the high dose group greater than the controls, but none of these differences was significant The differential count revealed a dose-related increase in percentage of segmented neutrophils (segs), but this difference was significant only for the high dose group. There was a significant reduction in percentage of lymphocytes for the two highest doses The percentage of eosinophils was increased in all treated groups, with the differences being significant for the low and high dose groups Other differential values did not show a dose-related trend and were not statistically significant (except that the low dose showed a significant reduction in bands but was not consistent with a dose-related response). These data are included in Table IX.

An examination of organ to body weight ratios did not reveal any to be significant ($p \leq 0.05$) except in the high dose group, in which the ratio was less than for the controls for the brain but greater for the heart, kidneys, and liver. Although not all of the values were significant, there was an indication that the mean ratios for the heart, liver, and spleen increased with an increase in dose of epichlorohydrin. While the mean body weight of the high dose group at 12 weeks was 8.5% less than for the controls, the organ to body weight ratios for the heart, kidneys, liver, and spleen in the high dose group ranged from 29.1 to 38.1% more than for the controls. On the other hand, the ratio for the brain of the high dose group was 27% less than for the controls. Thus, simple retardation in growth would not be expected to account for these differences (Table X).

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ACKNOWLEDGMENTS AND ADDRESSES

Received April 10, 1972, from the *Materials Science Toxicology Laboratories, College of Pharmacy and College of Dentistry, University of Tennessee Medical Units, Memphis, TN 38103*

Accepted for publication June 27, 1972.

Supported in part by Research Contract PH-43-68-1315 from the National Institute of Dental Research, Bethesda, Md.

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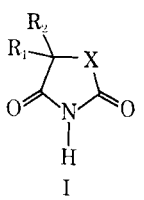
Binding of CNS Active Drugs to the Peptide Bond: A Model System and Mode of Action Hypothesis

P. R. ANDREWS

Abstract □ The effects of pentobarbital, bemegride, and trimethadione on the NMR spectrum of formanilide show that when possible the *cis*-isomer of the amide forms a doubly hydrogen-bonded complex with the drugs but the *trans*-isomer is favored for the formation of a single hydrogen bond. It is suggested that these CNS active drugs behave as molecular glues, linking together functional groups in protein molecules and thus opposing changes in protein conformation or association state. This hypothesis could account for the seemingly inconsistent structure–activity relationships of drugs with convulsant and anticonvulsant actions.

Keyphrases □ CNS drugs—hypothesis and model for binding to peptide bond □ Anticonvulsants—hydrogen binding to peptide bond, mode of action hypothesis □ Convulsants—hydrogen binding to peptide bond, mode of action hypothesis □ Hydrogen bonding—CNS drugs to peptide bond, hypothesis □ Structure–activity relationships—hypothesis for binding of CNS drugs to proteins □ NMR spectroscopy—determination, hydrogen bonding of drugs to peptide bond

Molecules of many structural types display anticonvulsant properties (1), but most drugs used clinically in epilepsy have similar structural features (2), which include two or more groups capable of forming hydrogen bonds. Molecular orbital calculations on 24 drugs of the general structure I indicated that hydrogen-bonding ability is similar for all such drugs (3).



I

Cyclic hydrogen-bonded complexes have been observed between several barbiturates and a model com-

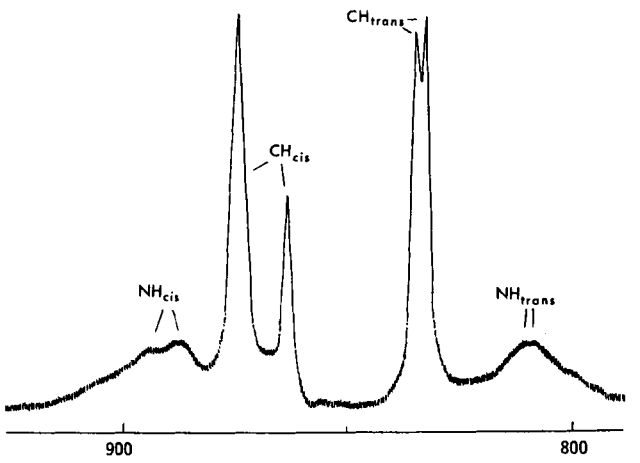


Figure 1—*Part of the 100-MHz. NMR spectrum of 0.6* M *formanilide measured in deuterated chloroform at 35°.*

pound, 9-ethyladenine, and the complexes are stronger than those formed by self-association of either species (4). Similar complexes are formed (5) between 9-ethyladenine and β,β-disubstituted glutarimides, whose activity passes from convulsant to anticonvulsant as extra methylene groups are added (6); the strength of the association is the same for both convulsant and anticonvulsant drugs (5). It appears that hydrogen bonding is relevant to both types of activity, although the available results do not define a specific receptor in either case (5). Adenine itself, in the form of flavin adenine dinucleotide (FAD), has been suggested (7) as a receptor for the barbiturates, which could explain their inhibition of the respiratory chain (8). This view is in accord with the barbiturate inhibition of some flavoenzymes (9), but the alternative of direct action on respiratory apoproteins is favored by the fact that several nonflavoenzymes are also inhibited (9). On the same basis, binding to protein rather than adenine seems likely to mediate convulsant and anticonvulsant activities.

The allosteric theory of drug action suggested by Changeux *et al.* (10) and Karlin (11) postulates that the influence of a drug is determined by its relative affinity for two conformational states of the receptor, where these two conformations are in equilibrium with each other and also with associated species. For drugs that act by hydrogen bonding, a simple model for this

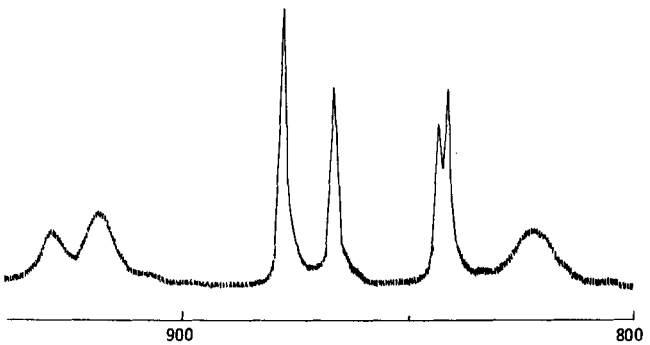


Figure 2—*Part of the 100-MHz. NMR spectrum of 0.6* M *formanilide plus 0.6* M *pentobarbital measured in deuterated chloroform at 35°.*

Table I—Effect of CNS Active Drugs on the *cis–trans* Isomerism of 0.6 *M* Formanilide in Deuterated Chloroform

Drug, 0.6 *M*	Mole Fraction of *cis*-Isomer	[*cis*]/[*trans*]	Chemical Shifts—*cis* CH	*cis* NH	*trans* CH	*trans* NH
None	0.53	1.13	868	890	833	810
Pentobarbital	0.62	1.63	872	924	842	822
Bemegride	0.57	1.32	869	906	835	837
Trimethadione	0.44	0.79	868	~872	834	~826

situation may be provided by formanilide, which incorporates protein backbone hydrogen-bonding groups and is in equilibrium between the *trans*-configuration (II) and the *cis*-configuration (III) (12). As a result of

II III

various intermolecular associations, the position of this equilibrium is dependent on concentration (13); while at a given concentration, the bonding of a drug to either or both forms is reflected in a shift in the equilibrium. This note presents the results of a preliminary study of the interactions between formanilide and three CNS active drugs: pentobarbital (anticonvulsant sedative), bemegride (convulsant), and trimethadione (anticonvulsant).

EXPERIMENTAL

Because of restricted rotation, the equilibrium between the *cis*- and *trans*-isomers of formanilide results in two sets of peaks for the protons bound to the peptide bond (13). No further splitting occurs with the formation of intermolecular complexes, but their presence is reflected in the extent of the downfield shifts of the amino proton signals and the relative areas of the *cis*- and *trans*-peaks, which provide a quantitative measure of the amount of each isomer present. The peak assignment (13) is shown in Fig. 1.

NMR spectra were run on a spectrometer[1] equipped with a variable temperature probe. Deuterated chloroform was used as the solvent with tetramethylsilane as an internal reference. Spectra were recorded for solutions of 0.6 *M* formanilide at various drug concentrations and temperatures. Representative of the results are the spectral data reported here for drug concentrations of 0.6 *M*, which are all mean values from several determinations at 35°.

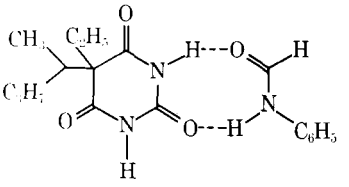
RESULTS AND DISCUSSION

The relevant portion of the NMR spectrum of 0.6 *M* formanilide in deuterated chloroform is given in Fig. 1, and Fig. 2 shows how this spectral region is affected by 0.6 *M* pentobarbital. The influence of pentobarbital and the other two drugs is recorded quantitatively in Table I.

The mole fraction of the *cis*-isomer in 0.6 *M* formanilide changes from 0.53 to 0.62 in the presence of 0.6 *M* pentobarbital. If no binding occurred between the drug and the *trans*-isomer, this result would indicate that pentobarbital constrains 20% of the total formanilide to the *cis*-configuration. However, downfield shifts of 34 and 12 Hz. for the NH proton signals show that both *cis*- and *trans*-isomers are bound to pentobarbital, so binding of the former isomer actually exceeds 20% of the total amide concentration. The stability of the bound *cis*-isomer may be explained by postulating the formation of a series of doubly hydrogen-bonded complexes (*e.g.*, IV) with the

[1] Varian HA 100.

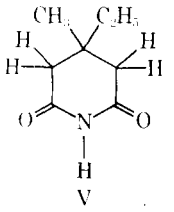
drug, as observed previously between 9-ethyladenine and barbiturates (4) or glutarimides (5).



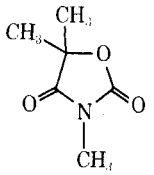
IV

Similar complexes appear to be formed between formanilide and bemegride (V), which at 0.6 *M* leads to an increased mole fraction of the *cis*-isomer despite strong downfield shifts which are indicative of extensive bonding for both the *cis*- and *trans*-isomers.

Doubly hydrogen-bonded complexes between formanilide and trimethadione (VI) are not possible, but CPK models of possible interactions indicate that either isomer could readily form a single hydrogen bond to the drug. However, at a trimethadione concentra-



V



VI

tion of 0.6 *M*, the mole fraction of *cis*-formanilide is reduced to 0.44, and there is a corresponding downfield shift in the *trans* N—H signal. The *cis*-peak is shifted to a higher field as a result of the decrease in concentration of this isomer, suggesting that little or no bonding occurs between the drug and the *cis*-configuration of the amide. The *trans*-isomer is thus strongly favored for formation of a single hydrogen bond with trimethadione, providing further evidence that the *cis*-complexes with pentobarbital and bemegride involve two hydrogen bonds. The comparatively weak hydrogen bonding of the *cis*-proton is in agreement with the observation by Green (14) that the *trans*-proton in formamide is favored by about 0.7 kcal./mole in the formation of hydrogen bonds to bromide ions[2].

The foregoing results demonstrate preferential binding of three CNS active drugs to the peptide bond, of which neither configuration should be ruled out as a possible site of action (15). However, if the present results are taken in conjunction with those for 9-ethyladenine (4, 5), it becomes apparent that the drugs are capable of forming strong hydrogen-bonded complexes with quite diverse structures, particularly if two bonds can be formed simultaneously. Their CNS activity could, therefore, involve multiple hydrogen bonding at a number of sites. Speculating on this basis, the author suggests that the barbiturates and related drugs may act on proteins (or other macromolecules) by linking together two or more hydrogen-bond-forming functional groups from chemically distinct portions of a protein molecule, or from two separate protein molecules, and thus stabilize a specific conformation or association state. The existence of many suitable and influential binding sites of this type may be anticipated if changes in protein conformation or association state participate in transmitter release or membrane depolarization, in the action of enzymes concerned with membrane transport or transmitter destruction, or in the enzyme systems controlling carbon dioxide or other metabolite levels. The ability to occupy such sites would depend on the nature, size, and shape of the substituent groups, so the activity of an individual drug would result from the summation of the convulsant and anticonvulsant effects of bonding to a large number of receptors. If this is the case, the drugs may be simply described as molecular glues, displaying a multicomponent action which is relatively insensitive to structural modifications.

By contrast, since each receptor is likely to be structurally specific, binding to a single site may occasionally dominate the overall activity of a drug, so small structural changes could produce compounds that exhibit quite different activity. Thus, for example,

[2] The bromide ion has both sedative and anticonvulsant actions (2).

Table II—Examples of Substituent Effects on Anticonvulsant Action

Drug	Activity	Reference
5,5-Diethyloxazinedione	Convulsant	17
5,5-Diisopropyloxazinedione	Anticonvulsant	17
3-Methyl-5,5-diethyloxazinedione	Anticonvulsant	17
3-Methyl-5,5-diisopropyloxazinedione	Anticonvulsant	17
β-Methyl-β-ethylglutarimide	Convulsant	18
β-Methyl-β-*n*-propylglutarimide	Dual action	18
β-Methyl-β-*n*-butylglutarimide	Anticonvulsant	18
N-Ethyl-β-methyl-β-ethylglutarimide	Dual action	6
N-Ethyl-β-methyl-β-*n*-butylglutarimide	Dual action	6
5-Ethyl-5-(1-methylbutyl)barbituric acid	Anticonvulsant	19
5-Ethyl-5-(1,3-dimethylbutyl)-barbituric acid	Convulsant	19

N-alkylation might have little effect if the dominant action of a drug were the linkage of amine hydrogens, but it would drastically alter activity if a favored site involved bonding to a carbonyl group. As it happens, such dichotomies occur among the experimentally observed structure–activity relationships of the barbiturates and similar drugs, including the following three examples related to their convulsant and anticonvulsant actions.

1. The activities of a series of anticonvulsants were correlated with *n*-octanol–water partition coefficients alone (16), *but* very slight changes in substituent groups can result in dual action (6) or convulsant activity (6, 17, 18). Some examples are given in Table II.

2. *N*-Alkylation often has little effect on activity [sometimes because of demethylation in the liver (19)], *but* it can cause significant activity differences (6) (Table II).

3. Some pairs of optical isomers display the same activity (20), *but* others antagonize each other (21).

Although speculative in nature, the hypothesis that the drugs act as molecular links opposing changes in protein conformation or association state is thus in accord with the otherwise inconsistent structure–activity relationships of convulsant and anticonvulsant compounds. It attributes these to a diversity of suitable receptor sites, which follows naturally from the capacity of the barbiturates and related drugs, possibly including the bromide ion, to form multiple hydrogen bonds. The hypothesis is also consistent with the observation that several modes of action may contribute to anticonvulsant properties (22) and with the variety of neural effects observed for both convulsant (21) and anticonvulsant (23) drugs. It could also account for other physiological actions of the barbiturates.

Several approaches which provide a satisfactory means of testing this general hypothesis are being undertaken in conjunction with a study of preferred drug conformations, and the present work will be extended to define the thermodynamic and geometric properties of the drug–amide complexes.

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ACKNOWLEDGMENTS AND ADDRESSES

Received December 6, 1971, from the *Department of Physical Biochemistry, The John Curtin School of Medical Research, Australian National University, Canberra, A.C.T., 2601, Australia.*

Accepted for publication June 27, 1972.

The experimental work was completed in the Chemistry Department, University of Melbourne.

Kinetics and Mechanisms of Monolayer Interactions I: Cetyl Sulfate and Cetrimonium Ions Interacting with Dipalmitoyl Lecithin and Dipalmitoyl Glycerol

FEDERICO A. VILALLONGA▲, EDWARD R. GARRETT, and MARCELINO CEREIJIDO*

Abstract □ The interaction of cetyl sulfate and cetrimonium (cetyltrimethylammonium) ions with dipalmitoyl lecithin and dipalmitoyl glycerol monolayers spread at the air/water interface was followed by the variation of the surface pressure and surface potential. The kinetics and the final equilibrium varied with the nature of the injected surfactant. An approximative method was devised to calculate the energies of activation, which are comparable with others obtained on a classical thermodynamic basis for cetyl alcohol monolayers interacting with sodium lauryl sulfate. The presence of the phosphoryltrimethylethanolamine group in the dipalmitoyl lecithin molecule decreases to some extent the interaction with cetyl sulfate and cetrimonium ions. The values of the energies of activation obtained indicate that the ionic groups of the polar moiety of dipalmitoyl lecithin are not equivalent in the perturbation that an attached hydrocarbon chain produces in the surface pressure of the monolayer. This difference may be explained by the different mobilities that a hydrocarbon chain would have when attached to one or the other ionic attraction centers because of the unrestricted movement of the positively charged trimethylammonium group around the phosphate linkage of the dipalmitoyl lecithin. Some implications of this observation to microstates of biomembranes are suggested.

Keyphrases □ Cetyl sulfate and cetrimonium ions—interactions with dipalmitoyl lecithin and dipalmitoyl glycerol monolayers, kinetics, mechanisms □ Monolayers, dipalmitoyl lecithin and dipalmitoyl glycerol—interactions with cetyl sulfate and cetrimonium ions, kinetics, mechanisms □ Phospholipid monolayers—interaction with long-chain surfactants, kinetics, mechanisms □ Surfactants, long chain—interaction with phospholipid monolayers, kinetics, mechanisms

The interaction of an insoluble monolayer spread at an interface with a soluble surface-active species injected in the subphase has been termed monolayer penetration (1–4). This interaction presents two aspects: the kinetics of approach to and the resultant equilibrium. The equilibrium has been studied from the two formally different aspects of the application of a modified Gibbs adsorption equation (5, 6) and the postulation of an osmotic equilibrium (7) between two presumed phases: dissolved surfactant in the bulk solution and surfactant molecules already penetrated into the monolayer.

Monolayers of dipalmitoyl lecithin at the air/water interface may be considered as suitable models to use in the study of certain properties which can be associated with the outer lipidic layer of cell membranes. For example, the discrimination in their interaction with monovalent cations such as sodium and potassium is similar to that exercised by the phospholipids that form the epithelial cell membrane (8–11).

The present study was carried out on the premise that the kinetics and the mechanisms of the interaction of phospholipid monolayers with ionic long-chain surfactants may yield useful information to further the understanding of the role of phospholipids in the cell membrane and in drug absorption.

EXPERIMENTAL

Reagents—Dipalmitoyl lecithin[1] was chromatographically homogeneous by TLC (12). The dipalmitoyl glycerol[1] was known to be a mixture of 1,2- and 1,3-isomers. Sodium cetyl sulfate[1] and cetrimonium bromide[2] gave no minima in the curves of surface tension against the logarithm of concentration.

Deionized and triple-distilled water was used throughout. Its pH after air equilibration was consistently between 5.6 and 6.0, and its surface tension was always, in approximately 200 experiments, between 99.8 and 100.2% of the value calculated from the Harkins equation (13). The air/water interfacial potential was −470 mv. (± 20 mv.). The hexanes[2,3] were spread (0.2 ml.) on 42.56 cm.^{2} of air/water interface and gave no significant variation on the surface tension of water (less than 0.02 dyne cm.$^{-1}$).

[1] Mann Research Laboratories, Orangeburg, N. Y.
[2] Eastman Kodak, Rochester, N. Y.
[3] Spectroquality, Matheson, Coleman and Bell, East Rutherford, N. J.