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The Perturbation of Lipid Bilayers by General Anesthetics: A Quantitative Test of the Disordered Lipid Hypothesis

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SUMMARY

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The ability of a wide range of general anesthetics to perturb the order reported from spin-labeled phospholipid:cholesterol (2:1) bilayers has been examined. The change in order induced by increasing concentrations of the following were examined: ethanol, butanol, trichloroethanol, α- and β-chloralose, urethane, pentobarbital, thiopental, ketamine, and phenytoin. All except the latter and β-chloralose caused marked decreases in order. The bilayer/buffer partition coefficients of phenobarbital, phenytoin, and urethane were measured. The change-in-order parameter as a function of total anesthetic concentration varied widely but when the agents were compared at constant concentration in the bilayer all the anesthetics examined gave very similar values. Phenobarbital was somewhat more effective at disordering than the other barbiturates. Phenytoin's weak disordering ability was probably due to solubility limitations rather than an inability to disorder. When the general anesthetic and nerve-blocking potency of these agents were compared to their membrane disordering ability, fair correlations were obtained, but the barbiturates tended to deviate and this deserves further attention. Furthermore the change-in-order parameter at general anesthetic concentrations is only 0.6% which is small compared to the variation to be expected in the physiological temperature range. Thus although the disordered lipid hypothesis is fairly successful at correlating the anesthetic potency data over a dose range of four orders of magnitude, some problems remain. How far these can be overcome by developing more realistic models within the framework of the hypothesis remains to be seen.

INTRODUCTION

Circumstantial support for the lipid theories of general anesthetic action has remained strong since the turn of the century (1). In the last decade advances in the understanding of membranes have been reflected in a renewed interest in these theories. The advent of lipid bilayers in particular has enabled the lipid–anesthetic interaction to be examined in greater detail. Gaseous, volatile, alcohol, steroid, amine, and barbiturate general anesthetics have all been shown to disorder lipid bilayers (2–7), although the last two classes of anesthetics only do so if certain proportions of cholesterol are included in the phospholipid bilayer (6). These changes are opposed by pressure (5, 8, 9), as is anesthesia with these agents (10, 11), and do not occur with the lipid-soluble nonanesthetic long-chain alcohols (4). The measured order parameter changes at anesthetic concentrations are close to the limit of detection of the method (5) but can be shown to be real (12).

In spite of this qualitative success few attempts have been made to quantitatively test the disordered lipid hypothesis of anesthetic action. In the case of gaseous and volatile agents the gas phase may be used as a reference state and some quantitative comparisons with anesthetic partial pressure have been attempted (9). In other cases where quantitative correlations have been attempted (7) considerable doubt remains because in the absence of lipid/buffer partition coefficients the distribution of anesthetic between the lipid dispersion and that free in solution is undefined. With this in mind we have measured the ability of nine general anesthetics, ranging from alcohols to amines, and one anticonvulsant

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to disorder egg phosphatidylcholine:cholesterol (2:1) bilayers. This level of cholesterol was chosen because we, and others, have previously found bilayers with less cholesterol are not always disordered by anesthetics (6, 13, 14). For some of these compounds the partition coefficients are known, but we have determined them for some of the others. For these compounds we are thus in a position to approach two quantitative questions. First, since we can calculate the free aqueous concentration of anesthetic, we are able to compare the aqueous concentrations required to cause general anesthesia or to block axonal conduction. Good correlations are found for most of this structurally diverse group of agents. Second, since the actual amount of anesthetic in the lipid is known, the ability of all the agents to change the order parameter at a given membrane concentration, a quantity we defined as disordering efficacy (6), can be examined. In this lipid bilayer the anesthetics all have roughly equal efficacies, and the apparently lower efficacy of the nonanesthetic phenytoin probably arises from solubility limitations.

MATERIALS AND METHODS

The effect of various general anesthetics and the anticonvulsant on membrane structures was generally monitored from the electron spin resonance (ESR) spectra of 1-acyl-2[8-(4,4-dimethyloxazolidine-*N*-oxyl)]palmitoylphosphatidylcholine [PC[1] (7, 6)], which was synthesized in this laboratory by Dr. M. Pringle according to the method of Hubbell and McConnell (15). Egg yolk phosphatidylcholine and phosphatidic acid were from Lipid Products, Surrey, United Kingdom, and used without further purification. Cholesterol (Sigma Chemical Co., St. Louis, Mo.) was recrystallized in methanol. Phenobarbital, thiopental, and phenytoin were from Sigma. Ketamine hydrochloride was a gift from Dr. R. M. Wheelock, Parke–Davis and Company, Detroit, Michigan. Octanol was purchased from Applied Science, State College, Pennsylvania. Trichloroethanol, chloralose and urethane were Aldrich products (Milwaukee, Wisc.).

The method for preparing vortexed phosphatidylcholine:cholesterol liposomes for spin-label studies has been reported previously (13). Drugs were codeposited with lipid before addition of buffer solution (0.01 M Tris–HCl, pH 7.0, in 0.1 M KCl). For the highly lipophilic agents, such as phenobarbital and phenytoin, their low solubility in the aqueous phase made it necessary to pay special attention to ensure that all drug was incorporated into the dispersion. In some cases radiolabeled tracers were employed for this purpose. The highly water-soluble agents, for example, urethane, were dissolved in the buffer as well as added to the lipid film to form liposomes of the desired total lipid to drug ratio. Volatile agents were only added in the buffer. Four percent of the phospholipids were always phosphatidic acid. The order parameter was calculated as previously (13) with polarity and $T_\perp$ corrections from the ESR spectra determined at 25°C on a Varian E-109 spectrometer. The disordering efficacy of an anesthetic is defined as the negative of the change-in-order parameter per unit concentration of anesthetic in the membrane (units of mM^{-1}).

[1] Abbreviation used: PC, phosphatidylcholine.

Partition coefficients of phenobarbital and phenytoin (^{14}C labeled, New England Nuclear) were measured using the ultrafiltration method of Miller and Yu (16). Incubations were carried out at 25°C with 2 mg/ml of lipid and 0.07 mM total concentration of drug. The pH was adjusted to give 90–95% association of these weak acids. Urethane's partition coefficient is too small for the ultrafiltration technique. The centrifugation method of Katz and Diamond (17), including corrections for nonsolvent water in the pellet, was used. Samples containing lipid, [^{14}C]urethane, and [^{3}H]sucrose (New England Nuclear) were incubated overnight at 4°C and then for 6 h at 25°C and pH 7.0 before centrifugation. All partition coefficients are expressed as (moles of anesthetic per milliliter of lipid/moles of anesthetic per milliliter of buffer). The ED_{50} for loss of righting reflex in tadpoles was determined as previously described (18).

RESULTS

The results of the spectroscopic measurements are presented in Figs. 1a and b, as plots of the change-in-order parameter of the spin-label PC (7, 6) as a function of the ratio of total moles of drug to phospholipid. The control order parameter had an average value of 0.62 but varied ±4% from preparation to preparation. In spite of this variation, which may arise from lipid oxidation, the value of ΔS observed in the presence of anesthetic remained relatively constant (4). In most cases two or more independent experiments were performed on each anesthetic with several different concentrations. Error bars are omitted from the diagram for clarity, but reproducibility in the ΔS values from day to day was generally within ±0.01.

Phenobarbital produced the strongest disordering effect at a given total concentration, although the maximum effect attained was less than with many other agents. Thiopental was similar but its effect seemed to saturate, probably due to limited solubility (the point at highest concentration was not included in the regression in Fig. 1a). Ketamine and trichoroethanol had similar slopes, but the alcohol exerted the larger maximum effect (Fig. 1b). α-Chloralose and butanol also had similar but smaller slopes, and the alcohol again produced the larger maximum effect. β-Chloralose was examined, but this nonanesthetic had such limited solubility that no significant changes were recorded. Urethane and ethanol had the lowest slopes of all the anesthetics. Although the alcohol once more exerted the highest maximum effect, this was only attained at concentrations so high that the data do not even appear on the extended scale of Fig. 1b. They are therefore given in the legend. Finally for comparison the data for the anticonvulsant, phenytoin, are shown. It had a slope between those of butanol and urethane, but the largest decrease in order parameter it produced was smaller than that of any other agent. Concentrations higher than those examined could not be dissolved.

Thus when considered on a total concentration basis the anesthetics fail to produce a consistent decrease in lipid order. This is probably an artifact of the method of comparison, because the slopes of the regressions in Fig. 1 tend to increase with increasing lipophilicity. Indeed

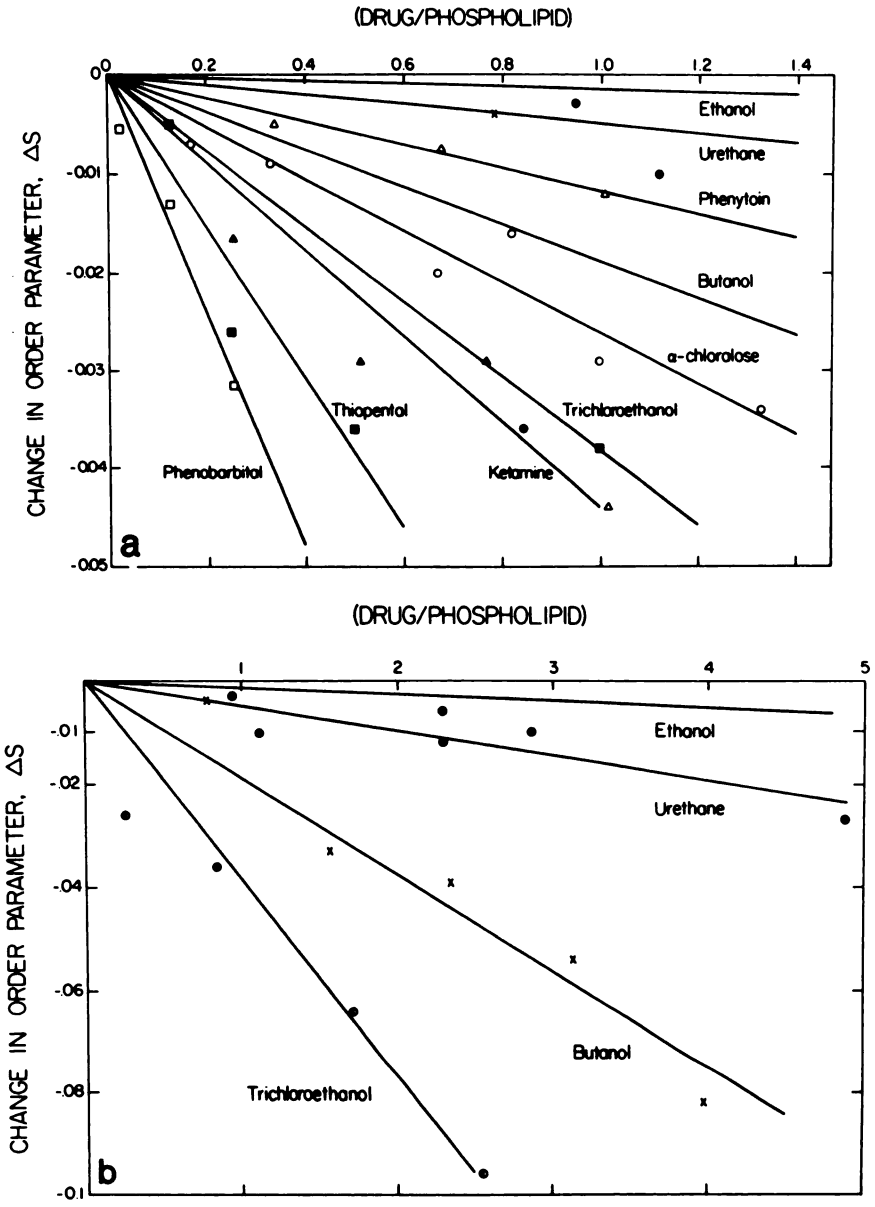

FIG. 1. *Plots of change-in-order parameter of the spin-label PC*

(a) The change-in-order parameter reported by PC (7, 6) as a function of the mole ratio of drug to phospholipid in vortexed lipid dispersions (64.3 mol% egg phosphatidylcholine; 2.7 mol% egg phosphadic acid; 33 mol% cholesterol). Phospholipid concentration was usually 40 mM. The lines are least-squares fits through the origin, but not all the data necessarily are on the graph (see Fig. 1b). Key (agent, symbol, slope ± SD (no. of points)): ethanol, +, −0.0013 ± 0.00022(3); butanol, ×, −0.019 ± 0.0013(5); trichloroethanol, ◐, −0.038 ± 0.0030(4); α-chloralose, ○, −0.026 ± 0.0016(6); phenobarbital, □, −0.120 ± 0.0085(3); phenytoin, △, −0.012 ± 0.0012(4); thiopental, ■, −0.076 ± 0.011(3), highest point omitted, see text; ketamine, ▲, −0.044 ± 0.0040(4); and urethane, ●, −0.0048 ± 0.00059(6). For ethanol and butanol 8-doxylstearic acid was used instead of PC (7, 6). (b) As in (a) but the scale is extended to include all data omitted in that figure except those for ethanol which were: 15.6, −0.0017; 31.3, −0.057; and 47, −0.053 (drug/phosphate, ΔS).

ethanol has a lipid/buffer partition coefficient of less than one and thus most of the ethanol will be in the aqueous phase because only a few percent by volume of our suspensions are lipid. Even with the more lipophilic agents examined about a fifth of the anesthetic remains in the aqueous phase. Thus although Fig. 1 is a convenient way of presenting the data, knowledge of the partition coefficients would enable a more meaningful analysis to be made.

The partition coefficients of phenytoin and phenobarbital in 2:1 egg phosphatidylcholine:cholesterol were found to be 330 and 83, respectively (for the completely associated acids). In the absence of cholesterol these values rose to 657 and 125, respectively, an effect similar to that noted previously with pentobarbital and thiopental. The overall accuracy of these figures is about 5% (16). Using the centrifugation assay the partition coefficient of urethane in the mixed lipids had a mean value of 1.09_5 with a variation of 1% between the two runs.

These partition coefficients enable some of the data in Fig. 1 to be recalculated to allow for the partitioning of anesthetic between lipid and buffer. A partition coeffi-

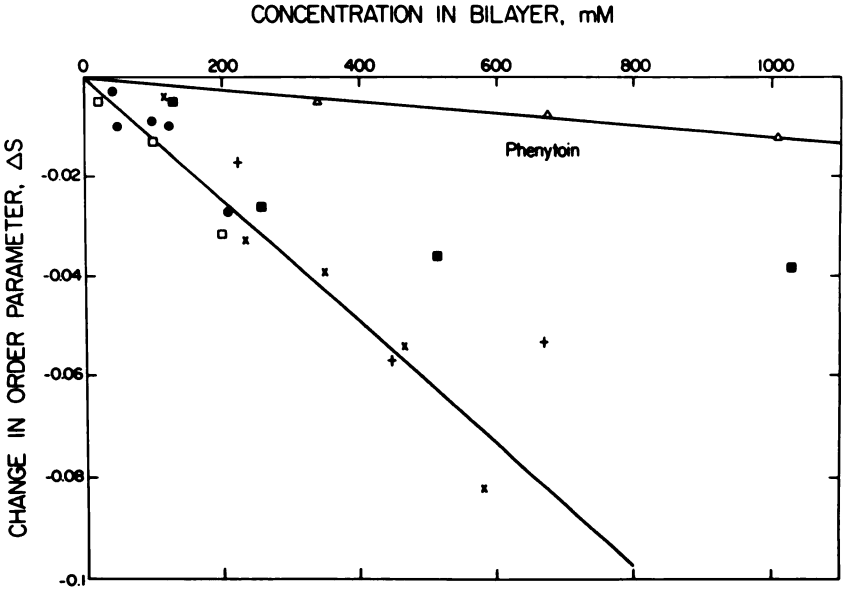

FIG. 2. *The change-in-order parameter for five anesthetics and phenytoin presented as a function of the concentration of the anesthetic in the bilayer*

Key as in Fig. 1. Sources of the partition coefficient data used in the calculation of bilayer concentration are given under Results and in Table 1. The slope of the line is $-1.2 \pm 0.13 \times 10^{-4}$ mM^{-1} for the five anesthetics and $-0.12 \pm 0.01 \times 10^{-4}$ mM^{-1} for phenytoin. The two highest concentrations of thiopental, ■, were supersaturated and therefore omitted from the regression (see Results).

cient for thiopental is also available (Korten and Miller, unpublished data) and we have used data (17) for ethanol and butanol in dimyristoylphosphatidylcholine above its phase transition to extend our calculations approximately to these agents. The results of these calculations are given in Fig. 2. A number of points must be considered in assessing these data. First, the data for the alcohols are least reliable. Not only are the partition coefficients in a different lipid, but the spectroscopic data were obtained with 8-doxylstearic acid instead of PC (7, 6). Therefore the very good fit of the alcohol data with the other anesthetics should not be emphasized. We have included the data to underscore the importance of considering membrane concentration for these agents of low partition coefficient. Second, the calculation shows that the final aqueous concentration exceeds the experimental solubility limit which we determined in our buffer system in some cases. The two highest concentrations of thiopental were omitted from the regression for this reason, they actually exceed the saturation limit by two and a half and five times. It is probable that some of the biphasic effects reported previously for thiopental (19) resulted from exceeding the solubility limit. The nonanesthetic, phenytoin, exhibits a different slope in Fig. 2. However, at all concentrations saturation was again exceeded. Since the spread of the data is small compared to our errors, the apparent trend with concentration could be fortuitous and actually represent a saturated solution in each case with the excess phenytoin undissolved. The concentration in lipid at saturation is 25–30 mM, which would bring the data close to the regression line for the other agents. On the other hand phenytoin might be incorporated into the lipid above the saturation limit if some form of phase separation were to occur. The observed effects were too close to the resolution of the method to make further investigation worthwhile.

ED_{50}'s for tadpole loss of righting reflex were found as follows (±SE); 170 ± 22 μM for ketamine; 115 ± 11 mM for ethanol; 12 ± 1.4 mM for butanol; and 1.8 ± 0.23 mM for α-chloralose. Phenytoin and β-chloralose failed to cause anesthesia even at saturation.

DISCUSSION

Disordering efficacy. Comparison of Figs. 1 and 2 demonstrates for the first time that agents with a 400-fold range of lipid to buffer partition coefficients are equally effective at disordering phospholipid:cholesterol (2:1) bilayers when compared on a membrane concentration basis. The only exception is phenytoin, but this is more apparent than real since all the data were obtained above the solubility limit. It must be emphasized that this conclusion applies only to the perturbation of order at the eighth acyl carbon that we have measured. It seems unlikely, for example, that 1.5 M ethanol (our highest free aqueous concentration) would fail to modify dipolar interactions in the lipid–aqueous interface.

The conclusion for the five anesthetic agents in the present work (Fig. 2) can be extended to include six more agents (Table 1) as follows. Order parameter data for xenon and halothane using the same lipid bilayer and spin label and using the vapor phase as standard state have been given by Trudell and colleagues (9, 12). The data for xenon were determined at elevated pressure, but this can be corrected (9). Using a partition coefficient for halothane (12) and xenon (Smith and Miller, unpublished data) the disordering efficacies in Table 1 were calculated by plotting ΔS versus membrane concentration. Similarly we were able to add data for pentobarbital (13, 16).

For the remaining three anesthetics we examined, no partition coefficient data are available. However, we noticed for a number of agents for which we have partition coefficients in egg phosphatidylcholine:cholesterol (2:1)

TABLE 1

Disordering Efficacy of Eleven General Anesthetics

The disordering efficacy was calculated as described in the Discussion. It is defined as the negative of the change-in-order parameter per unit concentration of anesthetic in the membrane. For agents 1–6 the bilayer/buffer partition coefficients are known in phosphatidylcholine: cholesterol (2:1). For the subsequent agents the partition coefficients were approximated by using for agents 7 and 8 partition coefficients in dimyristoylphosphatidylcholine (17) and for the remaining agents octanol/buffer partition coefficients (see Discussion). Order parameter data are from this work, except those for pentobarbital (13), xenon (9), and halothane (12).

Number	Agent	Disordering efficacy, ΔS (10^{-4} mM)	Partition Coefficient (pH 7.0) (Source)
1	Thiopental	0.85	172 (Unpublished)
2	Phenobarbital	1.53	51 (This work)
3	Pentobarbital	1.02	56 (16)
4	Urethane	1.15	1.1 (This work)
5	Xenon	0.91	14 (Unpublished)
6	Halothane	1.75	50 (12)
Mean ± SD (1–6)		1.2 ± 0.36	
7	Ethanol	0.93	0.44 (17)
8	Butanol	1.27	3.2 (17)
Mean ± SD (1–8)		1.2 ± 0.32	
9	α-chloralose	0.39	40 (20)
10	Ketamine	0.40	400 (calculated (21))
11	Trichloroethanol	1.66	7.1 (calculated (21))
Mean ± SD (1–11)		1.1 ± 0.46	

that the ratio of the octanol/buffer to the lipid/buffer partition coefficient varies from 0.3 to 2.4 with values for 80% of the agents between 0.7 and 1.8. We have therefore used the octanol partition coefficients (20, 21) of these three anesthetics to estimate their membrane concentration and hence their disordering efficacy.

Thus for six anesthetics, including an inert gas, a carbamate, and barbiturates, we have summarized in Table 1 the measured value of the disordering efficacy. It has a mean value of 1.2×10^{-4} mM^{-1} with a standard deviation of only 27%. The total range of efficacies is 2-fold between halothane and thiopental. Within the present work it is 1.8-fold between phenobarbital and thiopental. Such a range is probably higher than the combined errors of measuring order parameters (individual regressions of ΔS versus concentration generally had a standard deviation of 10%), phospholipid concentration (2%) and partition coefficients (5%), together with errors due to failure to incorporate anesthetic or lipid into the suspension. Thiopental and pentobarbital, differing only in the substitution of a sulfur for an oxygen atom at the second carbon in the pyrimidine ring, have efficacies which differ by no more than the expected errors. On the other hand phenobarbital with its bulkier substituent on the fifth carbon in the ring has a significantly higher disordering efficacy. Urethane (ethyl carbamate) has an efficacy similar to that of the former two barbiturates. The precision with which the xenon and halothane data can be expected to compare to our data is unclear, but it is surprising that halothane is a more effective perturber than either xenon or most of the agents we examined. Whether this is caused by its high polarity, the weakness of fluorocarbon–hydrocarbon interactions or other causes is uncertain.

The disordering efficacies of the two alcohols hardly differ from each other or from the preceding six agents in spite of the approximation made in the partition coefficient used, but the agreement must be regarded as partly fortuitous. The three remaining anesthetics in Table 1 all have disordering efficacies which probably do not differ from those of the other agents when the crude method for estimating their bilayer concentration is taken into account.

Thus, the overall conclusion to be drawn from Table 1 is that the disordering efficacy of a diverse range of anesthetics in phospholipid:cholesterol bilayers varies little. Two additional points are of interest with respect to this conclusion.

First, the conclusion is not independent of membrane composition. For example, in the absence of cholesterol pentobarbital has a negative disordering efficacy (it orders) while halothane still has a positive disordering efficacy (6). We have discussed possible reasons for the change in sign of the efficacy that addition of cholesterol produces in a previous paper (13). It appears here that 33 mol% cholesterol is sufficient to completely mask any such effects exhibited by these agents at lower cholesterol contents. For example, pentobarbital changes from ordering to disordering at 14 mol% cholesterol (13).

Second, do the lipid-soluble nonanesthetics and partial anesthetics have low disordering efficacy, as has been suggested (4), or are they simply solubility limited? Our data show that for the nonanesthetic, phenytoin, solubility limits the absolute decrease in order. The maximum effect is too small to be reliably measured, and therefore we are unable to comment on its disordering efficacy which may be less than, comparable to, but not much larger than that of the other agents. A similar situation holds for hexadecanol (Pringle and Miller, unpublished data). Thus solubility limits are a sufficient explanation for the lack of potency for these two agents. In other cases this may not be so. Thus for the partial anesthetic, tetrahydrocannabinol, we found that order increased for total drug to phospholipid ratios of 0.06 to 0.20 with a maximum increase in order parameter of +0.02. This is the only agent for which we have recorded ordering at such high cholesterol content in egg lecithin, and the only one for which the efficacy appears to differ from the other agents.

The disordered lipid hypothesis of anesthetic action. We now turn to using the information summarized in Table 1 to test the hypothesis that anesthetic action correlates with changes in lipid order reported from the eighth acyl carbon of phosphatidylcholine incorporated in a bilayer with 33 mol% cholesterol. This means that the product of disordering efficacy and the membrane concentration which causes anesthesia is constant for all anesthetics. To the extent that disordering efficacy is constant we are simply testing the relation between

bilayer solubility and anesthetic action. This is analogous to the problem of distinguishing the lipid solubility and expansion theories, where, as here, it is necessary to invoke the pressure reversal of anesthesia to justify the inadequacy of the solubility theory (22). It is unfortunate that none of the full anesthetics examined has such an anomalous disordering efficacy that the lipid solubility and disorder hypotheses might be distinguished on this basis alone.

To perform this test conveniently we have calculated from our data the aqueous concentration of each anesthetic which at equilibrium causes an arbitrary change-in-order parameter of −0.01. In Fig. 3 this quantity is seen to correlate well both with the concentration required to anesthetize tadpoles and with a set of data for block of a compound action potential in nerve. Sources of data are given in the legend. Each set of data was fitted by the best least-squares line both freely (dashed line) and also constrained to a slope of 1.0 (solid line) as required by the hypothesis.

The data for 10 anesthetics freely fitted to the tadpole anesthesia results yielded a slope of 0.8 ± 0.11. There are two major deviations from the hypothesis line; thiopental is eight times more, and phenobarbital five times less, potent than predicted. If these two agents are omitted the mean change-in-order parameter for general anesthesia is −0.0035 ± 0.0014, which is similar to a previous estimate for halothane alone (12). Thus for 8 out of 10 anesthetics a satisfactory fit is achieved covering four orders of magnitude of potency; these agents include an inert gas, two alcohols, an amine, a barbiturate, and a volatile anesthetic, as well as a carbamate and α-chloralose. Although this correlation could arise by chance, the fact that no such correlation would be found if cholesterol were omitted from the bilayer tends to argue against this. Similar correlations have been presented for a homologous series of alcohols (7). The barbiturates as a class, however, deviate markedly from our correlation and deserve further attention.

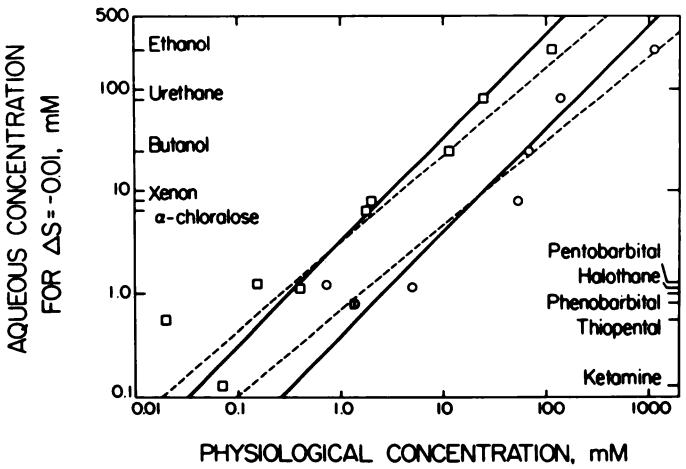

FIG. 3. *Correlations between physiological potency and disordering effect*

The aqueous concentration corresponding to a change-in-order parameter of −0.01 was calculated from the data in Table 1. The nerve block data, ○, were for sciatic nerves from Refs. (23, 24). Tadpole anesthesia data, □, from (23, 25) and this work. All concentrations are for the uncharged form of the drug. The dashed lines are least-squares fits and the solid lines were fitted similarly but with the slope constrained to one.

The set of data for block of the compound action potential in sciatic nerve fitted the order parameter data with a slope of 0.8 ± 0.11. The mean change-in-order parameter at nerve block is −0.03 ± 0.023. The largest deviation from the hypothesis line (slope = 1.0) is fourfold for pentobarbital. There is one other deviation, however, which is not shown. Phenytoin blocks at close to saturation (24) where we expect the order parameter change to be less than −0.01. Thus overall the data fit the model for nerve block fairly well but the use of the compound action potential which represents the sum of several processes (26), and does not take into account the frequency dependence of block noted in some instances (27), is less than ideal. However, no other uniform set of data is available for such a wide range of agents.

It would be premature to reject the model on the basis of the deviations noted above. Thus the ability of barbiturates, but not the volatile agents, to perturb bilayers is sensitive to both their cholesterol and negatively charged lipid content (6, 13). Probably the incorporation of protein into the bilayer would further modulate their disordering efficacy. The model might thus be fine-tuned by adjustments of the membrane composition.

Some other indications that the model would benefit from such fine tuning are that the partial anesthetic tetrahydrocannibinol orders this bilayer, has little influence on a bilayer with 50 mol% cholesterol (Pang and Miller, unpublished data), but does disorder a bilayer with the saturated phospholipid, dipalmitoylphatidylcholine, and cholesterol (1:1) (28). Similarly nitrogen does not disorder the present model (9) but does disorder red cells under some circumstances (29), an indication that membrane protein may also modulate disordering efficacy.

One other problem of the model deserves mention. The order parameter change equivalent to anesthesia is only −0.0035, or a relative change of 0.6%, which is similar in magnitude to the percentage volume change found for the critical volume hypothesis (30), as might be expected for two interrelated variables (8). Our control experiments show that a similar change-in-order parameter can be produced by raising the temperature 0.32°C. This clearly is a problem for the disordered lipid hypothesis, as has been recently emphasized (31). However changing lipid order by introducing an anesthetic into the lipid bilayer is qualitatively different from doing so by introducing thermal energy. The latter perturbant will be sensed by all parts of the system, and the change in order might well be compensated for elsewhere in the perturbation–response chain. However, this remains to be demonstrated. It is interesting to note that the critical volume hypothesis does not suffer from this disadvantage. Its critical volume is equivalent to an increase in temperature of over 10°C (30). It is thus possible that membrane volume changes are a more appropriate model for anesthesia than lipid order.

Thus, we conclude that the disordered lipid hypothesis of anesthetic action correlates the activity, or lack of it (phenytoin and β-chloralose), of a diverse set of agents

whose potencies range over four orders of magnitude. The hypothesis has the least success with the barbiturates. Although there are grounds to believe that refinement of the model might remove the latter anomalies, this remains to be proven. Since the disordering efficacies of all the agents examined were similar our data fail to distinguish between a disordered lipid and a simple lipid solubility model, although other criteria, such as pressure reversal of anesthesia, could do so. The importance of directly determining lipid solubility is emphasized by our findings for phenytoin. Had we found the latter to be present in bilayers that were little disordered the disordered lipid hypothesis would have been supported. As it was we found solubility to be a sufficient criterion for lack of anesthetic potency. Thus the supposed support for the disordered lipid hypothesis based on other nonanesthetic lipophilic agents which did not disorder bilayers (4) may need to be reassessed. This will require the measurement of appropriate partition coefficients. These arguments do not detract from the overall success of lipid-based unitary theories of anesthesia in general. They do leave open the question whether disorder in lipid acyl chains is an appropriate operator, particularly in view of its temperature dependence. Any other variable which is proportional to lipid solubility can satisfy pressure reversal by hydrostatic pressure and helium, and is less sensitive to temperature could also be appropriate. We have reviewed a number of possibilities in a previous paper (32).

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