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Inhalational Anesthetics Stereochemistry: Optical Resolution of Halothane, Enflurane, and Isoflurane

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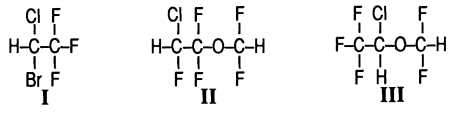
Halothane (I), enflurane (II), and isoflurane (III), which are among the most important inhalation anesthetics, are currently administered as racemic mixtures. The pure enantiomers have not been described, and no analytical method for resolving the commercially available racemic mixtures has been reported. Complete optical resolution of (±)-I and (±)-III on per-*n*-pentylated α-cyclodextrin (Lipodex A) and of (±)-II and (±)-III on octakis(6-O-methyl-2,3-di-O-pentyl)-γ-cyclodextrin capillary columns has been achieved, making rapid and convenient determination of enantiomeric ratios in samples of all three of these anesthetics possible.

INHALATIONAL ANESTHETICS CAN BE used to induce unconsciousness, analgesia, amnesia, and muscle relaxation. In the decades following W. J. G. Morton's demonstration in 1846 that diethyl ether could be used to induce a state of reversible insensibility (*1*), most available volatile chemicals were tested for anesthetic potential. Among these were chloroform and nitrous oxide, the latter of which remains an important supplemental anesthetic (*1*). By 1930, it was understood that anesthetics with the desired characteristics of noncombustibility, volatility, potency, low toxicity, and stability would likely be organic fluorides (*2*). This realization led eventually to the synthesis of halothane (I) in 1951 (*3*), enflurane (II) in 1963 (*4*), and isoflurane (III) in 1965 (*4*). Together with the weaker agent nitrous oxide, these three compounds are the most important agents in use today (*5*); interestingly, all three have chiral structures.



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Although the physiological effects of these drugs have been studied in detail, the definition of a molecular mechanism for general anesthesia has remained an elusive goal (*6*). Since a wide variety of molecules, including some inert gases, possess anesthetic properties, it is suspected that all such agents depress the nervous system by nonspecific perturbation of the fluid characteristics of nerve membranes (*6*). A more stereoselective interaction with the protein components of such membranes has not, however, been excluded. That the enzyme activity of luciferase is inhibited by clinically effective concentrations of anesthetics (*7*), and that saturable rat brain binding sites for halothane have been demonstrated by ^{19}F nuclear magnetic resonance (NMR) (*8*), strongly suggest direct protein binding. Early experiments in which halothane of modest enantiomeric enrichment was used failed to demonstrate differences between the two optically active preparations in their ability to depress conduction in a rat cervical ganglion or to increase disorder in a lipid bilayer (*9*). It is likely, however, that putative stereoselective interactions are weak; differences between anesthetic enantiomers may only be revealed with optically pure compounds. Thus if stereochemically homogeneous anesthetics were available, they would at the least be valuable research tools. It also is apparent that a convenient analytical technique for determining enantiomeric enrichments would in itself be extremely useful. We report chromatographic methods for the rapid resolution of all three of these racemic anesthetics on a microscale.

Preliminary gas chromatographic experiments were discouraging, although not surprising in view of the expected weak interactions of a chiral stationary phase with such lipophilic substrates. Experiments with chiral NMR shift reagents were similarly unrewarding. Our first success was with a capillary column using per-*n*-pentylated α-cyclodextrin (Lipodex A) (*10*) as the liquid phase (*11*). (±)-Halothane was completely resolved on this phase. We found that this same column gave complete resolution of (±)-isoflurane as well. (±)-Enflurane, however, was not resolved at all (Fig. 1A). By switching to another cyclodextrin derivative, octakis(6-O-methyl-2,3-di-O-pentyl)-γ-cy-clodextrin (*12*), we were also able to resolve (±)-II as well as (±)-III (Fig. 1B). With this column, the previously resolved (±)-I showed no separation. Thus (±)-III is readily resolved on both of these columns, while I and II are resolved on one or the other of the chiral liquid phases. The physical basis for the selectivity of these resolutions is unclear.

These results promise to be useful in several contexts. They provide a method of determining the enantiomeric purity of synthetic samples of I, II, and III. This method is rapid, convenient, and sensitive. Stereochemical analyses of many other similarly constituted volatile, lipophilic racemic substances that have hitherto resisted resolution may be possible with the use of these columns. It should now be possible to determine whether there is stereochemical selectivity in the enzymatic or metabolic deg-

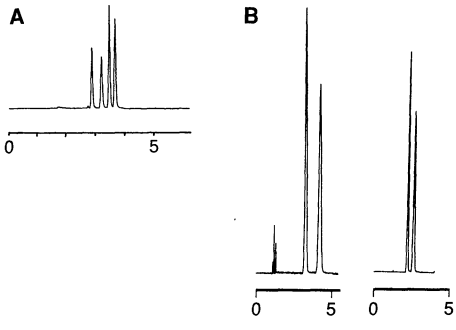

Fig. 1. Enantiomeric resolution of (**A**) halothane (I, longer retention times) and isoflurane (III, shorter retention times) on a 25-m fused-silica column with hexakis(2,3,6-tri-O-pentyl)-α-cyclodextrin and (**B**) of isoflurane (III, longer retention times) and enflurane (II, shorter retention times) on a 25-m Pyrex glass capillary column with octakis(6-O-methyl-2,3-di-O-pentyl)-γ-cyclodextrin. Retention times are in minutes. Capillary columns were coated according to the static procedure (*15*) as described earlier (*16*). A Carlo Erba Model 4160 gas chromatograph with split injection and flame-ionization detection was used. Conditions: headspace injection; column temperature, 30°C; carrier gas, 0.4 bar of hydrogen.

radation of these anesthetics in in vitro and in vivo experiments. Whether individual enantiomers of I, II, and III may have clinical advantages with respect to potency, toxicity, times of onset of action or recovery, or olfactory properties remains to be seen. While we have developed efficient syntheses of each of the enantiomers of halothane and enflurane (*13*), the remaining problems associated with carrying out the necessary in vivo studies, including the task of preparing sufficiently large quantities of each pure isomer, are not trivial. Nevertheless, it is important to establish whether any clinical advantage may be gained by the use of one particular stereoisomer of these anesthetics. This question is of particular interest in view of the increasing awareness of the potential significance of drug chirality within the chemical, medical, and governmental regulatory communities (*14*).

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Regulation of the Apolipoprotein AI Gene by ARP-1, a Novel Member of the Steroid Receptor Superfamily

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Apolipoprotein AI (apoAI) is a lipid-binding protein that participates in the transport of cholesterol and other lipids in the plasma. A complementary DNA clone for a protein that bound to regulatory elements of the apoAI gene was isolated. This protein, designated apoAI regulatory protein–1 (ARP-1), is a novel member of the steroid hormone receptor superfamily. ARP-1 bound to DNA as a dimer, and its dimerization domain was localized to the COOH-terminal region. ARP-1 also bound to a thyroid hormone–responsive element and to regulatory regions of the apoB, apoCIII, insulin, and ovalbumin genes. In cotransfection experiments, ARP-1 down-regulated the apoAI gene. The involvement of ARP-1 in the regulation of apoAI gene expression suggests that it may participate in lipid metabolism and cholesterol homeostasis.

CHOLESTEROL HOMEOSTASIS IS ACcomplished by the integration of pathways that govern cellular biosynthesis, uptake, and excretion of cholesterol (*1*). The high-density lipoproteins (HDL) and their major protein constituent, apoAI, participate in the excretion process (*1*). High-density lipoprotein removes cholesterol from peripheral tissues and transports it, either directly or via other plasma lipoproteins, to the liver, where it is excreted. It is thought that, because of their involvement in cholesterol excretion, apoAI and HDL are important in protection against coronary heart disease (*2*). Indeed, genetic deficiencies in apoAI and HDL are associated with excessive intracellular cholesterol accumulation and premature atherosclerosis (*2*).

The apoAI gene is expressed primarily in liver and intestine (*3*) and is regulated by diet (*4, 5*), estrogen (*5, 6*), thyroid hormone (*7*), and temporal factors during development (*3, 5*). The −222 to −110 DNA region upstream of the human apoAI gene functions as a liver-specific transcriptional enhancer (*8*). To identify the factors that regulate the apoAI enhancer in liver cells, we used a DNA fragment from the apoAI gene that included the region between −256 to −80 bp upstream of the transcription initiation site as a probe in deoxyribonuclease I (DNase I) protection experiments. Nuclear extracts from rat liver contained proteins that bound to four sites: A (−222 to −193), B (−169 to −149), C (−135 to −118), and D (−114 to −108, corresponding to the CCAAT homology) (Fig. 1). In contrast, extracts from tissues and cells that do not express apoAI (rat spleen, kidney, brain, and HeLa), protected only site A (Fig. 1) (*9*). Mutagenesis of sites A, B, or C indicate that protein binding to all three sites is essential for maximal expression of apoAI in human hepatoma (HepG2) cells (*8*).

To characterize the proteins that bound to site A, an oligonucleotide that corresponded to the A site (oligo A) was used as a probe to screen several λgt11 cDNA expression libraries (*10*). One positive clone (λHP-1) was isolated from a human placenta library, and binding-specificity spot tests (*10*) showed that the fusion protein produced by λHP-1–infected bacteria bound specifically to oligo A. The sequence of the insert was determined and its reading frame was established (Fig. 2A). The sequence of a clone that contained additional 5′ sequences (λHP-16) (*10*) revealed an upstream in-frame termination codon (underlined in Fig. 2A), thus placing the initiator methionine at position 343/345 and predicting a 414–amino acid protein, which we named apoAI regulatory protein–1 (ARP-1).

Comparison of ARP-1 with proteins in the GenBank database (*10*) revealed a similarity to human Ear-3 and Ear-2 "orphan" steroid hormone receptors (*11*) and the *Drosophila* Seven-up proteins (types 1 and 2), which regulate retinal cell differentiation (*12*). A cysteine-rich region of ARP-1 that corresponds to the DNA binding domain of the steroid hormone receptors (amino acids 79 to 144) (Fig. 2A), shares 98.5, 89.4, and 92.4% identity with the Ear-3, Ear-2, and Seven-up, respectively. The ARP-1 COOH-terminal domain (amino acids 145 to 414) is 95, 67, and 84.4% similar to Ear-3, Ear-2, and Seven-up (type 1), respectively, whereas the NH_2-terminal domain (amino acids 1 to 78) shows limited similarity. Thus, it appears that these proteins belong to an ancestral subfamily of nuclear receptors, which we refer to as the ARP subfamily (*13*). The high degree of evolutionary conservation of this subfamily suggests functional conservation and implies regulation of its members by similar ligands. The Ear-3 gene has been localized to human chromosome 5 (*11*), whereas ARP-1 is on chromosome 15 (*9*),

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