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## Molecule-Receptor Specificity

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*Phil. Trans. R. Soc. Lond. B* 1975 **272**, 75-85

doi: 10.1098/rstb.1975.0071

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## Molecule–receptor specificity

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The specificity of binding between small molecules and macromolecular receptors may be studied by comparing theoretically calculated conformational potential energy surfaces of a series of chemically similar molecules which have a range of receptor binding energies and biological activities. In this way essential requirements for binding may be highlighted, including the necessity of the small molecule adopting, or passing through, conformations which are not at energy minima and not found either in the solid state or in aqueous solution. In particular the conformational demands of the adrenergic  $\beta$ -receptor and histamine H1 receptor are considered.

## INTRODUCTION

Biological recognition at a molecular level involves the binding of the two recognizing molecular species. For a large class of such cases the binding is between a macromolecule, frequently a protein, and a small molecule or hapten group. Included in this category of small molecules are transmitters, hormones and most drugs, the majority of which are conformationally flexible molecules.

The detailed structure of the macromolecular receptors for transmitters or drugs is unknown, as is the nature of the environment in which these large molecules are found. It is thus possible that the small flexible molecule may be required to adopt a conformation which is different from that which it is most prone to adopt either in the solid state or in bulk solution.

This possibility becomes more of a probability when one considers the fact that many of the small molecules such as acetylcholine or histamine perform more than one distinct biological role and interact with different receptors. Drugs may block one activity but not another. Added to this is the crystallographic evidence that substrates when bound to enzymes seem to be distorted from their equilibrium forms.

Ideally we should like to know the conformation and electronic distribution of the small molecule in the environment of the receptor. Since this may be a non-equilibrium conformation the standard and powerful physical techniques such as X-ray crystallography of the solid or nuclear magnetic resonance spectroscopy of solutions may be unable to provide the full picture. To supplement physical information with details of unstable forms of molecules one is forced to consider the use of the methods of theoretical chemistry.

## THE USE OF THEORETICAL METHODS

In the realm of small gas-phase molecular problems, the admittedly brute-force techniques of accurate molecular orbital calculations have reached a high degree of sophistication. For the type of molecule of interest to the astrophysicist, theoretical calculations can be as accurate or even more accurate than laboratory experiment.

Extension to molecules of biological interest involves a number of additional complications which limit the confidence which can be placed on the results. Most obviously the molecules of interest are larger and since the integral evaluation increases roughly as the fourth power of the number of electrons in the molecule then the computational requirements become vast. Added to this is the fact that if the molecule is flexible, then a large number of calculations may be needed for each molecule, covering a wide range of conformations. This problem to a large extent restricts the theoretical methods to so-called semi-empirical molecular orbital calculations.

The second complicating feature is the fact that the gas-phase properties of the molecule may not necessarily be the same as the solution properties or the properties in the unknown conditions in the neighbourhood of the macromolecular receptor. None of the methods of including the effect of water as solvent in theoretical calculations is wholly satisfactory and there is no direct means of including factors which take into account the presence of a receptor molecule and the environment appropriate to such a site.

These difficulties suggest some limitations within which theoretical methods should be employed. Since the methods are likely to be approximate then it is essential that wherever possible the calculations should be checked by experimental measurement. For instance if conformation is the property under investigation, then stable conformations which are observed should be indicated as such and population ratios measured perhaps by n.m.r. spectroscopy should be reproduced by the calculations.

Nothing can be done directly about allowing for the biological environment so we are confined to studying the differences between similar molecules. In particular we will be on the safest ground if we study a series of molecules with very similar structures and physical properties, such as molecular mass, basicity and oil-water partition coefficient, but which at the same time manifest a range of biological activities or binding constants.

The difficulty of using published biological data from a variety of sources which may not be directly comparable also makes it desirable that any theoretical study of a series of molecules be done in direct collaboration with the experimental team which has made the biological measurements and can synthesize and test further examples.

## METHODOLOGY OF THE PRESENT WORK

The approach outlined above is applied here to two pharmacological problems. In both cases the problem consists of attempting to see whether there is a particular conformational requirement for a flexible molecule to interact with a receptor in order to produce a particular agonist effect.

For the atomic geometry of the molecules considered, bond lengths and angles are taken from standard tables (Sutton 1958, 1965), but two twist angles are taken as variables: each is varied over the full range of  $360^\circ$  in steps of  $15^\circ$ . For each conformation the energy is computed

by the molecular orbital method. The molecular orbital approximation chosen is the simplest, the extended Hückel method (Hoffmann 1963). In general this crude method gives a reasonable indication of preferred conformations of substituted aliphatic hydrocarbons and in the case of the work on histamine discussed later the results are supported by n.m.r. studies. In other cases this particular molecular orbital method exaggerates the energy differences between different conformations of a given molecule, but here we focus attention on the relative effects of substitutions on conformational preferences for which the simple method is adequate.

The results could be presented as conformational energy maps; two dimensions sufficing as we have only two variable angles. However, these energy contour maps contain such a wealth of information that it is difficult to compare a series of such diagrams. In order to be able to compare the conformational flexibility of members of a series, the directly computed

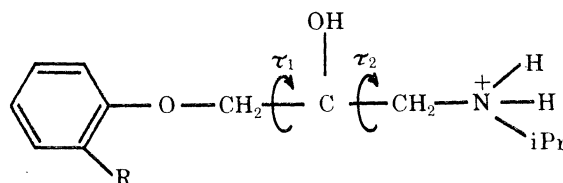


FIGURE 1. The skeleton of the phenoxypropanolamine cation showing the variable torsion angles  $\tau_1$  and  $\tau_2$  and position of variable substituent R.

energy map is transformed into an exponential map. For each point  $(\tau_1, \tau_2)$  on the surface  $\exp[E(\tau_1, \tau_2)/kT]$  is calculated:  $k$  is the Boltzmann constant and  $T$  the absolute temperature. Summations of these exponential factors are then normalized to yield conformational partition functions which can then in turn be used to calculate the percentage populations in any given form (say, for example, *trans* or *gauche*) (Farnell *et al.* 1974).

The exponential maps can also be used to compute so-called 'population maps' (Richards & Genellin 1974). These contain contours defining the region of conformation space which contains a given percentage of molecules at any specified temperature. This latter type of map has the advantage of showing at a glance the relative conformational flexibilities and preferences of the molecules in a series.

#### CONFORMATIONAL REQUIREMENTS FOR $\beta$ -ADRENOCEPTOR AGONISTS

We give preliminary results of a study of the partial agonist activity among *ortho*-substituted phenoxypropanolamines which have been made in collaboration with I.C.I. Pharmaceuticals Division. The basic structure of the molecules is shown in figure 1. We have considered the series with R=H, Me, Et, OMe allyl and COCH<sub>3</sub>. The sympathomimetic activities were measured as increases in the heart rates (beats per minute) of catecholamine depleted rats following a fixed dose of 2.5 mg/kg i.v.

For the calculations all torsion angles except  $\tau_1$  and  $\tau_2$  were fixed, either by the use of models or in cases where there is any serious doubt, by careful *ab initio* molecular orbital calculations on fragments of the molecule. (The side chain is taken as perpendicular to the aromatic ring and other fixed torsion angles are all in staggered *trans* conformations.)

The conformational preferences for four of the series are depicted in figures 2-5 in which three contours are shown; those encompassing 99%, 95% and 70% of the molecules at 37 °C. The central area ( $\tau_1 = 180^\circ$ ;  $\tau_2 = 180^\circ$ ) represents an all *trans* conformation with the side

chain nitrogen atom and the aromatic ring at maximum separation, while the subsidiary region ( $\tau_1 = 300^\circ$ ;  $\tau_2 = 180^\circ$ ) represents a *gauche* form.

Although the maps are, not surprisingly, very similar, nonetheless there is a definite discernable trend. If the maps are put into a logical sequence, either by ranking the areas of the

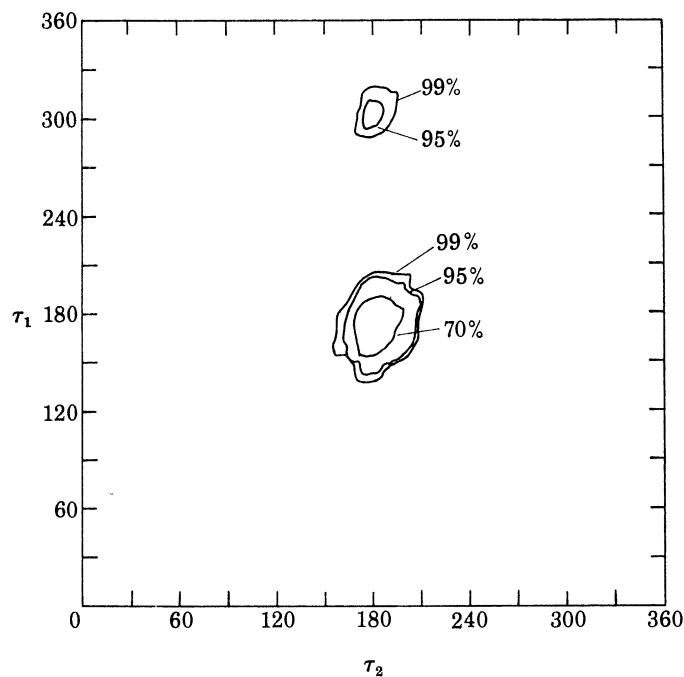


FIGURE 2. Percentage map for substituted phenoxypropanolamine. R = ethyl; activity =  $29 \pm 7$ .

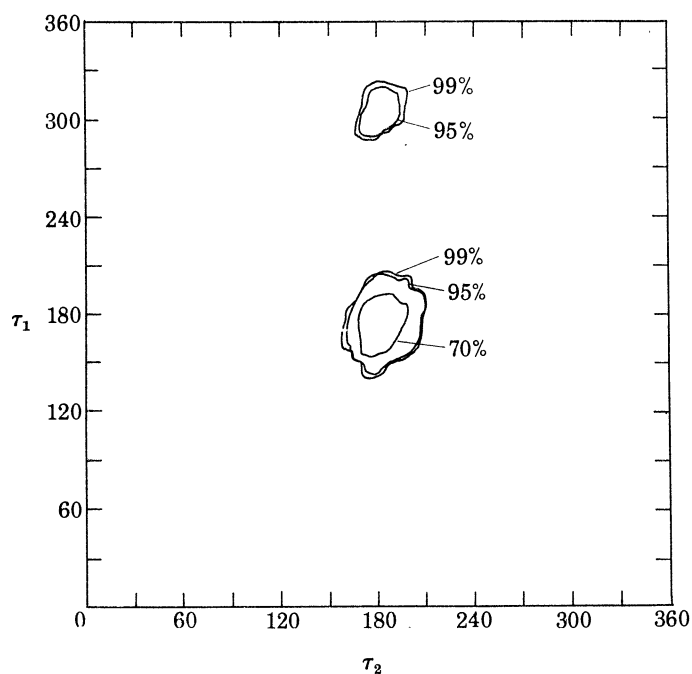


FIGURE 3. Percentage map for substituted phenoxypropanolamine. R = methyl; activity =  $65 \pm 11$ .

subsidiary *gauche* region or the main all *trans* region, then this order parallels the order of biological activities.

This ordering can be put on a quantitative basis by plotting the area of the *gauche* region (a measure of the population of this conformer) against the activity. The resulting correlation is shown in figure 6.

While these results are of a preliminary nature and some of the obvious extensions and

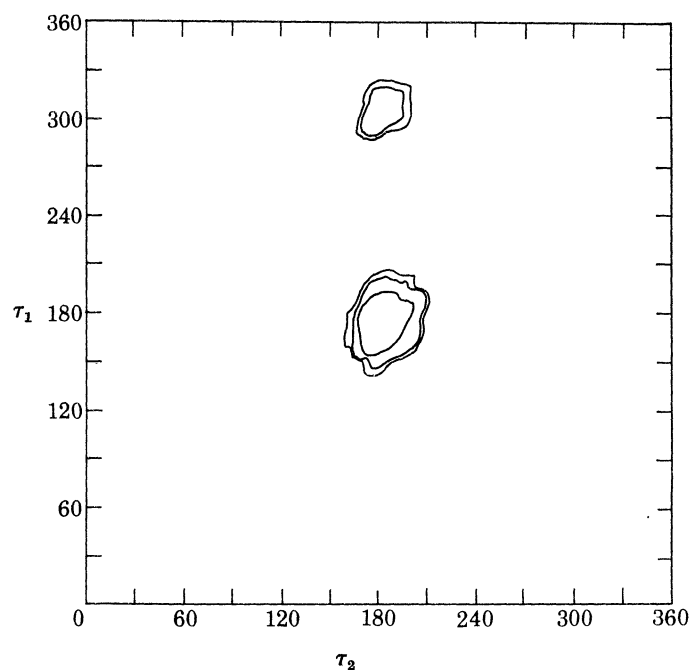


FIGURE 4. Percentage map for substituted phenoxypropanolamine. R = acetyl; activity =  $74 \pm 5$ .

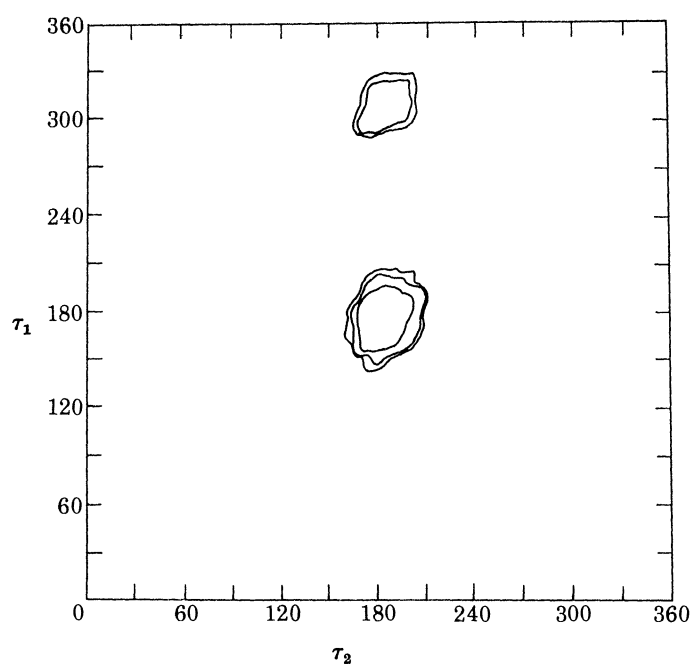


FIGURE 5. Percentage map for substituted phenoxypropanolamine. R = H; activity =  $104 \pm 7$ .

verifications are incomplete, the quality of the correlation is striking, particularly since the correlation is a direct one, not involving a logarithmic plot.

A possible implication to which this comparative study of conformational preference leads is that a conformational form which is only a minor constituent in solution is the one which interacts with the appropriate receptor.

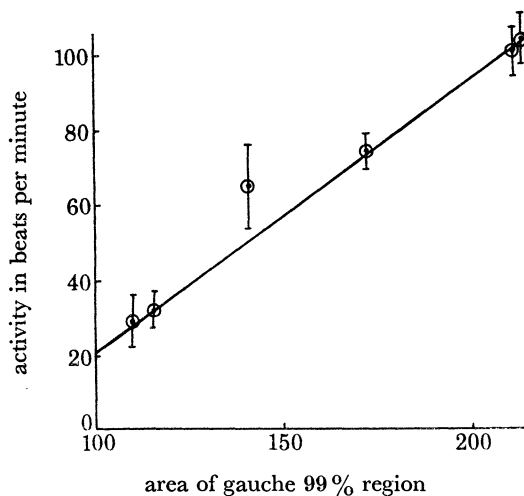


FIGURE 6. Correlation between measured activity and population of *gauche* form of substituted phenoxypropanolamine.

#### CONFORMATIONAL REQUIREMENTS FOR HISTAMINE H<sub>1</sub> ACTIVITY

In collaboration with Smith Kline & French Laboratories Ltd, we have made a similar study of the effects of methyl substitution on the conformation of histamine (figure 7), in relation to the agonist activity of the molecule at the H<sub>1</sub> receptor. The details of this work are given in a series of published articles (Ganellin *et al.* 1973*a, b*; Richards & Ganellin 1974).

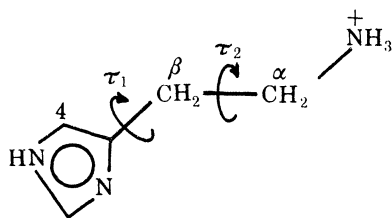


FIGURE 7. Histamine monocation showing the variable torsion angles  $\tau_1$  and  $\tau_2$  and the positions of methyl substitution.

In this case the most striking feature of the biological data is the fact that 4-methylhistamine is only weakly active at the H<sub>1</sub> receptor but active at the H<sub>2</sub> receptor. Consideration of space-filling models led to the suggestion (Ganellin 1973) that a non-equilibrium conformation may be essential for this type of receptor recognition.

The percentage maps calculated as described above again provide a clear correlation with activity. They are also supported by n.m.r. studies (Ganellin *et al.* 1973*a*). Compounds which are active as both H<sub>1</sub> and H<sub>2</sub> agonists have maps indicating predominantly *gauche* forms. H<sub>1</sub> active compounds are all able to approach very close to a planar form and have low

barriers to internal rotation. A selection of the examples studied are given in figures 8–13. The maps suggest a low-barrier to rotation about  $\tau_1$  for the histamine cation itself but a high barrier for the 4-methylhistamine cation. In general barriers derived from extended Hückel calculations are very suspect but in this case they are supported by *ab initio* calculation (Richards *et al.* 1975).

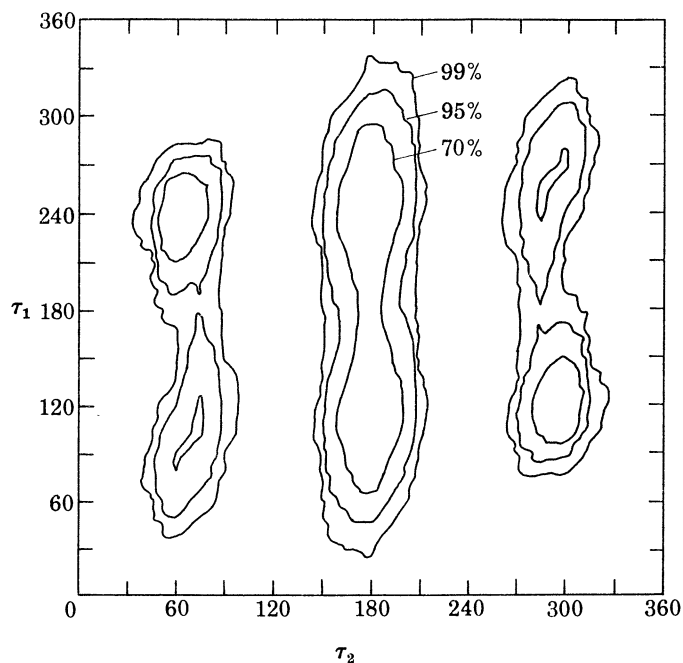


FIGURE 8. Percentage map for histamine.

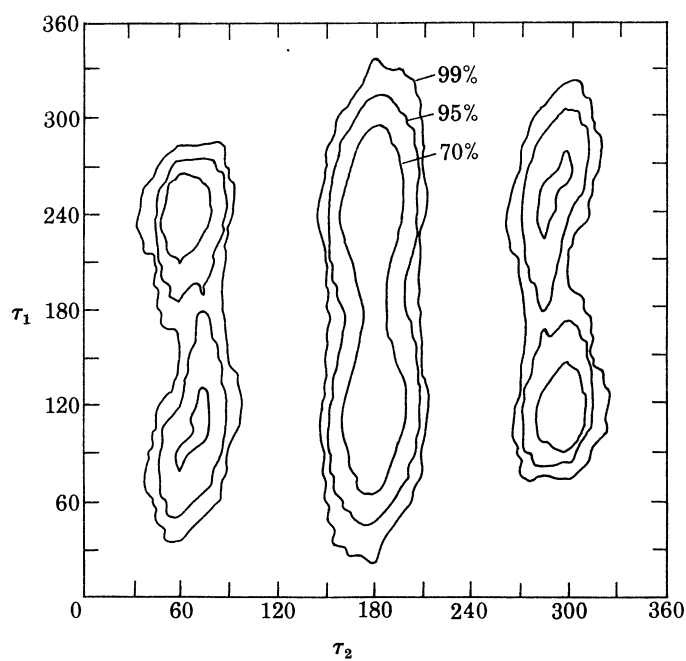


FIGURE 9. Percentage map for *N*-methylhistamine.



The maps provide a necessary but not sufficient test for H<sub>1</sub> agonist activity. Their interpretation suggests that the non-equilibrium planar conformation is a prerequisite for interaction with the H<sub>1</sub> receptor. Such analysis will not, however, distinguish whether this conformation is required to fit the active site of the receptor or alternatively whether it is merely a distorted conformation through which the molecule must pass to gain access to the receptor.

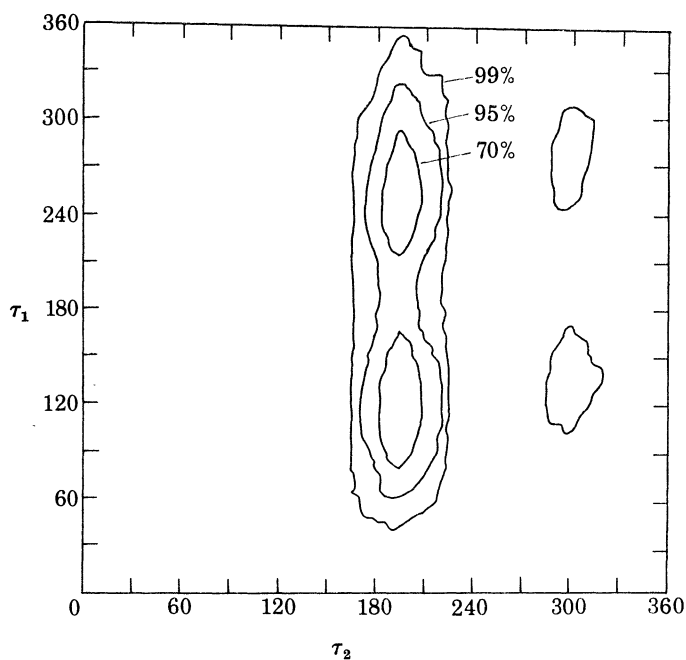
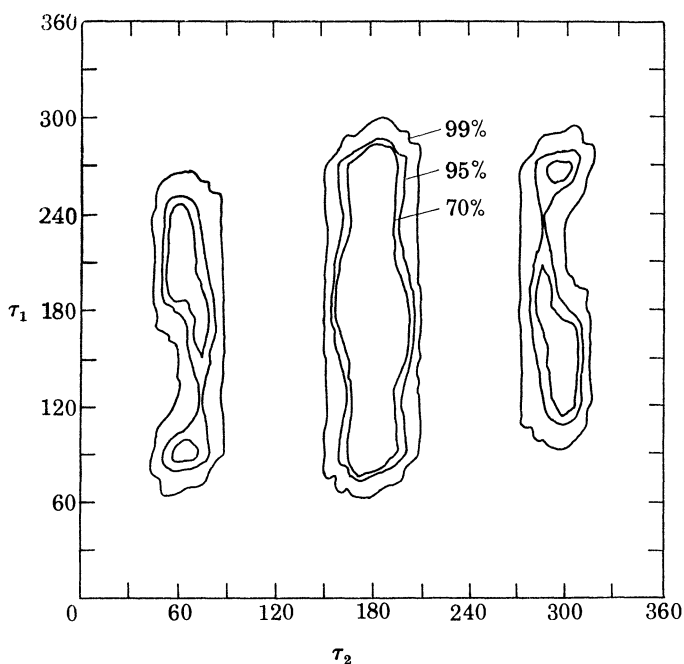
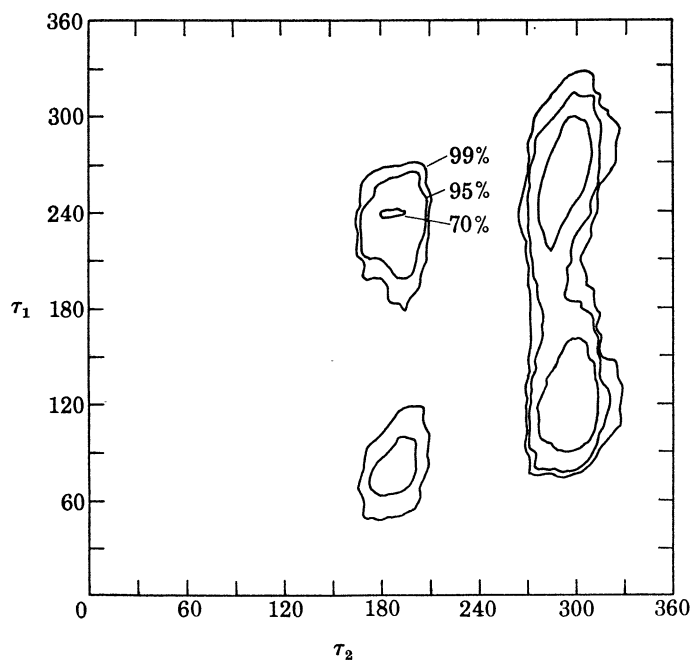
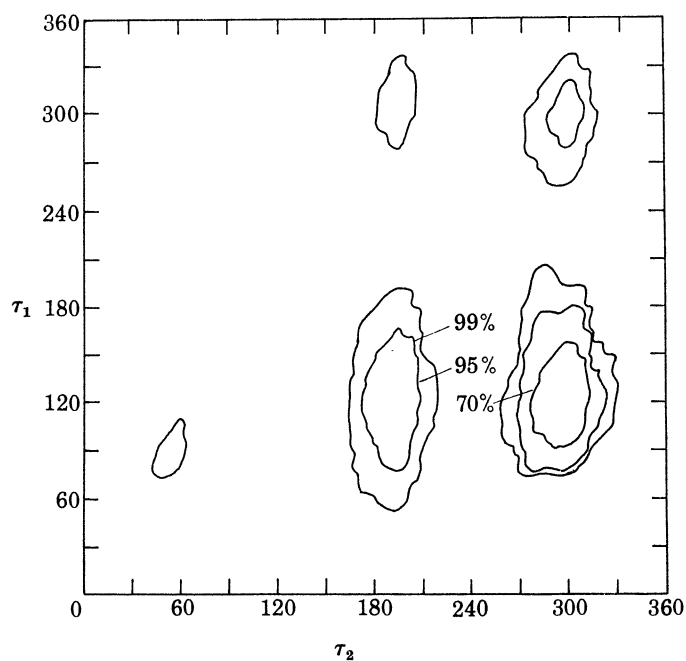
FIGURE 10. Percentage map for *N,N*-dimethylhistamine.

FIGURE 11. Percentage map for 4-methylhistamine.

## DISCUSSION

The two systems studied here are very different and yet in both cases the results suggest that the small flexible molecule has to adapt its conformation from its most stable form to one less stable, in order to produce a biological effect.

FIGURE 12. Percentage map for  $\alpha$ -methylhistamine.FIGURE 13. Percentage map for  $\beta$ -methylhistamine.

Rigid analogues are much used in the search for new drugs. If these have the correct rigid conformation they should be effective, but the sense of the theoretical work described here is that the inactivity of a rigid analogue cannot lead to any positive conclusions.

It seems unlikely that the use of conformational flexibility is restricted to the two series of compounds described above and it may be a much more general phenomenon. If this is so then the methods of molecular quantum chemistry have a place in the study of biological recognition alongside techniques similarly derived from physics such as the experimental structural methods.

The authors would like to express their gratitude to the research department of I.C.I. Pharmaceuticals Division and The Research Institute, Smith Kline & French Laboratories Ltd for their collaboration and active involvement in this work and to the Lord Dowding Fund for Humane Research whose generous grant to W.G.R. has enabled the theoretical calculations to be performed by Jane Hammond and David Aschman using a grant of computer time from the Science Research Council.

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#### Discussion

E. G. STEWARD (*The City University, London*)

Most endogenous and exogenous neurotransmitters are small, highly flexible, molecules. Structure–activity studies in these fields should therefore take into account the possible conformational modes which molecules can adopt (Warner, Player & Steward 1973). While quantum classical calculations may give reliable indications of the conformational modes in free space, they do not allow for the effect of solvent. This effect may be small for relatively rigid molecules, or those with low dipole-moment, but may be of overwhelming importance where there is a large dipole-moment in polar solvents, notably water. This is true for acetylcholine (Beveridge, Kelly & Radna 1974) and for the centrally acting inhibitory transmitter  $\gamma$ -aminobutyric acid (GABA) and analogues studied in our laboratory. In the GABA-like compounds, which are zwitterions with large dipole-moments, the electrostatic interaction energy with water is by far the largest single factor influencing molecular conformation in aqueous solution.

However, an aqueous environment may not be relevant to interaction of transmitter and receptor since, for instance, the receptor may well be buried within the lipid phase of the

neuronal membrane. Under these conditions, the free-space approximation may not be so far from the truth. In fact, in our studies of structure-activity relations in the GABA system (Steward & Clarke 1975), using very simple potential-energy calculations as in the work of Gill (1959, 1965), we have found correlations ( $r > 90\%$  for the twelve compounds considered) between potency and conformational population. We are investigating whether such correlations could also be obtained using the results of c.n.d.o./2 type molecular orbital calculations (Warner & Steward 1975; Warner, Borthwick & Steward 1975), with or without the inclusion of solvent effects.

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A. D. B. MALCOLM (*Department of Biochemistry, Glasgow University*)

You emphasized that the slide of heart beat against percentage of drug in the *gauche* conformation showed a linear relation between them, and that it was particularly significant that this was a plot of the actual data and not of their logarithms. *A priori* it might be expected that heart beat was a reflexion of the amount of drug bound, while percentage of drug in a particular conformation is more likely to reflect a free energy change. Since it is known that it is the logarithm of amount bound which is proportional to the free energy, would it not be more significant if you had shown a linear relation in a semi-logarithmic plot rather than in a direct plot?

K. DALZIEL (*Biochemistry Department, Oxford*)

Presumably the rotomer conformations of different probability will be interconvertible. Can Dr Richards say what the activation energy for such interconversions will be? It seems to me that they would be fast processes compared with the response time of most biological assay systems, and therefore that the different conformations would not be recognized.