

Cardiac Toxicity of Local Anesthetics in the Intact Isolated Heart Model: A Review

James E. Heavner, D.V.M., Ph.D.

An editorial in 1979 by George Albright about sudden cardiac arrest after regional anesthesia spawned an era of intense research focusing on what local anesthetics do to the heart and how they do it. The ultimate goal of the research was to bring to the clinician long-acting local anesthetics that are less cardiotoxic than ones available before 1979, bupivacaine and etidocaine, in particular. In this article, I will review results of studies of local anesthetic cardiotoxicity using the intact mammalian heart in vitro published after the Albright editorial through 2001. *Reg Anesth Pain Med* 2002;27:545-555.

Key Words: Toxicity, Local anesthetic, Cardiac, In vitro.

Local anesthetic cardiotoxicity studies using the isolated, intact mammalian heart in vitro are reviewed. Thirteen studies¹⁻¹³ published after the Albright editorial¹⁴ that focused attention on cardiac deaths associated with the use of bupivacaine and etidocaine were identified. The purpose of the studies included determination of relative toxicity of 2 or more local anesthetics, factors predisposing to toxicity, treatment of toxicity, mechanisms of toxic effects, and stereoselective action of local anesthetics. Consistent conclusions are that bupivacaine (1) has an exceptionally potent depressant effect on electrical conduction in the heart; (2) predisposes the heart to reentrant types of arrhythmias; and (3) exhibits stereoselective action with the S(-) isomer (levobupivacaine) being less cardiotoxic than either the racemic mixture or the R(+) isomer. The primary target for bupivacaine's cardiotoxic action is generally considered to be voltage-gated sodium channels. However, results from isolated heart studies, as well as results from other studies, suggest that actions of bupivacaine on other than voltage-gated sodium channels probably contribute to the cardiotoxic effects.

From the Department of Anesthesiology, Texas Tech University Health Sciences Center, Lubbock, Texas.

Accepted for publication July 19, 2002.

Presented in part at the American Society of Regional Anesthesia and Pain Medicine Conference on Local Anesthetic Toxicity, November 17-18, 2001, Miami Beach, Florida.

Reprint requests: James E. Heavner, D.V.M., Ph.D., Department of Anesthesiology, Texas Tech University Health Sciences Center, 3601-4th St, Rm. 1C-258, Lubbock, TX 79430. E-mail: james.heavner@ttmc.ttuhscc.edu

© 2002 by the American Society of Regional Anesthesia and Pain Medicine.

1098-7339/02/2706-0002\$35.00/0

doi:10.1053/rapm.2002.36458

The Isolated Heart In Vitro

In the intact human and animal, heart function is controlled by factors extrinsic, as well as intrinsic, to the heart. The heart will continue to function when extrinsic factors are removed by excising the heart and placing it in vitro in a suitable milieu. The isolated heart is used to investigate effects of e.g., local anesthetics on intrinsic properties of the heart. In vitro, as in vivo, the heart exhibits automaticity, i.e., beats spontaneously due to alternating "pace-maker" electrical depolarization and repolarization of elements of the sino-atrial (S-A) node. The depolarization spreads from the S-A node via right atrial cells to the atrioventricular (A-V) node and to the left atrium, and continues from the A-V node via Purkinje fibers that form the His Bundle, to right and left ventricular cells (Fig 1). The depolarization spreads so as to initiate sequential contraction (electromechanical coupling) of muscles of the walls of the 4 chambers of the heart starting with the right atrium and ending with the left ventricle. The electrical and mechanical activity require energy provided by metabolic activity of heart cells.

An electrocardiogram (ECG), similar to the standard ECG recorded from patients can be recorded from the isolated heart (Fig 2).¹⁵ Depolarization of the atria generates a P wave and ventricular muscular depolarization produces a QRS complex. During the PR interval, depolarization of atrial muscles, components of the atrioventricular node and of the common His Bundle occurs. Conduction delays or failure in one or more of these structures will produce PR interval prolongation or A-V conduction block.

The shape of action potentials recorded in different components of the heart vary (Fig 3) depending

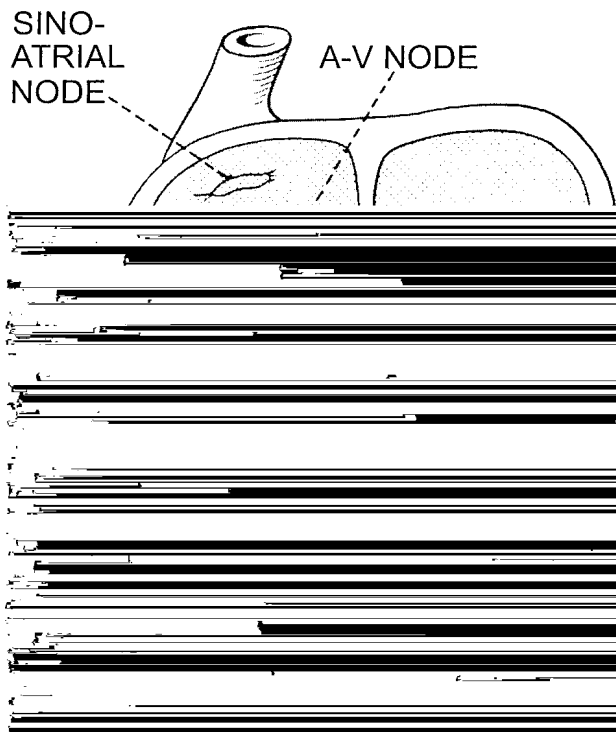


Fig 1. Sketch of cardiac conduction system. The sinoatrial node lies at the junction of the superior vena cava and the right atrium. The atrioventricular (A-V) node lies in the intraventricular septum just above the tricuspid valve. The A-V node gives rise to the common bundle leading to the single right bundle and branched left bundle. The bundles run into the middle and apical portions of the 2 septal surfaces on the endocardial surfaces of the septum. Purkinje fibers arise from the terminations of the bundles and run across the cavities and, in part, along the endocardium to the free ventricular walls and to the papillary muscles.

on ionic currents (Fig 4) that generate the potentials.^{16,17} Rapid influx of sodium ions into myocytes produces the initial rapid upstroke of action potentials in, e.g., atrium and ventricular muscle. In pacemaker (S-A node cells), inward calcium flux produces the initial depolarization that is less rapid than the depolarization due to sodium influx. As discussed later, selective actions of local anesthetics on ionic mechanisms determine to a large extent the susceptibility of different components of the cardiac conduction system to toxic effects of these drugs.

Two different isolated heart preparations are generally used for intact isolated heart studies, the Langendorff preparation and the working heart. The primary difference between the 2 preparations is how pressure in the left ventricle is controlled. For the Langendorff preparation, a balloon is placed in the left ventricle and inflated to an initial diastolic pressure (e.g., 0 to 5 mm Hg). A pressure transducer

connected to the balloon may be used to record changes in ventricular pressure (i.e., dp/dt) during the cardiac cycle. The coronary arterial system is perfused by retrograde perfusion of the aorta. In the working heart model, perfusate is introduced from a reservoir at a fixed height (e.g., 20 mm Hg) into the left atrium and is ejected by the left ventricle through the aorta into a chamber, e.g., 80 cm above the heart. Aortic pressure may be measured. Simple or complex arrays of electrodes placed on the heart may be used for recording electrical activity and/or for intermittent or continuous electrical pacing of the heart. Oxygen delivered to the coronary artery and coming from the coronary arterial system can be measured to determine O_2 consumption. What local anesthetics do to electrical, mechanical, and/or metabolic function of isolated mammalian hearts *in vitro* has been studied.

Local Anesthetic Studies

The 13 articles reviewed were identified by citation analysis and other computerized literature

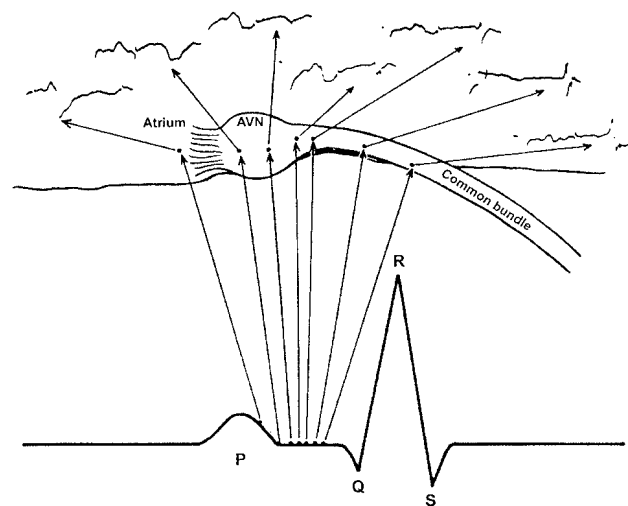


Fig 2. Potentials recorded extracellularly at 7 sites near A-V nodal and common bundle regions. Potential at far left is recorded from atrial muscle upstream from A-V node. As can be seen, it occurs during downstroke of P wave. Second potential from left is from head, or upper end, of A-V node. It shows an atrial potential followed by a large negative-going A-V nodal potential. Third potential from left is recorded in the center of the A-V node. It shows a positive-negative atrial potential followed by a rapid negative-going common bundle potential. Farther downstream, common bundle potentials show more positivity, becoming positive-negative at the far right. As can be seen, a large part of the interval between the end of the P wave and the beginning of the QRS complex is occupied by events in the A-V nodal region. (Data from Scher.¹⁵)

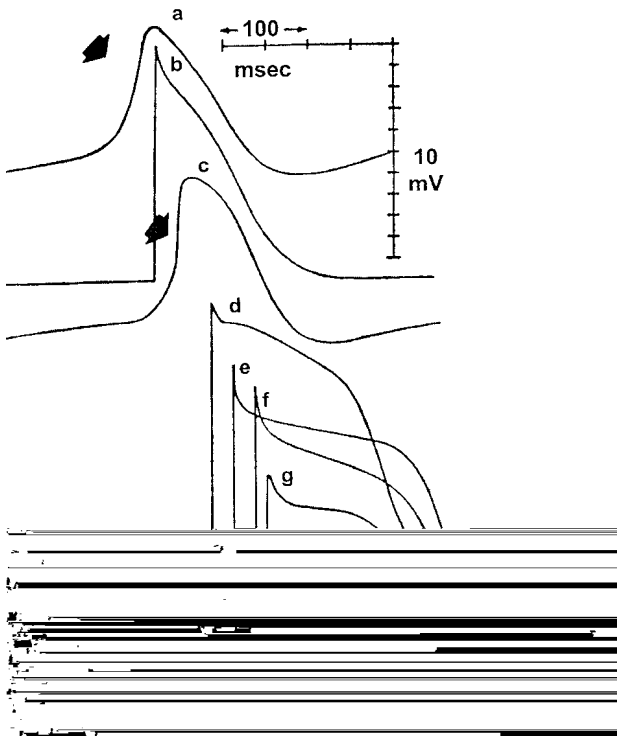


Fig 3. Comparison of action potentials recorded from different areas of the heart. (a) sinoatrial node; (b) atrium; (c) A-V node; (d) bundle of His; (e) Purkinje fiber in a false tendon; (f) terminal Purkinje fiber; (g) ventricular muscle fiber. (Data from Gadsby et al.¹⁶)

searches. All but one of the studies was published in *Anesthesiology*, *Anesthesia & Analgesia*, or *Regional Anesthesia and Pain Medicine*. The first publication appeared in 1981 and the most recent one appeared in 2000.

Study objectives and protocols varied considerably. The working heart was used in one study, and the Langendorff preparation was used in all other studies. Rabbit hearts were used in 8 of the studies, guinea pig hearts in 3 studies, and rat hearts in 2 studies. Bupivacaine was used in all studies, sometimes as the only drug (Table 1). The range of concentrations of some local anesthetics used in the studies varied considerably, e.g., bupivacaine 0.2 to 30 $\mu\text{g}/\text{mL}$; equivalent to plasma concentrations of approximately 2 to 60 $\mu\text{g}/\text{mL}$. At 1 $\mu\text{g}/\text{mL}$, bupivacaine is 90% bound in plasma with a marked decrease in percent bound as the plasma concentration exceeds 4 to 5 $\mu\text{g}/\text{mL}$.¹⁸ The conversion factor used to calculate approximate plasma concentration equivalent to bupivacaine concentration in protein-free perfusate was 90% for 0.2 $\mu\text{g}/\text{mL}$ and 50% for 30 $\mu\text{g}/\text{mL}$.

None of the conclusions reached in the isolated heart studies contradicts, to any significant degree, conclusions reached from other local anesthetic toxicity studies. The conclusions can be summarized as follows: (1) there is a positive correlation between the cardiotoxic potency of local anesthetics, lipid solubility, and nerve blocking potency; (2) the S(-) isomer of bupivacaine (levobupivacaine) is less cardiotoxic than the R(+) isomer; (3) the effects of bupivacaine on conduction in the heart promote induction of reentrant ventricular arrhythmias; (4) hyperkalemia enhances the cardiotoxicity of local anesthetics; (5) K^+_{ATP} channel openers, β -adrenergic agonists, and Ca^{++} channel blockers may have value in treating bupivacaine cardiotoxicity; and (6) rank order (from lowest to highest) of the cardiotoxic potency of local anes-

Fig 4. Ionic currents forming pacemaker action potentials (left) and ventricular action potentials (right) in the heart. Note that the initial upstroke of pacemaker potentials is due to calcium flux and the initial upstroke of ventricular action potentials is due to sodium flux. (Data from Block and Covino.¹)

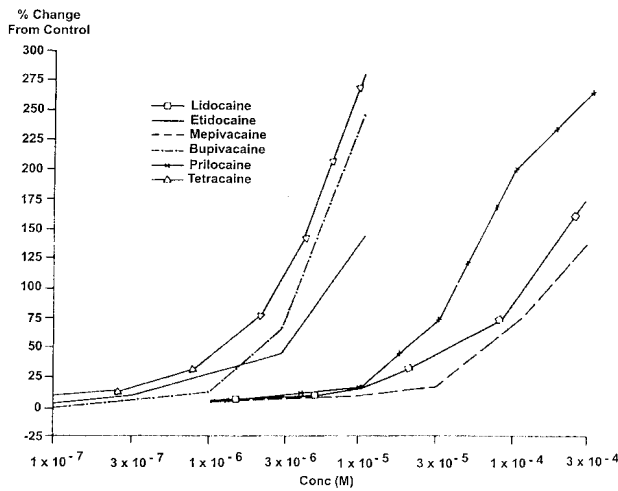


Fig 5. Effects of various local anesthetic agents on QRS duration. (Data from Ferrari and Opie.¹⁷)

thetics studied is prilocaine < lidocaine < mepivacaine << ropivacaine < levobupivacaine < racemic bupivacaine < R(+) bupivacaine < etidocaine < tetracaine.

Relative Toxicity

Lipid Solubility, Protein Binding, and Local Anesthetic Potency (n = 1)

Block and Covino¹ conducted the first systematic study of the effects of different local anesthetics on cardiac conduction and contractility in the intact mammalian heart in vitro (paced rabbit heart, Langendorff preparation) after the Albright editorial. The local anesthetics were grouped according to lipid solubility, protein binding, and potency and duration of action; prilocaine, lidocaine, mepivacaine versus bupivacaine, tetracaine, etidocaine. The grouping of the local anesthetics on the *a priori* criteria proved to correlate with grouping by effects on the heart based on all of the measures used in the study (intra-atrial conduction, A-V node conduction, intraventricular conduction, ventricular refractory period, and ventricular contractility) (Fig 5). Bupivacaine, tetracaine, and etidocaine groups were consistently more potent than local anesthetics in the other group on all measured variables. All of the agents produced a concentration-related depression of intra-atrial, A-V nodal, intraventricular conduction, and myocardial contractility. Within the high lipid solubility group, potency (on a molar basis) on all measured parameters was tetracaine > bupivacaine > etidocaine except for QT interval where bupivacaine > etidocaine < tetracaine (etidocaine = bupivacaine on myocardial contraction (dp/dt)). The conduction parameters varied in sen-

sitivity to the local anesthetics with QRS duration being most sensitive and A-V nodal conduction time being least.

The 3 less lipid-soluble local anesthetics were about 10 times less potent than the 3 most lipid-soluble ones in terms of detrimental effect on all measured parameters. For the less lipid-soluble drugs, the order of potency and sensitivity of the different parameters to effects of these local anesthetics was more variable and different from the highly lipid-soluble drugs. For example, QRS duration was least sensitive to prilocaine, the second most sensitive of the conduction-based parameters to mepivacaine and the most sensitive to lidocaine, as it was for all of the highly lipid-soluble local anesthetics. Noteworthy is that QT interval and contractility were generally quite resistant to the effects of all 6 local anesthetics.

Tetracaine was 30 times more potent in terms of negative inotropic action than lidocaine, whereas bupivacaine and etidocaine were 20 times more potent. In terms of nerve blocking action (50% decrease in C-fiber compound action potential amplitude of desheathed rabbit vagus nerve), tetracaine was shown by Gissen et al.¹⁹ to be approximately 25 times more potent than lidocaine, whereas bupivacaine and etidocaine were approximately 4 times more potent. Based on these findings, it was concluded that etidocaine and bupivacaine appear to be more cardiodepressant than lidocaine at equivalent anesthetic concentrations.

Bupivacaine Versus Lidocaine (n = 5)

Block and Covino¹ (see above; paced rabbit heart, Langendorff) reported that the rank order of sensitivity to bupivacaine, from highest to lowest, was

Table 1. Frequency of Drug Testing

Drug	No. of Studies
Local anesthetics	
Bupivacaine	13
Lidocaine	5
Levobupivacaine	3
Ropivacaine	2
R(+) bupivacaine	2
Mepivacaine	1
Prilocaine	1
Etidocaine	1
Tetracaine	1
β -adrenergic agonists	
Isoproterenol	1
K^+ ATP channel openers	
Pinacidil	1
Bimakalim	1
Cd^{++} channel blocking drugs	
Verapamil	1
Nimodipine	1

QRS duration (0.62 $\mu\text{g}/\text{mL}$) > intra-atrial conduction time (0.74 $\mu\text{g}/\text{mL}$) > His-Purkinje conduction time (0.87 $\mu\text{g}/\text{mL}$) > QT interval (1.36 $\mu\text{g}/\text{mL}$) > A-V nodal conduction time (0.89 $\mu\text{g}/\text{mL}$) > dp/dt (1.38 $\mu\text{g}/\text{mL}$). Conversely, the rank order for lidocaine was intra-atrial conduction time (6.56 $\mu\text{g}/\text{mL}$) > His-Purkinje conduction time (9.37 $\mu\text{g}/\text{mL}$) = QRS duration (9.37 $\mu\text{g}/\text{mL}$) > QT interval (11.24 $\mu\text{g}/\text{mL}$) > A-V nodal conduction time (26.4 $\mu\text{g}/\text{mL}$) > dp/dt (16.4 $\mu\text{g}/\text{mL}$). The numbers in parentheses are drug concentrations that produced 50% increase except for dp/dt (28% decrease) and A-V nodal conduction time (25% increase). Because of drug solubility limitations, 50% changes in dp/dt and A-V nodal conduction time were not determined. The potency ratio of bupivacaine to lidocaine was 15.1:1 for 50% increase in QRS duration and between 8.3:1 and 10.8:1 for 50% increase in the other 3 conduction measures.

Komai and Rusy² (see hyperkalemia section; spontaneously beating, working rat heart) reported that under normokalemia conditions, bupivacaine (1.25 to 30 $\mu\text{g}/\text{mL}$) and lidocaine (10 to 150 $\mu\text{g}/\text{mL}$) had little effect on mean aortic pressure. Bupivacaine was 14 times more potent than lidocaine with respect to 50% decrease in ventricular rate (median effective dose [ED₅₀] 7 $\mu\text{g}/\text{mL}$ bupivacaine v 100 $\mu\text{g}/\text{mL}$ lidocaine). The concentration ratio for slowing atrial rate was 6:1 (bupivacaine 20 $\mu\text{g}/\text{mL}$; lidocaine 120 $\mu\text{g}/\text{mL}$). The concentration of bupivacaine (6 $\mu\text{g}/\text{mL}$) and lidocaine (100 $\mu\text{g}/\text{mL}$) and potency ratio for 50% prolongation of PR interval were about the same as for 50% decrease in ventricular rate.

Tanz et al.³ (guinea pig heart, paced and unpaced, modified Langendorff) compared the effects of 0.3 and 3 $\mu\text{g}/\text{mL}$ bupivacaine to the effects of 10 and 30 $\mu\text{g}/\text{mL}$ of lidocaine on heart rate, df/dt, coronary flow, and myocardial oxygen consumption (MVO₂). df/dt was measured by connecting a thread attached to the epicardial apex to a force transducer. The lower concentration of both local anesthetics generally had little, if any, effects on any of the measured parameters. The higher concentrations had detrimental effects on all of the parameters. Heart rate was decreased by 53% by 3 $\mu\text{g}/\text{mL}$ bupivacaine and by 82% by 10 $\mu\text{g}/\text{mL}$ lidocaine. There was no significant difference between decrease in contractility (df/dt) produced by 3 $\mu\text{g}/\text{mL}$ bupivacaine (53%) and 30 $\mu\text{g}/\text{mL}$ lidocaine (40%). Decrease in MVO₂ was significantly different, 45% (bupivacaine) versus 18% lidocaine as was reduction in coronary flow, 42% (bupivacaine) versus 16% lidocaine. Arrhythmias were produced only by 3 $\mu\text{g}/\text{mL}$ bupivacaine (6 of 12 treated hearts). The most common arrhythmias were 2:1 heart block,

bigeminy, pulsus alternans, or trigeminy. Two preparations developed preventricular contractions at some time. Based on results from paced hearts, the investigators concluded that decreases in coronary flow and MVO₂ were secondary to depression of heart rate and df/dt. The potency ratio for depression of the measured values was greater than 10:1 for bupivacaine.

Pitkanen et al.⁴ (see below; rabbit heart, Langendorff, spontaneously beating and paced) compared the effects of 1, 6, and 13 $\mu\text{g}/\text{mL}$ bupivacaine and 6, 20, and 40 $\mu\text{g}/\text{mL}$ lidocaine. All hearts ceased functionality when perfused with 13.6 $\mu\text{g}/\text{mL}$ bupivacaine, and all hearts continued to function when perfused with 40 $\mu\text{g}/\text{mL}$ lidocaine. None of the hearts exposed to lidocaine had ECG conduction disturbances. All 6 hearts treated with 6 $\mu\text{g}/\text{mL}$ bupivacaine and with 13 $\mu\text{g}/\text{mL}$ bupivacaine showed ECG abnormalities. The nature of the abnormalities are described in the ropivacaine, bupivacaine, lidocaine section. All preparations treated with lidocaine could be electrically paced; 17% and 83% of the preparations exposed to 6 and 13 $\mu\text{g}/\text{mL}$ bupivacaine, respectively, could not be paced. The investigators concluded that 6 and 13 $\mu\text{g}/\text{mL}$, respectively, of bupivacaine were more cardiotoxic than 20 and 40 $\mu\text{g}/\text{mL}$, respectively, of lidocaine.

Mazoit et al.⁵ compared the pharmacokinetics and pharmacodynamics of lidocaine and bupivacaine in rabbit hearts (paced; Langendorff preparation). Lidocaine was delivered at 40 $\mu\text{g}/\text{mL}$ for 5 minutes then at 10 $\mu\text{g}/\text{mL}$ for 15 minutes. Bupivacaine was delivered at 4 $\mu\text{g}/\text{mL}$ for 5 minutes and at 1 $\mu\text{g}/\text{mL}$ for 15 minutes. Four hearts received bupivacaine at concentrations equal to the lidocaine concentrations. In 3 of these 4 hearts, electrical activity disappeared within 2 to 4 minutes after the start of bupivacaine infusion; the fourth heart had transient ventricular tachycardia.

Lidocaine and bupivacaine exhibited almost similar myocardial uptake and disposition kinetics. Lidocaine increased QRS duration by 25.8 ± 5.1 msec and bupivacaine increased QRS duration by 386 ± 147 msec.

Ropivacaine Versus Bupivacaine (n = 1)

Pitkanen et al.⁴ compared the inotropic and chronotropic effects of ropivacaine, lidocaine, and bupivacaine in the spontaneously beating and electrically paced rabbit heart (Langendorff model). Results of bupivacaine versus lidocaine comparisons are reported earlier in this review. There were no significant differences between reduction in oxygen consumption and pulmonary artery flow by bupivacaine and ropivacaine (1, 6, or 13 $\mu\text{g}/\text{mL}$).

None of the hearts “survived” the planned 30-minute exposure to 13 $\mu\text{g}/\text{mL}$ bupivacaine, only 1 of 6 “survived” 30-minute exposure to 6 $\mu\text{g}/\text{mL}$ bupivacaine, and 1 of 6 did not “survive” the planned 30-minute exposure to 13 $\mu\text{g}/\text{mL}$ ropivacaine. Both local anesthetics increased the voltage required to pace the heart via the atria. However 2 times as much ropivacaine (13 $\mu\text{g}/\text{mL}$) as bupivacaine (6 $\mu\text{g}/\text{mL}$) were required to prevent electrical pacing of half of the hearts. With 13 $\mu\text{g}/\text{mL}$ bupivacaine, none of the hearts could be paced. Bupivacaine (13 $\mu\text{g}/\text{mL}$) caused the greatest decrease in heart rate ($64\% \pm 4\%$ after 5 minutes of exposure). At 5 minutes, there was no significant difference between the magnitude of depression produced by 6 $\mu\text{g}/\text{mL}$ bupivacaine and 13 $\mu\text{g}/\text{mL}$ ropivacaine. Too, dp/dt was equally depressed by 6 $\mu\text{g}/\text{mL}$ bupivacaine and 13 $\mu\text{g}/\text{mL}$ ropivacaine ($72\% \pm 8\%$ v $71\% \pm 4\%$, respectively). Similar degrees of depression of left ventricular systolic pressure were produced by 6 $\mu\text{g}/\text{mL}$ bupivacaine and 13 $\mu\text{g}/\text{mL}$ ropivacaine ($62\% \pm 14\%$ v $61\% \pm 5\%$, respectively).

ECG conduction disturbances were produced in 2 of 6 hearts perfused with 6 $\mu\text{g}/\text{mL}$ ropivacaine, in 6 of 6 hearts perfused with 6 $\mu\text{g}/\text{mL}$ bupivacaine; 5 of 6, and 6 of 6 hearts exposed to 13 $\mu\text{g}/\text{mL}$ ropivacaine and bupivacaine, respectively, had ECG conduction disturbances.

Ropivacaine, Levobupivacaine, and Bupivacaine (n = 1)

Mazoit et al.⁶ compared the effects of equal concentrations of these 3 drugs on QRS duration and cardiac rhythm in electrically paced rabbit hearts (Langendorff preparation). The pharmacokinetic properties of the drugs in this model were also studied. Drug was infused into the inflow perfusate at 20 $\mu\text{mol}/\text{L}$ for 5 minutes and at 5 $\mu\text{mol}/\text{L}$ during 15 minutes (corresponding to 0.6 $\mu\text{mol}/\text{L}/\text{min}$ and 0.15 $\mu\text{mol}/\text{L}/\text{min}$ [approximately], 0.17 $\mu\text{g}/\text{mL}/\text{min}$, 0.04 $\mu\text{g}/\text{mL}/\text{min}$). Concentrations of local anesthetic in the perfusate exiting the heart were measured. Bupivacaine produced significantly greater prolongation of the QRS duration than did levobupivacaine and ropivacaine (ratio of 1:0.4:0.3, respectively). The estimated free concentration of bupivacaine, levobupivacaine, and ropivacaine necessary to double the basal QRS duration at 210 beats/min was 2.4, 7.2, and 14.4 $\mu\text{g}/\text{mL}$ for racemic bupivacaine, levobupivacaine, and ropivacaine. Arrhythmias were produced in 6 of 7 hearts by bupivacaine and only 3 of 7 hearts exposed to ropivacaine and in 3 of 7 hearts exposed to levobupivacaine. QRS widening showed marked rate depen-

dence. The rate dependence of QRS widening (slope of the QRS duration–heart rate relation) was significantly different between the 3 drugs, with an approximate ratio of 1:0.5:0.02 for racemic bupivacaine, levobupivacaine, and ropivacaine, respectively. Bupivacaine did not accumulate in the myocardium, and thus the toxic effect of long-acting local anesthetics is not a consequence of drug accumulation in heart tissue.

Arrhythmias

Two studies have been performed using isolated hearts with the specific goal of understanding arrhythmias produced by bupivacaine.^{7,8} In both studies, the rabbit heart (Langendorff preparation) was used. The conclusion from each study was that bupivacaine promotes induction of reentrant ventricular dysrhythmias.

Lacombe et al.⁷ infused hearts with 0.3, 1.5, and 3.0 $\mu\text{g}/\text{mL}$ bupivacaine. A statistically significant increase in PR and A-V interval duration occurred at both 1.5 and 3.0 $\mu\text{g}/\text{mL}$ bupivacaine. Duration of the P wave, AA interval, QTc interval, and QRS increased. Pacing thresholds were increased at 1.5 and 3.0 $\mu\text{g}/\text{mL}$ for the right atrium and left ventricle. No spontaneous tachyarrhythmia occurred or could be induced. Spontaneous A-V block occurred in 2 hearts (1 with 1.5 $\mu\text{g}/\text{mL}$, 1 with 3.0 $\mu\text{g}/\text{mL}$ bupivacaine). Based on the results of the study, Lacombe et al.⁷ concluded that automaticity was unchanged by bupivacaine. On the other hand, bupivacaine markedly impaired conduction of the electrical impulse, and this depression affects the different cardiac structures in heterogeneous fashion. They concluded that the mechanism involved in bupivacaine-induced tachyarrhythmias was dispersion of conduction and dispersion of refractoriness. Lack of spontaneously occurring reentrant tachyarrhythmias and failure to induce them in the presence of bupivacaine was explained by the absence of an anatomic substrate in the form of scar tissue.

de LaCoussaye et al.⁸ used high-resolution ventricular mapping to study the effects of 0.2, 0.5, 1.0, and 5 $\mu\text{g}/\text{mL}$ bupivacaine. Their hypothesis was that bupivacaine promotes the occurrence of reentrant ventricular dysrhythmias. Parameters measured included spontaneous sinus cycle length, ventricular effective refractory period, and longitudinal and transverse ventricular conduction velocity. During administration of 5.0 $\mu\text{g}/\text{mL}$ bupivacaine, spontaneous ventricular tachycardia occurred in 3 of 5 hearts, but in no hearts exposed to the 0.2, 0.5, or 1 $\mu\text{g}/\text{mL}$ bupivacaine. Ventricular fibrillation was induced by programmed electrical stimulation in all hearts during the

control. During administration of 0.2 $\mu\text{g}/\text{mL}$ bupivacaine, ventricular fibrillation was induced in 3 of 5 hearts. In the remaining 2 hearts, only sustained monomorphic ventricular tachycardia and nonsustained monomorphic ventricular tachycardia could be induced by programmed electrical stimulation. During administration of 0.5 $\mu\text{g}/\text{mL}$ bupivacaine, the spectrum of dysrhythmias induced by programmed electrical stimulation was completely different. In 1 of 5 hearts, ventricular fibrillation was still inducible, but in the remaining 4 hearts, sustained monomorphic ventricular tachycardias were the only dysrhythmias induced. At 1.0 $\mu\text{g}/\text{mL}$ bupivacaine, pacing induced a sustained polymorphic ventricular tachycardia in 1 of 5 hearts and in 2 of 5 hearts, nonsustained polymorphic ventricular tachycardia. During administration of 5.0 $\mu\text{g}/\text{mL}$ bupivacaine, premature electrical stimulation could not be tested because the hearts became inexcitable.

Spontaneous sinus cycle length was not modified at 0.2, 0.5, and 1.0 $\mu\text{g}/\text{mL}$. At 5.0 $\mu\text{g}/\text{mL}$, bupivacaine induced a marked and significant bradycardia.

In addition to using the standard Langendorff preparation, de La Coussaye et al.⁸ also studied the effects of bupivacaine using the frozen heart model. In this model, the right ventricle, interventricular septum, and the endocardial and intramural layers of the free wall of the left ventricle are frozen. This model allows precise analysis of the complete sequence of activation of the left ventricular epicardium during regular pacing and during the initiation of ventricular dysrhythmias. No spontaneous ventricular arrhythmias were observed in the frozen heart, but one heart had sustained monomorphic ventricular tachycardia after rapid pacing during control. At 0.2 $\mu\text{g}/\text{mL}$ bupivacaine pacing produced sustained monomorphic ventricular tachycardia in 3 of 4 hearts. Epicardial mapping demonstrated these were due to reentry. Rapid pacing produced no ventricular arrhythmias at bupivacaine concentrations greater than 0.2 $\mu\text{g}/\text{mL}$. Ventricular effective refractory period (VERP) was slightly, but significantly, prolonged by bupivacaine. There was a significant dose-dependent impairment of both longitudinal and transverse ventricular conduction velocities with no change in anisotropic ratio.

Results of the study by de La Coussaye et al.⁸ demonstrated that bupivacaine decreases ventricular conduction velocity and induces arcs of functional ventricular conduction block in a dose-dependent and use-dependent fashion. Epicardial mapping showed that inducible ventricular tachycardias were based on re-entry. Noteworthy is that spontaneous ventricular dysrhythmias occurred

only at the largest concentration of bupivacaine (5 $\mu\text{g}/\text{mL}$).

Stereoselectivity

The isolated heart was used in 2 studies addressing questions regarding stereoselective influences on cardiac toxicity of local anesthetics. The objective of one study was to determine if there is a stereoselective affect of bupivacaine isomers on A-V conduction.⁹ The objective of the second study was to compare the pharmacokinetics and pharmacodynamics of bupivacaine enantiomers.¹⁰ Results from both studies demonstrated that the S(-) isomer was significantly less detrimental to the heart than R(+) bupivacaine.

Graf et al.⁹ (guinea pig heart, Langendorff preparation) found the only significant differences between racemic and (+) and (-) forms of bupivacaine were on A-V conduction. Each heart was perfused with 0.5, 1, 5, and 10 μm of each drug. Atrial heart rates, coronary flow, O_2 extraction, and isovolemic systolic left ventricular pressure (LVP) were depressed to a similar extent by all 3 test substances. A-V conduction was more significantly prolonged by (+) bupivacaine than by (-) or racemic bupivacaine and (+) bupivacaine (10 μm ; 2.8 $\mu\text{g}/\text{mL}$) produced significantly more second degree A-V conduction blocks than either of the other 2 drugs. At 10 μm , A-V time was $54\% \pm 6\%$ longer with the (+) isomer and $30\% \pm 4\%$ longer with the racemate than with the (-) isomer. Type II (Mobitz) second degree A-V conduction block (normal A-V conduction delay with an occasional nonventricular-conduction atrial beat) occurred in 9 of 12 hearts, 1 of 12 hearts, and 0 of 12 hearts treated with (+), racemic, and (-) bupivacaine, respectively. Second-degree type I (Wenckebach [progressive lengthening of the A-V interval leading to nonventricular-conduction atrial beat]) A-V dissociation occurred in 1 of 12, 3 of 12, and 1 of 12 hearts treated with (+), racemic, and (-) bupivacaine (Fig 6). The investigators concluded that the bupivacaine isomers probably have differential effects on one or more ion-specific channels regulating A-V conduction. Other measured direct cardiac effects of bupivacaine appear to be independent of the isomeric form.

Because atrial slowing was similar for the different test substances, sinus rate influence on A-V conduction does not appear to be a factor in the A-V conduction differences exhibited by the isomeric form.⁹

Graf et al.⁹ pointed out that it appears to be an anomaly that bupivacaine delays only A-V conduction time in a stereoselective way because A-V delay is thought to be mediated primarily through

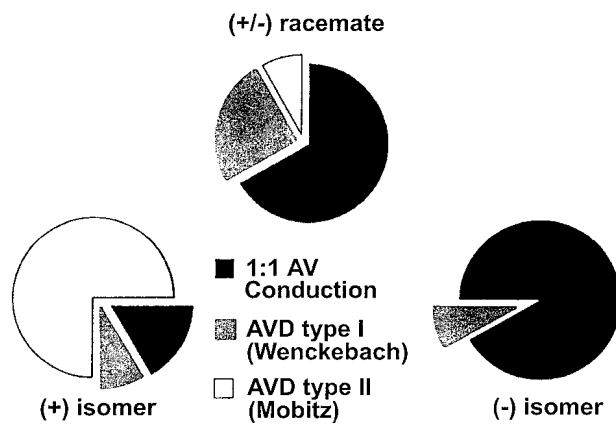


Fig 6. Effects of 10 μmo (+), (-), and (\pm) bupivacaine on second-degree atrioventricular (A-V) dissociation of the Wenckebach (type I) and Mobitz (type II) classification in 12 isolated guinea pig hearts. Each pie chart comprises 12 hearts. Significance of A-V dissociation is indicated in the text. Third-degree A-V dissociation and dysrhythmias and other than A-V dissociation were not observed. Atrioventricular dissociation reverted to sinus rhythm without A-V prolongation during the drug-free washout periods. (Data from Graf et al.⁹)

L-type Ca^{++} channels. Other variables measured that depend on L-type Ca^{++} channels were not stereoselectively altered.

The study by Mazoit et al.¹⁰ with paced rabbit hearts (Langendorff model) generally agrees with the pharmacodynamic findings of Graf et al.⁹ This group of investigators compared the pharmacokinetic and pharmacodynamic properties of racemic, (+), and (-) bupivacaine. QRS duration and pharmacokinetic modeling based on drug concentrations in perfusate exiting the heart were used as outcome measures. Local anesthetic infusion rate was 160 $\mu\text{g}/\text{min}$ for 5 minutes followed by 40 $\mu\text{g}/\text{min}$ for 15 minutes. These rates corresponded to 8 and 2 $\mu\text{g}/\text{mL}$ of local anesthetic in buffer. S(-) bupivacaine produced markedly less QRS widening and "severe" A-V conduction blocks, ventricular fibrillation, and asystole, than did (+) or racemic bupivacaine. Pharmacokinetic parameters (update and disposition kinetics) were similar for the 3 different drugs. However, the pharmacokinetic modeling indicated differences between (-) bupivacaine, and (+) and racemic bupivacaine with respect to intracardiac distribution. At steady state, one third of S(-) was distributed in the peripheral compartment; one fifth of S(+) and racemic bupivacaine was in this compartment. The differences between the effects of the drugs on A-V conduction were attributed to pharmacodynamic, not pharmacokinetic factors.

Hyperkalemia and Local Anesthetic Cardiotoxicity

Komai and Rusy² compared the cardiotoxicity of bupivacaine (1.25 to 7.5 $\mu\text{g}/\text{mL}$) and lidocaine (10 to 60 $\mu\text{g}/\text{mL}$) in the presence of normokalemia (5.9 mEq/L) and hyperkalemia (8 and 9.0 mEq/L) in the spontaneously beating, working rat heart. Their studies were preceded by other in vitro studies demonstrating that the cardiotoxicity of lidocaine is dependent on the concentration of extracellular K^+ .²⁰⁻²²

Hyperkalemia potentiated the negative effects of lidocaine and bupivacaine on heart rate and conductivity. Ventricular rate and A-V conduction (PR interval) were about equally sensitive to the local anesthetics in the presence of normokalemia, and hyperkalemia about equally affected their sensitivity. The ratio of the lidocaine to bupivacaine concentrations producing the same endpoint was highest for PR interval doubling (ratio = 17; normokalemic) and lowest for decreasing atrial rate by 50% (ratio = 6; normokalemic). At the highest concentrations tested, lidocaine (40 $\mu\text{g}/\text{mL}$) and bupivacaine (5.0 $\mu\text{g}/\text{mL}$) had little effect on mean aortic pressure in the presence of normal or elevated concentrations of K^+ (9.0 mEq/L). Komai and Rusy² concluded that bupivacaine, by virtue of a more potent effect on A-V conduction, has higher cardiotoxicity than lidocaine. Hyperkalemia selectively potentiated A-V block by relatively low concentrations (up to 5.0 $\mu\text{g}/\text{mL}$) of bupivacaine, but not by relatively low concentrations of lidocaine.

Treating Cardiotoxic Effects

Isoproterenol

Based on the demonstrated effects of bupivacaine on electrical conduction in the heart, Lacombe et al.¹¹ tested the hypothesis that isoproterenol, a catecholamine with predominate β -adrenergic properties known to facilitate conduction, might antagonize the toxic effects of bupivacaine. Their experiments were performed using isolated rabbit hearts and the Langendorff model. The concentrations of bupivacaine and isoproterenol used in the study were 1 and 1 to 2 $\mu\text{g}/\text{mL}$, respectively. Bupivacaine increased electrocardiographic intervals (P waves [30%], QRS complex [45%], PR [43%], A-V [45%], and QTc interval [13%]) and refractory periods of the myocardium (321%) and atrioventricular junction (45%), as well as the Wenckebach cycle (61% anterograde, 38% retrograde) and pacing thresholds (ventricular 270%, atrial 458%). Isoproterenol returned all of the values to levels not significantly different from baseline except the PR

interval, which was only partially corrected. No tachyarrhythmias occurred except for sinus tachycardia after isoproterenol. The effect of isoproterenol was observed despite the continued administration of bupivacaine. This suggests the sites of action of isoproterenol and bupivacaine differ.

Lacombe et al.¹¹ postulated that isoproterenol could exert its action by opposing either the calcium channel blocking effects of bupivacaine or its sodium blocking effects. They rationalized that isoproterenol promoted formation of intracellular cyclic adenosine monophosphate (cAMP). The subsequent activation of cAMP-dependent phosphorylases allows calcium channels to be open longer or in greater numbers, thus “replacing” in part the blocked sodium channels. Calcium dependent action potentials may be produced in cells normally dependent on fast sodium channels.

Ca⁺⁺ Channel Blocking Drugs

Adsan et al.¹² investigated whether pretreatment with either verapamil or nimodipine protects against bupivacaine cardiotoxicity. Based on evidence that calcium channel blockers may inhibit not only calcium channels, but other ion channels as well, such as fast sodium channels²³ and potassium channels,²⁴ Adsan et al.¹² rationalized there may be drug interaction between calcium channel blockers and bupivacaine which blocks sodium channels²⁵ and slow calcium-dependent channels,^{26,27} and potassium channels.²⁴

In prior *in vivo* studies, verapamil and diltiazem enhanced bupivacaine cardiotoxicity.²⁸⁻³⁰ On the other hand, nimodipine and nifedipine reduced bupivacaine cardiotoxicity.^{31,32} Nifedipine potentiated the negative inotropic effect of bupivacaine on guinea pig myocardium *in vitro*.^{33,34} The data indicate the interaction between bupivacaine and calcium channel blocking drugs varies depending on the drug. Adsan et al.¹² found that *in vivo* (pentobarbital-anesthetized rats) pretreatment with verapamil (150 µg/kg intravenous [IV]) or nimodipine (200 µg/kg IV) slightly increased the bupivacaine LD₅₀ (bupivacaine 3.08 [2.82 to 3.37] mg/kg, bupivacaine + verapamil 3.58 [3.19 to 4.01] mg/kg, bupivacaine + nimodipine 3.50 [3.16 to 3.87] mg/kg; 95% confidence limits in parentheses). The pretreatments also shifted the bupivacaine dose-response curve to the right.

A modified Langendorff preparation (rat heart) was used by Adsan et al.¹² for their *in vitro* studies (no left ventricular balloon, contractile force determined by attaching suture between the apex of the heart and a strain gauge). The arrhythmic dose of bupivacaine was determined by injecting increasing

amounts of bupivacaine as boluses with the perfusate. Bradycardia, premature ventricular contractions, and ventricular tachycardia were the most frequent arrhythmias. Either verapamil (10⁻⁷ mol/L) or nimodipine (5 × 10⁻¹¹ mol/L) was added to the perfusion solution, then the previously determined arrhythmogenic dose of bupivacaine was injected into the perfusate. Verapamil alone caused ventricular fibrillation in 2 of 10 isolated hearts. Verapamil pretreatment did not modify the effects of bupivacaine on heart rate, contractile force, and coronary perfusion pressure, nor did it modify the incidence of bupivacaine-induced arrhythmias. Nimodipine reduced the negative chronotropic and incidence of arrhythmias produced by bupivacaine, but did not modify bupivacaine-induced decrease in coronary perfusion pressure and contractile force.

No theoretical explanation was presented to explain the *in vitro* findings. Results of this and other studies do not support the use of Ca⁺⁺ channel antagonists as drugs of choice for treating bupivacaine cardiotoxicity.

K⁺_{ATP} channel openers

Boban et al.¹³ used the isolated spontaneously beating guinea pig heart (Langendorff preparation) to test the hypothesis that K⁺_{ATP} channel openers attenuate bupivacaine-induced atrioventricular block. The rationale for the study was evidence that bupivacaine prolongs cardiac action potential duration by blocking delayed rectifier³⁵ and transient outward³⁶ K⁺ currents and prolongs action potential duration. ATP-sensitive K⁺ channel openers are known to accelerate repolarization in cardiac tissue.³⁷⁻⁴⁰

For this study, Boban et al.¹³ infused a constant concentration (1.2 µg/mL) of bupivacaine to induce first degree A-V block or a concentration (4.3 to 7.2 µg/mL) to induce second-degree block. Variables monitored were heart rate, A-V conduction time, LVP, coronary flow, and myocardial oxygen extraction. During stable block, hearts were perfused with pinacidil (10, 20, and 30 µm) or bimakelin (0.1, 1, and 2 µm). The primary beneficial effect of the K⁺_{ATP} channel openers was to attenuate prolongation of A-V conduction produced by bupivacaine. Second-degree A-V conduction block was converted to first-degree A-V block by each K⁺_{ATP} channel opener.

Bupivacaine, 1.2 µg/mL, prolonged A-V conduction by 53% and decreased heart rate by 13%, LVP by 26%, coronary flow by 6%, and percent O₂ extracted by 7%. Both K⁺_{ATP} channel blockers enhanced the depressant effect of bupivacaine on LVP and O₂ extraction, markedly increased coronary

flow, attenuated the prolongation of A-V conduction, and did not change heart rate. The investigators cautioned that treatment of bupivacaine-induced cardiotoxicity with K^+ _{ATP} channel openers, although improving A-V conduction, might worsen cardiac depression and cause excessive coronary vasodilatation.

Conclusion

The primary conclusion from studies of the cardiotoxicity of local anesthetics using the intact, isolated mammalian heart in vitro is that highly lipid-soluble, extensively protein-bound, highly potent local anesthetics (e.g., tetracaine, bupivacaine, etidocaine) are much more cardiotoxic than are less lipid-soluble, protein-bound, and potent local anesthetics (e.g., lidocaine, prilocaine, mepivacaine). Bupivacaine has a potent depressant effect on electrical conduction in the heart primarily via an action on voltage-gated sodium channels that generally govern the initial rapid depolarization (phase 0) of myocytes. The S(−) form is less cardiotoxic than the R(+) form of racemate. The inotropic effects of bupivacaine favor the induction of re-entrant arrhythmias. Bupivacaine actions other than on voltage-gated sodium channels probably contribute to the dose-dependent cardiotoxic effect of this local anesthetic.

References

- Block A, Covino BG. Effect of local anesthetic agents on cardiac conduction and contractility. *Reg Anesth Pain Med* 1981;6:55-61.
- Komai H, Rusy BF. Effects of bupivacaine and lidocaine on AV conduction in the isolated rat heart: Modification by hyperkalemia. *Anesthesiology* 1981;3:281-285.
- Tanz RD, Heskett T, Loehning RW, Fairfax CA. Comparative cardiotoxicity of bupivacaine and lidocaine in the isolated perfused mammalian heart. *Anesth Analg* 1984;63:549-556.
- Pitkanen M, Feldman HS, Authur GR, Covino BG. Chronotropic and inotropic effects of ropivacaine, bupivacaine, and lidocaine in the spontaneously beating and electrically paced isolated, perfused rabbit heart. *Reg Anesth Pain Med* 1992;17:183-192.
- Mazoit JX, Orhant EE, Boico O, Kantelip J-P, Samii K. Myocardial uptake of bupivacaine: I. Pharmacokinetics and pharmacodynamics of lidocaine and bupivacaine in the isolated perfused rabbit heart. *Anesth Analg* 1993;77:469-476.
- Mazoit JX, Decaux A, Bouaziz H, Edouard A. Comparative ventricular electrophysiologic effect of racemic bupivacaine, levobupivacaine and ropivacaine on the isolated rabbit heart. *Anesthesiology* 2000;93:784-792.
- Lacombe P, Blaise G, Loulmet D, Hollmann C. Electrophysiologic effects of bupivacaine in the isolated rabbit heart. *Anesth Analg* 1991;72:62-69.
- de La Coussaye JE, Brugada J, Allesie MA. Electrophysiologic and arrhythmogenic effects of bupivacaine. *Anesthesiology* 1992;77:132-141.
- Graf BM, Martin E, Bosnjak ZJ, Stowe DF. Stereospecific effect of bupivacaine isomers on atrioventricular conduction in the isolated perfused guinea pig heart. *Anesthesiology* 1997;86:410-419.
- Mazoit JX, Boico O, Samii K. Myocardial uptake of bupivacaine: II. Pharmacokinetics and pharmacodynamics of bupivacaine enantiomers in the isolated perfused rabbit heart. *Anesth Analg* 1993;77:477-482.
- Lacombe P, Blaise G, Hollmann C, Tanguay M, Loulmet D. Isoproterenol corrects the effects of bupivacaine on the electrophysiologic properties of the isolated rabbit heart. *Anesth Analg* 1991;72:70-74.
- Adsan H, Tulunay M, Onaran O. The effects of verapamil and nimodipine on bupivacaine-induced cardiotoxicity in rats: An in vivo and in vitro study. *Anesth Analg* 1998;86:818-824.
- Boban M, Stowe DF, Gross GJ, Pieper GM, Kampine JP, Bosnjak ZJ. Potassium channel openers attenuate atrioventricular block by bupivacaine in isolated hearts. *Anesth Analg* 1993;76:1259-1265.
- Albright GA. Cardiac arrest following regional anesthesia with etidocaine or bupivacaine. *Anesthesiology* 1979;51:285-287.
- Scher AM. The electrocardiogram. In: Patton HD, Fuchs AF, Hille B, Scher AM, Steiner R, eds. *Textbook of Physiology: Circulation, Respiration, Body Fluids, Metabolism, and Endocrinology*, 21st ed. Philadelphia, PA: Saunders; 1989:796-819.
- Gadsby DC, Karagueuzian HS, Wit AL. Normal and abnormal electrical activity in cardiac cells. In: Mandel WJ ed. *Cardiac Arrhythmias: Their Mechanisms, Diagnosis, and Management*, 3rd ed. Philadelphia, PA: Lippincott; 1995:55-87.
- Ferrari R, Opie LH. *Atlas of the Myocardium*. New York, NY: Raven; 1992.
- Tucker GT, Mather LE. Pharmacology of local anesthetic agents. Pharmacokinetics of local anesthetic agents. *Br J Anaesth* 1975;47:213-224.
- Gissen AJ, Covino BG, Gregus J. Differential sensitivity of mammalian nerve fibers to local anesthetic agents. *Anesthesiology* 1981;53:467-474.
- Singh BN, Vaughan Williams EM. Effect of altering potassium concentration on the action of lidocaine and diphenylhydantoin on rabbit atrial and ventricular muscle. *Circ Res* 1971;29:286-295.
- Parameswaran R, Goldberg H. Effects of lidocaine on the sinus node and sino-atrial conduction. *Circulation* 1973;48:109 (suppl IV).
- Saito S, Chen CM, Buchanan J Jr, Gettes LS, Lynch MR. Steady state and time-dependent slowing of conduction in canine hearts. Effects of potassium and lidocaine. *Circ Res* 1978;42:246-254.
- Yatani A, Brown AM. The calcium channel blocker, nitrendipine, blocks sodium channels in neonatal rat cardiac myocytes. *Circ Res* 1986;57:868-875.
- Gotoh Y, Imaizumi Y, Watanabe M, Shibata EF, Clark

- RB, Giles WR. Inhibition of transient outward K^+ current by DHP Ca^{2+} antagonists and agonists in rabbit cardiac myocytes. *Am J Physiol* 1991;260:H1737-1742.
25. Clarkson CW, Handeghem LM. Mechanism for bupivacaine depression of cardiac conduction: Fast block of sodium channels during the action potential with slow recovery from block during diastole. *Anesthesiology* 1985;62:396-405.
 26. Coyle DE, Sperelakis N. Bupivacaine and lidocaine blockage of calcium-mediated slow action potentials in guinea pig ventricular muscle. *J Pharmacol Exp Ther* 1987;242:1001-1005.
 27. Sanchez-Chapula J. Effects of bupivacaine on membrane currents of guinea pig ventricular myocytes. *Eur J Pharmacol* 1988;156:303-308.
 28. Edouard AR, Berdeaux A, Ahmad R, Samii K. Cardiovascular interactions of local anesthetics and calcium entry blockers in conscious dogs. *Reg Anesth Pain Med* 1991;16:95-100.
 29. Finegan BA, Whitting RW, Tam YK, Clanachan AS. Enhancement of bupivacaine toxicity by diltiazem in anaesthetized dogs. *Br J Anaesth* 1992;69:492-497.
 30. Tallman RD, Rosenblatt RM, Weaver JM, Wang Y. Verapamil increases the toxicity of local anesthetics. *J Clin Pharmacol* 1988;28:317-322.
 31. Matsuda F, Kinney WW, Wright W, Kamban R. Nicardipine reduces the cardiorespiratory toxicity of intravenously administered bupivacaine in rats. *Can J Anaesth* 1990;37:920-923.
 32. Hyman SA, Kinney WVV, Horn JL. Nimodipine reduces the toxicity of intravenous bupivacaine in rats. *Anesth Analg* 1992;74:851-855.
 33. Herzig S, Ruhnke L, Wulf H. Functional interaction between local anaesthetics and calcium antagonists in guinea pig myocardium. I. Cardiodepressant effects in isolated organs. *Br J Anaesth* 1994;73:357-363.
 34. Wulf H, Gödicke J, Herzig S. Functional interaction between local anaesthetics and calcium antagonists in guinea pig myocardium. II. Electrophysiological studies with bupivacaine and nifedipine. *Br J Anaesth* 1994;73:364-370.
 35. Courtney KR, Kendig JJ. Bupivacaine is an effective potassium channel blocker in heart. *Biochim Biophys Acta* 1988;939:163-166.
 36. Castle NA. Bupivacaine inhibits the transient outward K^+ current but not the inward rectifier in rat ventricular myocytes. *J Pharmacol Exp Ther* 1990;255:1038-1064.
 37. Smallwood JK, Steinberg MI. Cardiac electrophysiological effects of pinacidil and related pyridylcyanoguanidines: relationship to antihypertensive activity. *J Cardiovasc Pharmacol* 1988;12:102-109.
 38. Hirai S, Kotake H, Kurata Y, Hisatome I, Hasegawa J, Mashiba H. Effects of pinacidil on the electrophysiological properties in guinea-pig papillary muscle and rabbit sino-atrial node. *J Pharm Pharmacol* 1990;42:339-343.
 39. Gautier P, Bertrand JP, Guiraudou P. Effects of SR 44866, potassium channel opener, on action potentials of rabbit, guinea pig, and human heart fibers. *J Cardiovasc Pharmacol* 1991;17:692-700.
 40. Findlay I, Deroubaix E, Guiraudou P, Coraboeuf E. Effects of activation of ATP-sensitive K^+ channels in mammalian ventricular myocytes. *Am J Physiol* 1989;257:H1551-1559.