Lack of Stereospecific Effects of Isoflurane and Desflurane Isomers in Isolated Guinea Pig Hearts

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Background: Volatile anesthetics alter membrane channel proteins. It is controversial whether they act by nonspecifically perturbing lipid membranes or by directly binding to amphiphilic and usually stereoselective regions on channel macromolecules. Biologically relevant receptors are usually stereoselective. The stereochemical effect of isoflurane and desflurane can be used as a pharmacologic tool to investigate whether these drugs bind to specific target sites. The specific optical isomers of isoflurane and desflurane were used to examine whether they produce any differential effects on electrical, mechanical, and metabolic function in isolated hearts.

Methods: Isolated guinea-pig hearts were perfused with Krebs-Ringer's solution containing, in random order, both isomers of either isoflurane (n = 11) or desflurane (n = 6) for 10 min with a 15-min washout period. Either anesthetic was injected into a preoxygenated, sealed bottle of perfusate, which gave concentrations of 0.28 and 0.57 mM for isoflurane and 0.48 and 0.88 mM for desflurane, which are equivalent to 1 and 2 MAC multiples.

Results: Both isomers of isoflurane and desflurane decreased left ventricular pressure, heart rate, and percent oxygen extraction and increased atrioventricular conduction time, coronary flow, and oxygen delivery. Each change was significantly different from control at each concentration, and these effects were greater with the high compared to the low concentration of each anesthetic. There was no significant difference between the (+)- and the (−)-isomers for either anesthetic for any measured or calculated variable. Also, the effects of the stereoisomers were similar to those of the racemic mixture.

Conclusion: These data indicate that the optical isomers of isoflurane and desflurane are equipotent, as assessed by their effects on cardiac function in isolated guinea-pig hearts. Although both agents may ultimately influence hydrophilic domains of the protein channels, their major cardiac effect appears to result either from global perturbation of the membrane lipids and/or an interaction at nonstereoselective sites on channels modulating cardiac anesthetic effects. (Key words: Anesthetics, volatile: desflurane; isoflurane; stereoisomers. Animal: guinea pig. Heart: coronary flow; electrophysiology; isolated; left ventricular pressure; oxygen consumption; perfused.)

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Pasteur L: Recherches sur la dissymétrie moléculaire des produits organiques naturels. Paris, 1861.

CLINICALLY available volatile anesthetics isoflurane and desflurane are racemic mixtures of two optical isomers. Louis Pasteur# deduced that the phenomena of stereoselectivity must involve diastereomeric interactions between the enantiomers and a dysymmetric receptor site. Since then, the problems of chirality, namely the mechanisms and consequences of the enantioselective interactions with biologic systems, are receiving ever increasing attention in all fields of research.

Chirality is a structural characteristic that results in a molecule that is asymmetric and not superimposable with its mirror images. The physical characteristic that best distinguishes enantiomers in solution is rotation of the plane of polarized light in opposite directions but with the same magnitude. In all other respects, the molecules are identical, meaning that they cannot be distinguished by the usual (achiral) physical characteristics such as melting point and lipophilicity.[1] These differences would not be of pharmacologic consequence if it were not for the fact that the biologic en-

vironment in which the drugs are placed is itself chiral. Biologic macromolecules are able to distinguish between enantiomers. Most frequently, asymmetry is associated with a carbon to which four different functional groups are attached. Nearly all modern volatile anesthetics are used clinically as racemic mixtures of stereoisomers. We have used the functional effects of their stereoselectivity to infer interaction with cellular macromolecules. However, it should be noted that historically useful and potent anesthetic agents such as diethyl ether, chloroform, and cyclopropane do not demonstrate stereoisomerism. Thus it can not be implied that stereoselectivity is required for biologic activity.

Volatile anesthetics have been demonstrated to functionally alter neuronal ion channels,[2,3] but it is controversial whether the traditional view is correct, *i.e.*, that they act by nonspecific perturbation of lipid membranes[4,5] or that they bind directly to amphiphilic[6] sites on excitable macromolecules such as proteins or nucleic acids.[7-9] If specific macromolecular receptors for volatile anesthetics are similar to those for native ligands, a specific binding of the individual enantiomers to these molecules would be probable. For macromolecules that are themselves stereospecific, there may be not only quantitative but also qualitative stereoselective differences in binding, recognition, and activation. If, however, activation does not occur for one of the enantiomers, the inactive enantiomer may bind to the same site as the active enantiomer and function as an antagonist. In this way, the activity of the racemic drug is not necessarily the sum of the activities of the individual enantiomers. Consequently, optical stereoselectivity of many drugs, and especially anesthetics, can be used as a pharmacologic tool to investigate the nature of the site of action.

Stereoselective behavior has been observed previously for intravenous agents such as ketamine,[10-14] but it rarely has been examined with volatile agents. In an older study, no significant difference between the two stereoisomers of halothane was found for sympathetic ganglionic transmission.[15] However, isoflurane isomers were found to differ considerably in their effects on anesthetic-activated potassium current and in inhibiting currents mediated by neuronal nicotinic acetylcholine receptors.[9] However, the same isomers did not differentially anesthetize tadpoles.[16]

General anesthetics not only produce anesthesia but also have side effects, such as cardiac depression, that may be mediated through effects on multiple target sites. The stereoselectivity of modern volatile anesthetics has not been investigated in cardiac tissue. In this study, we used pure optical isomers of isoflurane and desflurane to examine for differences in their depressant effects on heart rate, atrioventricular conduction time, left ventricular pressure (LVP), coronary flow (CF), and oxygen extraction and consumption using the isolated heart.

Materials and Methods

After approval was obtained from the Animal Studies Committee of the Medical College of Wisconsin, 10 mg ketamine and 1,000 units of heparin was injected intraperitoneally into 17 English short-haired albino guinea pigs (300–350 g). The animals were decapitated, and the hearts were rapidly excised during continuous retrograde aortic perfusion with cold oxygenated, modified Krebs-Ringer's solution (equilibrated with 97% O_2 and 3% CO_2). A description of the surgical preparation for this model has been reported in detail previously.[17,18]

After placement in the Langendorff apparatus, hearts were perfused through the aortic cannula with non-recirculated and oxygenated Krebs-Ringer's solution at a constant perfusion pressure of 55 mmHg (75 cm fluid column). The perfusion solution had the following composition (mM): Na^+ 137, K^+ 4.5, Mg^{2+} 1.2, Ca^{2+} 2.5, Cl^- 134, HCO_3^- 15.5, $H_2PO_4^-$ 1.2, glucose 11.5, pyruvate 2, mannitol 16, EDTA (ethylene-diamine-tetraacetic-acid) 0.05, and insulin 5 units/l. Perfusate and bath temperature were maintained at 36.4 ± 0.2°C using a thermostatically controlled water circulation.

Left ventricular pressure was continuously recorded isovolumetrically with a transducer (Gould-Statham P23, Gould Electronics, Elk Grove, IL), connected to a thin, saline-filled latex balloon (Hugo Sachs Electronic KG, Germany), that was inserted into the left ventricle through the mitral valve from a cut in the left atrium. The balloon volume was primarily adjusted to maintain a diastolic LVP of 0 mmHg during the control period so that any increase in diastolic LVP reflects an increase in left ventricular wall stiffness or diastolic contracture. The volume of the balloon was unchanged during the experimental period. Two pairs of bipolar silver electrodes (Teflon-coated silver, diameter 125 μm, Cooner Wire Company, Chatsworth, CA) were placed in the right atrium, and the pulmonary conus to monitor atrio-atrial and atrioventricular time. Spontaneous atrial heart rate was determined from the right

atrial beat-to-beat interval. Atrioventricular conduction time was determined from the superior right atrial to right ventricular pulmonary conus beat-to-beat interval. Coronary flow (CF) was measured as detailed previously[17,18] under constant pressure and at constant temperature by an electromagnetic flow meter (Biotronix BL610-2A with Series 2000C extracorporeal transducer, 1.5 mm ID, Biotronix Laboratories, Kensington, MD). To determine maximal CF, adenosine (0.2 ml of a 2-mM stock solution) was injected directly into the aortic root cannula during the initial control period and after the last control reading. Coronary sinus effluent was collected by placing a small catheter into the right ventricle through the pulmonary artery after ligating both venae cavae. Oxygen tension of the coronary inflow and outflow were measured continuously on-line by temperature-controlled miniature Clark electrodes (Instech 203B, Instech Laboratories, Plymouth Meeting, PA), calibrated periodically with 21% and 97% O_2 to adjust oxygen tension (P_{O_2}) to 150 and 650 mmHg, respectively. These measurements were verified off-line with an intermittently self-calibrating gas analyzer (Radiometer ABL-2, Metron Chicago, Des Plaines, IL).

Oxygen delivery (D_{O_2}) was calculated as inflow oxygen tension in mmHg times oxygen solubility (24 μl per ml Krebs-Ringer's solution at 760 mmHg O_2 and 37°C) times CF (ml/min) divided by gram of wet heart tissue (1.90 ± 0.06 g). Oxygen tension of the inflow perfusate was held constant. Percentage oxygen extraction was calculated as 100 times the difference between inflow and outflow oxygen tensions, divided by inflow oxygen tension. Similarly, myocardial oxygen consumption (MV_{O_2}) was calculated as oxygen solubility times the difference between inflow and outflow oxygen tensions times CF per gram of wet heart tissue.

Atrial and ventricular electrocardiograms, heart rate, atrioventricular conduction time, both spontaneous and atrially paced at a constant frequency of 240 impulses/min, outflow oxygen tension, CF, systolic (SLVP) and diastolic LVP (DLVP), and perfusion pressure were displayed on a fast-writing (3 kHz), high-resolution eight-channel chart recorder (Astro-Med, West Warwick, RI).

Anesthetic Delivery and Determination of Concentrations

Isoflurane and desflurane isomers (generously supplied and tested by Anaquest, Murray Hill, NJ) had chemical purities of more than 99.8%. The optical purities for (+)- and (−)-isoflurane isomers were 95.4% and 99.5%, respectively, and for (+)- and (−)-desflurane isomers they were 83.6% and 89.0%, respectively. The anesthetics were not administered by vaporizers but were injected directly into hermetically sealed glass bottles containing preoxygenated Krebs-Ringer's solution (pH 7.41 ± 0.07, P_{O_2} 689 ± 28 mmHg, and P_{CO_2} 23 ± 0.9 mmHg). A detailed description of the delivery of desflurane and isoflurane has been published previously.[18] Briefly, isoflurane isomers were injected into a 4-l glass bottle containing 1 l of perfusate while desflurane isomers were injected into a 1-l bottle containing 0.5 l of perfusate. To obtain a perfusate concentration of approximately 1 MAC, 150 μl of each isoflurane liquid isomer was required to be dissolved into 1 l of solution, and 350 μl of each desflurane liquid isomer was required to be dissolved into 0.5 l of solution. This was determined by measuring anesthetic concentration using gas chromatography and calculating effective vapor concentration as detailed previously.[18] Based on the Krebs-Ringer's-solution/gas partition coefficient of 0.55 for isoflurane[19] and 0.225 for desflurane[20] at 37°C, we calculated the effective vapor concentrations as 1.2 and 2.3 vol% for isoflurane isomers, and 5.6 and 10.1 vol% for desflurane isomers.

The experimental interval consisted of a 10 min of cardiac perfusion. During this interval, 1 ml of perfusate solution was collected at the aortic inflow port in sealed 2-ml vials for a head-space analysis of anesthetic concentration by gas chromatography.[18] Each experimental interval was followed by a 15-min washout period, during which the same volume of anesthetic was injected again into the sealed bottles to obtain higher anesthetic concentrations, or about 2 MAC. A gas volume of 120 ml 97% O_2 and 3% CO_2 was injected into each sealed bottle to account for the volume of gas taken up by the heart during the 10-min period of cardiac perfusion. The concentration of anesthetic did not change during the 10-min experimental period.

Protocol and Statistical Analysis

After the initial control period, adenosine was injected (0.2 ml of 200 μM stock) into the aortic cannula, and at least 30 min was allowed for stabilization. Subsequently, each heart was exposed to either isoflurane or desflurane. In a randomized order, the (+)- or (−)-isomer of isoflurane or desflurane was administered at the lower and then the higher concentration for 10 min, with a 15-min anesthetic-free control period between each experimental phase. Measurements were

made during the last minute of exposure to each isomer at each concentration and during the last minute of each control (washout) period. After the last control period, adenosine was again injected at the same concentration into the aortic root to observe any change in the maximal CF response.

All data are expressed as mean ± SEM. The following statistical comparisons were made by two-way analysis of variance (for repeated measures) using software noted previously.[17,18] Comparison of values are as follows: low and high anesthetic concentrations, and the secondary and final controls, *versus* initial control values; low *versus* high concentrations of each isomer; (+)- *versus* the (−)-isomer of isoflurane; and (+)- *versus* the (−)-isomer of desflurane. Fisher's least significant difference test was used to compare means. Differences among means were considered statistically significant when $P \leq 0.05$. Using the true mean difference and the true standard deviation of the differences between the isomers of isoflurane and of desflurane at the same concentrations, the β-error and the power of the test for each parameter was calculated.** The β-error expresses the probability that values considered equal are actually significantly different.

Results

Table 1 shows that measured concentrations of the isomers of isoflurane were not significantly different at either low and high levels; this was also true for the isomers of desflurane. The electrophysiologic effects of both isomers of isoflurane and desflurane on heart rate and atrioventricular conduction time (table 1) were similar. Heart rate was significantly decreased and atrioventricular conduction time was significantly prolonged with increasing concentrations of the isomers of each anesthetic. There were no significant differences in these variables between the isomers of isoflurane and isomers of desflurane at each anesthetic level. During the washout periods, heart rate and atrioventricular conduction time returned to initial control values.

Systolic left ventricular pressure decreased significantly in a concentration-dependent manner for both isomers of each anesthetic (fig. 1). The two isomers of isoflurane and of desflurane exhibited no differences in their effects on SLVP. During the anesthetic-free

** Hassard TH: Understanding Biostatistics, St. Louis, Mosby-Year Book, 1991, pp 167–182.

Table 1. Effects of Measured Concentrations of Isomers of Isoflurane and Desflurane on Heart Rate and Atrioventricular Conduction Time

	Isoflurane						Desflurane					
	Heart Rate (beats/min)		AV Time (ms)		Conc (mM)		Heart Rate (beats/min)		AV Time (ms)		Conc (mM)	
	(+)	(−)	(+)	(−)	(+)	(−)	(+)	(−)	(+)	(−)	(+)	(−)
C1	222 ± 4	218 ± 4	63.1 ± 1.5	63.5 ± 1.5	0.00	0.00	212 ± 6	217 ± 5	65.8 ± 1.8	64.8 ± 2.1	0.00	0.00
Low	205 ± 3*	202 ± 5*	65.5 ± 1.3*	66.9 ± 1.1*	0.28 ± 0.01	0.29 ± 0.01	196 ± 5*	201 ± 3*	68.2 ± 1.8	70.0 ± 3.0	0.48 ± 0.01	0.49 ± 0.02
C2	218 ± 2	217 ± 4	63.3 ± 1.5	63.6 ± 1.1	0.00	0.00	211 ± 7	212 ± 8	65.2 ± 2.0	65.6 ± 2.3	0.00	0.00
High	192 ± 3†	196 ± 3*	77.7 ± 1.5†	78.9 ± 0.9†	0.57 ± 0.01	0.54 ± 0.01	190 ± 4†	188 ± 3†	75.8 ± 4.0†	77.8 ± 6.0†	0.86 ± 0.03	0.90 ± 0.03
C3	218 ± 4	219 ± 4	63.9 ± 1.5	64.0 ± 1.6	0.00	0.00	213 ± 6	218 ± 7	65.6 ± 1.8	66.2 ± 2.1	0.00	0.00

Concentrations (Conc) measured by gas chromatography gave effective vapor concentrations of 1.2 and 2.3 vol% for both isoflurane isomers and 5.6 and 10.1 vol% for both desflurane isomers.
All data are expressed as mean ± SEM.
AV = atrioventricular; (+) = right turning isomer; (−) = left turning isomer; C1, C2, C3 = drug free controls.
* $P < 0.05$ *versus* C1.
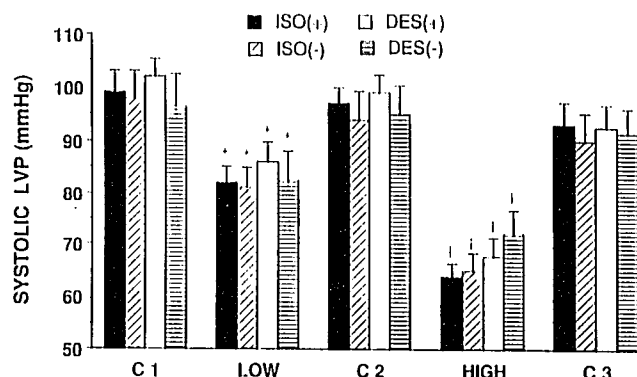† $P < 0.05$ *versus* C1 and low.

Fig. 1. Effects of two concentrations (LOW and HIGH) of optical isomers of isoflurane (ISO) and desflurane (DES) on systolic left ventricular pressure (SLVP) in 17 isolated perfused guinea-pig hearts. C1, C2, and C3 are control values (initial and after washout periods); LOW, equivalent to approximately 1 MAC (1.2 vol% ISO, 5.6 vol% DES); HIGH, equivalent to 2 MAC (2.3 vol% ISO, 10.1 vol% DES). *$P < 0.05$ versus C1. †$P < 0.05$ versus C1 and Low.

Fig. 3. Effects of two concentrations (LOW and HIGH) of optical isomers of isoflurane (ISO) and desflurane (DES) on myocardial oxygen consumption (MV_{O_2}). See figure 1 for legend and statistical symbols.

control periods (C2, C3), SLVP returned to initial control levels (C1) for both isomers of both anesthetics. Diastolic left ventricular pressure was unchanged by anesthetic exposure (data not shown). Assuming equivalent minimum alveolar concentration multiples for isoflurane and desflurane, linear regression analysis showed SLVP decreased by 17.1%/MAC for the (+)-isoflurane isomer and 17.2%/MAC for the (−)-isoflurane isomer and decreased by 16.3%/MAC for the (+)-desflurane isomer and 15.8%/MAC for the (−)-desflurane isomer. Both isoflurane and desflurane increased CF in a concentration-dependent fashion (fig. 2). The two isomers of isoflurane and of desflurane exhibited

no differences in their effects on CF. The increase in the CF with isoflurane or desflurane was less than the maximal CF elicited by adenosine (10.0 ± 0.6 $ml \cdot g^{-1} \cdot min^{-1}$ initially, and 9.3 ± 0.6 $ml \cdot g^{-1} \cdot min^{-1}$ after C3).

Myocardial oxygen consumption (MV_{O_2}; fig. 3) decreased for both isomers of isoflurane and desflurane compared with the control values. The decrease was more pronounced at the high concentrations. There were no significant differences in MV_{O_2} between the isomers of isoflurane or between the isomers of desflurane for each concentration. Similarly, percent oxygen extraction decreased in a concentration-dependent manner as SLVP and heart rate decreased with exposure to isoflurane and desflurane (fig. 4). There were
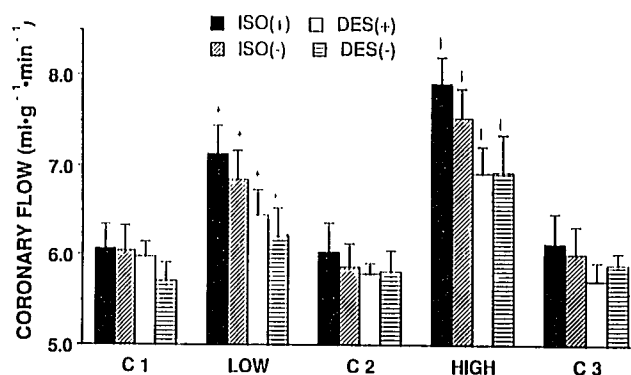

Fig. 2. Effects of two concentrations (LOW and HIGH) of optical isomers of isoflurane (ISO) and desflurane (DES) on coronary flow. See figure 1 for legend and statistical symbols.
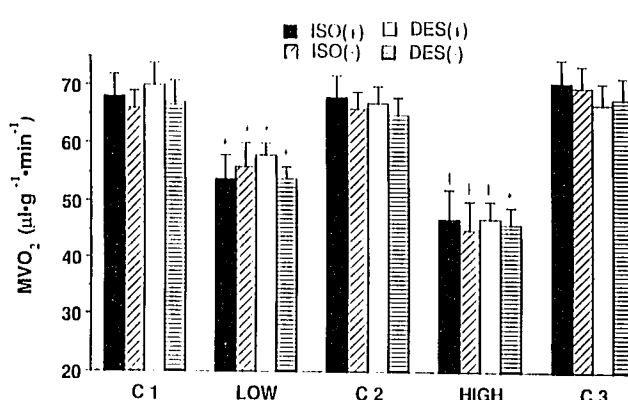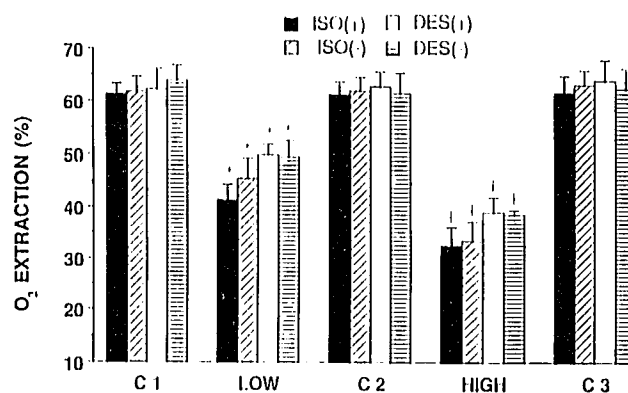

Fig. 4. Effects of two concentrations (LOW and HIGH) of optical isomers of isoflurane (ISO) and desflurane (DES) on percentage oxygen extraction. See figure 1 for legend and statistical symbols.

no significant differences in percent oxygen extraction between the isomers of isoflurane or between the isomers of desflurane for each concentration. Oxygen supply (D_{O_2}) relative to oxygen demand (MV_{O_2}) increased in a concentration-dependent manner for isoflurane isomers and for desflurane isomers (fig. 5). As for the other variables, there were no significant differences in D_{O_2}/MV_{O_2} between the isomers of isoflurane or between the isomers of desflurane for each concentration.

Using a significance level of $P \leq 0.05$ for true mean differences of the isoflurane isomers at the low and at the high concentration, the β-errors were ≤ 0.15 for all measured parameters with the exception of the D_{O_2}/MV_{O_2} ratio, for which the β-error was 0.3 for the low concentration. The powers of the tests for the isoflurane isomers consequently were between 0.85 and 0.99, again with the exception of the D_{O_2}/MV_{O_2} ratio at the low concentration, for which the power of the test was 0.7. For the desflurane isomers, the β-values were ≤ 0.25 and the powers of the tests were between 0.75 and 0.98 for the low and the high concentrations based on six experiments. Exceptions to these β-values and powers for 1 MAC equivalent desflurane were found for CF, oxygen extraction, and the D_{O_2}/MV_{O_2} ratio, the β-values for these variables were between 0.32 and 0.39 and the powers were between 0.68 and 0.61.

Discussion

We compared the direct chronotropic, dromotropic, inotropic, and coronary vasodilatory effects of the both isomers of isoflurane and desflurane in the isolated perfused heart. Effects examined were independent of neuronal and metabolic influences and without variations in pre- and afterloading factors. We found that, after exposure to approximately equivalent minimum alveolar concentrations of the (+)- and the (−)-isomer of isoflurane and desflurane, there were no significant differences between the isomers on electrophysiologic variables (heart rate and atrioventricular conduction time) or on mechanical (isovolumetric LVP) and metabolic variables (CF, percent oxygen extraction, MV_{O_2}). If autoregulation were not attenuated by anesthetics, a decrease in metabolic demand (MV_{O_2}) would be matched by a decrease in CF. However, because isomers of isoflurane and isomers of desflurane had similar effects to increase CF and decrease oxygen extraction, this indicates that the isomeric forms of these anesthetics are similarly effective direct vasodilators.
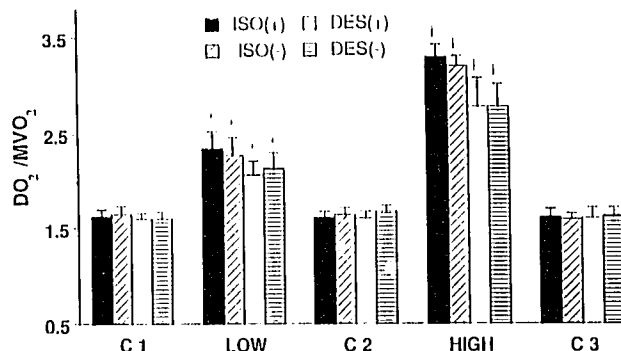

Fig. 5. Effects of two concentrations (LOW and HIGH) of optical isomers of isoflurane (ISO) and desflurane (DES) on oxygen supply to demand ratio (D_{O_2}/MV_{O_2}). See figure 1 for legend and statistical symbols.

Overall, our study suggests that stereoselective anesthetics have only nonspecific effects on several different tissues of the heart, *i.e.*, conduction system, myocardium, and vasculature.

Results of the current study also compare qualitatively with our results of an earlier study comparing isoflurane with desflurane administered only as racemic mixtures.[18] Using only pure optical stereoisomers of isoflurane and desflurane in the current study, we obtained results showing nearly the same electrophysiologic, mechanical, and metabolic effects as in the earlier report. Therefore, because we obtained nearly the same degree of effect with racemic and isomeric isoflurane and desflurane, a competitive antagonism by the two isomers to receptors appears unlikely. It should be noted, however, that the measured low and high concentrations of desflurane were slightly lower in the current study as compared to our previous study.[18] This may explain the slightly lower depressant effects of desflurane in our study.

Since Pasteur's report# to the French Academy of Science that stereoisomers of arginine tasted differently, we have become aware of the importance of optical stereoisomers. Chiral drugs, *i.e.*, drugs that have stereoisomeric forms, are most often prepared as racemates (1:1 mixture of isomers), because of the high costs involved in separation and purification of stereoisomers. However, today there is an increasing interest in stereoisomerism because biologic macromolecular receptors are often able to differentiate between the two possible rotational forms of a chiral drug.[21] Pharmacologically, racemic drugs may be considered to contain 50% impurity. Ariëns[22] has named this impurity

the "isomeric ballast," whereby isomers are potential participants in pharmacokinetic and pharmacodynamic interactions. For example, one optical isomer may be pharmacologically active at one site, whereas the other isomer is inhibitory to the first isomer at the same or different sites. Therefore, optical isomers are important as tools for understanding the location of the action of chiral drugs, *i.e.*, in an agonist/antagonist receptor interaction. The binding of optical isomers to a chiral biomolecule such as a macromolecule or an enzyme results in diastereomeric complexes and, at least in principle, in chiral recognition.[23]

Most modern volatile anesthetics are optically active so that their isomers can be used as pharmacologic tools to investigate whether they have specific macromolecular target sites such as membrane receptors or channel proteins. Stereospecific effects of volatile anesthetics have not previously been investigated in the isolated heart. Franks and Lieb[9] investigated the effects of both isomers of isoflurane on selectively sensitive ion channels in isolated molluscan CNS neurons, at 0.006 to 0.031 atm for each isomer. They found that the (+)-isomer of isoflurane was about twofold more effective than the (−)-isomer in eliciting the anesthetic-activated potassium current, $I_{K(An)}$, and in inhibiting a current mediated by neuronal nicotinic acetylcholine receptors. In inhibiting the much less sensitive transient potassium current I_A, the (−)-isomer of isoflurane was marginally more potent than the (+)-isomer, but both isomers at 0.05 atm were equally effective at disrupting phospholipid bilayers. Because the later effect was at a higher concentration, an equal effect of both isomers on potassium currents and bilayer phospholipids cannot be absolutely excluded. However, in an *in vivo* study of both optical isomers of isoflurane, Firestone *et al.*,[16] using *Rana pipiens* tadpoles, found that these isomers were equipotent in their ability to obstruct stimulation of tadpoles. Their results showing no stereoselectivity are in agreement with an older study[15] in which both (+)- and (−)-isomers of halothane had equivalent effects both on decreasing synaptic transmission in isolated cervical sympathetic ganglion of the rat and on increasing the motility of fatty acid chains in artificial phospholipid bilayer membranes. Most of these studies suggest that stereoselective volatile anesthetics have little effect on specific macromolecular complexes; rather they appear to exert primary physical effects on neural tissue and lipid bilayer membranes. However, the lack of stereoselectivity alone does not absolutely discriminate between protein *versus* lipid

binding because small optically active molecules can interact nonselectively with proteins. For example, isomers of halothane have been shown to induce conformational changes in hemoglobin in a nonstereoselective manner.[24]

Our study is the first investigation of effects of optical isomers of volatile anesthetics on the isolated perfused heart. We chose this model because, in former studies, we observed a good correlation between anesthetic concentration and cardiac depression.[17,18] We also have demonstrated that the inhibitory effect of volatile anesthetics on the atrial response to catecholamine stimulation is not competitive.[25] From this we inferred indirectly that other pathways are involved, *i.e.*, that volatile anesthetics do not compete directly for binding at beta adrenergic receptors. Also, anesthetics might exhibit different effects in a mammalian model than in other animal families or even in different tissues of the same species. Therefore, a limitation of our examination of isomeric effects of anesthetics is that we have used cardiac tissue, whereas the desired effect of an anesthetic is on neuronal tissue. Moreover, the concentration of an anesthetic that produces anesthesia may not be proportional to its direct cardiac effects, so our results are limited to how they alter cardiac function on the basis of vapor pressure concentrations equivalent to minimum alveolar concentration *in vivo*.

A limitation of a stereoselective model to infer whether there are specific binding sites for anesthetics is that three levels of interactions of stereoselectivity must be considered: penetration, recognition, and activation.[26] The term penetration means that there is a partitioning of the drug between body compartments, cells, and fluids. This distribution may be thought of as being an essentially nonchiral, physiochemical process, especially for anesthetic gases. Indeed some chiral effects may be anticipated as a result of the differences in the solubility of the isomers in solutions containing solutes like chiral proteins. In our study, the effect of penetration was eliminated by using solutions free of proteins and macromolecules. Recognition, on the other hand, involves the binding of a specific isomer to a specific macromolecular binding site. This stereoselective binding may be not only qualitative but also quantitative. After binding, activation may or may not follow recognition of the isomer. If a difference is observed between the isomers, this isomeric, selective pharmacologic effect may be a direct consequence of stereoselectivity in binding affinities. If, however, activation does not occur for one of the isomers, the in-

active isomer may be binding as an antagonist to the same site as the active isomer. The last step of stereo-selectivity for a drug is its metabolism because enzymes are usually chiral macromolecules. Indeed, we have observed a lack of stereoselectivity by volatile anesthetics on the heart. There was approximately neither a stereoselective recognition nor a stereoselective activation. A difference relative to the metabolism of the isomers could not be excluded because isomers of anesthetics may be differently metabolized. However, very little, if any, metabolism of anesthetics probably occurs in the isolated heart.

In summary, our study indicates that the optical isomers of the volatile anesthetics isoflurane and desflurane show no stereoselective effects in the isolated guinea-pig heart. It generally is accepted that these anesthetics influence cardiac function ultimately by altering the macromolecular structure of membrane proteins as shown for calcium channels,[2] which may lead to alterations in intracellular calcium content.[3] Assuming that any specific macromolecular receptors for volatile anesthetics act like those for native ligands, it is likely that there would be a specific binding site and a specific action of the individual anesthetic enantiomers on these molecules; therefore, we should have seen differential cardiovascular effects. Based on our results, we suggest that the major cardiac depressant effects of volatile anesthetics are primarily caused by global and unspecific perturbation of the membrane lipids with secondary effects on membrane macromolecules. Another probability is a direct interaction of volatile anesthetics at the nonstereoselective sites located on channel proteins.

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