Journal of Medicinal and Pharmaceutical Chemistry

VOL. 3, No. 2 (1961)

Chemical Models of Drug–Receptor Interaction—I. Preliminary Studies

ALEXANDER GERO and LIDA A. SHROPSHIRE, *Department of Pharmacology, Hahnemann Medical College, Philadelphia 2, Pa.*

A few years ago, Gero and Reese[1] reported orienting experiments on the interaction of 2-*N*,*N*-diethylaminoethanol—considered as a 'stripped-down' drug molecule—with amino acids and peptides, as an initial test of a working hypothesis according to which drug molecules capable of hydrogen bonding might act by intruding on the internal hydrogen bonding of a receptor protein. In these experiments, it was determined how the presence of an amino acid affects the distribution of diethylaminoethanol (henceforth designated as DEA) between water and chloroform. Since, even when pH effects were allowed for, in all cases more DEA went into the aqueous phase when the latter contained amino acid than when it did not, it was concluded that the results demonstrate association between DEA and amino acids in aqueous solution and that hydrogen bonds are likely to be responsible for the association.

Without committing ourselves to the correctness of the original working hypothesis, we still felt that there was merit in this line of study. Even if we look to the side chains of the α helix, rather than to the helix itself, as the binding site for drugs, NH_2 and COOH groups are prominent functional groups in these side chains, and we entertained the hope that further experiments might throw light on some of the generally accepted, but mainly hypothetical, modes of attachment of drugs to receptors; namely ionic interactions, hydrogen bonds, and van der Waals' forces. We therefore continued the investigation, using carbon tetrachloride rather than chloroform as the hydrophobic solvent, since CCl_4 is more convenient to handle and realizes better than chloroform the ideal of a non-polar solvent, at the opposite extreme from the polar water. The distribution of DEA between these two

solvents as a function of pH is shown in Fig. 1—a composite graph which also shows the pH titration curve of DEA. It is seen that, when the proper scale is chosen, the straight portions of the two curves are parallel, signifying that about one-third of the DEA base is in the CCl_4 and two-thirds of the base, and all of

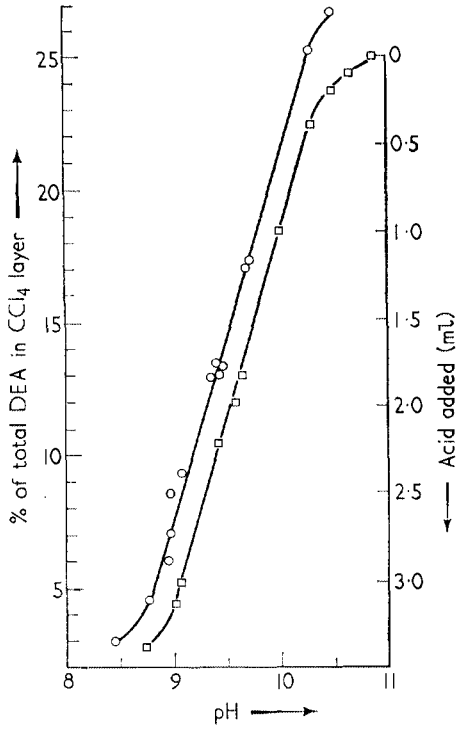


Fig. 1.

—○— water–CCl_4 distribution curve for DEA
—□— pH titration curve of DEA

the DEA cation (to be designated by the symbol $DEAH^+$), in the water. It is also seen that the pK_a of DEA is 9·58.

In the earlier experiments, the effect of the presence of an amino acid on the water–chloroform distribution coefficient was determined only at whatever pH happened to result from the addition of each particular amino acid to the DEA–water–chloroform system. In the experiments now being reported, we investigated

this effect for a number of simple amino acids over the pH range of 8·5 to 10·2, the limits of the range being dictated by experimental necessity: at higher pH it is extremely difficult to elude the disturbing action of atmospheric CO_2, while at a pH lower than 8·5 at least 97·5 per cent of the DEA is in the aqueous phase anyhow, so that the demonstration of any displacement of DEA from CCl_4 to water under the influence of amino acids becomes problematic.

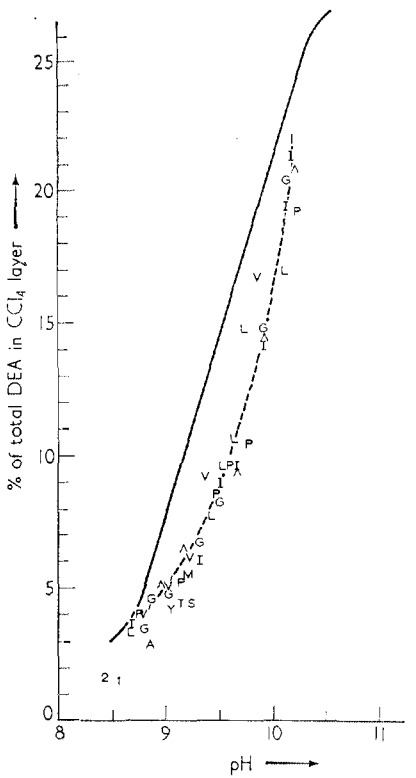


Fig. 2.

Symbols for amino acids

G	Glycine	P	Phenylalanine	A	Asparagine
A	Alanine	M	Methionine	1	Alanyl glycine
V	Valine	S	Serine	2	Alanyl norvaline
L	Leucine	T	Threonine		
I	Isoleucine	Y	Lysine		

However, our limited pH range was quite satisfactory because the greatest effect of amino acids on the distribution of DEA between water and CCl_4 occurred just in the pH range of 8·5 to 10·2. Fig. 2 reproduces the water–CCl_4 distribution curve for DEA from Fig. 1, and also shows how this distribution is affected by the presence of amino acids. The symbols for the several amino acids (see legend to Fig. 2) are so placed as to indicate the percentage of DEA in the CCl_4 phase when that particular amino acid is present, each single symbol being placed at a point which represents the average of all experiments conducted within a range of 0·1 pH unit. As found before, in all cases the amino acids draw more DEA into the aqueous phase; also, all points obtained for the simple amino acids glycine, alanine, valine, leucine, isoleucine, and phenylalanine, lie approximately on one and the same curve (shown as a broken line) while a single point for methionine also lies on the same curve but single points for several amino acids with additional hydrogen bonding groups (serine, threonine, asparagine, lysine, alanylglycine, and alanylnorvaline) lie deeper in the aqueous territory. The broken line deviates most from the blank line for the water–CCl_4 distribution of DEA around the middle of the pH range, and least at its extremes, indicating that the magnitude of the DEA–amino acid binding is pH dependent.

All of this is brought out more clearly when equilibrium constants are calculated for the reaction of DEA with amino acids. These association constants necessarily contain an element of arbitrariness because the formation of a DEA–amino acid complex is only inferred from our results and no such complex has ever been isolated; nor do we have kinetic data to indicate the order of the reaction. It is therefore to be considered only as an exploratory step when we arbitrarily assume the interaction to involve one molecule of DEA and one molecule of amino acid, and when we calculate equilibrium constants for the reaction:

$$\text{DEA} + \text{AA} \leftrightharpoons \text{DEA–AA}$$

where AA stands for amino acid, and DEA–AA for the DEA–amino acid complex.

As expected, the equilibrium constants were found to vary with the pH. We avoid therefore the term 'constant' and prefer

to speak of a 'binding coefficient' B, defined as the pH-dependent quotient

$$\frac{[\text{DEA–AA}]}{[\text{DEA}]\,[\text{AA}]}$$

Binding coefficients were calculated as follows: from the determination of the blank curve the distribution of DEA between water and CCl_4 at each pH was known. Having brought DEA to

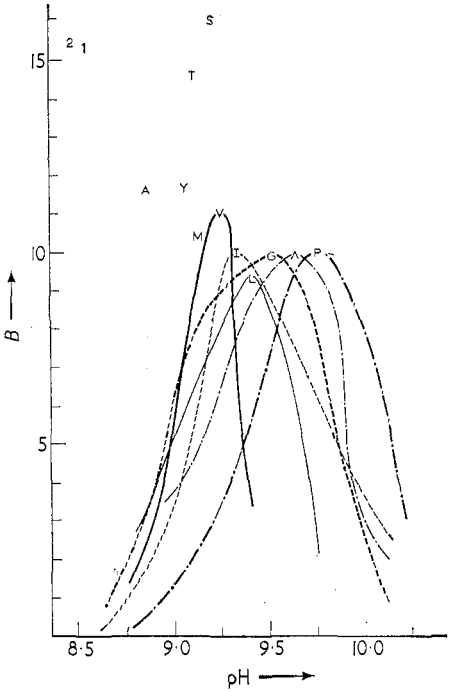


Fig. 3.

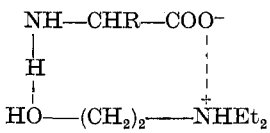
Symbols for amino acids as in Fig. 2. The symbols in the curves show the maximum of each curve.

equilibrium with the same two solvents in the presence of an amino acid, first the pH of the aqueous phase was measured in order to establish with which point of the blank curve the result should be compared, then the DEA content of the organic layer was determined. It was always lower than when no amino acid was present. From the known distribution coefficient at the particular pH measured and from the DEA content of the CCl_4

phase, the amount of free DEA in the aqueous layer was calculated. Subtracting from the known total amount of DEA, the amount of bound DEA was obtained, corresponding to an equivalent amount of bound amino acid; this in turn was subtracted from the known total amount of amino acid to obtain the amount of free amino acid.

From the superficial aspect of the broken line in Fig. 2, we expected the pH dependence of B to be described by one and the same bell-shaped curve for all six simple amino acids which we investigated systematically. On close scrutiny, however, we found that each of the amino acids requires a separate curve to describe this functional relationship. Fig. 3 shows these curves as well as the values of B at single values of pH for six more complex amino acids.

The binding coefficients of the simple amino acids go through maxima between pH 9·2 and 9·7, i.e., in an area where both the amino acid and the DEA are partially, but not completely, cationic. This is the basis of the explanation offered to account for our results: since the isoionic points of all the simple amino acids are around 6, their carboxyl groups are surely completely ionized at all pH values at which we worked. A positive charge on the nitrogen of the DEA molecule should therefore facilitate binding through the ionic attraction AA^{-}–$DEAH^{+}$:



With rising pH, binding decreases because the transition $DEAH^{+}$ → DEA removes the coulombic attraction between DEA and amino acid. By the same token, decreasing pH would increase the binding but for the fact that with increasing availability of hydrogen ions not only the DEA but also the amino groups of the amino acids become cationic. Thus coulombic repulsion between two ammonium groups is superimposed on the coulombic attraction between the $DEAH^{+}$ cation and the carboxylate anion, and binding decreases again as both species become increasingly cationic. It should be noted that, except for phenylalanine, the maxima of the binding coefficients appear to be roughly correlated

with the basicities of the amino groups of the several amino acids: binding is at its peak when about two-thirds of all NH_2 groups are ionized.*

While it appears that the pH dependence of B in simple amino acids can thus be reasonably interpreted as a consequence of ionic attraction and repulsion, the very much higher B of amino acids with additional hydrogen bonding groups (OH in serine and threonine, $CONH_2$ in asparagine, an ϵ-amino group in lysine, and a peptide group in alanylglycine and alanylnorvaline) clearly demonstrates the rôle of hydrogen bonds. It may seem somewhat surprising that lysine, within the limits of our experiments, is inferior to serine and threonine: one would expect an ionizable amino group to play a more prominent part in hydrogen bonding than an alcoholic hydroxyl group. However, as one of us has shown,[2] the ϵ-amino group of lysine is sterically well placed for chelation with the carboxyl group, thus decreasing both the availability of the ϵ-amino group for hydrogen bonding to another molecule, and the effective negative charge of the carboxylate anion.

We may sum up the results of our model experiments by saying that we believe two of the three accepted factors in drug-receptor interaction to have been demonstrated, namely, ionic forces and hydrogen bonding. One must, of course, beware of overworking a model: it is questionable just how far the interaction between amino acids and DEA in a water–CCl_4 system represents drug–receptor interactions in the body. Still, there may be relevance in the fact that the importance not only of ionic attraction but also of ionic repulsion clearly emerges from our data, especially when a group is considered (such as the NH_2 groups in our examples) which, charged or uncharged, can serve as a bonding agency by hydrogen bonds but is a repelling ion when charged. Applied to actual drug–receptor interactions, this observation points up the importance of allowing for possible coulombic repulsion and of looking for maximum interaction when some definite fraction of a particular ionizable group is charged.

* The anomalous position of phenylalanine is possibly due to some complicating effect of the benzene ring, an effect which could be attributable to the π electrons of the ring, to van der Waal's bonding, or to both. However, we do not have sufficient experimental data to decide this question and therefore prefer to leave it open for the time being.

We realize that our model is beset by two particularly bothersome imperfections: it restricts us to an unphysiological pH range, and it does not provide us with the possibility of demonstrating also van der Waals' bonding. It was because of these inherent limitations of DEA that we carried out complete studies on the pH dependence of binding only with the simple amino acids and contented ourselves with observations at a single pH with the more complex amino acids. We hope that experiments now in progress with a different drug model will remedy these deficiencies.

Experimental

The blank water–CCl_4 distribution curve for DEA was determined by placing 10 ml of a $\frac{1}{20}$ molar solution of DEA in CCl_4 (i.e. one-half millimole of DEA) in a 50-ml glass-stoppered bottle, together with 10 ml of water containing varying amounts of acid. The stopper was taped down tightly and the bottle shaken mechanically for 5 min. (It was found that longer shaking, up to 35 min, did not alter the results.) Then the layers were separated, the aqueous phase, after determination of its pH, titrated with N/20 acid or alkali, and the CCl_4 layer extracted with 10 ml of N/20 acid and then back-titrated with N/20 alkali. The titrations were carried out with microburettes permitting $\frac{1}{100}$ ml to be read accurately, using methyl red as an indicator.

Shaking the bottles was carried out in a box holding 28 bottles. Each individual experiment was performed four to fourteen times, depending on how reproducible it was. With well-fitting stoppers, losses were negligible when the titrations were carried out the same day. If, however, the bottles were kept overnight, there was clear interference from atmospheric CO_2, which had diffused in through the tape and past the stopper. Fast work is therefore very important for good results.

Experiments on binding followed the pattern of the blanks except that now the 10 ml of water in each bottle contained one millimole of an amino acid, plus—in the case of the simple amino acids—also varying amounts of acid or alkali so as to obtain data on binding at various pH values. After the layers were separated, the pH of the aqueous phase was measured, and the CCl_4 layer extracted with 5 ml of N/20 acid and the excess acid back-titrated

with N/20 alkali. The aqueous phase, which contained both amino acid and DEA, presented a more complicated analytical problem; therefore we chose to base our experiments entirely on the DEA content of the CCl_4 layer from which the DEA content of the aqueous layer was calculated. Each of these experiments, too, was repeated four to fourteen times.

Summary. In a model study of the drug–receptor interaction, one millimole of an amino acid (representing the receptor) was allowed to act on one-half millimole of diethylaminoethanol (representing the drug) in a two-phase system consisting of water and CCl_4. The amino acid always displaced the equilibrium distribution of the drug model in the direction from CCl_4 to water, and the magnitude of the displacement under the influence of various amino acids was interpreted to demonstrate the presence of ionic attraction and repulsion, and of hydrogen bonding, between the amino acids and the drug model.

Acknowledgement. This investigation has been supported by a grant from the American Cancer Society which we gratefully acknowledge.

(*Received 27 August, 1960*)

References

[1] Gero, A. and Reese, V. J. *Science*, **123**, 100 (1956)
[2] Gero, A. and Withrow, C. L. *Nature, Lond.*, **180**, 1354 (1957)