Anesthesiology 2002; 96:1427–34

Differences in Cardiotoxicity of Bupivacaine and Ropivacaine Are the Result of Physicochemical and Stereoselective Properties

Bernhard M. Graf, M.D., Ingo Abraham, M.D.,† Nicole Eberbach, M.D.,† Gudrun Kunst, M.D.,‡ David F. Stowe, M.D., Ph.D.,§ Eike Martin, M.D.‖*

Background: **Ropivacaine is believed to have a lower incidence of clinical cardiac side effects than bupivacaine. The aim of this study was to compare the direct cardiac effects of the optically pure *S*(−)-ropivacaine isomer and its nonclinically used *R*(+)-isomer with both optically pure bupivacaine isomers in isolated hearts. The hypothesis was that differences in direct cardiac effects are distinguished not only by stereoselective actions of local anesthetic molecules to specific receptors, but also by physicochemical differences triggered by replacing the butyl- by a propyl-residual on pipecoloxylide.**

Methods: **Guinea pig hearts (n = 31) were excised and perfused by the Langendorff method. Atrial and ventricular bipolar electrodes were placed to measure heart rate and atrioventricular conduction time. Left ventricular pressure, coronary flow, and oxygen tensions were measured. Twelve hearts were perfused with increasing concentrations (0.5, 1.0, 5.0, and 10 μM) of both isomers of bupivacaine, and 13 hearts were perfused with the same concentrations of ropivacaine isomers. Six hearts were perfused with higher concentrations (20, 30, 40, and 50 μM) of both isomers of ropivacaine. The order of isomers and anesthetic chosen were randomized.**

Results: **Both anesthetics had negative inotropic and chronotropic effects without evidence of stereoselectivity. Equal concentrations of both isomers of bupivacaine had negative inotropic effects greater than that of ropivacaine isomers. Atrioventricular conduction time was prolonged by both anesthetics in a concentration-dependent manner, but bupivacaine isomers increased atrioventricular conduction time more than ropivacaine isomers. In contrast to other variables, atrioventricular conduction time showed evident stereoselectivity for bupivacaine at the lowest concentration (0.5 μM) but only at higher concentrations for ropivacaine (> 30 μM). The *R*(+)-isomer was more potent than the *S*(−)-isomer on increasing atrioventricular conduction time for both bupivacaine and ropivacaine.**

Conclusions: **The results confirm that stereoselectivity can be demonstrated by a lengthening of atrioventricular conduction time for the more fat-soluble bupivacaine. However, for the less fat-soluble ropivacaine, the *S*(−)-isomer has no advantage over the *R*(+)-isomer for preventing slowing of atrioventricular conduction in clinical concentrations. Neither anesthetic showed stereoselective inotropic effects, but ropicavaine isomers had lesser cardiodepressant effects than bupivacaine isomers because of the replacement of the butyl- by a propyl-terminal group.**

* Associate Professor, † Resident, ‡ Assistant Professor, ‖ Chairman and Professor, Department of Anesthesiology, University of Heidelberg. § Professor, Departments of Anesthesiology and Physiology, Medical College of Wisconsin, Milwaukee, Wisconsin.

Received from the Department of Anesthesiology, University of Heidelberg, Heidelberg, Germany. Submitted for publication January 29, 2001. Accepted for publication January 15, 2002. Support was provided solely from institutional and/or departmental sources. Presented in part at the annual meeting of the American Society of Anesthesiologists, Orlando, Florida, October 20, 1998.

Address reprint requests to Dr. Graf: Department of Anesthesiology, University of Heidelberg, Im Neuenheimer Feld 110, 69120 Heidelberg, Germany. Address electronic mail to: bernhard_graf@med.uni-heidelberg.de.

THE reported advantage of ropivacaine and levobupivacaine over racemic bupivacaine, all long-acting, amide-type local anesthetics, is a lower incidence of cardiotoxicity. For this reason, these isomers were recently introduced into clinical practice. Although both levobupivacaine and ropivacaine are pure optical *S*(−)-isomers, the butyl-group of bupivacaine is replaced by a propyl-group for ropivacaine. The latter change of molecular structure affects major physicochemical characteristics such as molecular weight and lipophilicity, while not significantly affecting the pKa value. In addition, both local anesthetics are characterized by similar pharmacokinetic profiles on protein binding and metabolism.

Higher concentrations of bupivacaine and ropivacaine can block voltage-gated ion channels and intracellular enzyme systems, leading to reduced cardiac membrane potential[1–6] and intracellular metabolism.[7–9] Cardiac output may decrease because of ventricular dysrhythmias, contractile failure, or veno-vasodilation. Supraclinical concentrations of bupivacaine can result in death because of cardiorespiratory collapse.[10,11] Anesthetic morbidity and mortality may also be dependent on species and individual variability.[12] To best investigate direct cardiac effects of the isomers of the pipecoloxylide derivatives of ropivacaine and bupivacaine, we used the isolated heart model.

Isolated organ and *in vivo* studies have indicated that there are isomer-specific differences in local anesthetics, particularly on those variables that are regulated by cardiac sodium channels.[13–17] The isomer-specific interaction of local anesthetics with sodium channel receptor sites gave rise to the belief that clinical use of the lesser sodium channel depressant *S*-isomers might reduce the potential for cardiotoxicity. In addition to effects on voltage-gated cardiac channels, local anesthetics also interact with intracellular enzyme complexes, such as the mitochondrial respiratory chain, to lower intracellular adenosine triphosphate concentrations; this effect is dependent on intracellular concentration and lipophilicity of the drug but is independent of its stereoselectivity.[7,8]

It has not been shown clearly whether cardiac effects of long-acting amide-type local anesthetics are dependent on their stereoselectivity or on other physicochemical properties. This study tested for any direct observable cardiac effects of local anesthetics based on stereospecific binding and physicochemical characteristics. The isolated perfused heart preparation is frequently used to directly assess stereospecific effects of

multiple anesthetics as reported in recent studies.[14,15,18,19] This model was used to demonstrate cardiac effects that are independent, not only of central neuronal and humoral interactions, but also of dynamic effects caused by changes in preload and afterload. To make a precise distinction between cardiotoxic effects, whether caused by stereospecific binding or physicochemical properties, both isomers of bupivacaine and ropivacaine were examined. Isomers of each anesthetic have identical physicochemical profiles except that ropivacaine and bupivacaine have different molecular weights and fat solubilities.

Materials and Methods

After obtaining approval from the Institutional Animal Care Committee of the University of Heidelberg (Heidelberg, Germany), 30 mg ketamine and 1,000 units of heparin were injected intraperitoneally into each of 31 English short-haired, albino guinea pigs (weight, 250–350 g). After decapitation and sternotomy, the aorta was rapidly cannulated and the heart excised during continuous aortic root perfusion with a cold, oxygenated, modified Krebs-Ringer solution equilibrated with 95% oxygen and 5% carbon dioxide. A detailed description of the methods has been reported previously.[18,20] After placement in the Langendorff apparatus, each heart was perfused through the aortic cannula with non-recirculated and oxygenated Krebs-Ringer solution at a perfusion pressure of 55 mmHg (75-cm fluid column). The perfusate had the following composition: 137 mM Na^+, 4.5 mM K^+, 1.2 mM Mg^{2+}, 2.5 mM Ca^{2+}, 134 mM Cl^-, 15.5 mM HCO_3^-, 1.2 mM $H_2PO_4^-$, 11.5 mM glucose, 2 mM pyruvate, 16 mM mannitol, 0.05 mM ethylene-diamine-tetraacetic-acid, and 5 units/l insulin. Perfusate and bath temperatures were maintained at 37 ± 0.2°C using a thermostatically controlled water circulator.

Isovolumetric left ventricular pressure (LVP) was measured continuously with a transducer (Gould-Statham P23; Gould Electronics, Elk Grove, IL) connected to a thin, saline-filled latex balloon (Hugo Sachs Electronik KG, March-Hugstetten, Germany) inserted into the left ventricle through the mitral valve from a cut in the left atrium. The balloon volume was adjusted to maintain a diastolic pressure of 0 mmHg during the initial control period so that any increase in diastolic pressure reflects an increase in left ventricular wall stiffness or diastolic contracture. The volume of the balloon was unchanged during the experiment. Developed LVP was the difference between peak systolic and diastolic LVPs.

Two pairs of bipolar silver electrodes (Teflon-coated silver; diameter, 125 μm; Cooner Wire Co., Chatsworth, CA) were placed on the right atrium and the pulmonary conus to monitor atrioatrial and atrioventricular time. As detailed previously, spontaneous atrial heart rate was determined from the right atrial beat-to-beat interval. Atrioventricular conduction time was determined from the right atrial to the right ventricular pulmonary conus beat-to-beat interval by an electronic timer. Atrioventricular conduction time includes conduction not only through the atrioventricular node but also across Purkinje fibers and ventricular myocytes. First-degree atrioventricular dissociation was defined as a regular atrioventricular interval greater than 70 ms. Second-degree atrioventricular dissociation (slowed ventricular response) was characterized as type I (Wenckebach), a progressive lengthening of the atrioventricular interval leading to a nonventricular-conducted atrial beat, or type II (Mobitz), a regular atrioventricular conduction delay with an occasional nonventricular-conducted atrial beat. Third-degree atrioventricular dissociation was defined as a completely asynchronous atrioventricular interval.

Coronary inflow was measured at constant pressure and temperature by a transit-time, in-line ultrasound flowmeter (Research Flowmeter T106; Transonic Systems, Ithaca, NY). To determine maximal coronary flow and the possible effect of drugs on altering coronary flow reserve, adenosine (0.2 ml of a 200-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. Coronary inflow and outflow oxygen tension were measured continuously on-line by temperature-controlled miniature Clark electrodes (Instech 203B; Instech Laboratories, Plymouth Meeting, PA) calibrated periodically with 0, 21, and 97% O_2 to adjust oxygen tension to 0, 150, and 650 mmHg, respectively. The oxygen electrodes were placed directly into the aortic inflow cannula and pulmonary artery (coronary sinus) cannula after ligating the inferior and superior vena cava. These measurements were verified off-line with an intermittently self-calibrating gas analyzer (Radiometer ABL-2; Metron Chicago, Des Plaines, IL). Oxygen delivery, oxygen consumption, and percent oxygen extraction were calculated as described previously.[18] Inflow oxygen tension was kept constant (543 ± 34 mmHg). The ratio of oxygen delivery to oxygen consumption is an index of coronary perfusion corrected for metabolic rate. Heart weight was expressed as grams of wet heart tissue.

Atrial and ventricular electrograms, heart rate, spontaneous atrioventricular conduction time, outflow oxygen tension, coronary flow, systolic and diastolic LVP, and perfusion pressure were displayed on a screen and stored on the hard drive on-line in digital form each 30 s for statistical analysis. All measurements were taken at the last minute of each 10-min experimental period.

Maximal vasodilation was tested with adenosine, and at least 30 min was allowed for stabilization before initial control measurements were obtained. Anesthetic and its

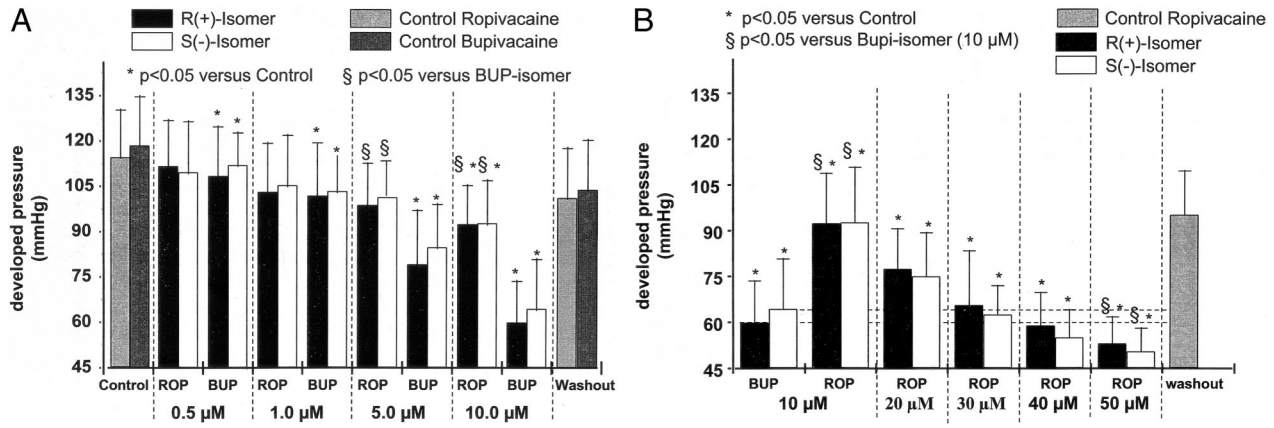

Fig. 1. (*A*) Effect of four concentrations of the pure optical isomers of ropivacaine and bupivacaine on left ventricular developed pressure (LVP) in 13 and 12 guinea-pig isolated perfused hearts, respectively. Values are shown as mean ± SD of initial and final control and individual concentrations. Control values between the individual concentrations are not displayed. Significant stereoselectivity for LVP was not observed between isomers of bupivacaine or isomers of ropivacaine at a given concentration, but LVP was different between bupivacaine and ropivacaine for concentrations greater than 1.0 μM. (*B*) Comparison of five increasing concentrations of the pure optical isomers of ropivacaine (n = 6) to a high concentration of isomers of bupivacaine (n = 12) on LVP in six hearts. Values are mean ± SD at 10 μM for both isomers of bupivacaine and final control and individual concentrations of isomers of ropivacaine (10–50 μM). Control values between the individual concentrations are not shown. There were no significant differences among the isomers of ropivacaine at a given concentration. ROP = ropivacaine; BUP = bupivacaine.

isomers were randomly assigned and tested in ascending concentrations. After random selection of an anesthetic and the order of its isomers, each isomer of the chosen anesthetic was perfused at the same concentration before proceeding to the next higher concentration. These were 0.5, 1, 5, and 10 μM *R*(+)- and *S*(−)-isomers of bupivacaine in 12 hearts and the same concentrations of *S*(−)- and *R*(+)-ropivacaine (provided by AstraZeneca, Södertälje, Sweden) in 13 hearts. Concentrated solutions of each drug were directly added into the preoxygenated Krebs-Ringer solution. An additional six hearts were perfused with 10-, 20-, 30-, 40-, and 50-μM concentrations of both isomers of ropivacaine.

Each heart was perfused for 10 min with each concentration of bupivacaine or ropivacaine isomers. Each experimental interval was followed by a 15-min drug-free washout period, after which the next highest concentration was given. The drug-free washout period between the different isomers was 20 min, after which the measured values returned to control. Measurements were obtained during the last minute of exposure to each concentration and during the last minute of each control (washout) period. Because all variables returned to control during the drug-free periods, these data are not presented in detail. After the last control period, adenosine was again injected at the initial concentration into the aortic root to observe any change in maximal coronary flow response.

Statistics

Data are presented as mean ± SD. Individual statistical comparisons of the mean values before and during administration of the isomers of ropivacaine and bupivacaine were performed by analysis of variance with repeated measures by student *t* tests with Bonferroni correction for multiplicity. The following comparisons were made: increasing concentrations of bupivacaine isomers and ropivacaine isomers *versus* the initial control (paired student *t* test), subsequent controls during washout *versus* initial control values (paired student *t* test), and each concentration of the isomers *versus* the enantiomer (paired student *t* test). The corresponding isomer of the other local anesthetic was also similarly compared (unpaired student *t* test) with Bonferroni correction. Differences among mean values were considered significant at $P < 0.05$.

Results

Isomers of bupivacaine depressed developed LVP from 0.5 μM upward in a concentration-dependent manner from baseline; in contrast, isomers of ropivacaine only demonstrated significant depression from 10 μM upward, with no evidence of stereoselectivity for ropivacaine or bupivacaine on mechanical function. Both isomers of ropivacaine had significantly smaller negative inotropic effects than either isomer of bupivacaine at 5 and 10 μM (figs. 1A and B). At 10 μM, *R*(+) bupivacaine significantly ($P < 0.001$) decreased developed LVP (by 49.3 ± 8.4%), which was similar to that of *S*(−) bupivacaine (by 48.8 ± 10.9%). *R*(+)- and *S*(−)-ropivacaine, at 10 μM, reduced developed LVP similarly (by 13.1 ± 17.1% and 12.1 ± 15.8%, respectively), which was less that that produced by 10 μM bupivacaine ($P < 0.05$).

As shown in figure 1B, 20–40 μM of the ropivacaine isomers had a similar depressant effect as that of the bupivacaine isomers at 10 μM; this indicates that bupiv-

Table 1. Change in Atrial Heart Rate (beats/min) during Perfusion at Increasing Anesthetic Concentrations

	Ropivacaine		Bupivacaine	
	R(+)-Isomer	*S*(−)-Isomer	*R*(+)-Isomer	*S*(−)-Isomer
Control	222 ± 25		227 ± 16	
0.5 μM	216 ± 26	215 ± 25	223 ± 16	222 ± 15
1.0 μM	217 ± 19	213 ± 22	219 ± 16	217 ± 14
5.0 μM	200 ± 21	208 ± 22	206 ± 18	200 ± 14
10.0 μM	196 ± 22	190 ± 21	191 ± 19	188 ± 14
Washout	221 ± 23		229 ± 12	
20 μM	190 ± 9	181 ± 11		
30 μM	180 ± 7	168 ± 9		
40 μM	172 ± 7	161 ± 8		
50 μM	161 ± 7	151 ± 8		
Washout	221 ± 10			

Control values are not shown between the individual concentrations. There were no significant differences between single isomers and both local anesthetics at equimolar concentrations.

acaine was a twofold to fourfold more negative inotropic anesthetic than ropivacaine. During the anesthetic-free control period after ropivacaine, developed LVP returned nearly to initial control levels, *i.e.*, 94.3 ± 9.8% for bupivacaine and 93.2 ± 12.1% for ropivacaine. Diastolic LVP (initially set to 0 mmHg) increased in all experiments to the same extent to a maximum of 3 ± 2 mmHg at the highest bupivacaine and ropivacaine concentrations (data not shown). During control washout periods, diastolic LVP returned to 0 mmHg.

Atrial automaticity was not significantly different between the isomers or between the anesthetics (table 1). Atrial heart rate decreased in a concentration-dependent manner by 15.9 ± 8.4% ($P < 0.001$) at 10 μM *R*(+)-bupivacaine and by 17.2 ± 6.2% ($P < 0.001$) at 10 μM *S*(−)-bupivacaine. The same concentrations of *R*(+)-ropivacaine depressed heart rate by 11.8 ± 9.9% ($P < 0.001$) and, similarly, by 14.7 ± 9.5% ($P < 0.001$) for *S*(−)-ropivacaine. Stereoselectivity between the isomers was not observed for atrial rates. Atrial heart rate was reduced by 27.2 ± 4.5% ($P < 0.001$ *vs.* control) at the highest concentration of *R*(+)-ropivacaine and by 32.0 ± 3.6% [$P < 0.001$ *vs.* control and $P = 0.06$ *vs.* *S*(−)-ropivacaine] at the highest concentration of *S*(−)-ropivacaine.

Both anesthetics significantly prolonged atrioventricular conduction time in a concentration-dependent manner (figs. 2A and B). For bupivacaine isomers, stereospecificity was demonstrated at the lowest concentration, 0.5 μM. The *R*(+)-isomer delayed atrioventricular time more than the *S*(−)-isomer. At 1 μM *R*(+)-ropivacaine, and from 5 μM for both isomers of ropivacaine, atrioventricular conduction was blocked significantly less than for bupivacaine, so that at least

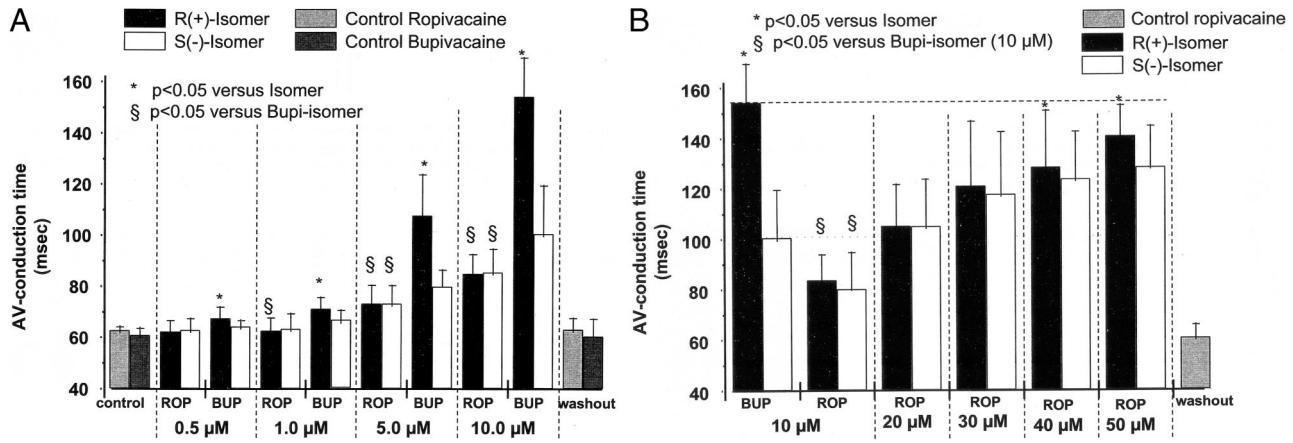

Fig. 2. (*A*) Effect of four concentrations of the pure optical isomers of ropivacaine and bupivacaine on atrioventricular conduction time in 13 and 12 hearts, respectively. Values are mean ± SD of initial and final control and individual concentrations. At the highest *S*(−)-bupivacaine concentration, 11 hearts, and for *R*(+)-bupivacaine, 2 hearts, remained in sinus rhythm (1:1 conduction). (*B*) Effect of five increasing concentrations of the pure optical isomers of ropivacaine (n = 6) compared with a high concentration (10 μM) of the isomers of bupivacaine (n = 12) on atrioventricular conduction time. Displayed are only hearts remaining in sinus rhythm (1:1 conduction). For *S*(−)-bupivacaine, 11 of 12 hearts, and for *R*(+)-bupivacaine, 2 of 12 hearts, remained in sinus rhythm. For both isomers of ropivacaine, all hearts at 10 μM and 20 μM maintained sinus rhythm. Six and five of six hearts, respectively, for 30 μM *R*(+)- and *S*(−)-ropivacaine remained in sinus rhythm. At 40 μM, five hearts in the *S*(−)-group and four hearts in the *R*(+)-group are shown. At 50 μM, three of six hearts treated with *R*(+)-ropivacaine and four of six hearts treated with *S*(−)-ropivacaine remained in sinus rhythm. AV = atrioventricular; ROP = ropivacaine; BUP = bupivacaine.

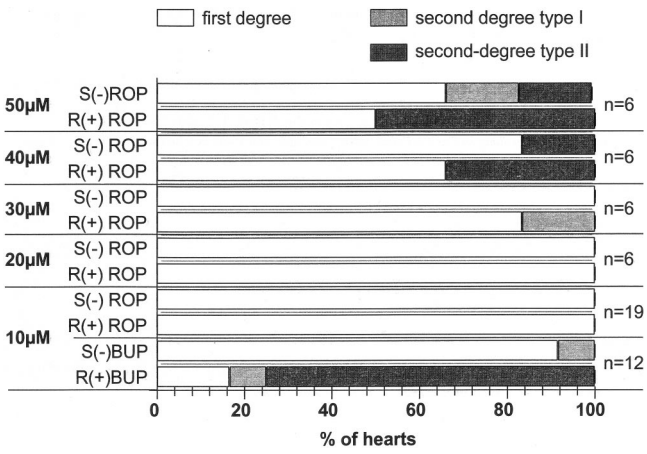

Fig. 3. Effect of 10 μM *S*(−)- and *R*(+)-bupivacaine and of 10, 20, 30, 40, and 50 μM *S*(−)- and *R*(+)-ropivacaine on second-degree atrioventricular dissociation of the Wenckebach (type I) and Mobitz (type II) classification on different numbers of hearts displayed in the graphs. All hearts with 1:1 atrioventricular conduction showed first-degree atrioventricular dissociation at 10 μM and higher. Significance of atrioventricular dissociation is given in the text. Third-degree atrioventricular dissociation and other dysrhythmias, with the exception of atrioventricular dissociation, were not observed. During drug-free washout periods, atrioventricular dissociation reverted to sinus rhythm. BUP = bupivacaine; ROP = ropivacaine.

double the concentration of *S*(−)-ropivacaine was required to produce an atrioventricular delay similar to that of *S*(−)-bupivacaine. A fivefold greater concentration of *R*(+)-ropivacaine was required to produce a similar delay in atrioventricular time by *R*(+)-bupivacaine. At concentrations greater than 30 μM, ropivacaine also showed stereoselectivity on atrioventricular delay.

The incidence of second-degree type II (Mobitz) and second-degree type I (Wenckebach) atrioventricular dissociation, respectively, caused by 10 μM bupivacaine (fig. 3) was 75% (9 of 12 hearts) and 8% (1 of 12 hearts) for the *R*(+)-isomer, and 0% (0 of 12 hearts) and 8% (1 of 12 hearts) for the *S*(−)-isomer. The incidence of combined types of atrioventricular dissociation was different from normal sinus rhythm for 10 μM *R*(+)-bupivacaine ($P < 0.001$) and for 10 μM *S*(−)-bupivacaine ($P < 0.05$). Bupivacaine did not produce third-degree atrioventricular dissociation. No second- or third-degree block occurred for either isomer at less than 30 μM ropivacaine. At 30 μM, the *R*(+)-isomer produced second-degree type I atrioventricular dissociation, whereas the 30-μM *S*(−)-isomer did not. *S*(−)-ropivacaine caused second-degree type II dissociation in 1 of 6 hearts at 40 μM and in 2 of 6 hearts at 50 μM. *R*(+)-ropivacaine caused second-degree atrioventricular dissociation in 2 of 6 hearts at 40 μM and in 3 of 6 hearts at 50 μM. Heart rate and atrioventricular conduction time returned nearly to initial control values during washout drug-free periods.

Initial and final maximal coronary flow responses to a bolus injection of adenosine were similar for all hearts (fig. 4A). Coronary flow, initially 7.0 ± 1.1 ml · g^{-1} · min^{-1} for bupivacaine and 8.1 ± 2.6 ml · g^{-1} · min^{-1} for ropivacaine, was decreased by both isomers of bupivacaine and ropivacaine by approximately 18 ± 13% at 10 μM. Only the bupivacaine isomers up to 10 μM reduced coronary flow significantly, and this effect was not statistically stereoselective. At 10 μM and higher, ropivacaine isomers reduced flow, but *S*(−)-ropivacaine tended to reduce it more than 40 μM *R*(+)-ropivacaine ($P < 0.01$; fig. 4B). The baseline ratios of oxygen supply to oxygen demand were similar: 1.59 ± 0.36 and 1.55 ± 0.45 for the bupivacaine and ropivacaine groups, respectively. At 10 μM *S*(−)- and *R*(+)-bupivacaine, the ratio of oxygen delivery to oxygen consumption increased to 1.88 ± 0.48, whereas for 10 μM *S*(−)-and *R*(+)-ropivacaine, this ratio was unchanged at 1.57 ± 0.43. At 50 μM *S*(−)-and *R*(+)-ropivacaine, the

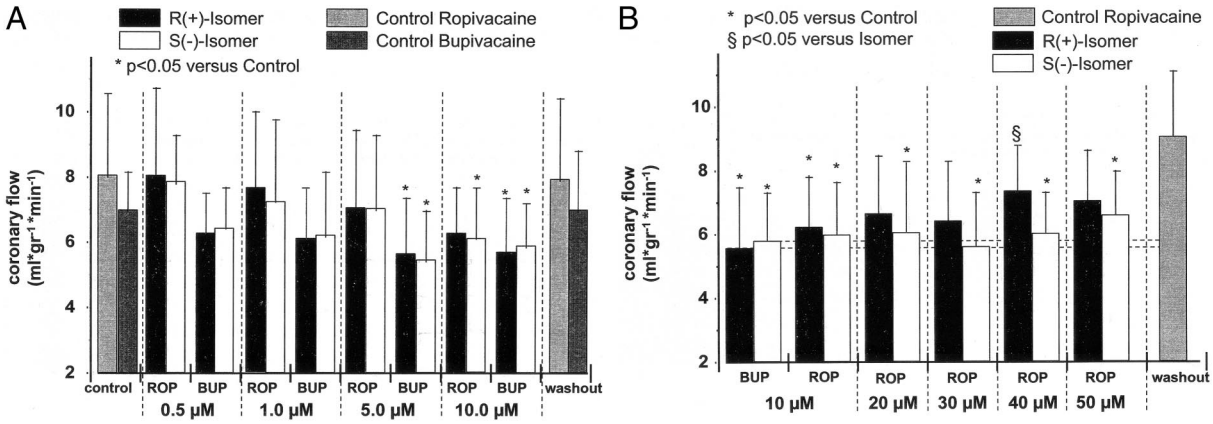

Fig. 4. (*A*) Effect of four concentrations of optical isomers of ropivacaine and bupivacaine on coronary flow in 13 and 12 hearts, respectively. Values are shown as mean ± SD for initial and final controls and individual concentrations. Control values between the individual concentrations are not displayed. There were no significant differences among the isomers of bupivacaine and of ropivacaine at a given concentration. (*B*) Effect of five high concentrations of the pure optical isomers of ropivacaine in six hearts compared with a high concentration of the isomers of bupivacaine in 12 hearts on coronary flow. Values are mean ± SD at 10 μM for both isomers of bupivacaine and ropivacaine and final control and individual concentrations (20–50 μM). Control values between the individual concentrations are not displayed. With the exception of the 40-μM concentration, there were no significant differences among the isomers of ropivacaine at a given concentration. ROP = ropivacaine; BUP = bupivacaine.

ratio of oxygen delivery to oxygen consumption was 1.86 ± 0.39 and 1.89 ± 0.34, respectively.

Discussion

Safer techniques for administering regional anesthesia arose after concern over serious complications[10] were reported in the 1980s. With the objective to further reduce these complications of regional anesthesia, pharmaceutical companies successfully developed and marketed the newer, pure *S*(−)-isomer compounds ropivacaine [*S*(−)-1-propyl-2'6'-pipecoloxylide] and levobupivacaine [*S*(−)-1-butyl-2'6'-pipecoloxylide]. Optical isomers, with the exception of rotation of the plane of light, share all physicochemical properties but have an asymmetric carbon atom. The *S*-isomers were recognized years ago as the less cardiotoxic of the two isomers.[21]

The replacement of the butyl-group in bupivacaine by a propyl-group in ropivacaine alters its physicochemical properties. After molecular weight, the principal difference is the lower lipid solubility of ropivacaine; both long-acting local anesthetics are characterized by similar pKa values. Differences between the isomers of the anesthetics are caused by different binding of the isomers to the target site, which itself must be optically active. Although the therapeutic effects between racemates (equimolar mixture of *R*- and *S*-enantiomers) and enantiomers of long-acting local anesthetics appear to be nearly similar,[22,23] it is necessary to approximate the therapeutic index of the *R*- and *S*-isomers to distinguish their potential clinical benefits over risk. In *in vivo* and *in vitro* studies, neuronal stereoselectivity depends on experimental conditions and the activation state of the channels.[17,24,25] Vladimirov *et al.*[17] summarized that clinically used concentrations of Na^+ channel blockers showed no stereoselectivity for duration and potency, whereas *in vitro* bupivacaine clearly demonstrated stereoselectivity ratios from 1.3 to 3 for *R*(+)-bupivacaine over *S*(−)-bupivacaine.[24] The relative potency between the *S*(−)-isomers of ropivacaine and bupivacaine, as well as between ropivacaine and racemic bupivacaine, is also uncertain. So-called minimal local anesthetic concentration studies using both pipecoloxylide-derivatives have shown, on the one hand, clear differences in obstetric studies,[26–28] but on the other hand have shown identical potency for peripheral neuronal blockade.[29] Because the desired therapeutic effect is the ED_{100} and dose–response curves typically fit a sigmoid curve, differences in potency may appear small at the higher clinically used concentrations than found by minimal local anesthetic concentration studies. In addition, the *S*-isomers of the piperidine ring local anesthetics were shown to be more vasoconstrictive than the corresponding *R*-enantiomers.[30,31] Thus, if *R*-isomers block neuronal impulse more potentially and longer on a molecular basis than its enantiomer, the stronger vasoconstriction and consequently the delayed washout of the *S*-isomers *in vivo* could also compensate for the weaker inhibition of the impulse by the *S*-isomer.

It was demonstrated years ago that cardiotoxicity is a primary risk factor of all local anesthetics. Local anesthetics cause direct cardiac collapse by blocking myocardial Na^+ channels and other voltage-gated channels. At higher concentrations, local anesthetics retard mitochondrial respiration in fast-metabolizing cells, thereby reducing adenosine triphosphate synthesis; these effects depend on the lipophilicity of the drug but not its stereoselectivity.[7,8,32,33] This effect on intracellular energy metabolism together with effects on Ca^{2+} channels may be related to effects on contractility, atrioventricular conduction, and the abilities of resuscitation after local anesthetics intoxication.[34]

The isolated, crystalloid-perfused guinea pig heart is a suitable preparation to examine direct cardiac effects independent of extrinsic mechanical, metabolic, and neurohumoral influence. The cardiovascular system is an important target for these drugs, because higher concentrations of lipophilic local anesthetic were found in cardiac and cerebral tissues compared with plasma.[35,36] We used this model to uncover stereoselective cardiac effects of different drugs at relevant clinical concentrations adjusted for protein binding.[14,15,18,19] Using this model, we showed that *R*(+)-bupivacaine caused a longer atrioventricular delay and a greater incidence of second-degree atrioventricular dissociation compared with *S*(−)-bupivacaine; racemic bupivacaine had an intermediate effect on delaying atrioventricular conduction and causing second-degree atrioventricular dissociation between the bupivacaine isomers.[14] For all other measured variables, there were no significant differences between the racemic and isomeric forms, thus characterizing *S*(−)-bupivacaine as a less cardiotoxic long-acting anesthetic compared with its *R*(+)- and racemic forms in causing atrioventricular dissociation. These findings for bupivacaine were confirmed in the current study. Importantly, the isomers of ropivacaine had no significant stereoselective effects on atrioventricular conduction at concentrations up to 30 μM; in contrast, bupivacaine markedly affected atrioventricular conduction at much lower concentrations. Clearly, stereoselectivity for atrioventricular conduction by ropivacaine was found, but only at higher, and therefore more toxic concentrations than those for bupivacaine. Even at concentrations five times higher, atrioventricular delay was significantly less affected by ropivacaine than by bupivacaine isomers. The stereoselective differences on atrioventricular delay can be explained by stereoselective effects on fast voltage-gated Na^+ channels. In contrast, there were no stereoselective effects observed for contractility by either anesthetic; the propyl-pipecoloxylide-derivative ropivacaine, however, reduced contractility significantly less when compared with the butyl-derivative bupivacaine

independently of its isomeric form. The current study allowed distinction between direct cardiotoxic effects caused by physicochemical properties between both long-acting local anesthetics from direct stereoselective binding to specific receptor proteins. Thus, stereoselectivity, based on our observation at altered atrioventricular conduction time, is not the only factor by which to address the cardiotoxic potential of long-acting local anesthetics.

Physicochemical characteristics, such as lipophilicity and molecular weight, are different between ropivacaine and bupivacaine and are caused by the replacement of the butyl- by a propyl-group. These appear to be significant factors that modulate potential cardiotoxic effects. The pKa values of these anesthetics are similar so they cannot account for the observed differences. With the addition of a larger group to the amine portion of the local anesthetic molecule, the pure *S*-isomer has the advantage over the racemic mixture to reduce atrioventricular dissociation. Consequently, despite an identically high lipophilicity, replacement of racemic bupivacaine by the pure optical levobupivacaine is an important factor for distinguishing the evident stereoselective effects on atrioventricular conduction. In contrast, the lighter and less lipophilic isomers of ropivacaine did not show stereoselective cardiac effects at clinically relevant concentrations.

A limitation of our model is that our heart was not paced at a constant heart rate, so use dependency of stereoselectivity for atrioventricular conduction could not be tested; this may be an important factor for stereoselectivity.[17] The ionized anesthetic concentrations examined in our crystalloid perfused heart model are effectively 10–15 times the plasma concentration that would give a similar effect because of plasma protein binding. Only the lower concentrations of the anesthetics we used are likely to be clinically relevant. We demonstrated that *S*(−)-ropivacaine has a greater coronary vasoconstrictor effect than its enantiomer at high concentrations, thus confirming that *S*-isomers increase smooth muscle tone more than *R*-isomers. A lower coronary flow may delay washout of the drug and consequently slow recovery, but lower flow could also enhance the effective concentrations at the target site. Nevertheless, after 2- or 3-min perfusion with an anesthetic, flow reached a steady state. Using constant-flow perfusion instead of constant pressure might eliminate this limitation, but as we showed earlier, cardiac variables are significantly more affected by constant flow.[37]

In summary, the pure *S*(−)-isomer of ropivacaine, compared with its racemic mixture, does not appear to offer any advantage in reducing atrioventricular dissociation, a known stereoselective effect. However, the shorter carbon chain on the amine portion of ropivacaine was associated with significantly reduced cardiotoxic effects on contractility and atrioventricular conduction without demonstrated stereoselectivity. Additional characteristics of these anesthetics, such as local anesthetic potency,[38,39] vasoregulation,[30,31,40] and sensoromotor differentiation,[41] should also be considered in future clinical trials to determine whether ropivacaine or levobupivacaine is the safer anesthetic for patients undergoing regional anesthesia.

References

1. Valenzuela C, Delpon E, Tamkun MM, Tamargo J, Snyders DJ: Stereoselective block of a human cardiac potassium channel (Kv1.5) by bupivacaine enantiomers. Biophys J 1995; 69:418- 27
2. Nau C, Wang S, Strichartz G, Wang G: Block of human hH1 sodium channel by the enantiomers of bupivacaine. ANESTHESIOLOGY 2000; 93:1022–33
3. Valenzuela C, Delpón E, Franqueza L, Gay P, Snyders D, Tamargo J: Effects of ropivacaine on a potassium channel (hKv1.5) cloned from human ventricle. ANESTHESIOLOGY 1997; 86:718–28
4. Olschewski A, Olschewski H, Brau ME, Hempelmann G, Vogel W, Safronov BV: Effect of bupivacaine on ATP-dependent potassium channels in rat cardiomyocytes. Br J Anaesth 1999; 82:435–8
5. Rossner KL, Freese KJ: Bupivacaine inhibition of L-type calcium current in ventricular cardiomyocytes of hamster. ANESTHESIOLOGY 1997; 87:926–34
6. Zapata-Sudo G, Trachez M, Sudo R, Nelson T: Is comparative cardiotoxicity of S(-) and R(+) bupivacaine related to enantiomer-selective inhibition of L-type Ca^{2+} channels? Anesth Analg 2001; 92:496–501
7. Sztark F, Malgat M, Dabadie P, Mazat J: Comparison of the effects of bupivacaine and ropivacaine on heart cell mitochondrial bioenergetics. ANESTHESIOLOGY 1998; 88:1340–9
8. Sztark F, Nouette-Gaulain K, Malgat M, Dabadie P, Mazat J: Absence of stereospecific effects of bupivacaine isomers on heart mitochondrial bioenergetics. ANESTHESIOLOGY 2000; 93:456–62
9. Tinker A, Williams AJ: Charged local anesthetics block ionic conduction in the sheep cardiac sarcoplasmic reticulum calcium release channel. Biophys J 1993; 65:852–64
10. Albright GA: Cardiac arrest following regional anesthesia with etidocaine and bupivacaine. ANESTHESIOLOGY 1979; 51:285–7
11. Reiz S, Nath S: Cardiotoxicity of local anaesthetic agents. Br J Anaesth 1986; 58:736–46
12. Bernards CM, Artru AA: Effect of intracerebroventricular picrotoxin and muscimol on intravenous bupivacaine toxicity: Evidence supporting central nervous system involvement in bupivacaine cardiovascular toxicity. ANESTHESIOLOGY 1993; 78:902–10
13. Valenzuela C, Snyders DJ, Bennett PB, Tamargo J, Hondeghem LM: Stereoselective block of cardiac sodium channels by bupivacaine in guinea pig ventricular myocytes. Circulation 1995; 92:3014–24
14. Graf BM, Martin E, Bosnjak ZJ, Stowe DF: Stereospecific effect of bupivacaine on atrioventricular conduction in the isolated perfused guinea pig heart. ANESTHESIOLOGY 1997; 86:410–9
15. Mazoit J, Decaux A, Bouaziz H, Edouard A: Comparative ventricular electrophysiologic effect of racemic bupivacaine, levobupivacaine, and ropivacaine on the isolated rabbit heart. ANESTHESIOLOGY 2000; 93:784–92
16. Morrison S, Dominguez J, Frascarolo P, Reiz S: A comparison of the electrocardiographic cardiotoxic effects of racemic bupivacaine, levobupivacaine, and ropivacaine in anesthetized swine. Anesth Analg 2000; 90:1308–14
17. Vladimirov M, Nau C, Mok W, Strichartz G: Potency of bupivacaine stereoisomers tested in vitro and in vivo: Biochemical, electrophysiological, and neurobehavioral studies. ANESTHESIOLOGY 2000; 93:744–55
18. Graf B, Boban M, Stowe D, Kampine J, Bosnjak Z: Lack of stereospecific effects of isoflurane and desflurane isomers in isolated guinea pig hearts. ANESTHESIOLOGY 1994; 81:129–36
19. Graf B, Vicenzi M, Martin E, Bosnjak Z, Stowe D: Ketamine has stereospecific effects on the isolated perfused heart. ANESTHESIOLOGY 1995; 82:1426–37
20. Boban M, Stowe D, Gross G, Pieper G, Kampine J, Bosnjak Z: Potassium channel openers attenuate atrioventricular block by bupivacaine in isolated hearts. Anesth Analg 1993; 76:1259–65
21. Aberg G: Toxicological and local anaesthetic effects of optically active isomers of two local anaesthetic compounds. Acta Pharmacol Toxicol 1972; 31:273–86
22. Cox CR, Checketts MR, Mackenzie N, Scott NB, Bannister J: Comparison of S(-)-bupivacaine with racemic (RS)-bupivacaine in supraclavicular brachial plexus block. Br J Anaesth 1998; 80:594–8
23. Cox CR, Faccenda KA, Gilhooly C, Bannister J, Scott NB, Morrison LM: Extradural S(-)-bupivacaine: Comparison with racemic RS-bupivacaine. Br J Anaesth 1998; 80:289–93
24. Lee Son S, Wang GK, Concus A, Crill E, Strichartz G: Stereoselective inhibition of neuronal sodium channels by local anesthetics: Evidence for two sites of action? ANESTHESIOLOGY 1992; 77:324–35

25. Nau C, Vogel W, Hempelmann G, Brau ME: Stereoselectivity of bupivacaine in local anesthetic, sensitive ion channels of peripheral nerve. ANESTHESIOLOGY 1999; 91:786–95

26. Lyons G, Columb M, Wilson R, Johnson R: Epidural pain relief in labour: Potencies of levobupivacaine and racemic bupivacaine. Br J Anaesth 1998; 81:899–901

27. Capogna G, Celleno D, Fusco P, Lyons G, Columb M: Relative potencies of bupivacaine and ropivacaine for analgesia in labour. Br J Anaesth 1999; 82:371–3

28. Polley LS, Columb MO, Naughton NN, Wagner DS, van de Ven CJ: Relative analgesic potencies of ropivacaine and bupivacaine for epidural analgesia in labor: Implications for therapeutic indexes. ANESTHESIOLOGY 1999; 90:944–50

29. Casati A, Fanelli G, Magistris L, Beccaria P, Berti M, Torri G: Minimum local anesthetic volume blocking the femoral nerve in 50% of cases: A double-blinded comparison between 0.5% ropivacaine and 0.5% bupivacaine. Anesth Analg 2001; 92:205–8

30. Newton D, Burke D, Kahn F, McLeod G, Belch J, MacKenzie M, Bannister J: Skin blood flow changes in response to intradermal injection of bupivacaine and levobupivacaine, assessed by laser Doppler imaging. Reg Anesth Pain Med 2000; 25:626–31

31. Khodorova A, Strichartz G: The addition of dilute epinephrine produces equieffectiveness of bupivacaine enantiomers for cutaneous analgesia in the rat. Anesth Analg 2000; 91:410–6

32. Sztark F, Tueux O, Erny P, Dabadie P, Mazat J-P: Effects of bupivacaine on cellular oxygen consumption and adenine nucleotide metabolism. Anesth Analg 1994; 78:335–9

33. Sztark F, Ouhabi R, Dabadie P, Mazat J: Effects of the local anesthetic bupivacaine on mitochondrial energy metabolism: Change from uncoupling to decoupling depending on the respiration state. Biochem Mol Biol Int 1997; 43:997–1003

34. Groban L, Deal D, Vernon J, James R, Buterworth J: Ventricular arrhythmias with or without programmed electrical stimulation after incremental overdosage with lidocaine, bupivacaine, levobupivacaine, and ropivacaine. Anesth Analg 2000; 91:1103–11

35. Chang D, Ladd L, Wilson K, Gelgor L, Mather L: Tolerability of large-dose intravenous levobupivacaine in sheep. Anesth Analg 2000; 91:671–9

36. Thomas JM, Schug SA: Recent advances in the pharmacokinetics of local anaesthetics: Long-acting amide enantiomers and continuous infusions. Clin Pharmacokinet 1999; 36:67–83

37. Graf B, Fischer B, Martin E, Bosnjak Z, Stowe D: Differential effects of rrginine vasopressin on isolated guinea pig heart function during perfusion at constant flow and constant pressure. J Cardiovasc Pharmacol 1997; 29:1–7

38. Kanai Y, Tateyama S, Nakamura T, Kasaba T, Takasaki M: Effects of levobupivacaine, bupivacaine, and ropivacaine on tail-flick response and motor function in rats following epidural or intrathecal administration. Reg Anesth Pain Med 1999; 24:444–52

39. Kanai Y, Katsuki H, Takasaki M: Comparisons of the anesthetic potency and intracellular concentrations of S(-) and R(+) bupivacaine and ropivacaine in crayfish giant axon in vitro. Anesth Analg 2000; 90:415–20

40. Guinard JP, Carpenter RL, Morell RC: Effect of local anesthetic concentration on capillary blood flow in human skin. Reg Anesth 1992; 17:317–21

41. Zaric D, Nydahl PA, Adel SO, Enbom H, Magnusson M, Philipson L, Axelsson K: The effect of continuous epidural infusion of ropivacaine (0.1%, 0.2% and 0.3%) on nerve conduction velocity and postural control in volunteers. Acta Anaesthesiol Scand 1996; 40:342–9