused a concentration-dependent disordering of SPM (Fig. 1). The slopes of the curves in Fig. 1, expressed as the change in anisotropy per millimolar drug, represent the potencies of the drugs *in vitro*. The potencies of the straight-chain fatty acids pentanoate through octanoate increased with chain length by a factor of 2.2 per additional methylene group (Fig. 2A). Ethanol and the two branched-chain acids, 2-ethylbutyrate and valproate, also disordered the membranes in a concentration-dependent manner (Fig. 1B), but these drugs did not show the same relationship between disordering potency and number of carbon atoms as did the straight-chain acids. Ethanol was more potent than the expected value for a two-carbon acid, and the branched-chain acids were less potent than their corresponding straight-chain acids. The disordering potencies of the straight-chain acids correlated with their reported octanol:water partition coefficients (10, 24), but ethanol was more potent and the two branched-chain acids were slightly less potent disordering agents than would be predicted from their relative solubilities in an isotropic solvent (Fig. 2B).

We also tested PTZ *in vitro* to determine whether its convulsant effect could be ascribed to a membrane-ordering action. Brain PTZ levels at the time of convulsion average about 0.5 μmol/g of brain (25). Membrane fluidity was not significantly affected by either 0.5 mM (Δr = +0.0004 ± 0.0008 SD, n = 3) or 5.0 mM PTZ (Δr = −0.0003 ± 0.0007 SD, n = 3). Thus convulsant concentrations of PTZ do not order SPM. However, at 50 mM,

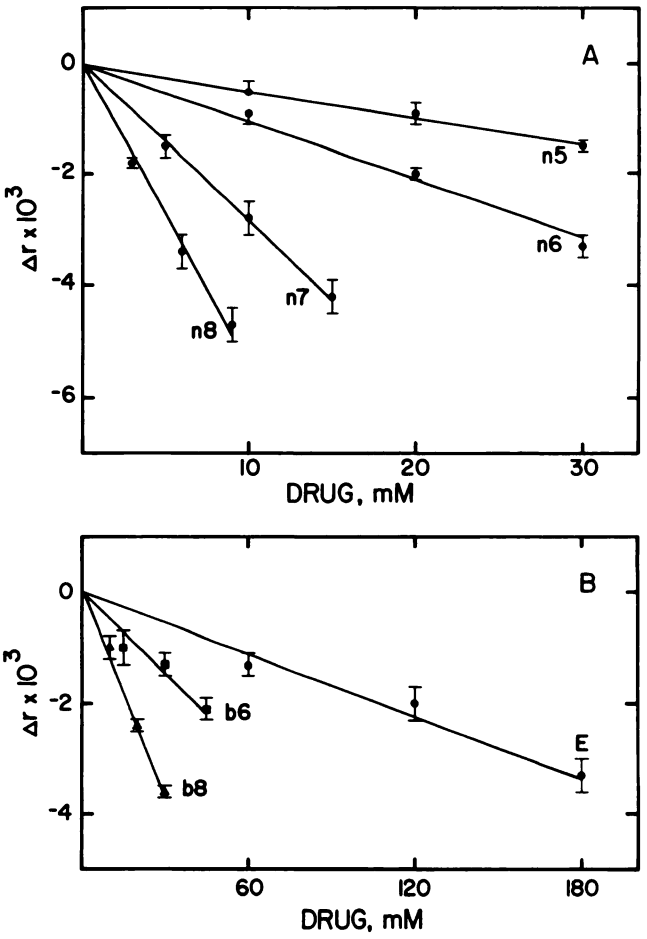

FIG. 1. *Membrane-disordering potency of ethanol and short-chain fatty acids*

The fluorescence anisotropy of DPH in synaptosomal plasma membranes was measured in the presence of ethanol and the short-chain fatty acids. A, The effect of straight-chain acids pentanoate through octanoate; B, the effects of ethanol, valproate, and 2-ethylbutyrate. Points represent the means of four to eight membrane preparations, and vertical bars represent the standard error. Membranes were prepared from the pooled brains of two to four mice. Membrane anisotropy in the absence of added drug averaged 0.2512 ± 0.0001 (SE, N = 51 samples). *n5*, Pentanoate; *n6*, hexanoate; *n7*, heptanoate; *n8*, octanoate; *b6*, 2-ethylbutyrate; *b8*, valproate; *E*, ethanol.

TABLE 1

Effect of ethanol and valproate on the fluorescence lifetime of DPH

Fluorescence lifetimes were determined for DPH in SPM in the presence and absence of added drug, as described under Materials and Methods. Values are the means of three to five determinations (S.E. = 0.1 nsec in all cases). At both temperatures the drug effects were nonsignificant using Student's t-test.

Temperature	Fluorescence lifetimes		
	Control	Ethanol, 200 mM	Valproate, 30 mM
	nsec		
37.0°	9.8	9.8	9.9
25.0°	10.1	10.1	10.0

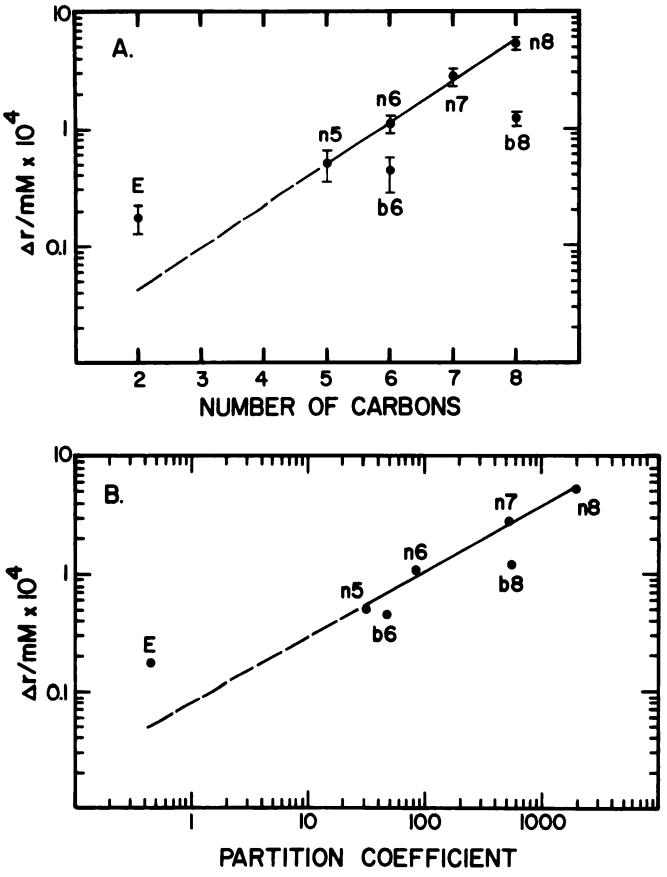

FIG. 2. *Influence of carbon number and partition coefficient on membrane-disordering potency*

The data of Fig. 1 were fitted to straight lines by linear regression analysis. In A, the disordering potencies, expressed as the change in anisotropy per millimolar drug, are plotted against number of carbon atoms in the drugs. Error bars show the 95% confidence limits of the disordering potencies. In B, the membrane-disordering potencies are plotted against their octanol:water partition coefficients taken from the literature (10, 24). Drugs are as defined in legend to Fig. 1.

PTZ can disorder membranes ($\Delta r = -0.0050 \pm 0.0006$ SD, $n = 3$).

Sedative potencies. The sedative potencies of the fatty acids, as measured by the brain levels that produce ataxia in mice (Table 2), increased with chain length, and the sedative potency of ethanol and the straight-chain acids hexanoate through octanoate showed a high correlation ($r^2 = 0.995$) with their ability to disorder SPM *in vitro* (Fig. 3). Pentanoate, the shortest acid tested, did not have measurable sedative action at the highest dose tested. More concentrated solutions were irritating to the mice and could not be tested for sedative action. The two branched-chain acids were more potent *in vivo* than would be expected from their membrane-disordering abilities.

The drug solutions were injected at hypertonic concentrations to minimize injection volumes (Table 2). Since injection of 2 M NaCl has been reported to produce muscle spasms and convulsions (9), it was necessary to consider the effect of high sodium concentrations. To estimate the effect of high ionic strength on sedative testing, we used ethanol as the test drug and injected it

TABLE 2

Sedative potency of the fatty acids and ethanol

Mice received i.p. injections of drug solutions at the concentrations and doses indicated in columns 2 and 3, and then were placed on a wooden dowel. When the mice fell off, they were immediately killed and brains were removed. Brain drug concentrations were assayed by gas chromatography (in the case of the fatty acids) or enzymatically (for ethanol). Pentanoate did not have measurable sedative potency in this test. Values are the mean fall times and brain drug concentrations for each drug tested (± standard error, $N = 6$–12).

Drug	Concentration	Dose	Fall time	Concentration in brain
	mM	*mmoles/kg*	*sec*	*μmoles/g*
Pentanoate	725	22	—	—
Hexanoate	700	14	169 ± 21	3.81 ± 0.31
Heptanoate	350	7	81 ± 7	1.85 ± 0.25
Octanoate	185	3.7	121 ± 11	0.82 ± 0.05
2-Ethylbutyrate	700	21	286 ± 49	4.29 ± 0.87
Valproate	350	7	116 ± 7	1.15 ± 0.10
Ethanol				
In 0.15 M NaCl	2170	43	61 ± 4	28.7 ± 1.5
In 0.80 M NaCl	2170	43	65 ± 4	34.3 ± 1.6

in either isotonic (150 mM) or hypertonic (800 mM) NaCl solutions. As shown in Table 2, the high ionic strength and added sodium did not significantly affect either the average fall time or the threshold brain ethanol level.

Anticonvulsant potencies. All of the tested drugs had measurable anticonvulsant activity against PTZ-induced seizures (Table 3). The anticonvulsant ED_{50} values for ethanol and the straight-chain fatty acids correlated well with their membrane-disordering potencies, but 2-ethylbutyrate and valproate appeared to be much more effective *in vivo* than would be predicted from their membrane

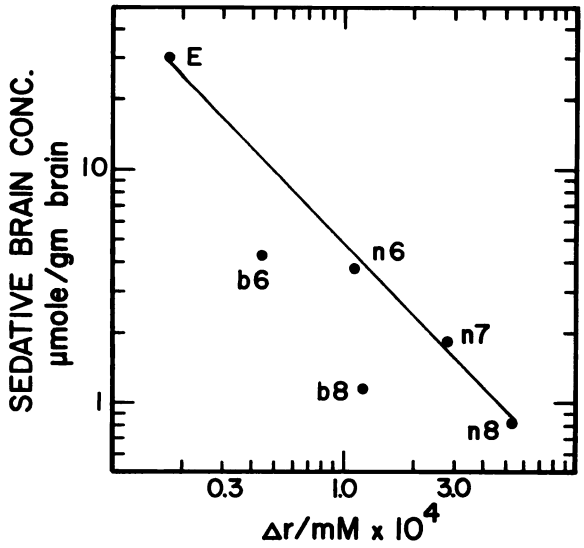

FIG. 3. *Relationship between sedative and membrane-disordering potencies*

The membrane-disordering potencies are shown plotted against the average brain concentrations of drug that caused the mice to fall from the wooden dowel (data listed in Table 2). Drugs are as defined in legend to Fig. 1.

TABLE 3

Protection against PTZ-induced seizures

ED_{50} values for protection against PTZ-induced seizures were determined for each drug by the Dixon up-and-down method (± standard error, N = 4–8 groups of mice, 4–6 mice per group). Mice received injections of drug solution, and then 2.5 min later a CD_{97} dose of PTZ. Mice were considered protected if a convulsion had not occurred by 11.5 min after the PTZ injection. Brain drug levels 10 min after an ED_{50} dose were determined by gas chromatography or enzymatically (mean ± standard error, N = 6–12 mice per drug).

Drug	ED_{50}	Concentration in brain
	mmoles/kg	*μmoles/g brain*
Pentanoate	7.0 ± 1.5	0.785 ± 0.072
Hexanoate	4.7 ± 0.6	0.265 ± 0.043
Heptanoate	3.01 ± 0.22	0.094 ± 0.038
Octanoate	2.96 ± 0	0.046 ± 0.015
Ethanol	11.3 ± 2.3	11.3 ± 0.3
2-Ethylbutyrate	2.42 ± 0.11	1.29 ± 0.04
Valproate	0.569 ± 0.087	0.35 ± 0.01

actions (Fig. 4). However, ED_{50} values are potentially misleading indicators of potency since they can be influenced by pharmacokinetic factors. We obtained a more accurate estimate of anticonvulsant potency by determining the brain levels of drug that were actually achieved at the time of seizure protection. When these were plotted against membrane-disordering potency (Fig. 5), an excellent correlation ($r^2 = 0.95$) was seen for all of the drugs tested.

DISCUSSION

The reported central nervous system depressant actions of short-chain fatty acids in animals include seizure protection (1, 5, 10) and loss of righting reflex (9). With respect to these actions and a variety of other pharmacological effects (26–29), longer fatty acids are more potent than shorter chain acids, indicating that lipid solubility is an important determinant. For homologous series of amphiphilic or lipophilic compounds, the octanol:water partition coefficients (one measure of lipid solubility) increase by a factor of 2–4 with each additional methylene group (24). This is due to the favorable free-energy change that occurs when the methylene group moves from the aqueous phase to the organic phase. Since the potencies of membrane-active agents depend upon their partition coefficients (7), the cell membrane-disordering potency and *in vivo* potency of a homologous series of sedative agents should increase by a factor of 2–4 per methylene group as well. In a recent study of aliphatic alcohols (13), membrane-disordering and hypnotic potencies increased by a factor of 2–3 with each additional methylene group, in agreement with predictions. A similar factor expressed the relative potency of fatty acids *in vitro* as inhibitors of $(Na^+\text{-}K^+)$-ATPase activity and *in vivo* as hypnotic agents (9, 29). In our study, the membrane-disordering, sedative, and anticonvulsant potencies of the straight-chain fatty acids increased by a factor of 2–3 per additional methylene group. Thus the potency of these agents both *in vivo* and *in vitro* show the same quantitative dependency on chain length, and we find an excellent correlation between membrane action and *in vivo* sedative and anticonvulsant potencies for the straight-chain acids. Ethanol may fit the same series, since its potency relative to the straight-chain fatty acids is about the same in all three tests.

Branching tends to decrease the partition coefficient of lipophilic compounds (24, 30). This probably explains why valproate and 2-ethylbutyrate were less potent mem-

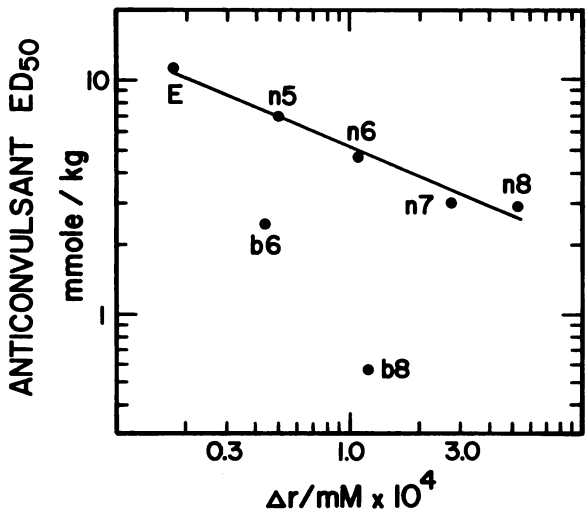

FIG. 4. *Relationship between membrane-disordering potency and anticonvulsant ED_{50} values*

Membrane-disordering potency is shown plotted against the ED_{50} values for protection against PTZ-induced seizures listed in Table 3. Drugs are as defined in legend to Fig. 1.

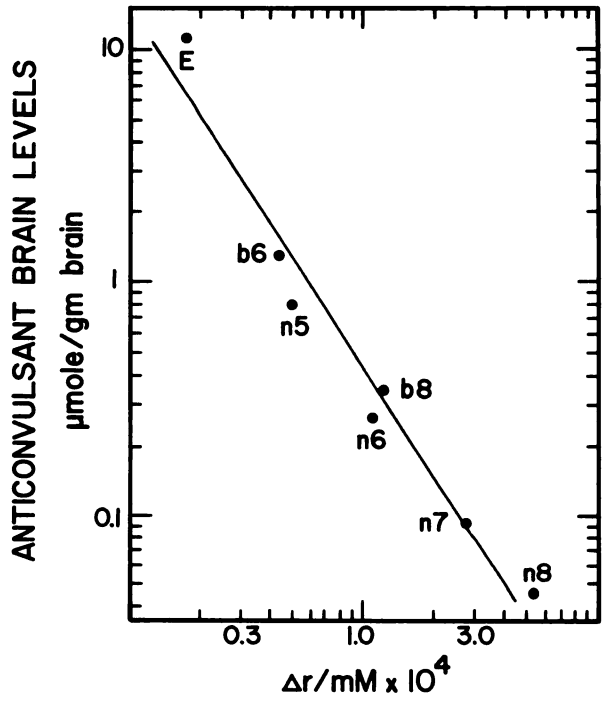

FIG. 5. *Relationship between membrane-disordering potency and anticonvulsant brain levels*

Membrane-disordering potency is shown plotted against the brain levels of anticonvulsant that were achieved at the time of seizure protection (listed in Table 3). Drugs are as defined in legend to Fig. 1.

brane-disordering agents than the corresponding straight-chain acids, octanoate and hexanoate, respectively (Fig. 2A). The actual partition coefficients of these compounds between aqueous phases and relevant brain membranes cannot be accurately predicted from octanol:water partition coefficients, since the structured lipid bilayer discriminates against certain compounds differently than does an isotropic organic solvent such as octanol (30). Thus it is not surprising that the membrane-disordering potencies of all of the drugs do not correlate perfectly with their reported octanol:water partition coefficients (Fig. 2B). The observed difference in membrane-disordering potency between the branched- and straight-chain acids is in the expected direction and of about the same magnitude as was seen in our earlier EPR studies of branched- and straight-chain alcohols (13). Similarly, *in vivo* the branched-chain acids were less potent anticonvulsant agents than the corresponding straight-chain acids, and the correlation between membrane-disordering potency and anticonvulsant potency holds for all of the agents tested (Fig. 5).

Our data agree with other reports that the anticonvulsant potency of short-chain fatty acids increases with chain length (5, 10) and lipid solubility (10), but in both of these studies only the branched-chain acids were found to be effective anticonvulsants; straight-chain acids did not have significant anticonvulsant action. This would appear to suggest that the membrane action of short-chain fatty acids cannot account for their anticonvulsant action, since a specific structural determinant is necessary for potency. These two studies dismissed the actions of straight-chain acids because the anticonvulsant ED_{50} values for these compounds were much higher than the branched-chain ED_{50} values. We also found, on the basis of ED_{50} values, that the branched-chain acids are more potent anticonvulsants than the straight-chain acids. However, ED_{50} values do not give a reliable measure of drug potency at the site of action. The actual brain concentrations of drug achieved at the time of seizure protection demonstrate that straight-chain acids are effective anticonvulsants; heptanoate and octanoate are actually more potent than valproate and 2-ethylbutyrate, as would be predicted from their relative membrane-disordering potencies. Thus there does not seem to be a structural determinant that is necessary for anticonvulsant action, although branching might enhance a fatty acid's ability to reach the brain after an i.p. injection.

We did see a difference between branched- and straight-chain acids in our test of sedative action. In comparison to the straight-chain acids and ethanol, valproate and 2-ethylbutyrate were more potent sedative agents than would be expected from their membrane-disordering potencies. However, the two branched-chain acids compared well with each other, in that valproate was about 4 times more potent than 2-ethylbutyrate both *in vivo* and *in vitro*. Thus, the correlation between membrane-disordering and sedative potency holds for these compounds as well, but for some reason the branched- and straight-chain acids acted somewhat differently. Possibly this is due to our method of evaluating sedative potency. By testing for an early sedative effect, we avoid the possibility that rapidly developing tolerance will confound the results. However, at these early time points the drugs are in the distribution phase, and differences in the rate of entry of drug into the brain or rate of equilibration among different areas of the brain might not allow different types of agents to be compared directly.

Our data show a correlation between membrane disordering and the *in vivo* effects of short-chain fatty acids. However, a causal relationship has not been demonstrated for these drugs or for anesthetic agents in general. We believe that the strongest argument for the importance of membrane disorder in the actions of central nervous system depressant drugs comes from our previous work with ethanol (31). We found that differences in sensitivity of mice to ethanol (whether due to innate genetic factors or to functional tolerance) matched the differential sensitivity of their brain membranes to disordering by ethanol *in vitro*. However, the membrane-disordering mechanism of anesthetic action is not universally accepted (32), and a different mechanism, such as one involving an interaction at a hydrophobic site on certain membrane proteins, might be responsible for the observed effects of these agents. One of the arguments against a lipid site of action is that the disordering effects are barely detectable at pharmacologically relevant concentrations. Although the bulk lipid, where most of the fluorescent probe resides, is only slightly disturbed by the drugs, larger effects might occur in some specific regions such as the boundary lipids of sensitive proteins. Regardless of the model, our data point to a hydrophobic site as the mediator of the actions of fatty acids in the brain and suggest that mere occupancy of such a site, without regard to chemical specificity, may suffice for anticonvulsant action.

In conclusion, these data show a strong correlation between the ability of short-chain fatty acids to disorder cell membranes and their ability to cause ataxia and protect against chemically induced seizures. This correlation suggests that the anticonvulsant action of valproate and other sedative agents might be mediated by cell membrane disordering.

ACKNOWLEDGMENTS

We thank Rodney R. Bucks and Steven G. Boxer for help with the fluorescence lifetime studies, and Rainer Zaechelein for technical assistance.

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