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REVIEW ARTICLE

Chirality: a blueprint for the future

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Chirality is a fundamental characteristic of nature and pervades the living world. We have been under its constant influence throughout evolution as a result of the asymmetrical nature of the environment and, while the origin of this phenomenon is a matter of speculation, the evidence surrounds us all. Most proteins, for example, are formed of L-amino acids while carbohydrates are composed of natural sugars, all D-isomers. Biological receptor systems comprise a complex structural organization of helices and sheets and display 'handedness'. This results in a profound effect on drug–receptor interactions.

The subject has fascinated scientists since the middle of the 19th century, when Louis Pasteur[65] first demonstrated the stereoisomeric forms of tartaric acid. Following earlier work by de la Provostaye, he undertook crystallographic studies on tartaric acid and its salts, and demonstrated the presence of hemihedral facets. In some instances, these were orientated to the left and in others to the right. By handpicking the crystals, he divided them into two groups and found that the solutions rotated light in equal but opposite directions. Pasteur recognized that the cause of this phenomenon lay in the molecular structure, and by extending these ideas he evolved the theory of the asymmetrical carbon atom.

Why is chirality relevant to anaesthesia? Advances in chiral technology are allowing the commercial synthesis of single-isomer compounds hitherto of academic interest only. By understanding the role of these stereoisomers in biological systems, the next logical step is the application of this knowledge to human physiology and pharmacology.

In addition, incentives have been provided by the drug regulatory authorities, who realize the potential benefits of single-isomer compounds in terms of simplification of the pharmacological profile and an elimination of so-called isomeric ballast.

International Union of Pure and Applied Chemistry (IUPAC) terminology

Isomers are unique molecular entities composed of the same molecular constituents with common structural characteristics. Stereoisomers are isomers whose atoms, or groups of atoms, differ with regard to spatial arrangement of the ligands, and they can be either geometrical or optical isomers. Geometrical isomers are stereoisomers without optically active centres and result from restricted rotation as a result of the presence of, for example, a carbon–carbon double bond (e.g. *cis*-2-butene, *trans*-2-butene). Geometrical isomers are not mirror images of one another. For these compounds, terminology such as *cis* (meaning 'together' or 'same side') and *trans* (meaning 'opposite side') are used to describe the spatial arrangement. Optical isomers are a subset of isomers that are optically active and are said to possess a 'chiral centre'. This term derives from the Greek *chiros*, meaning 'handed', and describes a molecule that is not superimposable on its mirror image. This is the necessary and sufficient definition for a molecule to be described as chiral. The majority of this review focuses on optical isomers, as the separation of geometric isomers is well established.

Optical isomerism can arise in a number of ways, but in its most familiar form a central carbon or sulphur atom is attached to four different groups, thus producing a tetrahedron. Van't Hoff first proposed this concept in 1874 and suggested that the four valencies of a carbon atom are directed towards the corners of a regular tetrahedron, thus enabling the concept of asymmetry to be realized (Fig. 1).

Optical isomerism enables the existence of two non-superimposable mirror images or enantiomers (Greek *enantios*=opposite, *meros*=part) that share identical physi-

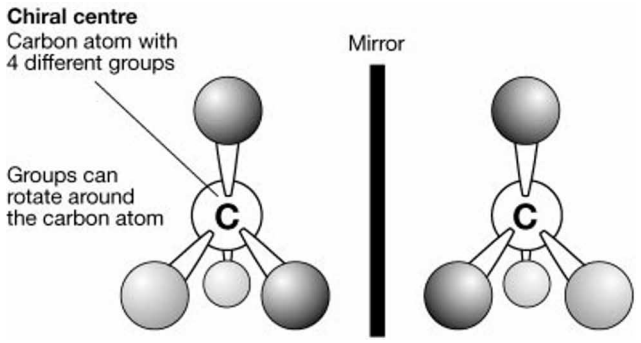

Fig 1 General depiction of a molecule with a chiral centre and its enantiomers.

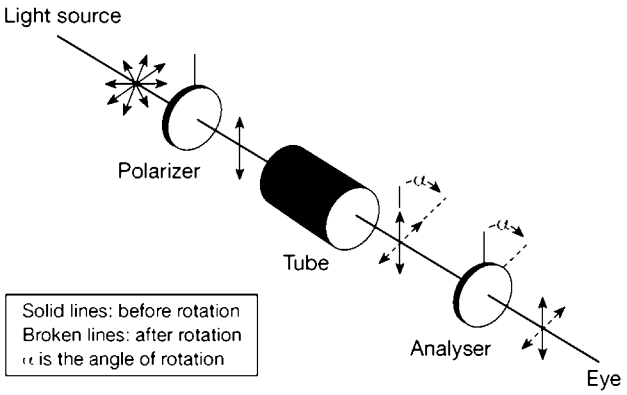

Fig 2 Measurement of optical activity using a polarimeter.

cochemical properties but differ in their rotation of plane-polarized light. Importantly, they can also differ in pharmacological profile, owing to highly stereospecific interactions at the receptor interface. A mixture of two enantiomers is called a 'racemate', a term popularized by Beilstein. A racemate is designated by the prefix (±) or rac-, or by the symbol RS or SR, and has no optical activity. The use of the term 'racemic mixture' is to be discouraged as it has been used as a synonym for both 'racemate' and 'racemic conglomerate'.

The nomenclature of chiral compounds has been clarified by the adoption of the International Union of Pure and Applied Chemistry (IUPAC) Recommendations 1996, which apply to basic terminology in stereochemistry.[42] Chiral compounds can be described by a combination of terms.

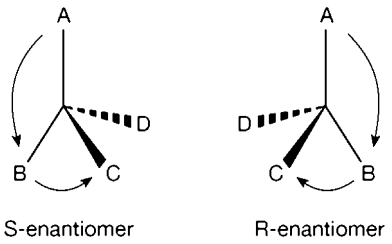

Fig 3 Sequence rule notation. Bonds represented as solid lines are in the plane of the paper, those drawn with dotted lines project away, and those represented by a wedge project towards the reader. Group priority (atomic size) is indicated as A>B>C>D, A representing the largest size. With the group of lowest priority projected away from the reader, the sequence is anticlockwise (S) in the enantiomer on the left and clockwise (R) in the enantiomer on the right.

Relative descriptors

The most common way to refer to the chirality of a molecule is still based on the effect it has on the rotation of optical light, with the descriptors (+) and (–) applied when the rotation is clockwise and anticlockwise respectively (Fig. 2). Isomers that rotate light clockwise and anticlockwise are termed 'dextrorotatory' and 'laevorotatory' respectively.

Optical rotation, although an unambiguous physical property, varies with measurement conditions and these are therefore standardized. The degree of rotation is measured at the sodium D line at 254 nm, as rotation varies with frequency. The actual property measured is an electronic transition between the orbitals in the molecule under study. The complete spectrum, the circular dichroism (CD) spectrum, differs from the ultraviolet spectrum in being both positive and negative. Rotation is determined by both electronic and magnetic moments. Achiral molecules, which have no magnetic moment, do not rotate plane-polarized light.

At very low rotation strengths, errors can occur as a result of the presence of contaminants. The type of solvent used and pH changes can also produce dramatic changes in the CD spectrum.

Absolute descriptors

The sequence rule notation, proposed by Cahn and colleagues, [12] is based on attaching an order of priority to substituent ligands attached to the central chiral atom. In this model, the ligands around the chiral centre are 'sized' according to their atomic number, placing the smallest to the back and looking at the remainder in terms of relative size (Fig. 3).

Consider a molecule Cabcd, where a, b, c and d are groups placed around a central atom C. If the sequence of the ligands in terms of size (largest to smallest) produces a clockwise progression, the arrangement is termed 'R' from the Latin *rectus* (right). Conversely, an anticlockwise order is termed 'S' from the Latin *sinister* (left). Any chiral molecule can be designated in this way. If two or more ligands are of equal size, the next atom along the chain is examined. Thus, a full description of a chiral compound may be given by an expression combining terms for the absolute descriptor, the relative descriptor and the chemical or generic name, e.g. S(–)bupivacaine.

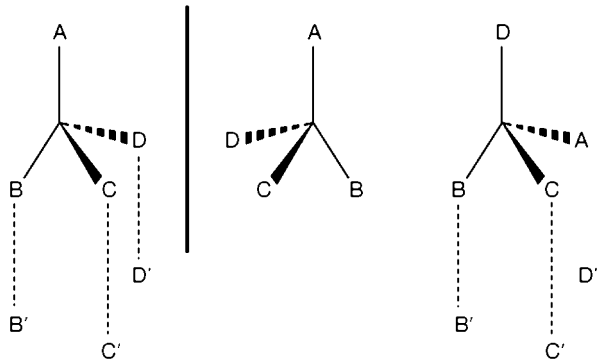

Fig 4 Easson–Stedman model. Enantiomeric interaction with a chiral biological macromolecule. The enantiomer on the left is involved in three simultaneous bonding interactions at the receptor, whereas that on the right interacts at two sites only. A, B, C and D represent ligands on the chiral compound and A′, B′, C′ and D′ are corresponding sites on the receptor.

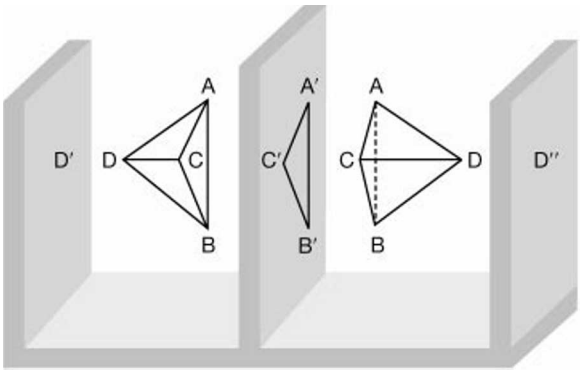

Fig 5 The four-location model. This is a four-point location model of the stereoselectivity of a protein. Groups A, B and C of different isomers occupy the same protein locations (A′, B′ and C′), whereas the D groups, which point in opposite directions, interact at different positions (D′ and D″).

Stereospecific biological interactions

Interaction of a drug with a biological system produces a cascade of events that ultimately leads to a point at which some physiological response can be measured. The initial interaction is highly stereospecific and a hypothesis to explain this was proposed by Easson and Stedman in 1933 (Fig. 4).[20] These authors adhered to the general principle that any three-dimensional molecule can be represented topologically by the simplest polyhedron, a tetrahedron. Attachment of the drug to the receptor was analogous to the 'attachment of a glove to the hand'. Maximal interaction in this model is derived from Aa+Bb+Cc+Dd. The interaction with the opposite enantiomer can be considered in a similar way, but here there are fewer congruencies at the binding site. The more each of the interactions tends to the maximum, the greater the separation in affinity between the enantiomers (Pfeiffer's rule).[67]

When comparing affinities, the enantiomer with the highest affinity is termed the 'eutomer' and that with the lowest affinity the 'distomer'. The pharmacological activity of the two enantiomers can therefore be compared by calculation of the 'eudismic ratio'. Eudismic analysis provides a powerful tool for drug design, by optimizing a series of enantiomer pairs by the comparison of the eutomers and distomers in a series of analogues. By providing a description of the increasing potency of the molecule as the affinity of the enantiomer with a receptor increases, a series of compounds can be analysed and information provided for molecular design. There can potentially be more than one eudismic ratio for a racemate if the compound has more than one pharmacological effect.

Recently, Mesecar and Koshland[61] have proposed a new 'four-location' model to account for stereospecificity (Fig. 5). The three-point attachment model described previously holds only if it assumed that the ligand can approach a flat protein surface from the top. If the binding sites on the protein molecule are in a cleft or on protruding residues, the three-point model will not be sufficient to allow discrimination. It is therefore suggested that a fourth point, whether a binding site or a location, is essential in order to distinguish between enantiomers in an actual protein structure.

To illustrate this, the enzyme isocitrate dehydrogenase (IDH) was used as an example. Its crystalline structure was examined using electron density mapping in the presence and absence of Mg^{2+}. When metal-free crystals of IDH were presented with rac-IDH, only the L isomer bound to the active site. In contrast, in the presence of Mg^{2+}, only the D isomer was seen at this site. A fourth group, the hydroxyl of the C-2 atom, varied its association according to conditions and accounts for the difference. The conclusion was that a minimum of four designated locations is needed in order to explain a protein's ability to discriminate between enantiomers.

Drug development

Until recently, the majority of single-isomer drugs available were those derived from natural sources (e.g. morphine, epinephrine, hyoscine), and racemates predominated. There is now clear evidence of a trend in the pharmaceutical industry towards the development of chiral drugs, either *de novo* or by deriving them from racemates marketed previously (i.e. 'chiral switching').[77] Several factors have influenced this trend, which has occurred independently and in parallel with a quest in the industry as a whole to develop more potent, selective and specific drugs. Chiral switching is a small component of this trend and relies on the existence of a racemate in the first place. The process is equivalent to developing a new active substance and requires a new application, but data on the racemate may be used as appropriate, together with 'bridging studies'. There is, however, limited potential in the market for the degree of

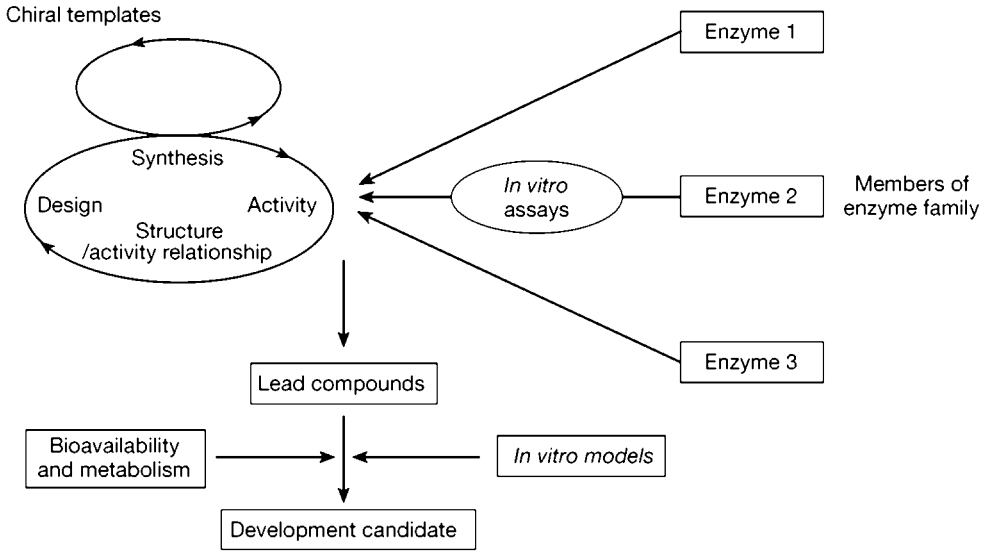

Fig 6 Chiral technologies to produce new drug candidates.

therapeutic benefit obtained to justify the degree of investment.

The real benefit of chiral technology lies in its application in the search for novel chemical entities. Regarding enantiomers as chemically distinct entities at an early stage in the research and development process is a valuable aid in the understanding of drug mechanisms. The key targets of selectivity and specificity can be pursued in an effort to improve drug efficacy while minimizing toxicity. Rational drug design (based on increased understanding of biological receptor systems) combined with chiral technology allows a 'chiral template' to be developed, as illustrated in Figure 6.

Coinciding with this has been the awakening of the drug regulatory authorities to the different pharmacological and toxicological profiles of enantiomers. The FDA policy statement for the development of stereoisomeric drugs, issued in 1992, made it more difficult to obtain approval for racemates.[25] This statement made it clear that approval could not be granted for a drug containing more than one isomer unless the pharmacokinetic and pharmacodynamic properties of each could be described and, more importantly, justified. In addition, the FDA offers a shortened approval process for the enantiomeric versions of approved drugs, with the promise of patent protection.

Drug regulatory authorities in other countries have followed this lead. Additional isomers in a compound are no longer considered as 'silent passengers' but as potential contaminants (so-called isomeric ballast). While it is unlikely that many racemates will be approved in future as a result of this, there are still situations in which their production is justified. These include situations in which (i) the enantiomers are configurationally unstable *in vitro* or undergo racemization *in vivo*; (ii) the enantiomers have similar pharmacokinetic, pharmacodynamic and toxicological properties; and (iii) it is not technically feasible to separate the enantiomers in sufficient quantity and/or quality.

Situations also exist in which an enantiomeric ratio other than unity may be justified if the ratio is expected to improve the therapeutic profile, as there is no reason to expect the optimum eudismic ratio to be necessarily 1:1 (i.e. the dose–response curves would not usually be expected to be congruent).

Methods of enantioselective synthesis

More than 50% of commercial drugs have at least one stereogenic centre. Research efforts since the mid-1980s have succeeded in producing a range of chiral technologies that aim to exert ultimate control over a chemical reaction by diverting its enantioselectivity. Indeed, where this is not the case, synthetic production of a racemate necessarily yields less than 50% of the desired drug. The reliable preparation of chiral molecules of very high enantiomeric purity therefore allows both biological evaluation and industrial application.

The key methods for the production of single-isomer drugs are described below.

Isomer separation

This falls into three main categories.

Separation by chromatography

Chromatography is the only method applicable to all stages of the pharmaceutical development chain from discovery to the full-scale process.

The first application of a countercurrent chromatographic process, of which the simulated moving bed (SMB) is an example, was based on Broughton's patent from 1961 and

was widely adopted in the petrochemical industry. Since the 1990s, the problems of scaling down the multi-ton systems used in this industry have been overcome, enabling laboratory-scale systems to be developed for application to medicinal chemistry.

The basic concept of SMB technology is the continuous countercurrent movement of stationary and mobile phases in which the movement of a stationary phase is simulated. The small particles in this component are packed into single columns and connected to form a circle. Four external valves allow the addition and subtraction of feed and effluent. The mobile phase is pumped through the circle and when it passes the stationary phase a slight separation occurs, the less absorbable compound running in front and the more absorbable compound staying behind. When steady state is reached, the system can be operated continuously. An example of a pharmaceutical compound separated by SMB chromatography is tramadol.

Crystallization

Two methods predominate. First, the racemic product or a simple chiral salt of the product (e.g. hydrochloride) may, under specific conditions, crystallize to give only one isomer. This method has been used in the resolution of α-methyl-L-dopa, methadone, asparagine and glutamic acid. These compounds are known as conglomerates; however, relatively few compounds (about 7%) exhibit this behaviour. The second crystallization method is to form a diastereomeric salt by mixing the racemate with a chirally pure compound. Again, under the correct conditions, only one diastereomer will crystallize out. This technique relies upon the ability of the two components to form a salt; however, a covalent bond could also be formed with a chiral auxiliary. This last example is not widely used (if at all) in the manufacture of chiral drugs.

Enzymic resolution (biotransformation)

Enzyme-mediated reactions are appealing to the developmental chemist as they produce a diverse range of transformations and avoid extreme reaction conditions with their concomitant inflated manufacturing costs and potential hazards. Enzymes have been used not only in the resolution of racemates but also to allow the introduction of new stereogenic centres. Their use for the preparation of chiral pharmaceuticals has increased only recently as the methods employed have been adapted to commercial production. Enzyme resolution allows the separation of a racemic drug or drug derivative (e.g. an ester) into its two enantiomers owing to the fact that the enzyme only reacts with one isomer. Two general approaches are used. The first involves incorporating in the synthetic design of the desired compound an enzyme resolution stage as a means of separating and recovering the isomer. Alternatively, the diversity of biotransformations that enzymes can produce is considered and synthesis is designed around that transformation which results in the desired chiral centre(s).

As the use of enzyme transformations in the pharmaceutical process expands, these two approaches may ultimately converge in the creation of the desired product. Enzymatic resolution has been used in the preparation of benzodiazepines, e.g. S-14 lotrafiban (Smith Kline Beecham), antibacterial drugs, e.g. levofoxacin, and antiinflammatory drugs, e.g. the S isomers of 2-aryl propionic acids [S-naproxen, S-suprofen].

Asymmetrical synthesis

This term refers to the process of taking an achiral drug (i.e. one containing no chiral centre) and synthetically converting it by one of a number of routes to one isomer of a compound with a chiral centre. These methods include asymmetrical hydrogenation (asymmetrical catalysis), asymmetrical dihydroxylation and hydroxamination etc. Computational toolkits for molecular design, visualization and analysis (e.g. SYBYL, created by Astra-Zeneca) can assist the process.

Chiral pools

The term 'chiral pool' refers to the many naturally available chiral molecules that exist in high enantiomeric purity and frequently at low cost. The most versatile chiral starting materials, in order of their industrial production per annum, are: carbohydrates, α-amino acids, terpenes, hydroxy acids and alkaloids. Other inexpensive chiral natural products are ascorbic acid, dextrose, ephedrine, limonene, quinidine and quinine, etc. Naturally occurring amino acids are readily available from bulk fermentation processes and these constitute the most important class within the chiral pool, with amounts available ranging from 10 to 10^5 tons per year. These compounds can be incorporated into the molecule to provide the desired chiral centre (e.g. synthetic peptides) or to induce the desired chiral centre during synthesis (chiral induction or diastereoselective synthesis). The term 'diastereoselective synthesis' refers to the formation of a new chiral centre into a single enantiomer when only one new isomer is formed. Chiral starting materials such as amino acids can be converted into antibacterials, cytotoxic agents and protease inhibitors (e.g. ritonavir) using this technology.

Overall, it is evident that tremendous advances have been made over the last few years in the utilization of modern asymmetrical synthetic tools that enable single-isomer drugs to be brought to commercial fruition. [43]

Practical example

The manufacture of ropivacaine.HCl.H_2O provides an example. [26] The technique is based on a resolution method published by Tullar [78] some 30 yr ago, when it was found that (±)-2′,6′-pipecoloxylide could be resolved by the use of (–)-dibenzoyl-L-tartaric acid (a natural isomer), thereby making virtually any member of the series of *N*-substituted enantiomeric derivatives available via a subsequent alkylation step. Ropivacaine.HCl.H_2O can be synthesized in three steps. (i) *Resolution* is achieved by fractional crystallization.

Optical purity and enantiomeric yield are dependent on both the crystallization time and the water content of the solvent. (ii) *Alkylation*: *N*-alkylation of the resolved pipecoloxylidide base is then performed and the HCl salt precipitated from the organic phase by adding hydrochloric acid. (iii) *Final optical purification*: final recrystallization of the crude hydrochloride from acetone/water (10:1) gives ropivacaine.HCl.H_2O. This process generates an overall yield of 50% and an optical purity of >99.5%.

Metabolite switches

There is currently much interest in the metabolites of known drugs as well as in their enantiomers.[77] The ability to produce the active metabolic moiety directly may simplify pharmacogenetic and other issues, and a variety of drugs are under investigation. These include the prokinetic agent cisapride (active metabolite norcisapride) and the antimalarial halofantrine (active metabolite desbutylhalofantrine). Desmethylzopiclone has also been studied; this is a metabolite of zopiclone in which an *N*-methylpiperazine ring has been demethylated.

Pharmacokinetics

The processes of absorption, distribution and metabolism are critical determinants of drug action and can assume equal relevance to the actual biological effect of the drug at its receptor site. The potential for discrimination between enantiomers at each of these stages is therefore important and highlights the need for stereospecific drug assays.

After administration of a racemate, the proportion of enantiomers (initially 1:1) changes continuously until a new steady state is reached. Significant differences may be seen in the rates of enzymatic conversion, carrier transport, protein binding, distribution and elimination, i.e. active processes. Passive processes, determined primarily by physicochemical characteristics, show smaller differences. The eudismic ratio thus changes constantly and is subjected to the confounding influences of genetic polymorphism and chiral inversion. Toxic side-effects may reside not in the parent isomer but in an isomer-specific metabolite.

It is therefore clear that pharmacokinetic data paying no heed to these features and simply extrapolated from multicompartmental modelling is at best misleading, and has been denigrated by Ariens as 'pseudoscientific nonsense'.[6] Ideally, analytical techniques allowing quantitative *in vivo* sampling should be developed and validated at an early stage of drug development. Some of the techniques used for this purpose include chiral high-performance liquid chromatography, chiral gas chromatography, nuclear magnetic resonance, optical rotatory dispersion and X-ray crystallography. Examples of how pharmacokinetic processes may be influenced by enantioselectivity are discussed below.

Absorption

Passive transfer across cellular membranes is dominated by lipophilicity and the extent of ionization at physiological pH. There is generally little enantiomer-specific difference as the lipid and aqueous solubilities are identical.

Active transport processes can discriminate between the enantiomers, with implications for bioavailability. For example, the inherent vasoconstriction characteristic of the S(–) isomer local anaesthetics results in the drug remaining at the site of injection longer and may influence the peak plasma concentration.[4]

Protein binding

Most drugs bind to plasma proteins to a varying degree. Stereoselectivity in binding can have a significant effect on the amount of drug in the plasma and this is species-dependent. Albumin is the most predominant plasma protein and dominates binding for acidic drugs, while α_1-acid glycoprotein (AGP), present to the extent of 3% of albumin, has a lesser overall effect. However, the elevation in AGP seen in acute illness increases the amount of drug bound to it. Variations in protein binding between enantiomers can offset their potency difference. A case in point is warfarin, in which the S(–) enantiomer is two to five times more potent than the R(+) enantiomer, but this is negated to a large extent by the greater plasma clearance of the former. [11]

Metabolism

Enzyme systems reliant on receptor–drug interactions are subject to stereoselective influences in a similar manner to other systems. The interactions between enantiomers can be complex, existing both within the species (i.e. pharmacogenetic differences) and transcending it. Extra complications arise as a result of inversion processes, whereby one enantiomer is converted into another. Processes such as these, which alter the eudismic ratio, can have toxicological implications, and it is equally important to realize that toxicity may not reside in the parent isomer but in an isomer-specific metabolite. Competition between enantiomers can also occur when more than one metabolic pathway for substrate breakdown exists, stereoselective factors determining which pathway is followed. Age, race, sex and disease all complicate the issue.

Chiral synthesis and its impact on anaesthetic practice

General anaesthetic agents

Inhalational agents

The majority of the inhalational anaesthetics used currently are chiral, with the notable exception of sevoflurane. Exploitation of stereochemical technological processes

Table 1 Relative potencies of anaesthetic isomers. ED_{50}=median effective dose; MAC=minimum alveolar concentration; EEG=electroencephalogram

		Isomer	
		S	**R**
Isoflurane			
Pond snail K^+ ion channel[16]	Ion channel current generation	S twice as effective as R	
Mouse, *in vivo*[70]	Sleep time (min)	9	6
Rat, *in vivo*[17]	MAC	1.06	1.62
Rat, *in vivo*[24]	MAC	1.44	1.69
Thiopental			
Animal[28]	EEG depression (% baseline)	10	40
Human $GABA_A$[32]	ED_{50} (μM)	26	52
Etomidate			
Tadpole[34]	ED50 (μM)	57	3.4

may allow potential clinical benefits to be achieved while simultaneously promoting a greater understanding of their mechanism of action. Recent work attempting to elucidate the mechanism of action of anaesthetic agents emphasizes the importance of specificity in action, whereas the more generalized earlier theories were based on physical characteristics such as lipid solubility. A difference in potency between the isomers of an inhalational anaesthetic agent would therefore lend support to specific anaesthetic–protein binding interactions.

Franks and Lieb[27] studied the effects of isomers of isoflurane on nerve ion channels derived from the pond snail and found the S(+) isomer to be twice as potent in triggering an anaesthetic activated potassium current as the R(–) enantiomer. Harris and colleagues[34] conducted *in vivo* studies and gave mice intraperitoneal injections of the isomers of isoflurane. They found a greater sleep time with the S(+) isomer. Similar evidence for greater potency of the S(+) isomer of isoflurane was demonstrated in the rat model by Lysko and colleagues.[53] The minimum alveolar concentration (MAC) of S(+)-isoflurane was 1.06 (SD 0.07)% compared with 1.62 (0.02)% for R(–)-isoflurane and 1.32 (0.03)% for the racemate. This was the first study to look at the stereoisomers of a volatile agent when given by a conventional route (inhalation) and to measure a clinically relevant outcome (MAC). While some groups have supported these findings,[34 35 62 64] there have been conflicting results.[31 46 63] In a repetition of the study of Lysko and colleagues, Eger and colleagues [22] found the MAC of the S(+) isomer to be 1.44% *vs* 1.69% for the R(–) isomer (racemate 1.59%). Although the S(+) isomer was found to be the more potent, this difference was not statistically significant. Overall, these findings suggest that any enantiomer-selective effect for volatile anaesthetics may be relatively weak. However, the evidence is much stronger for specific drug–receptor interactions when the modes of action of intravenous agents are considered (Table 1).

Intravenous anaesthetics

Thiopental, methohexital, ketamine and etomidate are all chiral compounds. While the first three are used as racemates, etomidate is given as the single R(+) isomer. Propofol is not a chiral compound.

S(+)-thiopental has a shorter terminal half-life than the R(+) isomer [13] because of its more rapid metabolism and clearance. These differences extend to the metabolite, pentabarbital. There have been few studies comparing the effects of separate administration of the two thiopental isomers, although in the early 1970s this was performed in the mouse model [33] and the S-enantiomer was found to be more potent. This was confirmed by Mark and colleagues[58] in a study in human volunteers.

More recent animal work[59] has confirmed that S-thiopental is more potent when assessed by depression of EEG activity, but is associated with a lower therapeutic ratio. In one study, three out of seven animals died in the S-thiopental group and recovery from EEG depression was slower. An earlier study[60] using a similar experimental model confirmed this better therapeutic ratio for the R enantiomer and suggested that it may be due to a greater distribution in CNS tissue than heart.

In vitro studies of possible CNS receptor target sites have been performed in order to explain the greater potency of the S-isomers. Minimal stereoselectivity was at α-amino-3-hydroxy-5-methylisoxazolepropionic acid (AMPA) receptor[44] and nicotinyl acetylcholine receptor[18] sites, making it unlikely that they are pivotal in producing barbiturate anaesthesia. Study of expressed human $GABA_A$ receptors[15] produced differences in median effective concentration (EC_{50}) for S-thiopental [26 (SD 3.2) μM], rac-thiopental [35.9 (4.2) μM] and R-thiopental [52.5 (5) μM], and these values are consistent with the differences in potency for CNS depressant effects found *in vivo*.

Methohexital, although no longer used in the UK, is of interest as it contains two asymmetrical centres, one at position 5 in the barbiturate ring and the other the initial carbon on the 1-methyl-2-pentynyl side-chain. This produces two pairs of stereoisomers.[13] One pair are enantiomers and the other pair are diastereomers (i.e. they are not mirror images). It was used clinically as a mixture of the two least excitatory isomers. Methohexital and thiopental also exhibit tautomerism (dynamic isomerism). In alkaline solution, sodium thiopental is highly water-soluble because of an ionized side-chain. When injected into plasma at pH 7.4, this becomes a non-ionized, non-dissociated form which rapidly isomerizes to its tautomer, a highly lipid-soluble compound.

Steroid anaesthetic agents, while not currently used in clinical practice, also exhibit chirality. Pregnanolone and allopregnan-3-ol-20-one both contain eight chiral centres (C-3, C-5, C-8, C-9, C-10, C-13, C-14 and C-17), which leads to complex stereochemistry. A recent study looked at two enantiomers of each compound.[16] Stereoselectivity was evaluated both *in vivo* (GABA receptor effects in rat brain

membrane preparations) and *in vitro* (loss of righting reflex in tadpoles and mice). The results showed that 5α-reduced steroids, but not 5β-reduced steroids, show a high degree of enantioselectivity/enantiospecificity in their actions as modulators of $GABA_A$ receptors and as anaesthetics. For all compounds studied, the effects on $GABA_A$ receptor function closely tracked the anaesthetic effects. These data show that the anaesthetic steroid recognition site is capable of distinguishing enantiomers, suggesting a protein-binding site of specific dimensions and shape.

Etomidate is used as a single isomer in clinical practice. The anaesthetic effect resides predominantly in the R(+) enantiomer, which is approximately five times as potent as the S-isomers.

Tomlin and colleagues[76] studied the EC_{50} required to induce loss of the righting reflex in tadpoles. R(+)-etomidate had an EC_{50} of 3.4 (SD 0.1) μM compared with 57 (1) μM for S(+)-etomidate. They also studied the isomeric effects on GABA-induced currents in mouse fibroblasts that had been stably transfected with bovine $GABA_A$ receptors, using a patch-clamp technique. The R(+) isomer was much more effective in potentiating GABA-induced currents, although the degree of stereoselectivity varied with anaesthetic concentration. The two isomers were equally effective in disrupting lipid bilayers. These findings are consistent with the theory that the effects on the $GABA_A$ receptor are central to the anaesthetic activity of etomidate.

Lambert's group[9] further examined the nature of the interaction between etomidate and the mammalian $GABA_A$ receptor. They used the *Xenopus laevis* oocyte expression system in conjunction with two-point voltage clamping to assess the effects of a single-point amino acid substitution in one of the GABA receptor subunits on the response to R(+)-etomidate. They found that a single-point mutation on the β_3 subunit completely abolished the allosteric regulation of the $GABA_A$ receptor by etomidate. This finding is further evidence for the specificity of anaesthetic action.

Harrison and colleagues have also investigated site-directed mutagenesis. In separate studies, specific mutations at serine 270 in the $GABA_A$ receptor α subunit were found to alter the sensitivity of the receptor to enflurane and isoflurane.[51 62] Harrison's recent editorial also expands on the theory of anaesthetic-binding pockets or cavities within target molecules such as the NMDA or the GABA receptor, and how specific mutations may affect areas which border the anaesthetic binding domains.[36 37]

Ketamine, a phencyclidine derivative, produces dissociative anaesthesia rather than the generalized CNS depression seen with other intravenous induction agents. It is also unique in that it has significant analgesic effects and does not depress the cardiovascular system. However, ketamine's adverse effect profile, such as emergence delirium and excessive cardiovascular stimulation, has meant that its use has been limited in general anaesthetic practice.

In vitro studies using guinea-pig brain homogenate have examined the binding affinity the chiral forms of ketamine with phencyclidine and opioid receptors.[41] Binding affinity was highest for the phencyclidine receptor and exhibited stereospecificity with a S(+)/R(–) ratio of 2.5 in favour of the S(+) isomer. The high binding affinity accords with the theory that the anaesthetic and analgesic effects of ketamine result from NMDA receptor blockade. Binding affinity for the κ opioid receptor was 20–30 times lower but still exhibited stereospecificity [S(+)/R(–) ratio 4.2]. Affinity for the μ opioid receptor was lower still and had an S(+)/R(–) ratio of 2.5. Enantiomer-specific effects have also been demonstrated for human sodium channels,[28] while studies of muscarinic receptors have produced conflicting results.[29 73]

Pharmacological data reveal that the metabolism of S(+)-ketamine by human liver microsomes[47] is 20% greater than that of the R(–) enantiomer and 10% greater than that of the racemate, giving a faster clearance of drug. The production of similar pharmacodynamic effects with a smaller dose of drug and faster clearance gives S(+)-ketamine an attractive pharmacological profile for further investigation.

In human volunteers, Adams and colleagues[1] compared intravenous rac-ketamine 2 mg kg^{-1} with S-ketamine 1 mg kg^{-1}. They found equivalent cardiovascular stimulation but more rapid recovery in the S-ketamine group. Doenicke and colleagues[17] compared equivalent doses of rac- and S-ketamine but added a third group, who were premedicated with intravenous midazolam. The expected increase in mean arterial pressure (MAP) was identical in the first two groups but abolished in the midazolam group. Recovery of visual attentiveness and sensorimotor performance was better in the S-enantiomer group, as was subjective assessment of mood. Midazolam prevented any unpleasant emergence sequelae at the expense of slower recovery of cognitive performance.

When comparing subanaesthetic doses, Pfenninger and colleagues[68] found equivalent analgesic effect and cardiovascular stimulation but less anterograde amnesia and better recovery of concentration with S-ketamine.

Clinical studies have also been performed examining the use of ketamine in coronary artery bypass surgery[83] and orthopaedic surgery.[10] The cardiac study compared rac- and S-ketamine at three time points: at induction, during steady-state fentanyl–midazolam anaesthesia, and at aortic cross-clamping during extracorporeal circulation. At each point, a bolus of racemate 3 mg kg^{-1} or S-enantiomer 1.5 mg kg^{-1} was given. Monitoring included invasive arterial blood pressure, right heart pressure and volume, left ventricular systolic and end-diastolic pressures, and maximum speed of left ventricular pressure increase. During intubation, heart rate and MAP increased to a similar degree in the two groups. Overall, there was no major difference in the haemodynamic profiles of rac- and S-ketamine.

During orthopaedic (knee) surgery, anaesthesia was induced with midazolam and either rac-ketamine 2 mg kg^{-1} or S-ketamine 1 mg kg^{-1}. A continuous infusion of racemate 1 mg^{-1} kg^{-1} h or S-enantiomer 0.5 mg^{-1} kg^{-1} h was then

given throughout surgery in addition to vecuronium and N_2O/O_2. The authors found no difference in cardiovascular variables. Clinically, therefore, the cardiovascular effects of the S-enantiomer are indistinguishable from those of the racemate.

Analgesics

The use of S-ketamine may have greater potential as an analgesic than as a general anaesthetic. At low rates of infusion, the cardiovascular side-effects are not seen and the advantages of avoiding opiate-induced side-effects are apparent. It can also be administered via a variety of different routes. S-ketamine, when given by caudal block in paediatric anaesthesia,[57] provides postoperative analgesia equivalent to that of bupivacaine. Its use in adult acute pain services could also be expanded. Arendt-Nielsen and colleagues[5] found that the S-isomers reduced temporal and spatial summation of pain in a post-burn model in human volunteers and also had the advantage of producing less slowing of reaction time than the racemate.

Tramadol

Tramadol hydrochloride is a chiral analgesic with a novel, dual mode of action. It has weak affinity for the μ opioid receptor (10 times less than codeine)[69] but, more importantly, increases central neuronal synaptic levels of 5-hydroxytryptamine and norepinephrine. These neurotransmitters are involved in antinociceptive descending pathways in the spinal cord. Tramadol has two chiral centres, in positions 1 and 2 in the cyclohexanol ring, giving rise to four stereoisomers.[13] Clinically, it is used as a mixture of two enantiomers, (1R, 2R)(+)-tramadol and (1S, 2S)(–)-tramadol.

The effects of the stereoisomers have been looked at in animal models. The actions of rac-, (+)- and (–)-tramadol on electrically evoked norepinephrine efflux and uptake, measured by fast cyclic voltammetry, were studied in the rat locus coeruleus brain slice.[79] Rac-tramadol and its enantiomers all significantly increased the stimulated level of norepinephrine efflux. However, only (–)-tramadol blocked norepinephrine reuptake, increasing the reuptake half-time to 499 (SD 63)% of the predrug value. These findings are in agreement with those of other studies.[19 74]

Stereoselectivity is also found with the effects on the serotonergic system. Raffa and colleagues[70] found the (+) enantiomer to be the most potent inhibitor of serotonin uptake in the mouse model. In addition, they found that both enantiomers produced an antinociceptive effect in the acetylcholine-induced abdominal constriction test. Rac-tramadol was significantly more potent than the theoretical additive effect of the enantiomers. Their finding of antinociceptive synergy in the rat Randall–Selitto yeast-induced inflammatory nociception model was also demonstrated in the mouse 55°C hotplate test.

These experimental findings indicated that, interestingly, the racemate would be the optimum formulation for clinical use. Grond and colleagues[32] confirmed this clinically by looking at the use of tramadol in patient-controlled analgesia after major gynaecological surgery. Patients were given an individualized loading dose of up to 200 mg and were randomized to (+)-, (–)- or rac-tramadol PCA with an on-demand dose of 20 mg. The primary endpoint was a decrease in pain score. Failure to reach this endpoint was most frequent in the (–) enantiomer group [53% *vs* 12% in the (+)-tramadol and 15% in the rac-tramadol group]. Of the patients who reached the primary endpoint, more were in the (+) enantiomer group [67% *vs* 48% in the rac-tramadol and 38% in the (–)-tramadol group]. The percentage of patients satisfied with their pain relief in the first 24 h after surgery was 82, 76 and 41% for the (+)-, (rac)- and (–)-tramadol groups respectively. Nausea and vomiting was the most common side-effect and was seen most frequently in the (+)-tramadol group. It was concluded that the racemate was superior to the enantiomers when efficacy and side-effects were considered together.

Dexmedetomidine

Medetomidine, an imidazoline compound, is a very potent, selective and specifically full agonist at both pre- and postsynaptic α_2-adrenoceptors.[82] It is used widely in veterinary anaesthesia. Its inhibition of sympathetic tone in the CNS leads to a characteristic pattern of pharmacodynamic responses, including hypotension, bradycardia, sedation, anxiolysis, analgesia and hypothermia. These effects can be inhibited or reversed by administration of the selective and specific α_2-antagonist atipamezole.[82] This finding forms a strong basis for the use of atipamezole as a reversal agent against medetomidine-induced effects in veterinary practice (e.g. at the end of surgical procedures).

In receptor binding experiments, the α_2/α_1 selectivity ratio of medetomidine is 1620 compared with 220 and 160 for clonidine and xylazine respectively. The α_2-adrenoceptor activity of medetomidine resides almost solely in its D-enantiomer, i.e. dexmedetomidine.[82]

In the rat brain,[55] dexmedetomidine causes sedation and hypothermia and induces a dose-dependent decrease in the release and turnover of norepinephrine, dopamine and 5-hydroxytryptamine, features characteristic of α_2-agonists. It has an MAC-sparing effect when given to beagles[81] anaesthetized with halothane and also decreases heart rate and cardiac output.

Volunteer studies have confirmed this MAC-sparing effect with isoflurane. Ebert's group[21] examined the cardiovascular, respiratory and endocrine effects of increasing doses of dexmedetomidine infusions. Monitoring included direct arterial, central venous and pulmonary artery pressures, cardiac output, oxygen saturation, end-tidal carbon dioxide, arterial blood gas analysis and catecholamine concentrations. The catecholamine concentrations were significantly reduced at the initial dose level and the increase in norepinephrine seen during the cold pressor test was abolished. There was a dose-dependent increase in sedation and reductions in heart rate, mean

arterial pressure, pulmonary artery pressure, cardiac output and stroke volume. Recall and recognition were reduced at a dose greater than 0.7 ng ml^{-1}. Respiratory variables were minimally affected and acid–base status was unchanged.

The effects of dexmedetomidine have been studied both during and after surgery. Aho and colleagues,[2] in a double-blind, randomized, controlled trial with 20 patients undergoing hysterectomy, compared dexmedetomidine infusion with placebo. The infusion was commenced 10 min before induction of anaesthesia and was then set at a maintenance rate that was given throughout. Isoflurane was administered according to predetermined criteria. In the dexmedetomidine group, the isoflurane requirement was reduced by >90%.

In another study of 41 patients undergoing major vascular surgery,[75] an infusion of dexmedetomidine or saline was started 20 min before induction of anaesthesia and continued until 48 h after surgery. Heart rate stability was greater in the dexmedetomidine group, and this group also had a significantly smaller increase in norepinephrine concentrations in the immediate postoperative period.

When dexmedetomidine or placebo was used for sedation and analgesia in 119 patients requiring ventilation after cardiac or general surgery,[80] the dexmedetomidine group required significantly less midazolam or morphine.

Further investigation of this compound will help define its role in intensive care and also in relation to anaesthesia[66] and acute pain.

Dextromethorphan

Dextromethorphan is the dextrorotatory isomer of the codeine analogue levorphanol. It is widely used for its central antitussive action and has weak affinity (10-fold less than codeine) for the μ opioid receptor.[69] In the normal dose range it has no analgesic or sedative effects and it is not a respiratory depressant. In addition, it is a non-competitive antagonist at the NMDA receptor. This property has led to its experimental use in a number of clinical areas, such as acute and chronic pain and neuroprotection after brain injury.

In acute pain studies, antagonism of the NMDA receptor should theoretically reduce central hypersensitization (wind-up) and so reduce analgesic consumption and/or pain scores. Dextromethorphan has been given before[30 38] and after[39] surgery and has had modest effects on both analgesic consumption and pain scoring. In chronic pain, it has been used alone and in combination with morphine (Morphidex).[45] There is evidence to suggest not only that it augments the analgesic effect of opioids but also that it blocks or reduces the development of tolerance after long-term opioid administration.[23]

NSAIDs

Non-steroidal anti-inflammatory drugs (NSAIDs) originate from a structurally diverse group of compounds and inhibit the synthesis of prostaglandin at one or more steps in the endoperoxide biosynthetic pathway. Some of the groups from which they arise are chiral and these include the largest group, the 2-arylpropionic acids (2-APAs) or 'profens'. Additionally, other arylalkanoic acids (e.g. ketorolac, etodolac) and some miscellaneous compounds (e.g. azapropazone) are also chiral.

It has been recognized for a number of years that the major or exclusive *in vitro* inhibition of prostaglandin synthesis is elicited by the S enantiomer.[24] This has resulted in the introduction into clinical practice of a number of single-isomer NSAIDs, including S-naproxen, S-ibuprofen and, more recently, S-ketoprofen. These drugs aim to achieve a faster onset of action, enhanced potency and the promise of diminished side-effects. From a theoretical standpoint, advantages exist in marketing single-isomer NSAIDs as the dose selection is simplified, pharmacokinetic profiles are less complex and drug interactions less likely to occur. This in turn enables more rational prescribing and better understanding of nociceptive and inflammatory processes. However, further evaluation is needed before an appropriate assessment of their comparative safety can be realized.

Neuromuscular blocking drugs

Pancuronium, vecuronium and rocuronium are all aminosteroid derivatives and, like other steroid molecules, contain multiple asymmetrical centres. However, the manufacturing process is such that a single stereoisomer is produced and used clinically.[13]

Atracurium and mivacurium possess four chiral centres and so theoretically they may exist as one of 16 possible stereoisomers.[13] Atracurium is synthesized in a non-selective manner but internal symmetry reduces the number of stereoisomers to 10. Each can be classified by its configuration at the two carbon atoms (R or S) and by its relative configuration at the two carbon–nitrogen bonds (*cis* or *trans*). The isomers can be divided into three groups of geometrical isomers: *cis–cis, trans–trans* and *cis–trans*. There are considerable differences in their pharmacokinetics and pharmacodynamics. The 1R-*cis*,1R-*cis* isomer (cisatracurium) is the most active and potent. Although the neuromuscular blockade it produces is similar to that given by atracurium, atracurium produces little or no histamine release and significantly lower laudanosine concentrations.[7] Mivacurium consists of three geometrical isomers. The predominant (94%) *cis–trans* and *trans–trans* isomers are equipotent, have a high clearance rate (4.74 litre h^{-1} kg^{-1}) and very short elimination half lives (approximately 2 min).[7] In contrast, the *cis–cis* isomer has a lower clearance rate, longer half-life and much lower potency.

Local anaesthetics

In 1969, Luduena first demonstrated that the enantiomers of 2′,6′-pipecoloxylide derivatives show significant differences in local anaesthetic action.[52] The S(−) enantiomers of both mepivacaine and bupivacaine were found to be longer-lasting, mirroring earlier experiments on the enantiomers of prilocaine. This early work also found that

S(−) bupivacaine was considerably less toxic than the R(+) isomer (both intravenously and subcutaneously) in small animal species, yet without any apparent loss of potency.

While interesting, these results were not relevant, given that the large-scale commercial synthesis of enantiomerically pure local anaesthetics was not feasible. In addition, the release of bupivacaine as a racemate had heralded many advantages over its predecessors. Its prolonged duration of action reduces the need for repeated administration, sensory block is more prominent than motor block (the so-called sensorimotor split) and the drug is highly potent. This last characteristic is also its Achilles heel, however, as highlighted by Albright's editorial in 1979.[3] Sudden cardiovascular collapse, occasionally with no premonitory symptoms, prompted a re-evaluation of the potential advantages to be gained by reformulating the drug as a single-isomer preparation, i.e. levobupivacaine. Commensurate with this has been the release of ropivacaine, the S(−) enantiomer of propivacaine. Both have been the subject of recent reviews[54 56] and, despite ongoing debate concerning their relative potencies, are long-acting agents with a wide spectrum of action. In addition, the evidence to suggest that they have a more benign side-effect profile is considerable.

Levobupivacaine has been shown consistently to be less toxic than rac-bupivacaine (bupivacaine) in all animal studies. It is less arrhythmogenic and shows a trend towards more effective resuscitation and has a higher convulsive threshold, and the lethal dose range is of about 1.3- to 1.6-fold higher.[14 40]

A study by Bardsley and colleagues[8] in human volunteers, using thoracic bioimpedence to evaluate cardiac contractility, compared the effects of intravenous levobupivacaine and bupivacaine. An infusion of each drug was administered at the rate of 10 mg min^{-1} until CNS symptoms appeared or 150 mg had been given. Despite a higher mean plasma concentration of levobupivacaine than bupivacaine (2.38 *vs* 1.87 μg ml^{-1}), levobupivacaine had less effect on the mean stroke index [–5.2 (SD 7.4) *vs* –11.9 (8.4) ml m^{-2}, P =0.001], the acceleration index [–0.09 (0.15) *vs* –0.2 (0.16) s, P=0.001] and the ejection fraction [–2.5 (3.3) *vs* –4.3 (3.9)%, P=0.02]. Another study in human volunteers examined the electroencephalographic (EEG) effects after an intravenous dose of levobupivacaine 40 mg, bupivacaine 40 mg or placebo.[72] Both drugs produced a characteristic slowing of the EEG, consistent with CNS depression, but levobupivacaine had effects that were less both in magnitude and in the amount of the brain involved.

A similar method was used in an earlier comparison of the effects on the CNS and cardiovascular system of an intravenous infusion of ropivacaine, bupivacaine and placebo in 12 healthy volunteers.[48] Objective CNS signs (e.g. muscular twitching, dysarthria) or symptoms were observed in 10 subjects in the ropivacaine group as opposed to all the subjects receiving bupivacaine. The mean time from cessation of the infusion to their disappearance was shorter in those receiving ropivacaine [13 (SD 11) *vs* 20 (16) min, P<0.05], despite a higher tolerated dose and a concomitant higher free plasma concentration (0.56 *vs* 0.30 mg $litre^{-1}$, P<0.001). At doses producing CNS symptoms, cardiovascular changes (depression of conduction, diastolic function) were less pronounced with ropivacaine than with bupivacaine.

The available animal data therefore point in the same direction: the single-isomer drugs are less toxic. The available human data, although for lower doses, follow this trend and it therefore seems logical to assume that the single-isomer drugs are safer in man. Supportive evidence is provided by recent case reports describing accidental intravenous administration of both drugs.[49 50] However, only their widespread introduction into clinical practice will allow us to truly ascertain their value.

Summary

The chirality that is inherent in the enzyme systems of living organisms results in an abundance of enantiopure organic molecules in the living world. In addition to the optical properties first noticed by Pasteur, stereospecific interactions at recognition sites result in differences in both biological and toxicological effects. This fact underlies the continuing growth in chiral chemistry, rooted as it is in fundamental biochemistry.

The pharmaceutical industry has undergone a strategic shift and embraced the wide spectrum of asymmetrical synthetic methods now available. The use of these processes in developmental synthesis and large-scale manufacturing has provided new challenges in drug discovery, motivated by a desire to improve industrial efficacy and decrease the time from the conception of a new drug to the market. The economic impact of the industrial production of chiral drugs is now huge—more than 50% of the 500 top-selling drugs were single-enantiomers in 1997. Sales have continued to increase by more than 20% for the past 6 yr and worldwide annual sales of enantiomeric drugs exceeded US$100 billion for the first time in the year 2000, chiral drugs representing close to one-third of all sales worldwide.

While some 'chiral switches' may be of less apparent benefit, or indeed detrimental in some cases, encouragement by the regulatory agencies and the ability to extend the life cycle of a drug coming off patent promotes the trend. However, it may turn out to be the ability to provide chiral templates, and thereby attack the key targets of selectivity and specificity, that will lead to the greatest benefits. Research into new chemical entities that can interact specifically with enzyme families may potentially lead to new therapies for complex disease

processes. As Richards[71] has stated, the approach is designed to create a *made to measure product*, rather than one *off the peg*.

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