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THE RELEVANCE OF CHIRALITY TO THE STUDY OF BIOLOGICAL ACTIVITY

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1. INTRODUCTION

One of the recurring themes in the history of chemistry has been that of the study of the shape of molecules and it is also a theme of increasing complexity. From X-ray and other studies a great deal is now known about the absolute configuration of molecules in terms of their 3-dimensional arrangements of atoms and bonds. Furthermore, a great deal is known about molecular conformation and the interconversion of conformations, largely by use of techniques such as nmr. This kind of study introduces the dimensions of time and energy into the description of molecules. More recently, the emergence of the techniques of molecular modelling enables these energies to be calculated easily and visualised even to the extent of models of enzyme-inhibitor complexes. Faced with such beguiling techniques it could be thought, therefore, that we know almost all there is to know about the appearance of molecules and yet this appreciation is all through the eye of the chemist.

We are only just starting to arrive at an appreciation of how one molecule may appear to another molecule. It certainly is not by means of the familiar collection of atoms and bonds which the chemist usually uses to convey his ideas. There is not as yet, and possibly never will be, a convenient representation of such an appearance because molecular recognition is a process of mutual interaction between molecules and this will change whenever there is a change in the observing molecule.

The closer study of molecules in three dimensions necessitated by consideration of molecular interaction has led to a better appreciation of the importance of absolute configuration and biological activity. This has long been recognised where diastereoisomers are concerned but is currently of most importance in the area of racemates and enantiomers. Most commonly enantiomers are related by a change in configuration at one centre but the application of the concepts generated by consideration of such is expandable into molecules with more than one chiral centre, or into those related by planes or rotational axes of chirality. The theoretical implications of chirality on molecular recognition and biological activity will be covered in this report and exemplified where possible. However this treatment is not intended to be an exhaustive review of all types of molecule-receptor interaction.

2. BIOASSAY

The normal physical techniques of measurement are inappropriate where molecular recognition is concerned for they usually involve the interaction of a molecule with electromagnetic radiation and/or ionising forces. Fortunately, in the very process of molecular recognition, nature has provided a very sensitive tool in the form of bioassays where, as the recognition process involved often provokes some biologically relevant event, the consequences of the normal responses between molecules can be observed indirectly. The many techniques of bioassay are beyond the scope of this report but, in general, by a cascade of events there is an amplification of the interaction between a molecule and its receptor into a measurable event. Unfortunately, in the course of this amplification, a good deal of detail is lost. This means that in order to generate the concept of interaction between a molecule and a receptor, called a pharmacophore, the effect of many similar molecules is generally studied. An evaluation of responses of these individual molecules, coupled with molecular modelling techniques can then be expressed in terms of more abstract concepts such as the distance of hydrogen bond donor groups above an aromatic plane and subtending such and such an angle to the centroid of the plane. An extension of this approach has led most recently to the creation of 3-D database systems which can be used to catalogue and search for such concepts.

Almost all of the examples considered here will involve the interaction of small molecules (drugs) with large, usually proteinaceous, molecules (receptors). It is necessary to consider first how this is expressed in bioassay.

2.1 *General terms and concepts used in bioassay*

In general terms, a drug D is supposed to interact reversibly with a receptor R to form a drug-receptor complex DR which then in some way produces a response (Fig. 1). Under these conditions the drug may be described as an **agonist** which will have an **affinity** for the receptor and also an **efficacy** or **intrinsic activity** at that receptor which results in the response. The **potency** of a drug is the ability it has to provoke the response: this is essentially a product of the **affinity** and the **efficacy**. So, a drug may have a poor affinity for a receptor but have a large effect there and appear to be very potent. It is important, therefore, when considering how the biological activity is influenced by absolute configuration, to separate these factors. Unfortunately this is not always possible.

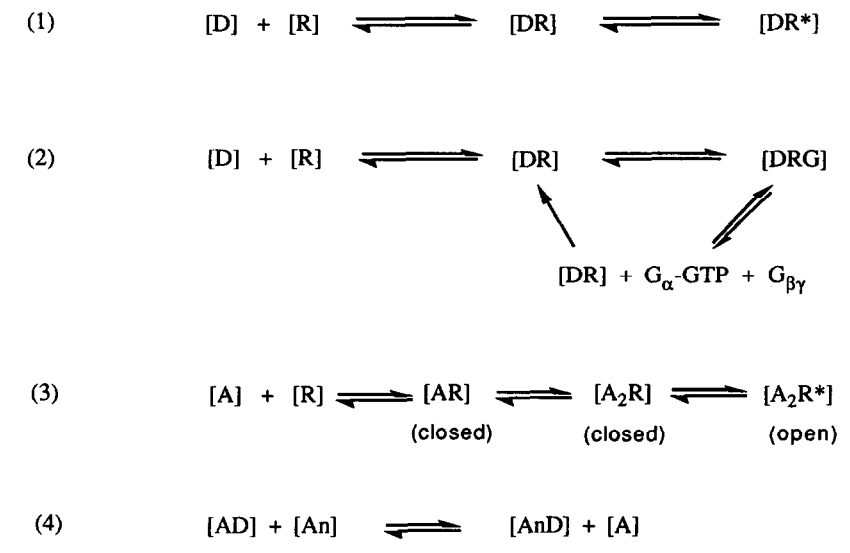


Fig. 1. Interactions of drugs with different receptor types. (1). Classical simplified interaction of a drug (D) with a receptor (R) to give a drug-receptor complex (DR) which undergoes a conformational change to an activated complex (DR*) and this then subsequently produces an effect. (2). G-protein coupled receptor family where the initial drug-receptor complex (DR) couples to a GTP-binding regulatory protein (G) causing it to be split into two mobile parts $G_{\beta\gamma}$ and G_α-GTP which in turn activates or inhibits second messenger systems, eg. adenylate cyclase, or opens ion channels directly. (3). Directly coupled receptor to ion channel where conformational change in the receptor is responsible for channel opening. Sometimes more than one agonist molecule (A) is necessary to activate the channel as in the case of the nicotinic acetyl choline receptor. (4). An antagonist molecule (An) may interact with the agonist-receptor complex displacing the agonist and preventing its effect.

If another molecule is able to compete with the drug for binding to the receptor and yet has no efficacy itself it can thereby prevent the response to the drug and is described as an **antagonist**. The term **partial agonist** also exists to describe drugs of low efficacy which do not provoke a full response. Antagonists may be described either in terms of their ability to prevent a response or in terms of the equilibrium constant of the equation (iv). In this case it is theoretically possible to describe the recognition in terms of the free energy and entropy of interaction although this is rarely done.

Ligand gated receptors can be divided into two families, the G-protein linked family and the ion channel family. These have been recently extensively classified.[1] In the case of the former, the intermediacy of the G-protein serves as a coupling mechanism for amplification mechanisms based on second messenger systems which may lead to the opening of ion channels in the cell membrane. Alternatively, a more specialised type of interaction may be found where the receptor constitutes an ion channel which is voltage gated. In these receptors, a change in membrane potential causes a conformational change which opens or closes an ion channel.The importance of ion channels in terms of molecular recognition lies in the extent to which they can be measured using the comparatively new technique of electrophysiology. On opening a channel, a large amount of cations (Na^+,K^+,Ca^{2+}) or anions (Cl^-) transit the membrane creating a current which can be measured with great accuracy. This, coupled with the ability to measure single channels, that is single recognition events between a molecule and channel, in real time provides data on the rates of the processes involved.

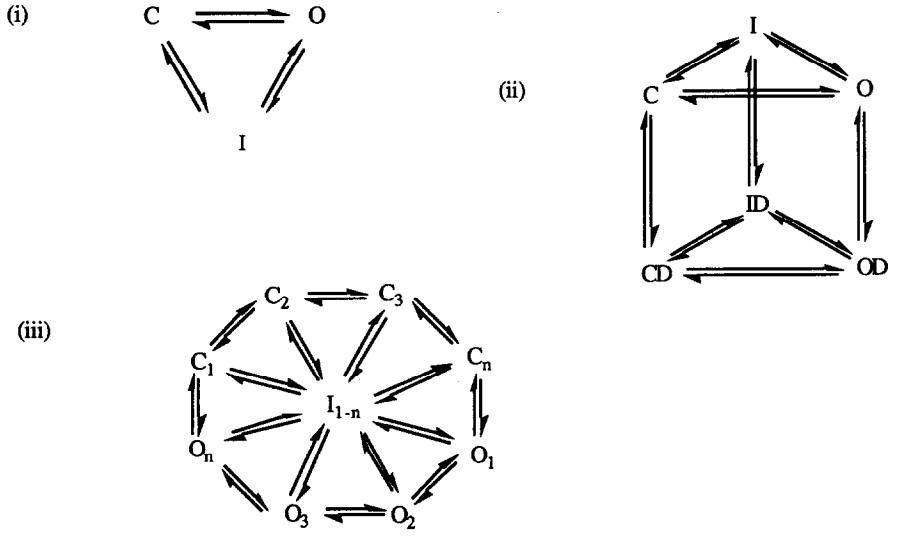


Fig. 2. Interconversion between ion channel states. (i). Simple three state model with equilibria between closed but activatable (C), open (O) and closed and inactive (I) states. (ii). Interactions of the three state model with a drug (D). (iii). More general model with many states.
Although in principle any interconversion between many states is possible, those shown tend to be the only ones measured.

Conceptually, ion channels can be viewed as existing in three states, open (O), closed (C) and inactive (I) with various rates of interconversion between these states (Fig. 2). The rate constants for these processes can be measured and the changes induced by interaction with drugs (D) also measured. Depending upon the particular channel and situation, it is then possible to generate models which indicate several open, closed and inactive states any of which may interact with drugs (Fig. 2 scheme (ii)).

A drug may interact to stabilise the closed or inactive channel and is termed a **blocker** (or **antagonist** in the case of, for example, calcium channels). A channel may have to open before it can be blocked or a drug may stabilise the open form to be a channel **opener** or **activator** or the rate of inactivation may be slowed down as has been elegantly shown by Aldrich.[2]

Following on from such receptor recognition events, there may be further transmission of the stimulus following recognition, such as effects on second transmitter substances, nerve firing or muscle tone and thence to effects on behaviour, blood pressure etc. As with any process of amplification, it is to be expected that the closer one is to the initial recognition event, the easier it is to relate subsequent measurements to the stereochemistry of the interaction.

3. REGULATORY CONSIDERATIONS

Without doubt, one of the major driving forces at present for the increasing awareness of the consequences of stereochemistry and biological activity is the increasing interest the drug regulatory authorities are taking in the subject. While there are no hard and fast rules as yet,[3] it is debateable that any new drug type should be marketed as a racemate and to do so, except under certain circumstances, could be seen as a competitive disadvantage. A complicating factor is that different approaches are taken in various countries but in general there is a growing consensus against the marketing of racemates. Indeed, in FDA guidelines[4,5,6,7] it is stated that, even in racemates, enantiomers may be considered as impurities and that safety and efficacy data need to be produced for each stereoisomer including physical/chemical information. This means that an "inactive" stereoisomer may be treated as a 50% impurity unless a huge amount of supporting work is available. It can be seen as a pragmatic matter, therefore, to develop a single enantiomer.

There are, however, perfectly reasonable situations where it would be possible or even desirable to develop a racemate. These may include a combination of technological, economic and even social factors such as in the development of a new AIDS drug[8]. There may be pharmacokinetic reasons where both enantiomers are active and the racemate has a more desirable profile than a single enantiomer.

A more common case would be where the molecule does not have sufficient configurational stability so rendering the element of chirality unstable. One such case is when a facile epimerisation can be demonstrated to occur in aqueous solution. However this reaction should be sufficiently fast compared with the biological half life. It is desirable to establish the rates and mechanisms of such inversions. One case in point is diethylpropion[9] where the inversion is slow compared to the half life. The mechanisms of inversion processes can be somewhat unexpected, for example, in ibutilide[10] where a carbenium ion intermediate and intramolecular nucleophilic attack at the chiral carbon has been postulated to account for 20% of the racemisation during storage in aqueous medium.

Apart from inversion at a centre of chirality, interconversion between enantiomers is also possible when axes or planes are the sources of chirality. Sometimes these can lead to surprises and unexpected configurational stability can result, as in the case of the muscarinic receptor antagonist telenzepine[11] where the free energy of activation for racemisation is 35 kcal/mol. This provides almost complete enantiomeric stability at physiological temperature and pH. Although not a racemisation, the aldose reductase inhibitor

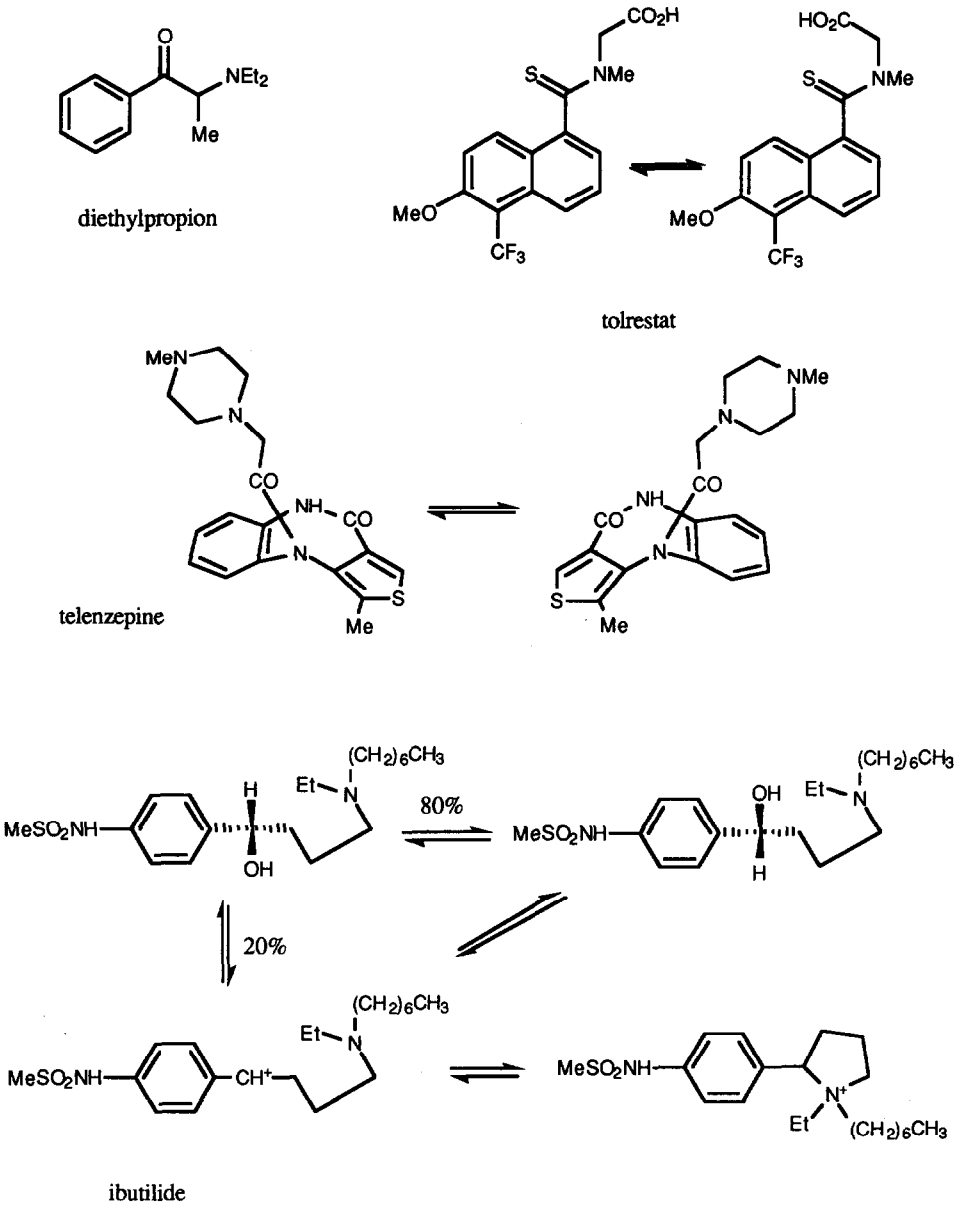
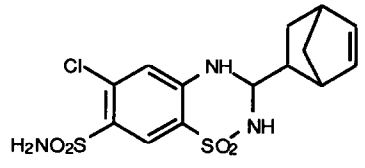


Fig. 3 Drugs which display processes leading to interconversion of conformational isomers and enantiomers.

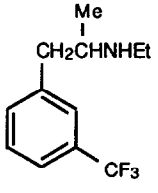
tolrestat, marketed as the thermodynamically more stable "trans" rotamer, in solution exists in equilibrium with the "cis" rotamer. This conformational isomerism has been extensively studied.[12] Coupled with speculation about the bioactive conformation,[13] this information has been used with imagination in the design of conformationally rigid analogues.[14]

As seen with ibutilide, chirality can have a significant effect on the dosage form used. So, in addition to data on the pharmacology of enantiomers, data have also to be presented for evaluation on physicochemical differences of a racemate and single enantiomer in terms of solubility, stability etc. This may also be crucial in drug development because a different approach to the regulatory authorities may be needed depending on the dosage form to be used.

Finally, in terms of regulation, comes the question as to what to do about drugs marketed at present. Many older drugs were introduced without adequate information on their stereochemical identity or composition. The antihypertensive agent cyclothiazide, for example, is marketed as a mixture of four racemates and small but significant differences in batches of the marketed drug have been observed.[15] Whether this is of therapeutic consequence is unclear, and may never be resolved, but it serves to highlight the lack of concern in the past on this issue. A more positive consequence of the re-examination of older drugs may be the marketing of a single enantiomer, where a racemate had been marketed previously, thus avoiding an undesirable side-effect profile and giving an old drug new life. This has happened in the case of *rac*-fenfluramine, originally produced as an amphetamine analogue without stimulant effects, for use as an anorectic agent. Side-effects are due to effects of the L-enantiomer on dopaminergic transmission whereas the D-enantiomer, dexfenfluramine, is anorectic because it is claimed that it increases presynaptic output and decreases postsynaptic uptake of serotonin.[16] This has recently led to the regulatory approval and marketing[17] of dexfenfluramine as "a turning point for your overweight patients".



cyclothiazide



fenfluramine

Fig.4 Older drugs recently re-examined from the point of view of stereoisomerism

4.THEORETICAL CONSIDERATIONS

There have been many theories about the origin of biomolecular handedness, the most intriguing being the neutral parity-violating electroweak interaction.[18,19,20] Once formed, biological systems developed in a way which amplified the importance of chirality as they became more and more complex. This led to the production of large molecules which produce a highly asymmetric environment. It is

almost axiomatic that, in order to interact most efficiently with this environment, drugs should also have a high degree of asymmetry. Why this observation has been largely ignored until recent years is a cause for speculation but the majority of chiral synthetic drugs currently marketed are as racemates, although this trend is changing.[21,22]

4.1 Pfeiffer's rule and eudismic analysis

Theoretical treatment of the interactions of enantiomers with biological systems dates back to Carl Pfeiffer who, in 1956,[23] observed that highly potent chiral compounds have a large difference in potency between enantiomers whereas weakly active compounds have little difference between enantiomers. This observation can best be rationalised by consideration of how molecules may recognise each other. For any ligand the possible non-covalent interactions can be separated into such terms as van der Waals, hydrogen bonding, charge transfer, electrostatic interactions and all these combine to produce a multi-dimensional property cloud. Any of these forces may predominate in a particular receptor interaction but at high affinity each should approach maximal. This is only possible when the molecular envelopes around ligand and receptor are highly complementary.[24,25,26]

There will be different affinities for each enantiomer in its interaction with a particular receptor. The enantiomer with the highest affinity is termed the **eutomer** and the one with the lowest affinity the **distomer**. The **eutomer** : **distomer** ratio is called the **eudismic ratio** and the logarithm of the eudismic ratio is the **eudismic index.** Often there is significant correlation when the eudismic index is plotted against the affinity of the eutomer producing straight lines. The slope of these lines, which are quantitative measures of the stereoselectivity of the system, is called the **eudismic-affinity quotient.**[24,27,28]

Eudismic analysis has led to quantification of the data from many series of compounds[24] and provides a validation of Pfeiffer's rule. At first glance it may seem remarkable, in view of the high 3-dimensionality of molecular interaction, that there could be much, if any, interaction between the distomer and receptor. Indeed, for many years the distomer was treated as inactive ballast: it was often assumed that the racemate should, in the worst case, have half the activity of the eutomer. That this is not the case was pointed out forcibly in a seminal paper by Ariens[22] where neglect of stereochemistry was described as a basis for sophisticated nonsense in pharmacokinetics and clinical pharmacology. Even though only one enantiomer may be therapeutically active it does not mean that the other is really inactive. It could contribute to side-effects but it also has all the functionality needed to interact with the receptor and so could antagonise the eutomer or even produce the opposite effect at the receptor.

4.2 Interactions of a chiral drug with a generalised receptor

In order to understand fully all the possibilities of enantiomer interaction, it is necessary to consider the effect of a chiral molecule with a generalised chiral receptor (Fig. 5). In this case, such a drug is assumed to have sites a b c d capable of interaction with specific complementary sites on a receptor A B C D. Thus Aa will be a significant interaction whereas Ab Ac and Ad are of minimal importance and possibly repulsive. If the receptor is considered as a fixed conformation then maximal interaction can be obtained from one combination of interactions: Aa+Bb+Cc+Dd. Considering all possible interactions (Fig. 5, scheme (i)), by keeping each group a,b,c and d fixed in turn, one finds that the R-enantiomer will have one four-way and eight one-way significant interactions. Similarly, the S-enantiomer will have six two-way significant interactions. There could, therefore, be some affinity of the S-enantiomer for the receptor and the magnitude of this will depend on the relative importance of the individual interactions and

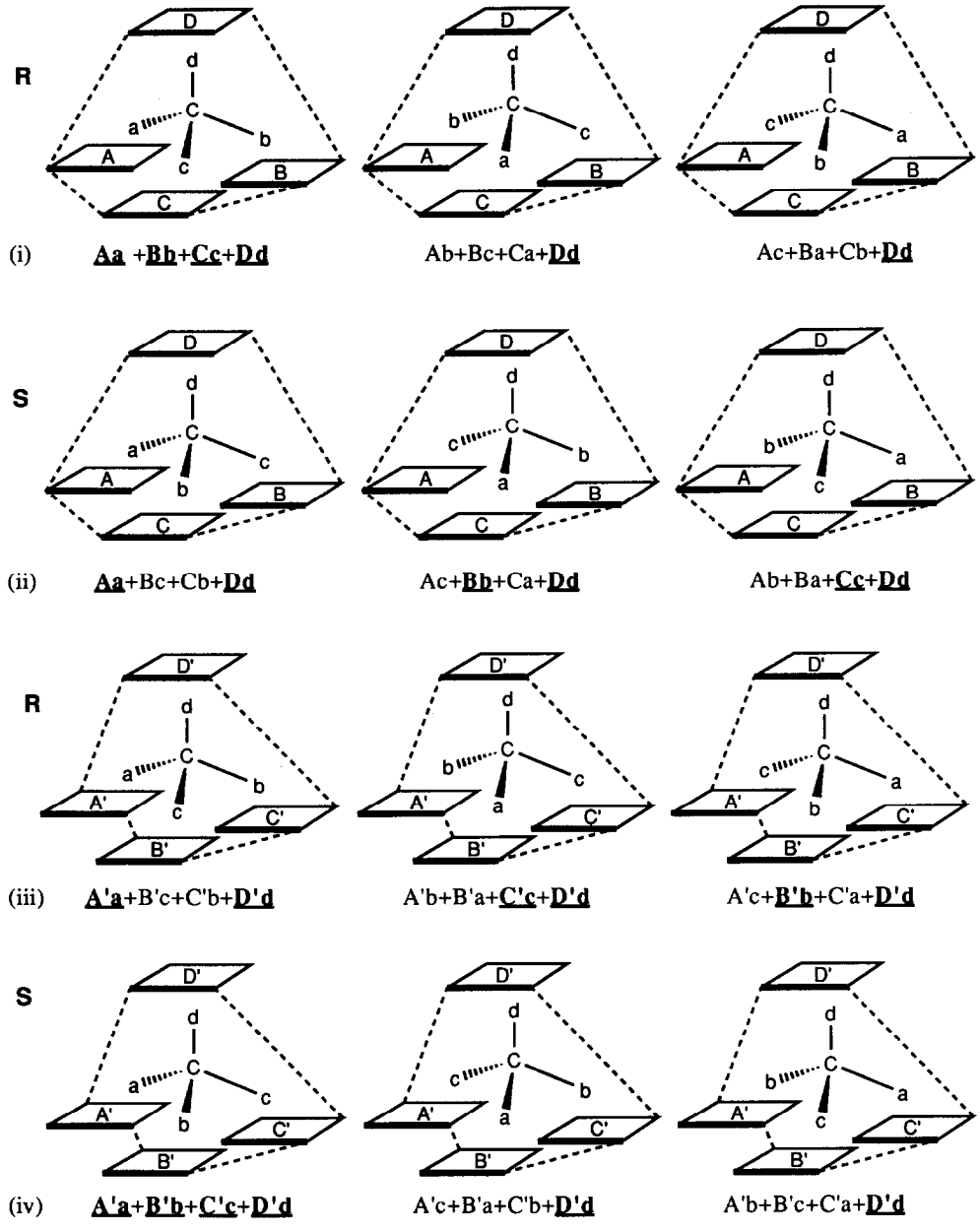


Fig.5 Chiral interactions with a receptor. Major interactions are indicated **Aa**. (i). With d fixed, the R-enantiomer can produce one 4-way and two 1-way major interactions and, allowing each group to be fixed in turn, there are a total of eight 1-way interactions (ii) the S-enantiomer produces three 2-way major interactions with d fixed.

Following a conformational change in the receptor (iii) the R-enantiomer now produces three 2-way major interactions and (iv) the S-isomer has one 4-way and two 1-way major interactions, again with d fixed. Modified from Lehmann et al.[28]

also on the co-operativity between them. Where such co-operativity is high in the eutomer, and there is a high degree of molecular fit at the receptor, then the S-enantiomer will be essentially inactive at this particular receptor. Whether the S-enantiomer produces a response depends on the efficacy at the receptor produced by the two-way significant interactions. In other words, for the S-enantiomer to produce the conformational changes in the receptor needed to activate ion channels or second messenger systems it may only be necessary to have one or two of these significant interactions. The S-enantiomer will then possess significant activity but, conversely, the activity of the R-enantiomer will not be maximal.

Even where the activity of the R-enantiomer is maximal, the S-enantiomer still has the possibility of a significant number of interactions with the receptor. Its affinity for the receptor may not therefore be zero and it will be able to antagonise the effect of the R form, at least to some extent.

This treatment so far assumes that there is no conformational change in the receptor but this is not necessarily the case. For all receptors conformational changes can produce more than one state in which a drug may interact, including inactive states. For G-protein coupled receptors an agonist (A) may interact with the receptor (R) in either the active form, when it is coupled to the G-protein (RG), or in an inactive state (R) in the absence of such coupling. Since the receptor site in R or RG is related by conformational change then an enantiomer of an agonist has two possible conformations with which it may interact, with possibly different consequences. The ion channel receptor family is known to have inactive conformations (I) distinct from closed (C) or open (O) forms and may, indeed, have more than one closed or open form leading to complex interconversion pathways (Fig. 2) related by conformational change. Such changes can be prompted by tissue changes or changes in membrane potential. Channel blockers or activators may have their effects by acting at one or more of these states . One may expect enantiomers to have opposite effects in these systems if a stereoisomer interacts with a closed channel, preventing its opening, or an open channel, preventing its inactivation.

In terms of our previous model of interaction, a conformational change may reorganise the sites on the receptor (Fig 5, schemes (iii), (iv)). In which case, the S-enantiomer may become more able than the R-enantiomer to bind to the conformationally modified receptor. Thus the S-enantiomer could become the eutomer in the promotion of an effect which may be the same or different to that provoked by the R-enantiomer.

5. THE EFFECTS OF STEREOCHEMISTRY ON BIOLOGICAL RESPONSE

5.1. Background to receptors

There are several types of receptor system with which a molecule may interact. These include metabolic enzyme systems, which are considered later, neurotransmitter receptors, which are linked to second messenger systems or ion channels by G-proteins,[29] and other ion channels which may be gated by ligands, voltage[30] and/or inorganic ions.[1] These systems typically occur in structurally related families which can be extensive. It can be predicted that there would be different selectivities of enantiomers for different members of the same receptor family.

One major problem in understanding stereochemical interactions with receptors is that the classification of receptors is usually pharmacological. This is based on response to standard agents, and is not based on structure. There are also tissue and species differences between receptors classified as being the same. This makes interpretation of the effects of stereoisomers on receptor sub-types difficult. Great advances in molecular biology have led towards classification based on sequence information[30] but the same protein expressed in different environments may still not have the same shape. When discussing receptors it is therefore important to consider tissue and species.

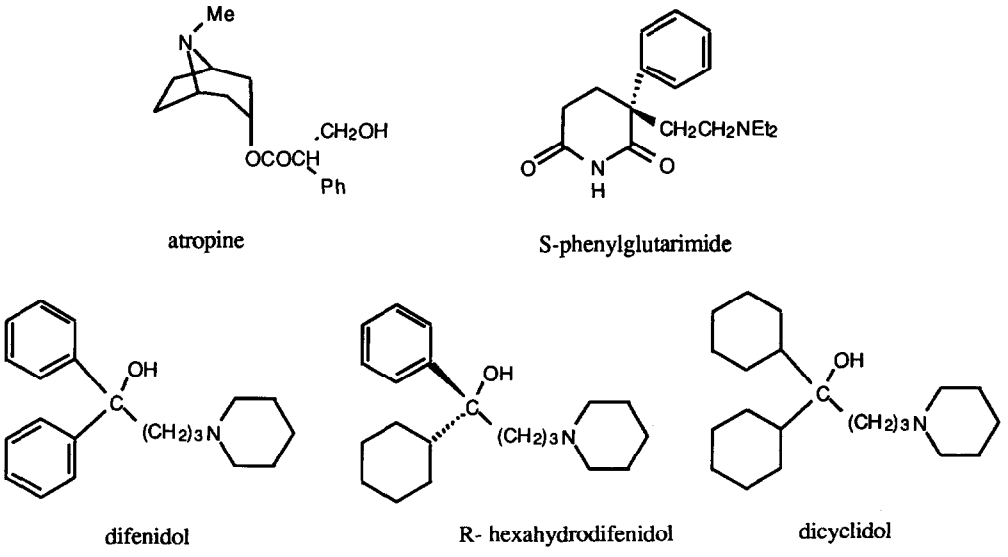
The same neurotransmitter may bind to receptors from different families. For example acetyl choline binds to muscarinic (G-protein linked) and nicotinic (ligand gated ion channel) receptors, so here one would expect vast differences in the molecular recognition processes.

However, some receptors, such as pre- and post-synaptic muscarinic receptors are quite similar and one may see selection between such receptors by enantiomers of a particular ligand. All G-protein related receptors, regardless of primary neurotransmitter, have a high degree of homology but are not quite the same and so it is possible that a compound may have an affinity for one receptor and its enantiomer for a different receptor. This could contribute to side-effects in the racemate.

5.2. Neurotransmitter receptors

There have been a number of reviews on the effects[31,32,33] of stereochemistry on neurotransmitter receptors and even a plea that a database be set up to catalogue such observations.[27] It is only intended here, therefore, to consider some recent examples which illustrate the basic principles of stereoisomer recognition. These are exemplified by three receptors, for acetylcholine, serotonin and dopamine, but the principles can be extended to other receptors. An extensive review has recently been published on the effects of chirality at the adrenergic receptor.[34]

5.2.1 Muscarinic acetylcholine receptors. Differentiation between muscarinic acetylcholine receptor sub-types has been demonstrated by stereoisomers of atropine-like compounds.[35] In the case of atropine itself, all the pre-synaptic muscarinic autoreceptor activity is due to the R-(+) enantiomer but the post-synaptic activity is mainly due to the S-(-) enantiomer with an eudismic ratio of 330. A nicely conceived study of difenidol, dicyclidol and hybrids[36] was able to separate the effects of the R- and S-hexahydro-isomers of difenidol and relate them to binding free energy decreases associated with substituent exchange. This was mirrored by partial selection between M_1 (neuroblastoma), M_2 (cardiac),



atropine S-phenylglutarimide

difenidol R- hexahydrodifenidol dicyclidol

Fig. 6. Agents active at the muscarinic acetylcholine receptor

M_3 (pancreatic) and M_4(striatum) muscarinic receptors. This suggests the possibility of tailoring the activity of drugs for different tissues, central and peripheral, by use of different stereoisomers.

Recognition is, of course, a two-way process and stereoselectivity works both ways. S-hexahydrodifenidol is non-selective between muscarinic receptor sub-types whereas the R-enantiomer selects for the M_1 receptor.[36] It is, however, just as correct to say[37] that the stereochemical demands made by M_1 (rabbit vas deferens) receptors between, for example, phenglutarimide enantiomers, are more stringent than $M_{2\alpha}$ (guinea pig atria) and $M_{2\beta}$ (guinea pig ileum) receptors. In this case S-(+)-phenglutarimide was found to be a potent M_1-"selective" antagonist (pA_2=8.5) whereas the much less potent R-enantiomer (pA_2=4.75) showed no discrimination. This observation demonstrates the principles underlying Pfeiffer's rule.

5.2.2. Serotonin receptors A receptor family of great current interest, the serotonin (5-hydroxytryptamine) receptor, has been divided, pharmacologically, into at least seven sub-types.[1,38] Many belong to the G-protein coupled superfamily. Although there is little evidence as yet for stereoisomer selection between these sub-types, there is a considerable effect of enantiomer stereochemistry. This is mainly observed in the antagonism of one enantiomer by another. Thus at the 5-HT_{1A} receptor, enantiomers of 11-hydroxy-10-methylaporphine have opposing pharmacological effects.[39,40] Both bind strongly to 5-HT_{1A} receptors, as evidenced by their ability to displace the prototype 5-HT_{1A} agonist [^{3}H]-8-hydroxy-2-di-n-propylaminotetralin (8-OH-DPAT) from rat forebrain membranes, with the R-enantiomer being an agonist. In contrast, the S-enantiomer has no agonist effect but blocks the actions of the R-enantiomer and 8-OH-DPAT.

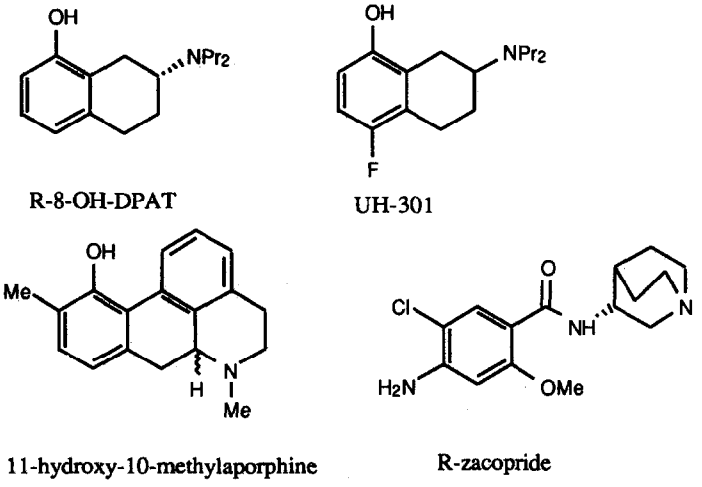


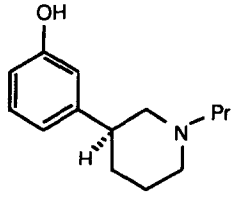
Fig. 7 Agents acting at the serotonin receptor

Such effects illustrate the danger inherent in the study of racemates, a point which is well illustrated by *rac*-UH-301.[41,42] This compound, a 5-fluoro analogue of 8-OH-DPAT, as a racemate appeared to

have no serotonergic activity whereas *rac*-8-OH-DPAT behaves as a potent 5-HT_{1A} receptor agonist *in vivo*. It was noticed that *rac*-UH-301 did displace [^{3}H]-8-OH-DPAT *in vitro* and so the enantiomers were separated. (R)-UH-301 proved to be an agonist at the 5-HT_{1A} receptor and was slightly less potent than 8-OH-DPAT. The S-enantiomer, however, binds to this receptor but lacks efficacy and so antagonises the effect of the R-enantiomer. Thus the racemate produces overall inactivity. Furthermore, the R-enantiomer antagonises 8-OH-DPAT at the 5-HT_{1A} receptor leading to the possibility that stereochemistry can be used to design partial 5-HT_{1A}-receptor agonists as anxiolytic agents.[41] Finally, it is noteworthy that 8-OH-DPAT, itself displays enantioselectivity in its intrinsic activity but not in its affinity.[43] This has led to a detailed study[43,44] of analogues where consideration of the activity of a series of enantiomers has provided a computer graphics derived model for 5-HT_{1A} agonism. This work illustrates well how an understanding of the interactions of stereoisomers can be used prospectively as a tool in drug design.

There is also evidence of enantiomeric differentiation at the 5-HT_3 receptor, a ligand gated ion channel. *Rac*-zacopride, a 5-HT_3 antagonist, is a potent anti-emetic agent in dogs but produces emesis in ferrets on oral dosing which is blocked by intra peritoneal dosing of *rac*-zacopride. These differences are presumably due to pharmacokinetic differences and the different locations of 5-HT receptor sub-types. Hence both emetic and anti-emetic properties are suggested to be due to agonist and antagonist actions of the S-enantiomer on different 5-HT_3 sites.[45] This is in contrast to actions in the central nervous system where zacopride, as the racemate or the R(+) enantiomer, has an anxiolytic profile in rats, antagonised by the S(-) enantiomer which, indeed, may be anxiogenic when 5-HT function is reduced.[46] There is, therefore, some evidence of species and tissue differences in stereoselectivities at the 5-HT_3 receptor but they appear to be complex.

5.2.3. Dopamine receptors. Closely related to the 5-HT receptor is the dopamine receptor. Indeed, several compounds are active at both 5-HT_{1A} and dopamine D_2 receptors and the stereoselectivity observed in these drug interactions can be the same.[47] Differentiation between serotonin and dopamine receptors by enantiomers of fenfluramine has already been discussed. Dopamine D_2 receptor isoforms exist both pre- and post-synaptically and this possibly corresponds to differences in the third cytoplasmic loop, a region supposed to couple to G proteins[48,49]. The cyclised dopamine derivative R-3-PPP is an agonist at both pre- and post-synaptic D_2 receptors whereas the S-enantiomer shows a different pattern of activity, being an agonist at pre-synaptic receptors and an antagonist at post-synaptic receptors.[41,50]



(S)-3-PPP

Fig. 8 3-PPP, an example of discrimination between between pre- and post-synaptic dopamine receptors

Evidently there is enough difference between the receptors for similar recognition processes for enantiomers pre-synaptically but, post-synaptically, the S-enantiomer cannot interact in a way to cause the conformational change needed for G-protein activation and consequently lacks efficacy.

5.3. Ion channels

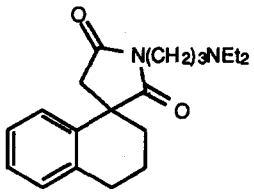
Ion channels are a class of drug receptors which are different from G-protein linked neurotransmitter receptors in that their opening and closing is effected directly by interaction with a ligand and/or the potential difference across the cell membrane.[51] Depending on the ions transported by the channels, they may be opened or closed by the rise in membrane potential during the depolarisation caused during neuronal firing or axonal conductance. A considerable amount of information is now available about the relationships between the families of these receptors.[30] Ion channels are especially relevant in generating an understanding of the effects of chirality on recognition[52] because they represent the class of biological receptors where the effects of recognition can be measured most directly. Moreover their conformation, and ability to change conformations, can be changed by changing their immediate environment. For example, as the change in membrane potential changes the conformation of ion channels so the apparent affinity of a drug will change. Conversely the binding of a drug will change the voltage activation curves for the ion channels.

5.3.1 Sodium channels. An example of the differentiation between enantiomers at a sodium channel is provided by the local anaesthetic drug RAC-109.[53] There is no discrimination between enantiomers at the resting potential of the cell membrane but the potency of the S-enantiomer increases when the membrane is repeatedly depolarised. Evidently the conformational changes which occur in the receptor, by changing the voltage difference along the length of the channel, favour interaction with the S-enantiomer. Such observations hold great promise for elucidating the factors responsible for molecular recognition once more is known of the actual structures involved.

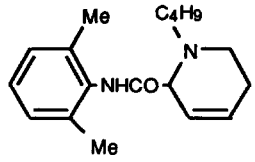
Some interactions can be very subtle indeed. When sodium channels are removed from intact tissues, where (+)-R-bupivacaine is more potent than the S-enantiomer, then reconstituted into an artificial membrane,then the S-enantiomer becomes approximately six times more potent than the R-enantiomer.[54] A similar inversion of potency is also observed with RAC-109 and cocaine. The enantiomers of these are essentially equipotent in intact tissues but excision produces a high degree of selectivity for the (-)-enantiomer. This work demonstrates not only how sensitive the conformation of receptors is to the environment around them but also how much care needs to be taken in the interpretation of such results.

Enantiomers can, of course, interact at different sites and ion channels are a convenient way to study this. Thus (S)-DPI 201,106 is a Na^+ channel activator which increases channel open time by slowing the rate of channel inactivation. In contrast, the (R)-enantiomer is a channel blocker.[55,56] Both agents are of similar potency and kinetic experiments suggest that they probably interact at different sites.

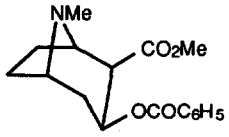
5.3.2 Calcium channels. There are well documented examples of enantiomers having opposite actions in the field of the calcium channel antagonists, 1,4-dihydropyridines, where the S-enantiomers of Bay K 8644, PN-202,791 and H-160/51 are activators and the R-enantiomers are antagonists of L-type Ca^{2+} channels. With 1,4-dihydropyridines, the antagonists are markedly voltage dependent[57,58] but the activators show little dependence. Although it is possible that the enantiomers interact with different sites, it is more plausible that the different stereospecificities observed reflect opposite stereoselective requirements of the open and inactivated channel conformations. This is reinforced by the voltage dependence of the process. Indeed, the activator properties of (S)-Bay K8044 and(S)-PN-202,791 are



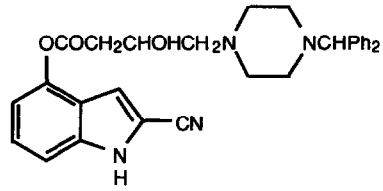
RAC-109



bupivacaine

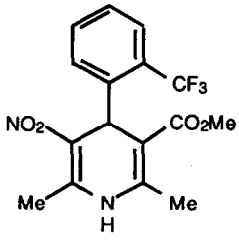


cocaine

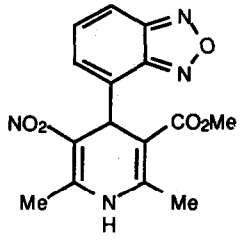


DPI-201,106

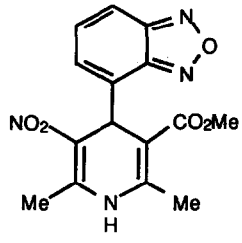
Fig. 9 Agents active at sodium channels



Bay K 8644



PN-202,791



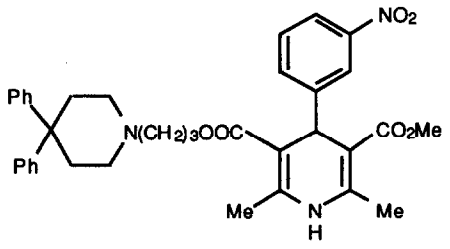
H-160/51

Fig. 10 Agents active at calcium channels

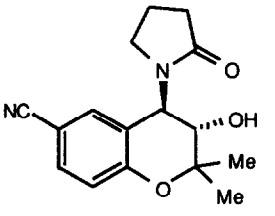
modified to antagonist by changing the membrane potential. An understanding of the nature of the conformational change of the receptor brought about by the change in potential difference across the membrane would greatly increase our knowledge of molecular recognition processes.

5.3.3 Potassium channels. There is significant homology between Ca^{2+}, Na^{+} and K^{+} channels. This means that the stereoselectivity of interaction can carry across channel types. The dihydropyridine Ca antagonists, (+)- and (-)-niguldipine are K^{+} channel activator and blocker respectively.[59]

Some ion channels are activated by endogenous ligands. The cardiac potassium channel K_{ATP} is one of these and is opened by a new class of drugs of which cromakalim is the prototype example. Originally tested as a racemate, it was subsequently found that the (-)-enantiomer lemakalim was some 20-fold more active because the distomer antagonises the effect of the eutomer but is not itself a channel blocker. The stereospecificity of the benzopyran K^{+}-channel activators is exhibited by the other compounds in the class.[60]



niguldipine



lemakalim

Fig. 11 Agents active at potassium channels

5.3.4. Chloride channels. Closely related structurally to the cation channels, perhaps surprisingly, are the anion channels which transport chloride ions across cell membranes. In contrast to the cation channels,and receptor gated chloride channels such as the the $GABA_A$ receptor, little is known about agents which interact with voltage activated chloride channels and most of the known agents are non-steroidal anti-inflammatory acids. In rat skeletal muscles the S-(-)-ienantiomers of analogues of one of these, clofibric acid, produce a dose related decrease of chloride conductance.[61] In comparison, the R-(+)-enantiomers increase conductance at low concentrations and decrease it at high. The exact reason for this is unknown but may reflect interaction with low and high affinity binding sites with the low affinity site being the same as that with which the S-enantiomer interacts.

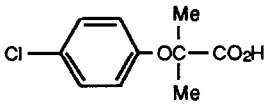


Fig. 12 Clofibric acid, analogues show stereoselectivity at chloride channels

6. METABOLISM AND DISTRIBUTION

A specialised type of receptor recognition where the drug is chemically modified by the receptor, leads one to predict that the presence of one enantiomer may affect the metabolism of the other leading to changes in pharmacokinetics. Where one enantiomer is an enzyme inhibitor this may possibly enhance the activity of the racemate over that of a single enantiomer. However, the large variability of metabolism between normal individuals and between normal and disease states may lead to an unpredictability of therapeutic effects.

Metabolic processes usually involve interaction with an enzyme system leading to functionalisation.[62] These include oxidative transformations by cytochrome P-450 isozymes, followed by chemical degradation or subsequent conjugation prior to elimination. The same recognition processes as for other types of receptor system also apply. Thus, for example, one stereoisomer may inhibit the metabolism of the other. The situation is also complicated by the differences in individuals in their ability to metabolise certain drugs which has led on occasion to the classifications of extensive metabolisers and poor metabolisers.63 The blood levels of an active enantiomer could, therefore, be vastly different if administered as the single enantiomer or racemate when the inactive enantiomer also interacts with a metabolic process. The extent of this will also depend significantly on how extensive a metaboliser an individual happens to be. In addition, metabolic processes may produce active or inactive metabolites by the use of several different enzyme systems. If the production of one of these is inhibited then others will take over and so the whole pattern of metabolites and rates of accumulation of these will vary. Where the profile of the activity of a drug is due to the presence of active metabolites then a different pharmacological outcome can result depending on whether a single enantiomer or racemate is administered. Metabolic processes often generate a chiral centre, by hydroxylation for example, producing diastereomeric mixtures from molecules with existing chiral centres administered as racemates. These are then expected to have different bulk chemical properties such as solubility, partition coefficient and pKa. It is such properties which govern transport across membranes and distribution into various body compartments. There are also, in some cases, differences between enantiomers in the transport across membranes and in generalised plasma-protein binding.

Many psychoactive drugs have at least one chiral centre. The implications of chirality and geometric isomerism on the activity and metabolism of several of these has been recently reviewed.[31] Behavioral effects of such compounds can critically depend on their distribution and rates of metabolism. Amphetamine, for example, has most of its stimulant and anorectic properties in the S-(+)-enantiomer. Some properties such as tolerance to anorexia and hypersensitivity to psychoses are found on repeated dosing and are presumed to be due to metabolism[64] to α-methyl-p-tyramine. Studies with *rac*-amphetamine have shown[65] that the rates of metabolism to this compound differs and that each enantiomer affects the metabolism of the other. Such inhibition of metabolism may lead to an increased effectiveness of a drug but the interactions can be quite complex. The anticoagulant warfarin has, as the eutomer, the S-enantiomer although it is marketed as the racemate. In this case metabolism primarily occurs by hydroxylation at the 6,7,8 and 4' positions with 7-hydroxylation predominating in man (60-70%). The R-enantiomer is a competitive inhibitor of the 6- and 7- hydroxylation but, the S-enantiomer is only a weak inhibitor of R-enantiomer for this process. This significantly contributes to the biological effect of the racemate[66] Although this might be thought to be beneficial, it may also be responsible for a number of other metabolic drug interactions that have been observed in the clinical use of *rac*-warfarin.

Sometimes, metabolic conversion of one enantiomer into another can be useful in enhancing the activity of a drug without any other liability. Perhaps the most intriguing example is the bioinversion of the R-enantiomer of ibuprofen and other similar anti-inflammatory agents into the S-form. Early reports[67]

indicated that the urinary metabolites of *rac*-ibuprofen were dextrorotatory and the process of inversion has subsequently been extensively studied[68] and shown to be enzyme catalysed[69,70] either systemically or in the gut. This inversion is thought to involve a stereospecific formation of a thioester of the R-enantiomer with co-enzyme-A followed by racemisation, and hydrolysis.[71,72] This sequence has been the basis of theoretical calculations which suggest that the bioavailability of the S-enantiomer is similar from a 150mg dose of S-ibuprofen or 200mg of the racemate.[73]

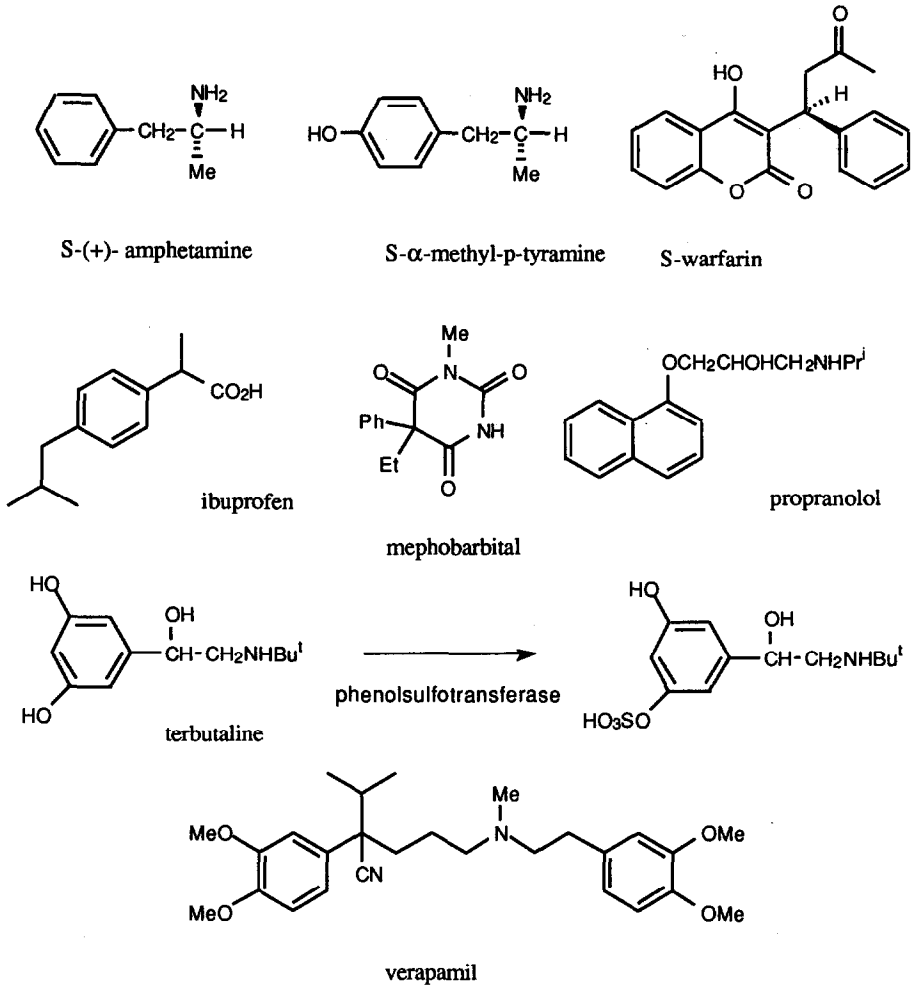


Fig. 13 Drugs which interact stereospecifically with metabolic and distributive processes

Conjugation, prior to excretion, may or may not follow initial metabolism of a drug. As would be expected, this has also been shown to be a stereospecific process in the conjugation with glucuronic acid and glutathione[26,62] Sulphation of phenolic drugs is a major route of metabolism and shows both regio- and stereo-specificity. Terbutaline, the sympathomimetic amine,[74] has recently shown stereospecificity in conjugation by phenolsulphotransferase, through the co-substrate 3'-phosphoadenosine-5'-phosphosulphate: the dextrorotatory enantiomer was metabolised some seven times faster than the laevorotatory enantiomer. This ratio was similar to that observed when the racemate was administered.

Measurement of stereospecific tissue distribution can be somewhat more troublesome. *Rac*-propranolol, for example, displays slight ((+):(-) = 1.2) stereospecificity for uptake into the central nervous system or in plasma. However, there is a higher level of (+)-propranolol in the liver which is presumed[75] to be a result of stereoselective metabolism of the (-) enantiomer in this tissue. In addition, the extent of binding also depends on the proteins under study. Thus mephobarbital shows only slight discrimination between enantiomers in *in vitro* plasma protein binding whereas there is a greater preference for binding the R-enantiomer to human serum albumin.[76] This is supposed to be the major source of enantioselectivity in plasma. It has been suggested[33] that acidic drugs commonly bind to human serum albumin in an enantioselective manner and that the binding of basic drugs, to α_1-acid glycoprotein, is relatively non-selective. This, however, is probably too great a generalisation and some basic drugs do show significant selectivities.[33]

Stereoselectivity in first pass metabolism also complicates the comparison of oral and intravenous drug delivery and makes monitoring difficult. The actual effectiveness of *rac*-verapamil, for example, is the result of a multiplicity of effects all of which combine to produce a total therapeutic effectiveness[52] The S-enantiomer generally seems to be around three to ten times more effective than the R-enantiomer depending on tissue type. However, the plasma clearance of the S-enantiomer is about twice that of the R-enantiomer making its bioavailability lower. Also, since there is significant first-pass metabolism of the S-enantiomer, the R:S ratio is greater at about 4 to 5 than after a single intravenous administration where it is 2.

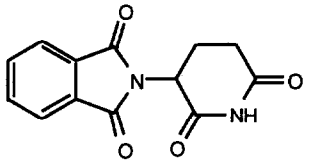
Combining all these metabolic considerations together with the activity at receptors produces the overall effectiveness of a drug in whole animals and patients.

7. TOXICITY

The factors which control the overall manifestation of toxic effects are the same as those which contribute to the total therapeutic effectiveness but it is in the area of lack of side-effects and low toxicity that the success of many drugs is determined. Just as there is stereoselectivity in the action at the primary target receptor there will also be undesired selectivity by the distomer at other receptors. This may be responsible for side effects as in the case of *rac*-fenfluramine[16]. In addition, where there is interaction with metabolic enzymes these can lead to the interactions with other drugs noted above for *rac*-warfarin which may severely limit the use of a drug. Also, the presence of an "inactive" enantiomer will lead to an increased load on the liver and other metabolic and excretory systems. Such metabolic products may in themselves be a cause for concern as with the anti-inflammatory 2-arylpropionates (profens) where the distomers of some of these form hybrid triglycerides[77] which are long lasting and potentially of concern.[3]

Perhaps the most well-known case and one used extensively in arguments against the marketing of racemates is that of thalidomide. The teratogenic differences observed between the enantiomers led initially to speculation[32] that early work,[78] which showed no difference in teratogenicity between single enantiomers and racemates, was in error. Subsequent work indicated that the S-enantiomer was the culprit

and led to assertions[79] that the teratogenicity could have been avoided if the R-enantiomer had been used. Subsequently, detailed reviews[6,80] maintain that definitive work still needs to be performed and it is probable that both enantiomers are indeed teratogenic. However even one of the reviews[6] has incorrectly been taken[3] to ascribe the teratogenic effects to the S-enantiomer thus reinforcing a probable myth. The case against the alleged association of teratogenicity only with the S(-)-enantiomer has been succinctly presented recently.[81]



thalidomide

Fig. 14 Thalidomide which is one example where toxicological problems would not have been overcome by use of a single enantiomer

This case illustrates the difficulty involved in ascribing toxic events to a single enantiomer where the mechanism of toxicity is not clear . The large practical difficulties involved in such toxicological studies provide a very compelling case for the marketing of single enantiomers rather than the marketing of racemates.

8. SUMMARY

The factors involved in molecular recognition are extremely complex but the use of stereoisomers and especially the use of enantiomers can help to shed light upon them. Biological assay methods are becoming more and more sophisticated and allow the study of such interactions at a molecular level. This has enabled more quantitative treatments to be made both of rates and free energies of interaction processes. A start can now be made at understanding the phenomenon of recognition in a quantitative manner.

Another consequence of this study is the observation of interactions between enantiomers for these recognition processes. The interactions include, amongst other things, the antagonism of one enantiomer by the other. Metabolic distinction between enantiomers is known and the "inactive" enantiomer could be responsible for side effects.

The consequence of the interactions between enantiomers can be most readily appreciated and measured when the response being measured is close to the initial receptor recognition event. Bioassay, though, may involve measurement a long way down a transduction pathway when the factors being measured become more and more distant. While it is possible, in animal behaviourial assays for example, to detect antagonism of one enantiomer by another, the production of side-effects and the consequences of altered metabolism and distribution make interpretation difficult as systems become more complex. At the extreme, the interpretations of toxic events and extrapolation between species become impossibly difficult. Nevertheless, all is not doom, as some valuable drugs owe their success to such interactions leading to an overall profile of activity.

There is still a long way to go before arriving at a full understanding of molecular recognition processes of relevance to biology. There are, nevertheless some generalisations[22,24,28] which are worth re-stating and amplifying:

(i) There is no such thing as an inactive enantiomer.

(ii) The greater the separation of activity between eutomer and distomer the more likely it is that the activity will be maximal. This can be seen to be a corollary to Pfeiffer's rule.

(iii) The highest affinity and selectivity will be obtained by molecules with the greatest degree of asymmetry or dissymmetry because interaction with the receptor will then be maximal. This follows because of the high degree of co-operativity of interactions with receptors.

(iv) Stereoselectivity between receptors may be restricted to only one enantiomer. In general the distomer will be less discriminating between receptor sub-types. Stereochemistry can be used to select between different members of the same receptor family.

(v) One enantiomer may produce the therapeutic action and the other enantiomer may be responsible for the side effects

(vi) The distomer will antagonise the binding of the eutomer except possibly at very high affinities where activity is maximal.

(vii) Stereoisomers can have opposite effects such as agonist/antagonist or channel opener/blocker activity at the receptor level which may be reflected in the final effects in the whole animal.

(viii) Conformational change in the receptor provoked by a different environment such as the localisation in a different tissue or by change in membrane potential can cause a switch in stereoselectivity between enantiomers. Conversely different enantiomers can select between the same receptor in different environments.

The consequences of these observations are profound and will, in time, have an impact on the way medicinal chemistry is practised. A far greater understanding of drugs and receptors will follow when it becomes routine to test single enantiomers and not racemates. This will ultimately produce the information needed to understand the key question of how molecules recognise each other. It does, however, take courage to separate and test enantiomers, when the racemate is inactive, but if the compound was worth making in the first place then that carries its own justification. It may still be argued that to consider stereoisomers at an early stage in drug research adds too much to the already difficult burden facing the medicinal chemist. It is argued that such considerations are correctly left to a later stage when candidate compounds have almost been decided upon but this can be far too late. Modern synthetic methods enable the design of stereospecific syntheses which are intrinsically better than racemic methods; they just take a little more thought. Common chiral intermediates can be involved at the design stage and separations using chiral hplc columns make the chemistry every bit as feasible. The results obtained from testing pure compounds are so much more valuable that chirality can and should be used aggressively as a tool in drug design.

Although this Report has been concerned with biological activity from the perspective of a medicinal chemist in the pharmaceutical industry, the same considerations apply in the agrochemical field. Here the "inactive" enantiomer has more freedom to interact with other ecological systems and could be described as an environmental pollutant.[82] In this area especially there is no such thing as an inactive enantiomer and the question needs to be asked if such a molecular contaminant is benign.

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