Electrophysiologic and Arrhythmogenic Effects of Bupivacaine

A Study with High-resolution Ventricular Epicardial Mapping in Rabbit Hearts

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It has been shown that administration of toxic doses of bupivacaine may induce ventricular dysrhythmias. However, the mechanism of these dysrhythmias is still unknown. The present study was designed to test the hypothesis that bupivacaine facilitates the occurrence of reentrant ventricular dysrhythmias. High-resolution ventricular epicardial mapping was used to study the effects of 0.2, 0.5, 1.0, and 5.0 µg/ml bupivacaine in 11 Langendorff-perfused rabbit hearts. Five hearts were kept intact (intact heart). In six other hearts, a thin layer of epicardium was obtained by an endocardial cryotechnique (frozen heart). Bupivacaine induced ventricular dysrhythmias in 3 of 5 intact hearts at 5.0 µg/ml. In 3 of 6 frozen hearts, 0.2 µg/ml bupivacaine facilitated the induction of ventricular tachycardia by programmed electrical stimulation. Epicardial mapping showed that all tachycardias were based on reentry of the impulse around an arc of functional conduction block. Moreover, bupivacaine significantly prolonged the ventricular effective refractory period and slowed longitudinal and transverse conduction velocity in a dose- and use-dependent manner. It is concluded that bupivacaine facilitates induction of reentrant ventricular dysrhythmias in isolated rabbit heart. (Key words: Anesthetics, local: bupivacaine; cardiotoxicity. Heart: electrophysiology; reentry; ventricular dysrhythmias; ventricular mapping.)

LARGE DOSES OF BUPIVACAINE are known to induce cardiovascular collapse and/or ventricular dysrhythmias.[1,2] It is well established in isolated preparations that bupivacaine decreases the fast inward sodium current and therefore the maximum upstroke velocity ($\dot{V}_{max}$) of ventricular and Purkinje action potentials.[3–5] Because ventricular conduction velocity is correlated with the $\dot{V}_{max}$,[6] it is postulated that bupivacaine is responsible for the slowing of ventricular conduction velocities.[6] Moller and Covino[5] demonstrated in rabbits that bupivacaine decreases $\dot{V}_{max}$, depolarizes the maximum diastolic potential in Purkinje fibers, and induces block of conduction between Purkinje fibers and ventricular muscle. These authors[5] and others[2–4,7] postulated that bupivacaine can

induce ventricular dysrhythmias by reentry. However, no direct evidence of ventricular reentry has yet been provided. Conversely, two reports[8,9] and more recently a report by Bernards and Artru[10] suggested that bupivacaine-induced ventricular dysrhythmias were at least partially mediated by the autonomic nervous system.

The aims of this study were 1) to investigate the electrophysiologic effects of bupivacaine, 2) to evaluate whether bupivacaine facilitates the occurrence of ventricular dysrhythmias in isolated hearts, and 3) to reveal the mechanisms of bupivacaine-induced ventricular dysrhythmias by using high-resolution epicardial mapping in rabbit hearts.

Materials and Methods

HEART PREPARATION

The experimental protocol followed the guiding principles of the American Physiological Society and was approved by the Animal Study Committee of the University of Limburg. Eleven Flemish rabbits weighing between 3.25 and 3.8 kg were used in this study. After anticoagulation with heparin (1,000 IU), the animals were killed by cervical dislocation. The thorax was opened by a midsternal incision, and the heart was rapidly removed and placed in cold perfusion fluid (10° C). The aorta was cannulated, and the heart was connected to a Langendorff perfusion system. The coronary arteries were perfused with a pressure of 50 mmHg, resulting in a flow of 42 ± 6 ml/min. The millimolar composition of the perfusion fluid was: NaCl 130, NaHCO$_3$ 20.1, KCl 4.0, CaCl$_2$ 2.2, MgCl$_2$ 0.6, NaH$_2$PO$_4$ 1.2, and glucose 12. The solution was saturated with a mixture of 95% O$_2$ and 5% CO$_2$, and the pH was adjusted at 7.35.

In six of the hearts, an endocardial cryotechnique was used to freeze the complete right ventricle, the interventricular septum, and the endocardial and intramural layers of the free wall of the left ventricle (frozen heart).[11,12] This cryotechnique was used to avoid epicardial breakthrough of longitudinal wavefronts from deeper layers and to allow complete mapping of electrical activation because no intramural wavefronts were present. Briefly, a cryoprobe was inserted through the pulmonary artery in the right ventricle, filled with liquid N$_2$ (−192° C), and maintained in place until the right ventricle was completely frozen. The heart was then immersed in a tissue bath containing perfusion fluid at 30° C. The cryoprobe

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was installed in the left ventricular cavity through the left atrium and the coronary circulation was temporarily interrupted. The cryoprobe was filled with liquid N_2 and maintained in place for 7 min. After this period, the coronary circulation was restored; the probe was removed; and the heart was withdrawn from the tissue bath. For the rest of the experiment, the temperature of the heart was kept constant at 37° C. As a result of this procedure, only a thin epicardial layer, about 1 mm thick, of the free wall of the left ventricle survived, the rest of the myocardium being completely destroyed.[11,12] We have demonstrated previously that in this thin surviving layer, refractoriness and conduction velocity are not affected by the procedure and remain stable for many hours, suggesting the circulatory condition in the epicardial layer was adequate.[12] At the end of the experiments, the hearts were dissected to verify the efficacy of cryoprocedure. In five of the hearts, the endocardial cryoprocedure was not applied, and the Langendorff-perfused rabbit heart was kept intact (intact heart).

PROTOCOL

Recording and Induction of Ventricular Dysrhythmias

High-resolution mapping of epicardial excitation was performed using a spoon-shaped electrode containing 248 unipolar electrodes at regular distances of 2.25 mm. The computerized mapping system allowed simultaneous recording, storage, and automatic analysis of all 248 electrograms and on-line presentation of color-coded activation maps.[11,13] Programmed electrical stimulation was performed using a programmable constant current stimulator delivering 2-ms square pulses at twice diastolic threshold for both regular stimulation and induction of premature beats. Bipolar stimulation could be performed through any pair of electrodes in the spoon electrode. Both in the intact and in the frozen hearts, the stimulation protocol consisted of 1) application of one, two, and three premature stimuli (S2, S3, and S4 respectively) delivered with a decreasing coupling interval after ten basic stimuli (S1–S1) at 300-ms intervals in the frozen heart, and at 10 ms shorter than the spontaneous sinus cycle length in the intact heart; and 2) application of trains of ten stimuli at a regular cycle length that was progressively decreased at 10 ms steps until one-to-one capture of the ventricle failed (maximum pacing).

After the inducibility of ventricular dysrhythmias was assessed during control, 0.2 (0.7 μM), 0.5 (1.8 μM), 1.0 (3.5 μM), and 5.0 μg/ml (18 μM), bupivacaine (bupivacaine HCl 0.5%, Roger Bellon, France) was successively infused into the aortic cannula. After 20 min of infusion, inducibility of dysrhythmias was tested again using the same protocol as during control. Once the protocol was com-

pleted, normal Tyrode's solution was infused for a 30-min period to return to control conditions, to rule out the possibility of deterioration over time. The occurrence of spontaneous ventricular dysrhythmias was also recorded and analyzed.

Electrophysiologic Measurements

The following parameters at control and 20 min after each dose of bupivacaine were measured: spontaneous sinus cycle length (milliseconds) in intact heart, ventricular effective refractory period (VERP, milliseconds), longitudinal ventricular conduction velocity (centimeters per second), transverse ventricular conduction velocity (centimeters per second), and the anisotropic ratio (longitudinal ventricular conduction velocity/transverse ventricular conduction velocity) in the frozen heart. VERP was defined as the shortest S1–S2 interval still resulting in a propagated premature impulse during regular pacing with a S1–S1 interval of 300 ms. VERP was determined by decreasing the coupling interval of the premature stimulus in steps of 2 ms. As previously described by Clerc[14] and Spach et al.,[15] cardiac tissue has a different axial resistance along and perpendicular to the fiber axis of the myocardial fibers. This different axial resistance results in direction-dependent differences in conduction velocity (anisotropic conduction). Therefore, pacing at the center of the thin surviving layer of the left ventricle produced an ellipsoidal spread of propagation, with fast conduction parallel to the fiber axis (longitudinal conduction) and slow conduction perpendicular to it (transverse conduction). Conduction velocity was defined as the distance travelled by the wavefront normal to the isochrones per unit time. In each experiment, both longitudinal and transverse conduction velocities and the anisotropic ratio were measured after ten basic stimuli (S1–S1) at 1,000-ms intervals. In addition, to test the use-dependency of the drug, longitudinal ventricular conduction velocity was measured after 10 basic stimuli at 800-, 700-, 600-, 500-, 400-, 300-, and 200-ms intervals.

DEFINITION OF VENTRICULAR DYSRHYTHMIAS

We defined ventricular dysrhythmias as ventricular fibrillation and sustained and nonsustained ventricular tachycardia. Nonsustained ventricular tachycardia was defined as ventricular tachycardia lasting more than three successive beats but less than 30 s before spontaneous termination. Sustained ventricular tachycardia was defined as ventricular tachycardia lasting longer than 30 s. Finally, a separation into monomorphic and polymorphic tachycardia was made. The term monomorphic implied a uniform beat-to-beat QRS morphology. The term polymorphic ventricular tachycardia was defined as the occurrence of continuous change in QRS configuration.

Statistical Analysis

Spontaneous sinus cycle length in the intact heart, and VERP, longitudinal and transverse ventricular conduction velocities, and anisotropic ratio in frozen hearts were expressed as mean ± SD. All of these parameters were analyzed by an two-way analysis of variance followed by a Neuman-Keuls test. The chi-square test was used to compare occurrence of dysrhythmias. $P < 0.05$ was considered statistically significant.

Results

Effects of Bupivacaine on Inducibility of Dysrhythmias and on Electrophysiologic Parameters in the Intact Heart

Table 1 shows the occurrence of spontaneous and inducible ventricular dysrhythmias in the intact Langendorff-perfused rabbit heart. No spontaneous dysrhythmias were observed during control and administration of 0.2, 0.5, and 1.0 μg/ml bupivacaine. However, during administration of 5.0 μg/ml bupivacaine, spontaneous ventricular tachycardia occurred in three of five hearts.

By programmed electrical stimulation using up to three premature beats and maximum pacing rate, ventricular fibrillation was induced in all hearts during the control. During administration of 0.2 μg/ml bupivacaine, ventricular fibrillation was induced in three of five hearts using the same protocol. In the remaining two hearts, only sustained monomorphic ventricular tachycardia and nonsustained monomorphic ventricular tachycardia could

be induced. During administration of 0.5 μg/ml bupivacaine, the spectrum of dysrhythmias induced by programmed electrical stimulation was completely different. Although in one of five hearts, ventricular fibrillation was still inducible, in the remaining four hearts, sustained monomorphic ventricular tachycardias were the only dysrhythmias induced. At 1.0 μg/ml bupivacaine, the same pacing protocol induced in one of five hearts a sustained polymorphic ventricular tachycardia, and in two of five hearts, nonsustained polymorphic ventricular tachycardias. During administration of 5.0 μg/ml bupivacaine, premature electrical stimulation could not be tested because the hearts became inexcitable.

Figure 1 shows an example of the effects of bupivacaine. In control, ventricular fibrillation occurred after maximal pacing rate at a cycle length of 110 ms. At 0.2 μg/ml bupivacaine, rapid pacing at a cycle length of 140 ms did not induce ventricular fibrillation but did induce nonsustained monomorphic ventricular tachycardia. At 0.5 μg/ ml bupivacaine, rapid pacing at a cycle length of 160 ms induced sustained monomorphic ventricular tachycardia. Finally, at 5.0 μg/ml, bupivacaine induced a spontaneous idioventricular rhythm.

Spontaneous sinus cycle length (table 1) was not modified at 0.2, 0.5, and 1.0 μg/ml bupivacaine; however, in one preparation, an intermittent atrioventricular block occurred at 1.0 μg/ml bupivacaine. At 5.0 μg/ml, bupivacaine induced a marked and significant bradycardia ($P < 0.05$). In one preparation, an intermittent idioventricular rhythm occurred (fig. 1). After 30 min wash-out, all preparations recovered to their control values.

TABLE 1. Spontaneous Cycle Length and Cumulative, Spontaneous and Programmed Stimulation Inducible Ventricular Dysrhythmias during Control and Administration of Bupivacaine in Intact Heart

| | Control | Bupivacaine (μg/ml) | | | |
		0.2	0.5	1.0	5.0
Spontaneous cycle length (ms)	302 ± 55	294 ± 63	310 ± 75	323 ± 78	557 ± 71 $P < 0.05$
Spontaneous dysrhythmia	0/5	0/5	0/5	0/5	3/5 (SPVT) (NSPVT) (NSMVT)
1 premature beat	0/5	0/5	0/4	0/4	—
2 premature beats	1/5 (1 VF)	3/5 (1 SMVT) (2 VF)	2/4 (1 SMVT) (1 NSMVT)	1/4 (1 NSPVT)	—
3 premature beats	4/5 (4 VF)	4/5 (1 SMVT) (3 VF)	2/4 (1 SMVT) (1 NSMVT)	1/4 (1 NSPVT)	—
F$_{max}$	5/5 (5 VF)	5/5 (1 SMVT) (1 NSMVT) (3 VF)	5/5 (4 SMVT) (1 VF)	3/5 (1 SPVT) (2 NSPVT)	—

Premature beats were applied during regular pacing at a cycle length of 300 ms or 10 ms shorter than the spontaneous cycle length. Note that at 0.5 and 1 μg/ml, only four of five preparations could be activated by regular pacing at 300 ms and premature beats and none at 5 μg/ml bupivacaine. Chi-square test is nonsignificant.

F$_{max}$ = maximal pacing rate; VF = ventricular fibrillation; SPVT = sustained polymorphic ventricular tachycardia; NSPVT = nonsustained polymorphic ventricular tachycardia; SMVT = sustained monomorphic ventricular tachycardia; NSMVT = nonsustained monomorphic ventricular tachycardia.
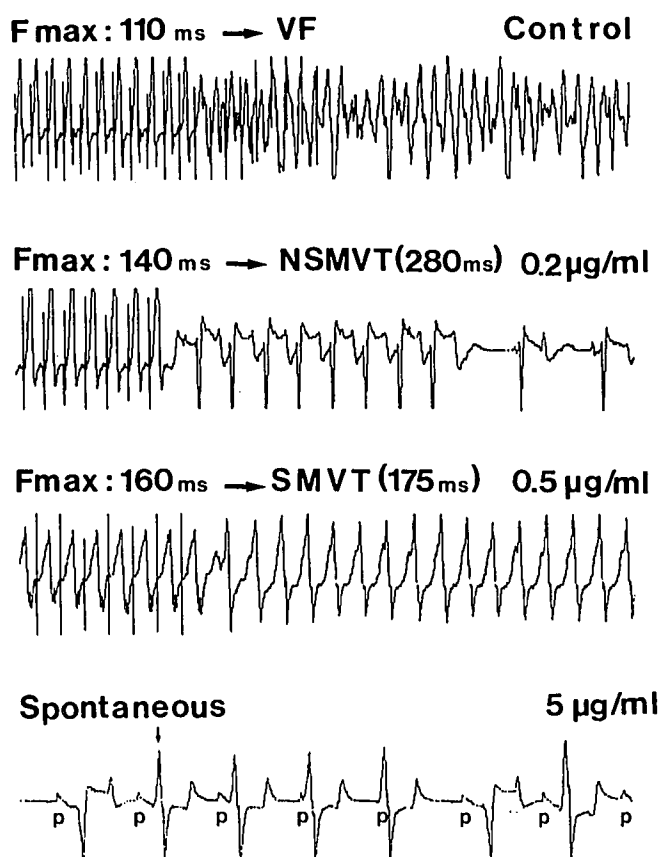
Fmax: 110 ms → VF   Control

Fmax: 140 ms → NSMVT(280ms) 0.2 µg/ml

Fmax: 160 ms → SMVT (175ms) 0.5 µg/ml

Spontaneous   5 µg/ml

FIG. 1. Effects of bupivacaine in intact heart. During control (*first panel*), maximal pacing rate (F_{max}) occurred at a cycle length of 110 ms and induced ventricular fibrillation (VF). At 0.2 µg/ml bupivacaine (*second panel*), F_{max} occurred at a cycle length of 140 ms and induced nonsustained monomorphic ventricular tachycardia (NSMVT) with a cycle length of 280 ms. At 0.5 µg/ml bupivacaine (*third panel*), F_{max} occurred at a cycle length of 160 ms and induced sustained monomorphic ventricular tachycardia (SMVT) with a cycle length of 175 ms. At 5.0 µg/ml bupivacaine (*fourth panel*), spontaneous idioventricular rhythm occurred. No pacing was obtained because the preparation was inexcitable.

EFFECTS OF BUPIVACAINE ON INDUCIBILITY OF VENTRICULAR DYSRHYTHMIAS AND ON ELECTROPHYSIOLOGIC PARAMETERS IN THE FROZEN HEART

After the endocardium was frozen, all measurements were performed during ventricular pacing because no spontaneous atrioventricular conduction was present. However, pacing with a cycle length shorter than 1,000 ms could not be done in one heart at 0.5 µg/ml, two hearts at 1.0 µg/ml, and three hearts at 5.0 µg/ml bupivacaine even using stimuli of high intensity (10 mA) and long duration (4 ms). No spontaneous ventricular dysrhythmias were observed. Table 2 shows that one preparation exhibited sustained monomorphic ventricular tachycardia after rapid pacing during control. However,

in three of six preparations, sustained monomorphic ventricular tachycardia was induced at 0.2 µg/ml bupivacaine. Epicardial mapping demonstrated that all these ventricular tachycardias were based on reentry. At concentrations greater than 0.2 µg/ml, no ventricular arrhythmia was induced.

In figure 2, initiation of a regular sustained monomorphic ventricular tachycardia with a cycle length of 271 ms by application of one premature stimulus (S2) during administration of 0.2 µg/ml is shown. During application of the premature stimulus (S2), the impulse encountered a long arc of conduction block extending almost from base to apex. The impulse, however, could turn around this arc of conduction block near the apex, and activation proceeded from apex to base. The impulse arrived at the area distal to the line of block at time 217 ms. The area proximal to the block that was activated at 20 ms could now be reactivated in the opposite direction at 220 ms, and a counterclockwise circular movement around the arc of functional block at a regular cycle length of 271 ms was initiated.

In table 3, the effects of bupivacaine on VERP and conduction velocities in the epicardium of the frozen heart are given. VERP was slightly but significantly prolonged by bupivacaine. Longitudinal and transverse ventricular conduction velocities and the anisotropic ratio were measured during regular pacing at 1,000 ms. There was a significant dose-dependent impairment of both longitudinal and transverse ventricular conduction velocities with no change of the anisotropic ratio. After 30 min washout, all preparations recovered to their control values. At 0.2 µg/ml bupivacaine, ventricular conduction velocities were not significantly impaired during regular pacing at 1,000 ms. However, longitudinal conduction velocity became significantly altered when regular pacing was shortened to 800 ms and less ($P < 0.05$).
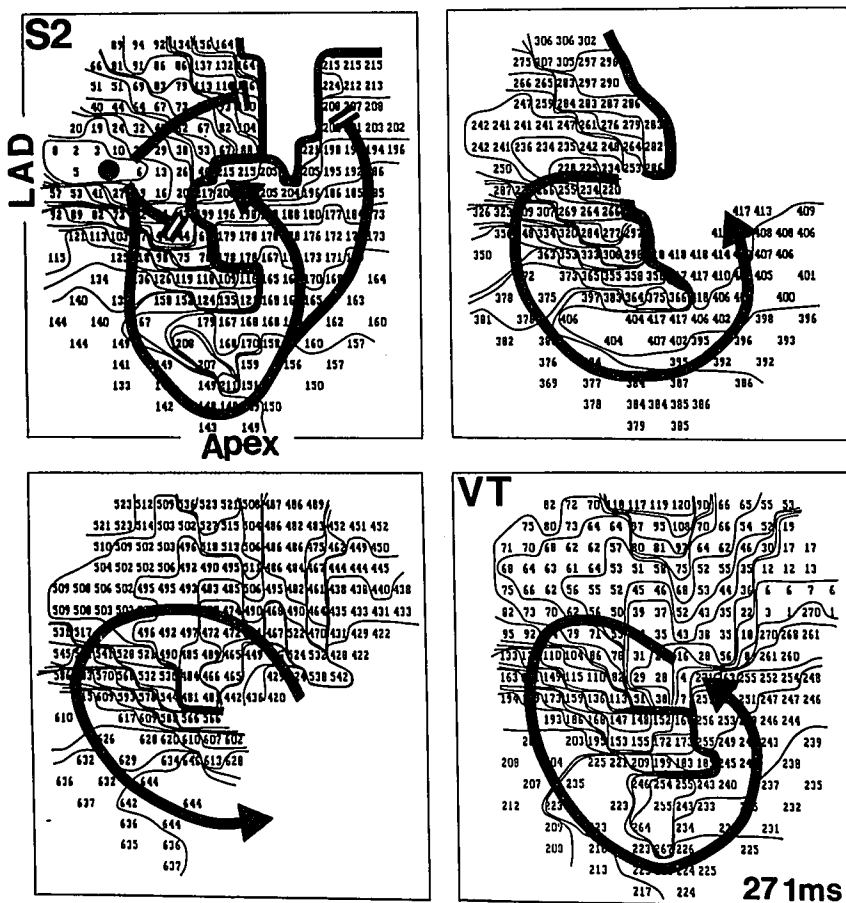
In figure 3, four different activation maps obtained during regular pacing at 500 ms in the same heart during

TABLE 2. Cumulative Inducibility of Ventricular Dysrhythmias during Control and Several Doses of Bupivacaine in the Frozen Heart

	Control	Bupivacaine (µg/ml)			
		0.2	0.5	1.0	5.0
1 premature beat	0/6	1/6 (SMVT)	0/5	0/4	—
2 premature beats	0/6	1/6 (SMVT)	0/5	0/4	—
3 premature beats	0/6	2/6 (SMVT)	0/5	0/4	—
F_{max}	1/6 (SMVT)	3/6 (SMVT)	0/5	0/4	—

One heart was inexcitable at 0.5 µg/ml, two hearts at 1.0 µg/ml, and all preparations at 5 µg/ml, when pacing at a cycle length shorter than 700 ms. Chi-square test is nonsignificant.

F_{max} = maximal pacing rate; SMVT = sustained monomorphic ventricular tachycardia.

BUPIVACAINE 0.2 μg/ml

FIG. 2. Initiation of sustained reentrant ventricular tachycardia during administration of 0.2 μg/ml bupivacaine in ventricular epicardium (after freezing). *Top tracing:* ECG recorded during induction of ventricular tachycardia with a premature beat (S2). *Panels:* Four consecutive activation maps are given showing spread of depolarization. Numbers indicate local activation times in milliseconds. Isochrones are drawn at 10-ms intervals. The thick isochrones indicate local conduction block. Arrows indicate direction of activation. Double bars indicate conduction block. LAD = left anterior descending coronary artery. Top left panel shows the conduction blockade resulting from the premature beat, and the first and second beat of the sustained ventricular tachycardia are shown by the top right and bottom left panels, respectively. The pattern of the depolarization during sustained ventricular tachycardia (VT) is shown in the lower left panel. All maps represent the same frontal view of the heart. See text for further description.

control, 0.2, 0.5, and 5.0 μg/ml bupivacaine are shown. Longitudinal ventricular conduction velocity, which during control was 77 cm/s, progressively decreased to 35 cm/s at 5.0 μg/ml bupivacaine. Moreover, during the largest dose of the drug, a long arc of conduction block extending from the base to the mid-left ventricular wall was present. Figures 4 and 5 show the use-dependent effects of bupivacaine on longitudinal ventricular conduction velocity. Figure 4 is a typical example of bupivacaine-induced use-dependent slowing of conduction. Four activation maps obtained during administration of 0.2 μg/ml bupivacaine at four different pacing cycle length are shown. Longitudinal ventricular conduction velocity, which was 60 cm/s at a pacing cycle length of 1,000 ms,

progressively decreased to 30 cm/s when the pacing cycle length was decreased to 200 ms. At the shortest pacing cycle, arcs of conduction blocks appeared in the mid-left ventricular wall. Figure 5 presents the average decrease in conduction velocity that was observed with various cycle length stimulation rates at various bupivacaine concentrations.

Discussion

This study demonstrates that bupivacaine decreases ventricular conduction velocity and induces arcs of functional ventricular conduction block in a dose-dependent and use-dependent fashion. Moreover, spontaneous ven-

TABLE 3. Effects of Bupivacaine on Electrophysiologic Parameters in Frozen Heart

Bupivacaine (µg/ml)	VERP	θ_L	θ_T	θ_L/θ_T
Control	141 ± 15 (n = 6)	67 ± 9 (n = 6)	30 ± 4 (n = 6)	2.2 ± 0.3 (n = 6)
0.2	163 ± 21 (n = 6)*	59 ± 8 (n = 6)	27 ± 6 (n = 6)	2.3 ± 0.6 (n = 6)
0.5	170 ± 17 (n = 5)*	49 ± 5 (n = 5)*	25 ± 2 (n = 5)*	2.1 ± 0.4 (n = 5)
1.0	172 ± 12 (n = 3)*	41 ± 6 (n = 4)*	21 ± 4 (n = 4)*	2.1 ± 0.5 (n = 4)
5.0	—	35 ± 12 (n = 3)*	18 ± 5 (n = 3)*	2.2 ± 0.9 (n = 3)
Wash-out	144 ± 16 (n = 6)	65 ± 8 (n = 6)	29 ± 5 (n = 6)	2.2 ± 0.4 (n = 6)

Data are expressed as mean ± SD.

VERP = ventricular effective refractory period (milliseconds) measured at regular pacing of 300 ms; θ_L = longitudinal ventricular conduction velocity (centimeters per second) measured at regular pacing

of 1,000 ms; θ_T = transverse ventricular conduction velocity (centimeters per second) measured at regular pacing of 1000 ms; θ_L/θ_T = anisotropic ratio measured at regular pacing of 1,000 ms.

* $P < 0.05$ versus respective control value.

tricular dysrhythmias were observed at the largest dose of bupivacaine (5.0 µg/ml). Finally, epicardial mapping showed that inducible ventricular tachycardias were based on reentry.

Four concentrations of bupivacaine were chosen. All of these solutions in our in vitro study were protein-free. Because bupivacaine is highly bound to plasma proteins,[16,17] it may be postulated that 0.2, 0.5, and 1.0 µg/

PCL: 500 ms

FIG. 3. Dose-dependent effects of bupivacaine in the frozen heart on the longitudinal ventricular conduction velocity at a pacing cycle length (PCL) of 500 ms. Closed circle represents the pacing site. Numbers indicate local activation time in ms. Isochrones are drawn at 10-ms intervals. The thick isochrones in the bottom right map indicate local conduction block. The circled activation times indicate the sites between which the ventricular conduction velocity was measured in all panels. θ = longitudinal ventricular conduction velocity; LAD = left anterior descending coronary artery. Increasing concentrations of bupivacaine progressively impaired longitudinal ventricular conduction velocity. At 5.0 µg/ml bupivacaine (bottom right), pacing site was modified because pacing at the center did not drive the preparation. Note the long line of conduction block extending from the base to the mid-left ventricular wall. Conduction block occurred at the region of the heart that showed the slowest conduction during control.
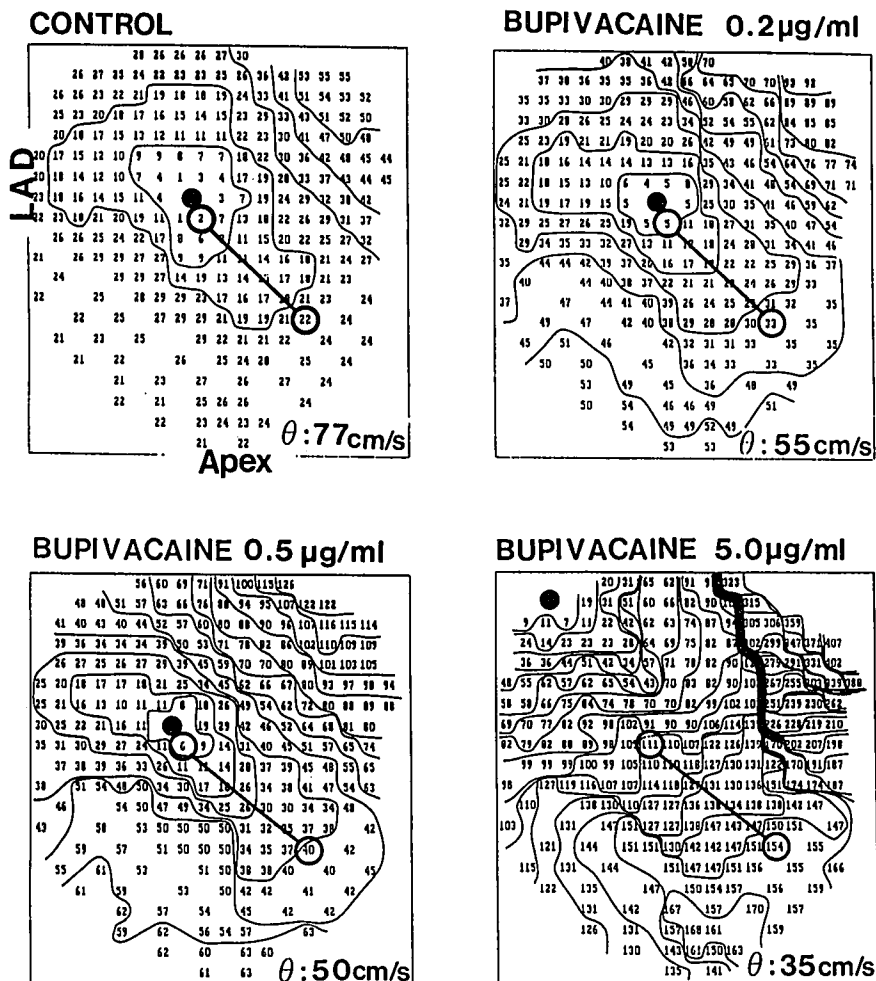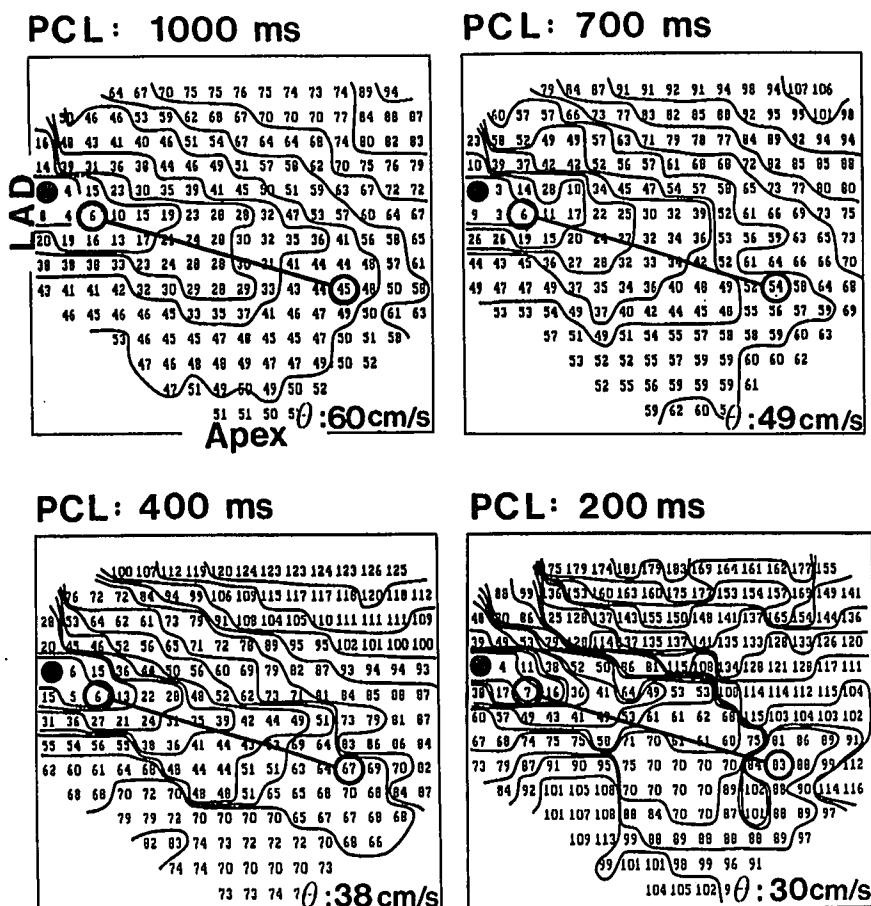
BUPIVACAINE 0.2μg/ml

FIG. 4. Activation maps showing the use-dependency of 0.2 μg/ml bupivacaine in frozen heart. The filled circles represent the pacing sites. Numbers indicate local activation time in milliseconds. Isochrones are drawn at 10-ms intervals. The thick isochrones in the lower maps indicate local conduction block. The circled activation times indicate the sites between which the ventricular conduction velocity was measured in all panels. θ = longitudinal ventricular conduction velocity; PCL = pacing cycle length; LAD = left anterior descending coronary artery. Note the progressive slowing of ventricular conduction velocity at decreasing pacing cycle lengths from 1,000 to 200 ms. Several arcs of conduction block appeared, especially at a pacing cycle length of 200 ms (bottom right).

ml correspond to plasma concentrations of approximately 2, 5, and 10 μg/ml in plasma, assuming 90% binding. However, the amount of plasma-binding decreases through this range so that appropriate values are probably represented by values of 2, 4, and 5 μg/ml. Finally, 5.0 μg/ml in vitro may correspond to about 14 μg/ml bupivacaine in plasma, assuming 65% binding.[16,18,19] Therefore, the concentration of 0.2 μg/ml bupivacaine we used is probably similar to the one obtained during regional anesthesia.[20–22] In contrast, the three other concentrations of bupivacaine we used should be considered to be in the toxic ranges.[18,23,24]

BUPIVACAINE-INDUCED VENTRICULAR DYSRHYTHMIAS

In the intact heart, bupivacaine was able to induce spontaneous ventricular dysrhythmias in three of five preparations—one sustained ventricular tachycardia and two nonsustained ventricular tachycardias. As the hearts

were denervated, a direct cardiotoxic effect of bupivacaine could be involved. This was previously demonstrated by Nath et al.,[25] who reported direct electrophysiologic disturbances when bupivacaine was injected in the coronary arteries.

Additional information was given by the frozen heart model. This model was used because it allows precise analysis of the complete sequence of activation of the left ventricular epicardium during regular pacing and during the initiation of ventricular dysrhythmias.[11,12] In three of six hearts, sustained monomorphic ventricular tachycardia was induced at 0.2 μg/ml bupivacaine, one ventricular tachycardia after one single premature beat, two ventricular tachycardias after three premature beats, and three after maximal pacing. However, one ventricular tachycardia was already inducible in one preparation at maximum pacing during control. The analysis of the complete sequence of activation during initiation of ventricular tachycardias showed a marked slowing of conduction, arcs of conduction block, and reentry around these arcs of
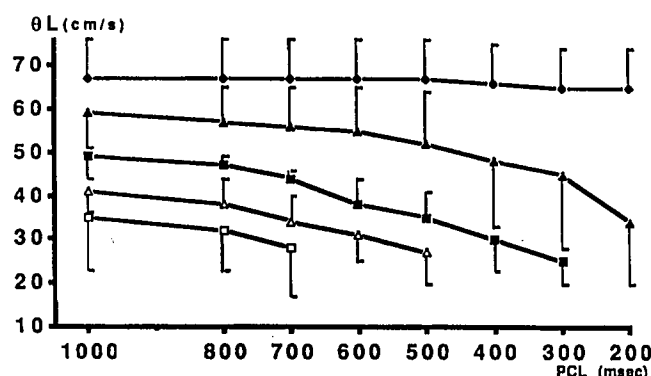
FIG. 5. Use-dependent effects of bupivacaine in frozen heart. Data are expressed as mean ± SD. Filled circles = control; filled triangles = 0.2 μg/ml bupivacaine; filled squares = 0.5 μg/ml bupivacaine; open triangles = 1.0 μg/ml bupivacaine; open squares = 5.0 μg/ml bupivacaine. Pacing could be realized in all preparations during control and at 0.2 μg/ml bupivacaine from 1,000 to 200 ms; in five of six preparations at 0.5 μg/ml bupivacaine from 1,000 to 300 ms; in four of six preparations at 1.0 μg/ml bupivacaine from 1,000 to 500 ms; and in three of six preparations at 5.0 μg/ml bupivacaine from 1,000 to 700 ms. PCL = pacing cycle length; θL = longitudinal ventricular conduction velocity. The effects of bupivacaine became significant from 0.2 μg/ml during regular pacing at 800 ms ($P < 0.05$).

conduction block. Although many reports have assumed that ventricular dysrhythmias induced by bupivacaine were based on reentry,[2,5,7] this is the first direct evidence of reentrant ventricular tachycardia induced by bupivacaine. No dysrhythmias could be induced at concentrations greater than 0.2 μg/ml bupivacaine in the frozen heart. This might be explained by the inability to rapidly pace the heart at greater concentrations of bupivacaine. This bupivacaine-induced inexcitability has been described previously in rabbit hearts.[5,7]

The morphology of ventricular dysrhythmias was different between the intact heart and the frozen heart. In the intact heart, two of three spontaneous ventricular dysrhythmias were polymorphic. Rapid pacing or multiple premature beats induced ventricular fibrillation and polymorphic and monomorphic ventricular tachycardias, whereas in the frozen heart, all ventricular tachycardias were monomorphic. This difference may be related to the fact that elimination of four fifths of the left ventricular wall by the cryoprocedure diminished the ventricular mass to such an extent that multiple reentering wavelets could no longer occur.[26,27]

BUPIVACAINE-INDUCED ELECTROPHYSIOLOGIC ALTERATIONS

At the smallest concentration (0.2 μg/ml), bupivacaine already induced a significant slowing of ventricular conduction velocity during regular pacing from 800 ms and below and VERP prolongation. A slowing in the param-

eters of ventricular conduction was previously reported in anesthetized dogs, in which a plasma concentration of 1.0 μg/ml bupivacaine induced a slight but significant prolongation of the His-Purkinje conduction time (HV interval) and a widening of the QRS complex.[24] Our results are also in accordance with previous *in vivo* and *in vitro* studies showing that bupivacaine induces QRS widening and HV prolongation[23,24,28] and a progressive and marked decrease in $\dot{V}_{max}$ of fast action potentials in guinea pig papillary muscle[3,4,29] and in rabbit Purkinje fibers and ventricular muscle.[5]

Bupivacaine impaired both longitudinal and transverse ventricular conduction velocities in a use- and dose-dependent fashion with no modification in the anisotropic ratio. These results are in accordance with our previous study on flecainide using the same model.[30] However, they differ from previous studies by Anderson *et al.*[31] and by Kadish *et al.*[32] These authors demonstrated that lidocaine and procainamide, respectively, have a predominant effect on longitudinal conduction velocity. It may be argued that differences in the experimental model (in both studies, canine myocardium was used) might account for the different results.

Our study was focused on the effects of bupivacaine on conduction velocities and therefore on fast sodium channels, but bupivacaine is known to interact with other cardiac channels like calcium and potassium channels.[29,33-37] The magnitude of these effects are conflicting or sometimes controversial in terms of animal species and techniques used. Nevertheless, it is now well established that bupivacaine also inhibits the transient outward current in rat ventricular myocytes.[35] The inhibition of the transient outward current prolongs the inactivated state of cardiac sodium channels.[37] Because bupivacaine preferentially blocks cardiac sodium channels in their inactivated states,[3] Castle[37] postulates that bupivacaine-induced inhibition of the transient outward current could enhance the depression of $\dot{V}_{max}$ induced by bupivacaine. It is also established that the transient outward current is present in the epicardium but not in the endocardium.[38] This could explain some of the differences in the effects of bupivacaine we observed between intact and frozen hearts. Nevertheless, how this effect could modify the overall proarrhythmic effects induced by bupivacaine, especially in the epicardial layer of tissue, cannot be answered with the experimental protocol we used.

CLINICAL IMPLICATIONS

Care must be taken in extrapolating these results to the clinical setting. Our study shows that bupivacaine at the free plasma concentration obtained during regional anesthesia (0.2 μg/ml) facilitated the induction of ventricular dysrhythmias by programmed electrical stimula-

tion. However, spontaneous ventricular dysrhythmias occurred only at the largest concentration of bupivacaine (5.0 μg/ml).

Toxic doses of bupivacaine also induce hemodynamic disturbances with a dramatic decrease in contractility.[3,29,39] These hemodynamic abnormalities, which could facilitate the occurrence of ventricular dysrhythmias, do not play a role in a Langendorff model. Furthermore, all the dysrhythmias observed occurred in denervated hearts. In the clinical situation, it is well known that the autonomic nervous system interferes with cardiac electrophysiology. In particular, the sympathetic nervous system facilitates the occurrence of cardiac dysrhythmias, specially in patients with coronary artery disease[40] and with proarrhythmic effects of other sodium channel blockers such as flecainide.[41] Moreover, it has been reported in humans that bupivacaine given intravenously induces an increase in plasma catecholamine levels.[42] Thus, taking into account the studies of Heavner,[8] Thomas et al.,[9] and Bernards and Artru,[10] it may be speculated that the autonomic nervous system could worsen the ventricular dysrhythmias induced by large doses of bupivacaine and could transform, for example, nonsustained ventricular tachycardia into sustained ventricular tachycardia or into ventricular fibrillation.

Our study also demonstrates that 0.2 μg/ml bupivacaine significantly slows ventricular conduction velocity during regular pacing from 800 ms and less. Although we previously reported that bupivacaine plasma levels obtained after lumbar epidural anesthesia do not enhance preexisting atrioventricular and ventricular conduction defects in humans,[43] caution must be taken in the use of bupivacaine in these patients.

In conclusion, using high-resolution ventricular epicardial mapping in rabbit hearts, the study shows that bupivacaine depressed both longitudinal and transverse ventricular conduction velocity in a dose-dependent and use-dependent fashion. Arcs of ventricular conduction block were induced with administration of high doses of the drug or during rapid pacing at lower dose. Finally, bupivacaine is able to induce spontaneous ventricular dysrhythmias in toxic ranges. All of the ventricular tachycardias induced in the frozen heart during bupivacaine administration were based on reentry.

References

1. Albright GA: Cardiac arrest following regional anesthesia with etidocaine or bupivacaine. ANESTHESIOLOGY 51:285–287, 1979
2. Reiz S, Nath S: Cardiotoxicity of local anaesthetic agents. Br J Anaesth 58:736–746, 1986
3. Clarkson CW, Hondeghem 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 62:396–405, 1985
4. Clarkson CW, Hondeghem LM: Evidence for a specific receptor site for lidocaine, quinidine, and bupivacaine associated with cardiac sodium channels in guinea pig ventricular myocardium. Circ Res 56:496–506, 1985
5. Moller RA, Covino BG: Cardiac electrophysiologic effects of lidocaine and bupivacaine. Anesth Analg 67:107–114, 1988
6. Nattel S, Jing W: Rate-dependent changes in intraventricular conduction produced by procainamide in anesthetized dogs: A quantitative analysis based on the relation between phase 0 inward current and conduction velocity. Circ Res 65:1485–1498, 1989
7. Lacombe P, Blaise G, Loulmet D, Hollmann C: Electrophysiologic effects of bupivacaine in the isolated rabbit heart. Anesth Analg 72:62–69, 1991
8. Heavner JE: Cardiac dysrhythmias induced by infusion of local anesthetics into the lateral cerebral ventricle of cats. Anesth Analg 65:133–138, 1986
9. Thomas RD, Behbehani MM, Coyle DE, Denson DD: Cardiovascular toxicity of local anesthetics: An alternative hypothesis. Anesth Analg 65:444–450, 1986
10. Bernards CM, Artru AA: Hexamethonium and midazolam terminate dysrhythmias and hypertension caused by intracerebroventricular bupivacaine in rabbits. ANESTHESIOLOGY 74:89–96, 1991
11. Brugada J, Boersma L, Kirchhof C, Brugada P, Havenith M, Wellens HJJ, Allessie MA: Double-wave reentry as a mechanism of ventricular tachycardia acceleration. Circulation 81:1633–1643, 1990
12. Allessie MA, Schalij MJ, Kirchhof CJHJ, Boersma L, Huybers M, Hollen J: Experimental electrophysiology and arrhythmogenecity: Anisotropy and ventricular tachycardia. Eur Heart J 10E:8–14, 1989
13. Allessie MA, Hoeks APG, Schmitz GML, Reneman RS: On-line mapping system for the visualisation of the electrical activation of the heart. Int J Cardiac Imaging 2:59–63, 1987
14. Clerc L: Directional differences on impulse spread in trabecular muscle from mammalian heart. J Physiol (Lond) 255:335–346, 1976
15. Spach MS, Miller WT Jr, Geselowitz DB, Barr RC, Kootsey JM, Johnson EA: The discontinuous nature of propagation in normal canine cardiac muscle: Evidence for recurrent discontinuities of intracellular resistance that affect the membrane currents. Circ Res 48:39–54, 1981
16. Denson D, Boyle D, Thompson G, Santos D, Turner P, Myers J, Knapp R: Bupivacaine protein binding in term parturients: Effects of lactic acidosis. Clin Pharmacol Ther 35:702–709, 1984
17. Tucker GT: Pharmacokinetics of local anaesthetics. Br J Anaesth 58:717–731, 1986
18. Rosen M, Thigpen J, Shnider S, Foutz S, Levinson G, Koike M: Bupivacaine-induced cardiotoxicity in hypoxic and acidotic sheep. Anesth Analg 64:1089–1096, 1985
19. Moller R, Covino BG: Cardiac electrophysiologic properties of bupivacaine and lidocaine compared with those of ropivacaine, a new amide local anesthetic. ANESTHESIOLOGY 72:322–329, 1990
20. Johnson MD, Mickler T, Arthur GR, Rosenburg S, Wilson R: Bupivacaine with and without epinephrine for intercostal nerve block. J Cardiothorac Anesth 4:200–203, 1990
21. Gin T, Chan K, Kan AF, Gregory MA, Wong YC, Oh TE: Effect of adrenaline on venous plasma concentrations of bupivacaine after interpleural administration. Br J Anaesth 64:662–666, 1990

22. Salevsky FC, Whalley DG, Kalant D, Crawhall J: Epidural epinephrine and the systemic circulation during peripheral vascular surgery. Can J Anaesth 37:160–165, 1990

23. Hotvedt R, Refsum H, Helgesen KG: Cardiac electrophysiologic and hemodynamic effects related to plasma levels of bupivacaine in the dog. Anesth Analg 64:388–394, 1985

24. Eledjam JJ, de La Coussaye JE, Brugada J, Masse C, Desch G, d'Athis F, Sassine A: Cardiac electrophysiological effects of bupivacaine in the anesthetized dogs: Relation with plasma concentration. Arch Int Pharmacodyn 295:147–156, 1988

25. Nath S, Häggmark S, Jokansson G, Reiz S: Differential depressant and electrophysiologic cardiotoxicity of local anesthetics: An experimental study with special reference to lidocaine and bupivacaine. Anesth Analg 65:1263–1270, 1986

26. Moore EN, Spear JF: Natural occurrence and experimental initiation of atrial fibrillation in different animal species, Atrial Fibrillation. Edited by Kulbertus HE, Olsson SB, Schlepper M. Molndal, Sw AB Hassell, 1989, pp 33–41

27. Garrey WE: The nature of fibrillary contraction of the heart. Its relation to tissue mass and form. Am J Physiol 33:397–414, 1914

28. Kotelko DM, Shnider SM, Dailey PA, Brizgys RV, Levinson G, Shapiro WA, Koike M, Rosen MA: Bupivacaine-induced cardiac arrhythmias in sheep. ANESTHESIOLOGY 60:10–18, 1984

29. Lynch C III: Depression of myocardial contractility in vitro by bupivacaine, etidocaine, and lidocaine. Anesth Analg 65:551–559, 1986

30. Brugada J, Boersma L, Kirchhof C, Allessie MA: Proarrhythmic effects of flecainide: Evidence for increased susceptibility to reentrant arrhythmias. Circulation 84:1808–1818, 1991

31. Anderson KP, Walker R, Lux RL, Ershler PR, Menlove R, Williams MR, Krall R, Moddrelle D: Conduction velocity depression and drug-induced ventricular tachyarrhythmias: Effects of lidocaine in the intact canine heart. Circulation 81:1024–1038, 1990

32. Kadish AH, Spear JF, Levine JH, Moore EN: The effects of procainamide on conduction in anisotropic canine ventricular myocardium. Circulation 74:616–625, 1986

33. Coyle DE, Sperelakis N: Bupivacaine and lidocaine blockade of calcium-mediated slow action potentials in guinea pig ventricular muscle. J Pharmacol Exp Ther 242:1001–1005, 1987

34. Sanchez-Chapula J: Effects of bupivacaine on membrane currents of guinea pig ventricular myocytes. Eur J Pharmacol 156:303–308, 1988

35. de La Coussaye JE, Masse C, Bassoul B, Eledjam JJ, Gagnol JP, Sassine A: Bupivacaine-induced slow-inward current inhibition: A voltage clamp study on frog atrial fibres. Can J Anaesth 37:819–822, 1990

36. Courtney KR, Kendig JJ: Bupivacaine is an effective potassium channel blocker in heart. Biochim Biophys Acta 939:163–166, 1988

37. Castle NA: Bupivacaine inhibits the transient outward K+ current but not the inward rectifier in rat ventricular myocytes. J Pharmacol Exp Ther 255:1038–1046, 1990

38. Krishnan SC, Antzelevitch C: Sodium channel block produces opposite electrophysiological effects in canine ventricular epicardium and endocardium. Circ Res 69:277–291, 1991

39. Eledjam JJ, de La Coussaye JE, Brugada J, Bassoul B, Gagnol JP, Fabregat JR, Masse C, Sassine A: In vitro study on mechanisms of bupivacaine-induced depression of myocardial contractility. Anesth Analg 69:732–735, 1989

40. Podrid PJ, Fuchs T, Caudinas R: Role of the sympathetic nervous system in the genesis of ventricular arrhythmia. Circulation [Suppl 2] 82:I 103-I 113, 1990

41. Myerburg RJ, Kessler KM, Cox MM, Huikuri H, Terracall E, Interian A, Fernandez P, Castellanos A: Reversal of proarrhythmic effects of flecainide acetate and encainide hydrochloride by propranolol. Circulation 80:1571–1579, 1989

42. Hasselström LJ, Morgensen T, Kehlet H, Christensen NJ: Effects of intravenous bupivacaine on cardiovascular function and plasma catecholamine levels in Humans. Anesth Analg 63:1053–1058, 1984

43. Eledjam JJ, de La Coussaye JE, Colson P, Viel E, Bassoul B, Bertinchant JP, d'Athis F: Is epidural anaesthesia using bupivacaine safe in patients with atrio-ventricular conduction defects? Acta Anaesthesiol Scand 33:402–404, 1989