Drug Chirality in Anesthesia

Carla Nau M.D.,* Gary R. Strichartz, Ph.D.†

MORE than one-third of all synthetic drugs are chiral and are marketed as racemates, 1:1 mixtures of two types of molecules bearing identical chemical constitution and atomic connections but with a different spatial orientation of their constituent atoms. Drug receptors may discriminate between stereoisomers in several ways; binding may favor one form over the other, and to a degree varying with the drug molecule and with the state of the receptor, or stereoisomers may have opposite effects, one form being an agonist at the same receptor where its mirror image is antagonist. Stereoselectivity is interesting from both a scientific and clinical point of view. Scientifically, stereoselective responses give evidence for specific receptor-mediated responses and information about the structure of the binding site. Clinically, the use of single stereoisomers of chiral anesthetic drugs has received increasing attention recently, because it might decrease side effects and increase drug-safety.

In this brief review we define some fundamental terms of stereochemistry, introduce the nomenclature for designating the different stereoisomers (enantiomers), present general principles of stereoselective drug action, and describe the possible contributions of pharmacokinetic and pharmacodynamic mechanisms to stereoselective advantages. Finally, three examples of chiral anesthetic drugs are discussed in the above context.

Chemistry

The molecular formula of a drug gives the number of the different types of atoms forming the drug molecule. Isomers are compounds with the same molecular formula, but with different molecular structures. Constitutional isomers differ structurally in the arrangement of bonds connecting their atoms. Although constitutional isomers can exhibit similar pharmacologic properties, they are completely different molecules with different physicochemical properties, and often quite different receptor affinities and specificities. Stereoisomers show an identical arrangement of bonds connecting their atoms, yet the relative orientation of their atoms in space differs. The pharmacologically relevant kind of stereoisomers are configurational stereoisomers that cannot be made alike by any rotations about single bonds, that are structurally stable, and can be separated from one another without interconversion.

Enantiomers are pairs of stereoisomers that, in their three-dimensional projection, are related to one another as an object to its mirror image, and thus are not superimposable (fig. 1). A typical example of familiar objects related in such a way are the right and left hand. Such nonsuperimposable objects are said to be chiral. The most common origin of chirality is the presence of an asymmetric carbon atom, a saturated carbon atom attached to four different substituents.

Enantiomers have identical physicochemical properties in an achiral spatially uniform environment, such as an aqueous solution. However, a solution containing only one enantiomer usually causes a measurable rotation of the plane of polarized light passing through the solution, and this rotation has the same degree but opposite direction (clockwise or counterclockwise) for corresponding enantiomers. Thus, enantiomers are said to be optically active.

Terminology

The simplest naming system for enantiomers is based on optical activity, using the appropriate sign indicating whether the rotation of plane polarized light passing through a solution of the drug is clockwise [dextrorotatory, d(+)] or counterclockwise [levorotatory, l(−)]. These observed signs of rotation, d(+) and l(−), are often confused with the designations D and L according to the Fischer Convention that is widely used in carbohydrate and protein chemistry and where the configuration about a chiral atom is assigned by comparison to a standard, (−)-glyceraldehyde, that is assigned the D configuration. The most rapidly applicable and unambiguous convention, however, is the R/S classification of the Cahn-Prelog-Ingold convention that specifies the absolute configuration in the name of the compound. The
four substituents around the chiral center are assigned priorities depending on atomic number and atomic mass. The \( R \) configuration is assigned if, looking down the bond from the chiral center to the substituent with the lowest priority, the other substituents are ordered from higher to lower priority in a clockwise direction, and the \( S \) configuration is assigned if these substituents are so ordered in a counterclockwise direction. This is the preferred International Union of Pure and Applied Chemistry (IUPAC)-endorsed method of naming and identifying stereoisomers, and is the one used in this review.

**General Principles**

Most pharmacologic responses are mediated through receptors. The critical element determining the specificity of the response is the recognition of the drug molecule by the receptor molecule. From this concept, stereoselective responses to drugs have been taken as strong evidence for specific receptor-mediated responses. In general, drugs that bind to their target receptor with higher affinity, a feature that is usually accompanied by greater specificity, are also drugs that show the largest stereoselectivity.

There are minimal structural requirements for a molecule to express stereoselectivity in a drug-receptor binding process. In 1933, Easson and Stedman suggested “three of the groups linked to the asymmetric carbon atom in an optically active (chiral) drug are concerned in its attachment to its specific receptor in the tissue.”¹ According to this oversimplified model, the substituents of the active or more potent enantiomer are oriented in a way that is complementary to corresponding groups of a static binding site (fig. 1). In a more dynamic model, taking into account the possible conformational changes of both receptor and drug that occur during a binding process, a three point-attachment to a specific receptor can be imagined as selectively induced by one of the enantiomers. In other words, the corresponding groups are preferentially aligned in their optimal orientation by one of the enantiomers in reciprocal, mutually complementary interactions during the binding process, one example of the phenomenon of “induced fit” that accompanies many protein-ligand binding events.

The “stereoselectivity ratio” indicates the relative affinity or potency between the two enantiomers of a chiral molecule. This ratio can range over three to four orders of magnitude, from \( 10^4 \) to less than 10. Inasmuch as stereoselectivity is often a reflection of specificity for a receptor, large stereoselectivity ratios for the actions of a chiral drug are usually interpreted as evidence for tight binding to a structurally well-defined site, usually assumed to be a protein. One must be cautious, however, in construing weak stereoselective responses as support for the existence of protein receptors. Phospholipids and cholesterol both contain chiral carbon atoms and could themselves mediate stereoselective effects, as examples of weakly stereoselective drug interactions with lipid bilayers have shown.² Conversely, lack of stereoselectivity does not necessarily imply nonreceptor-associated responses, as the chiral center could be located in a region of the drug molecule that is irrelevant for interaction with the receptor (silent chirality).

It is important to recognize that the clinical actions of
drugs, rarely at equilibrium between their external source and the target receptors, are strongly influenced by pharmacokinetic processes that themselves are often stereoselective. Receptor interactions are only one aspect of the overall effect.

**Clinical Aspects of Chirality**

More than one-third of all synthetic drugs are chiral. Most of them, however, are available as 1:1 mixtures of enantiomers, so-called *racemic mixtures or racemates*. Examples of chiral drugs used in anesthesia are ketamine, thiopentone, isoflurane, enflurane, desflurane, atracurium, mepivacaine, bupivacaine, tramadol, atropine, isoproterenol, and dobutamine.

Whether the clinical use of single stereoisomers (homochiral drugs) provides significant advantages depends on both pharmacokinetic and pharmacodynamic properties of the enantiomers (fig. 1). Unfortunately, detailed pharmacokinetic profiles of the enantiomers of racemic drugs are often unknown. Generally, several processes of drug disposition together determine the overall pharmacokinetic profile. Each of these processes may have different stereoselective preferences. Enzymic metabolism and protein binding, for example, are potentially highly stereoselective, while passive processes like diffusion or absorption are less likely to show stereoselectivity. As a result, even though the separate pharmacokinetic processes of enantiomers can be quite stereoselective, the overall stereoselectivity may be modest. Using a single enantiomer at least makes pharmacokinetics less complex.

In the pharmacodynamic evaluation, stereoselectivity of one or several therapeutic actions, or stereoselectivity of undesirable side effects resulting from interactions with other than the therapeutic target, or both, have to be taken into account. Theoretically, elimination from a racemic drug of an enantiomer that contributes less to the therapeutic action will increase the therapeutic index. If the side effect is stereoselective and arises from a less or equally potent enantiomer, its elimination will favorably reduce the side effect. Further advantages of using enantiomers include less complex and more selective pharmacologic profiles, and less complex, and importantly, more predictable concentration-response relationships.

**Three Examples of Chirality in Anesthetic Drugs**

**Local Anesthetics: S-(levo)bupivacaine and Ropivacaine.** Bupivacaine is widely used clinically as a potent, long-acting local anesthetic. Its known potential for central nervous system, and especially cardiovascular system toxicity, however, stimulated a search for new and safer agents, resulting in the introduction of ropivacaine (the S-enantiomer of a bupivacaine homolog, carrying a propyl-chain instead of a butyl-chain at the tertiary amine) and levobupivacaine (S-bupivacaine).

The most important molecular targets for local anesthetics are voltage-gated Na⁺ channels of excitable membranes, which control the permeability of Na⁺ ions. By binding to Na⁺ channels, local anesthetics prevent their normal function and consequently block the propagation of action potentials. Binding is dependent on the membrane potential and on the pattern of depolarizations, indicating that it is modulated by the “state” or conformation of Na⁺ channels. Open and especially inactivated Na⁺ channels show a higher affinity for local anesthetics than resting channels.

A variety of Na⁺ channel isoforms, including neuronal and cardiac types, has been investigated for bupivacaine stereoselectivity. From these studies it appears to be a common feature of Na⁺ channels to display only weak or moderate stereoselectivity toward bupivacaine enantiomers. Stereoselectivity also seems to be influenced by the channel state. For inactivated states, R-bupivacaine exhibits about a 1.5-fold higher potency compared to S-bupivacaine. Resting and open states show no significant stereoselectivity. The weak bupivacaine stereoselectivity is mirrored in all *in vitro* and *in vivo* studies investigating stereoselective differences by bupivacaine enantiomers in neuronal blockade.

Ropivacaine (a pure S-enantiomer) is less potent than S-bupivacaine or racemic bupivacaine to block Na⁺ channels.6 No studies of stereoselectivity for ropivacaine-related enantiomers have been reported.

Recent clinical studies comparing the action of S-bupivacaine to racemic bupivacaine have demonstrated that the anesthetic and analgesic effects of S-bupivacaine are largely similar to those of racemic bupivacaine. For extradural anesthesia, sensory block tended to be longer with S-bupivacaine compared with racemic bupivacaine. A study comparing ropivacaine with racemic bupivacaine for femoral nerve block found that both drugs produced equally effective sensory and motor block in equal concentrations. Extralumbar ropivacaine, however, produced significantly less motor block than racemic bupivacaine. In studies investigating minimum local anesthetic concentrations of epidural ropivacaine and racemic bupivacaine for pain relief in obstetric patients, ropivacaine was significantly less potent than bupivacaine, with a potency ratio of 0.6.

Animal toxicity studies have reported a 50% higher systemic toxicity for R-over S-bupivacaine, attributable to cardiotoxicity (direct effects on the myocardium or indirect, centrally mediated effects, or both) and CNS toxicity. When cardiovascular collapse was induced by intravenously delivered local anesthetics in dogs, resuscitation was more likely for S-bupivacaine than racemic bupivacaine, but most favorable for ropivacaine. Lower binding to plasma protein of S-bupivacaine compared with R-bupivacaine balances their differential dosing for cardiovascular collapse; at this toxic end-point...
the free plasma concentrations of the two enantiomers are equal.\textsuperscript{13}

\textit{S}-bupivacaine and ropivacaine are examples of single enantiomer drugs that suggest a clinical advantage over traditionally used racemic bupivacaine, supposedly caused by a significant decrease in side effects. However, two issues will require further evaluation before a sound judgment about their true advantage in clinical anesthesia is established: First, clinical data directly comparing the potency of \textit{S}-bupivacaine and ropivacaine are needed; if it turns out that potency ropivacaine has a lower potency than \textit{S}-bupivacaine, the therapeutic index argument may favor \textit{S}-bupivacaine. Second, accumulated clinical experience is required to confirm the safety advantage of \textit{S}-bupivacaine and ropivacaine over racemic bupivacaine.

\textbf{Ketamine.} Ketamine, a phencyclidine derivative, is an intravenous anesthetic producing dissociative anesthesia characterized by catalepsy, amnesia and analgesia, normal or slightly enhanced laryngeal reflexes and skeletal muscle tone, and respiratory stimulation. Ketamine’s central nervous system-derived sympathetic stimulation usually overrides its direct myocardial depressant effects. Postanesthetic excitatory and emergence phenomena, also of central origin, limit the usefulness of ketamine for single-drug anesthesia.

Clinically, the \textit{S}- and \textit{R}-enantiomers differ in their pharmacodynamic and pharmacokinetic effects. \textit{S}-ketamine is about three times more potent than \textit{R}-ketamine as an anesthetic and analgesic agent,\textsuperscript{15} whereas postanesthetic excitatory and emergence reactions are similar for the racemic mixture and the enantiomers. \textit{S}-ketamine is the primary contributor to cardiovascular stimulation, one mechanism being a more pronounced inhibition of reuptake of released catecholamines. The plasma clearance of \textit{S}-ketamine is significantly greater than that of \textit{R}-ketamine,\textsuperscript{15} most probably based on a enantiomeric selectivity in hepatic metabolism by microsomal enzymes.\textsuperscript{16}

Ketamine binds stereoselectively to the phencyclidine (PCP) binding site of the \textit{N}-methyl-D-aspartate (NMDA) type of glutamate-gated ion channels and thereby noncompetitively inhibits the action of this excitatory amino acid neurotransmitter.\textsuperscript{17} Other molecular mechanisms involve opioid receptors, nicotinic and muscarinic acetylcholine receptors, and monoaminergic signaling pathways. Although there is good correlation between ketamine’s actions on NMDA receptors and its clinical actions, the clinical relevance of effects on other potential targets is not apparent. Studies of the enantiomers of ketamine, however, could indicate which of these other receptors might be important for the various behavioral end-points of anesthesia \textit{in vitro}. Two other clinical actions of ketamine, preconditioning\textsuperscript{18} and neuroprotection,\textsuperscript{19} that may be important for postoperative outcome, also appear to derive from binding to the NMDA receptor.

Ketamine is an example of a drug that exhibits stereoselective actions in both the main-effect and the most important side effects, and thus may present additional advantages for the stereoselective use of this drug. Furthermore, the most unwanted side effect originates from the less potent enantiomer for the main-effect, exemplifying the most desirable balance of stereoselective effects for the actions of anesthetic drugs. \textit{S}-ketamine promises to be clinically advantageous over racemic ketamine in avoiding an unnecessary drug-load and improving postoperative recovery.

\textbf{Volatile Anesthetics: Isoflurane.} Enantiomers of volatile anesthetics have been of minor clinical interest but have been used to address two long-standing questions about the mechanisms of action of these drugs: First, is disruption of the normal function of ion channels by volatile anesthetics a primary result of binding to these proteins or a secondary result following nonspecific perturbation of lipid membranes? Second, which specific sites are the relevant targets for reaching the different end-points of general anesthesia?

Stereoselective actions of isoflurane isomers were first demonstrated on some ion channels of molluscan CNS neurons.\textsuperscript{20} At the same time, isoflurane isomers were equally effective in directly modifying the physical properties of lipid bilayers and partitioned equally between lipid bilayers of phosphatidylcholine and phosphatidic acid. The observations of stereoselectivity support the hypothesis that the functional effects of volatile anesthetics involve their binding to protein targets, indeed, to ion channels, rather than to bulk membrane lipids, as implied by theories of membrane perturbation.

The value of data about stereoselective actions of volatile anesthetics \textit{in vitro} is that they provide a basis for discrimination among relevant loci of anesthesia \textit{in vivo}. If stereoselectivity found \textit{in vivo} is not manifested at a putative target \textit{in vitro}, then that site is less likely to be involved in the anesthetic process than a locus that does exhibit this stereoselectivity.

However, there are contradictory reports about whether the enantiomers of isoflurane have different anesthetic potencies. Recently, Dickinson \textit{et al.}\textsuperscript{21} reported that intravenously administered \textit{S}-isoflurane was about 40\% more potent than \textit{R}-isoflurane at producing a loss of righting reflex in rats. In addition, \textit{S}-isoflurane induced about 50\% longer sleep times than \textit{R}-isoflurane. This observation is consistent with the demonstrated stereoselectivity of isoflurane anesthesia based on sleep time after intraperitoneal injection of isoflurane enantiomers in mice. Further, studies determining the minimum alveolar concentration (MAC) for isoflurane-isomers administered by the conventional inhalational route in rats either found \textit{S}-isoflurane to be about 50\% more potent than \textit{R}-isoflurane\textsuperscript{22} or found the enanti-
omers to differ only minimally in their anesthetic potencies. Three issues complicate the interpretation of these findings: First, the small number of animals that can be tested, and thus the power of most studies, is limited by the small quantities of enantiomers that are available. Second, the different modes of administration of isoflurane might allow for different, stereoselective, pharmacokinetic contributions. Third, different molecular targets might underly the different anesthesia-defining endpoints that were chosen in the studies. The second problem could be resolved by direct measurements of anesthetic concentrations in blood, if not in brain and spinal cord, at the time of behavioral end-points, although this is a challenging requirement for tissue from small animals.

Stereoselective actions may provide clues to determining the relevant targets involved in the anesthetic process. Among ion channels the most sensitive to anesthetics appear to be the fast, neurotransmitter-gated ion channels located at synapses, especially the γ-aminobutyric acid receptor type A (GABA<sub>A</sub>), glycine, 5-HT<sub>3</sub>, and neuronal nicotinic ACh receptors. Much work has focused on the GABA<sub>A</sub> receptor. GABA is the most important inhibitory neurotransmitter in the brain. Volatile and other general anesthetics like barbiturates, benzodiazepines, propofol, and anesthetic steroids all potentiate inhibitory postsynaptic currents evoked by low concentrations of GABA in vitro. The degree to which these in vitro effects lead to an enhancement of inhibitory synaptic transmission in vivo, and thus, to the phenomena of general anesthesia, remains to be shown. However, support for important functional involvement of the GABA<sub>A</sub> receptor is found in the correlation between the degree of stereoselectivity for isoflurane observed in these pharmacologic assays and that found in some studies of anesthesia in animals.

Voltage-gated ion channels have long been dismissed as probable targets of general anesthetics since apparently higher concentrations are needed for their tonic inhibition, neglecting their ability to engage in state-dependent drug-binding (discussed previously). More recent studies have shown suppression of Ca<sup>2+</sup> and Na<sup>+</sup> channels at clinically relevant concentrations. Experiments examining the stereoselective actions of volatile anesthetics on voltage-gated ion channels would provide useful data to determine whether and which of these targets might participate in the different end-points of general anesthesia.

Clinical advantages of chiral general anesthetics are unlikely. Isoflurane is one example of a drug that, to our current knowledge, exhibits only modest stereoselective actions in the main-effect, if at all. Although the use of the S-isoflurane may have limited clinical application in cases where side effects are particularly critical, the real advantage is probably quite marginal. Currently, the stereoselective actions of isoflurane are primarily of scientific interest.

Conclusions

The clinical use of chiral anesthetic drugs as 1:1 mixtures of their enantiomers has been the accepted practice for most of the history of anesthesia. This has been partly caused by an early general ignorance about the role of chirality in pharmacology, and later by the expense required to separate the stereoisomers on a large scale. With increasing knowledge about stereoselective advantages better methods have been developed to simplify the separation and preparation of stereoisomers. In response to growing concerns about the medical and legal ramifications of drug toxicity, future chiral anesthetic drugs will most likely be primarily developed and administered as the single more potent or less toxic enantiomer. However, the clinical advantages of single enantiomers must be balanced against the additional costs from using these markedly more expensive drugs. Although there are few scientific arguments for continuing to administer drugs as chiral mixtures when single, pure, and specific stereoisomers are both safer and more potent, economic factors must also be considered in the choice of anesthetic agent. Such decisions should account for differences in the longer-term postoperative outcomes, as well as the immediate perioperative safety of the drugs used in our practice.

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