

Proarrhythmic and Antiarrhythmic Effects of Bupivacaine in an In Vitro Model of Myocardial Ischemia and Reperfusion

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Background: Bupivacaine may have toxic cardiovascular effects when accidentally administered by intravascular injection. However, its electrophysiologic effects in the presence of myocardial ischemia remain unknown. The authors evaluated the electrophysiologic and anti- and proarrhythmic effects of bupivacaine in an *in vitro* model of the ischemic and reperfused myocardium.

Methods: In a double-chamber bath, a guinea pig right ventricular muscle strip was subjected partly to normal conditions and partly to simulated ischemia followed by reperfusion. The electrophysiologic effects of bupivacaine were studied at 1, 5, and 10 μM concentrations.

Results: Bupivacaine (5 and 10 μM) decreased the maximal upstroke velocity of the action potential (V_{max}) in normoxic conditions and further decreased (10 μM) the V_{max} decrease

induced by ischemic conditions. Bupivacaine reduced the mean occurrence time to the onset of myocardial conduction blocks (9 ± 3 min; mean \pm SD; $P < 0.005$ with 5 and 10 μM compared with 17 ± 6 min during simulated ischemia with no drug or control), and it increased the number of preparations that became inexcitable to pacing (55% of preparations with 1 μM and 100% with 5 and 10 μM , compared with 17% for the control group). The incidence of spontaneous arrhythmias was reduced by 5 and 10 μM bupivacaine during ischemia and reperfusion and was enhanced by 1 μM bupivacaine during the ischemic phase.

Conclusions: In guinea pig myocardium under ischemic conditions, bupivacaine induced a loss of excitability at concentrations of 5 and 10 μM . Proarrhythmic effects observed at 1 μM were considered as lower than the cardiotoxic range in normoxic conditions. The incidence of reperfusion arrhythmias was decreased at all concentrations. (Key words: Action potentials; cardiac toxicity; electrophysiology; guinea pig heart; local anesthetics; ventricular muscle.)

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BUPIVACAINE has been clearly implicated in the onset of ventricular arrhythmias and sudden cardiovascular collapse in humans.¹⁻⁴ *In vivo* laboratory studies have shown severe cardiac arrhythmias⁵⁻⁷ and also depression of cardiac output, myocardial contractility, and intracardiac conduction velocity^{5,8} subsequent to intravenous injection of convulsant (or higher) doses of bupivacaine. *In vitro*, bupivacaine depresses the action potential (AP) maximal upstroke velocity in a use-dependent manner; decreases contractile force, spontaneous sinoatrial activity, and intracardiac conduction velocity; and facilitates the induction of reentrant ventricular arrhythmias in isolated rabbit hearts.⁹⁻¹³ However, the effects of bupivacaine in the presence of myocardial ischemia remain largely unknown. In several studies in intact animals, bupivacaine may induce serious electrophysiologic changes and arrhythmias under acidotic or hypoxic conditions^{14,15} and may reduce the time required for ventricular fibrillation induction during coronary ischemia.¹⁶

On the other hand, during myocardial ischemia the

"border zone," which has been described as the intermediate zone separating normal and hypoxic or ischemic tissues¹⁷ and associated with inhomogeneous distribution of electrical properties, anatomic, and biochemical changes, has been established as a major site of arrhythmias.^{18,19} Thus injury currents, with a recognized origin in the border zone, as suggested by investigations of isolated porcine and canine hearts,²⁰ are thought to be a possible mechanism leading to arrhythmias such as automatic activity, focal reexcitation, or reentry arrhythmia.²¹

The aim of this study was to examine the electrophysiologic mechanisms underlying the ischemia-bupivacaine cardiotoxicity interaction. We evaluated in isolated guinea pig ventricular myocardium the electrophysiologic effects of bupivacaine and its effects on the incidence of conduction disturbances and arrhythmias in an *in vitro* model of ischemic and reperfused myocardium.²²⁻²⁵

Materials and Methods

Care of the animals conformed to the recommendations of the Helsinki Declaration, and the study was performed in accordance with the regulations of the official edict of the French Ministry of Agriculture.

Guinea pigs of either sex weighing 300–400 g were killed after brief anesthesia with ether. The hearts were quickly removed and placed in oxygenated Tyrode's solution at room temperature. A thin myocardial strip was dissected longitudinally from the free wall of the right ventricle and pinned with the endocardial surface upward in a special perfusion chamber.²²⁻²⁵ This chamber (5 ml) is bisected by a thin latex membrane containing a centrally located hole that allowed the preparation to be passed carefully through and divided into two zones called the normal zone (NZ) and the altered zone (AZ) (fig. 1). The two compartments were perfused independently at the rate of 2 ml/min with Tyrode's solution oxygenated with 95% oxygen and 5% carbon dioxide. The Tyrode's solution was composed of 135 mM Na⁺, 4 mM K⁺, 1.8 mM Ca²⁺, 1 mM Mg²⁺, 1.8 mM H₂PO₄⁻, 25 mM HCO₃⁻, 117.8 mM Cl⁻, and 5.5 mM glucose. The pH was 7.35 ± 0.05, and the temperature was maintained at 36.5°C with thermostated water circulation (Polystat 5HP; Bioblock, Illkirch, France). At the end of each experiment, absence of leak between the two compartments was tested by injecting methylene blue dye into one of the two compartments.

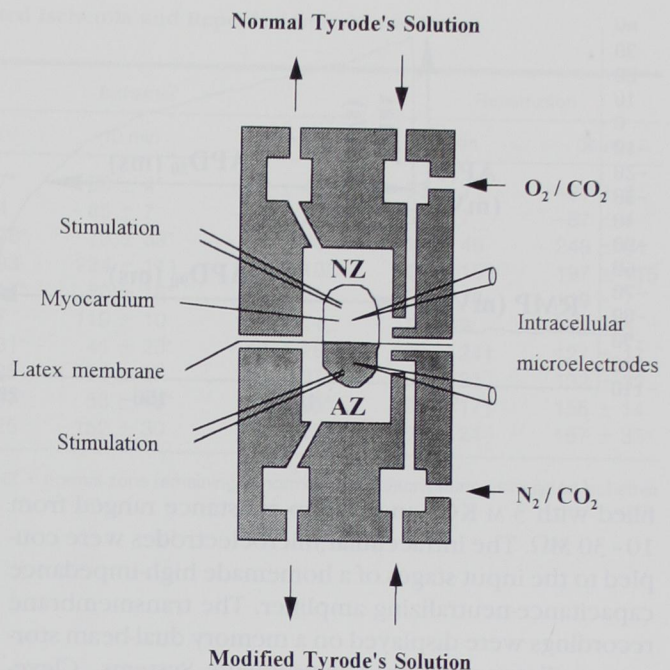


Fig. 1. The double-chamber bath. NZ, normal zone, in which myocardium was superfused with normal Tyrode's solution during the experiments. AZ, altered zone, in which myocardium was superfused with modified Tyrode's solution during simulated ischemia and with normal Tyrode's solution during reperfusion.

Data Acquisition and Analysis

The myocardial strips were stimulated at a frequency of 1 Hz by two bipolar Teflon-coated steel wire electrodes positioned in the NZ and the AZ. A commutator allowed us to apply the stimulation to the preparation by one or the other stimulating electrode. Stimuli were rectangular pulses lasting 2 ms and twice the diastolic threshold intensity delivered by a programmable stimulator (model SMP 310; Biologic, Grenoble, France). Preparations that needed pulses stronger than 5 V to elicit AP were discarded because there could be a conduction block at the level of the latex separating membrane. During the protocol, stimulation was stopped whenever sustained spontaneous arrhythmias occurred. An extrastimulus that lasted 2 ms and was twice the diastolic threshold amplitude was applied every four stimulations in an attempt to elicit extrastimulus-induced repetitive responses. The coupling time interval between the stimulus and the extrastimulus was divided into increments by 5-ms steps from the effective refractory period to the total repolarization duration. Transmembrane potentials were recorded simultaneously in both myocardial regions using glass microelectrodes

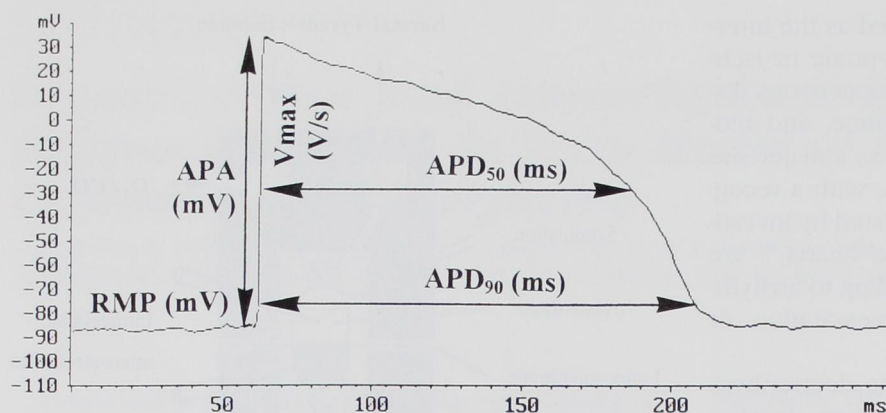


Fig. 2. Representative guinea pig right ventricular action potential and its parameters automatically measured during the experiments. RMP, resting membrane potential; V_{\max} , maximal upstroke velocity of action potential; APA, action potential amplitude; APD_{50} and APD_{90} , action potential duration measured at 50% and 90% of repolarization, respectively.

filled with 3 M KCl, and the tip resistance ranged from 10–30 M Ω . The intracellular microelectrodes were coupled to the input stages of a homemade high-impedance capacitance-neutralizing amplifier. The transmembrane recordings were displayed on a memory dual-beam storage oscilloscope (Gould Instruments Systems, Cleveland, OH). The following AP characteristics (fig. 2) were automatically stored and measured by a cardiac AP automatic acquisition system and processing device (DATA-PAC; Biologic): resting membrane potential, action potential amplitude, action potential duration at 50% of repolarization (APD_{50}) and at 90% of repolarization (APD_{90}), and maximal upstroke velocity (V_{\max}). Whenever possible, the same impalement was maintained throughout the experiment. When impalement was lost during measurement, readjustment was attempted. If the readjusted parameters deviated by no more than 10% from the previous ones, experiments were continued, otherwise they were terminated.

Experimental Protocol

During a 60-min equilibration period, the two compartments were perfused with normal Tyrode's solution. Thereafter, simulated ischemia was induced and maintained for 30 min in one compartment (AZ) by superfusion with a modified Tyrode's solution, whereas the other compartment remained in normal conditions (NZ; fig. 1). The modified Tyrode's solution differed from normal by an elevated potassium concentration (from 4 to 12 mM); decreased bicarbonate concentration (from 25 to 9 mM), leading to a decrease in pH (from 7.35 ± 0.05 to 7.00 ± 0.05); a decrease in oxygen tension by replacement of 95% oxygen and 5% carbon dioxide with 95% nitrogen and 5% carbon dioxide; and withdrawal of glucose. As previously reported,^{22–25} the

present modifications, which combined hypoxia, hyperkalemia, acidosis, and lack of substrates, are similar to those reported by Morena *et al.*,²⁶ who reproduced *in vitro* the electrophysiologic abnormalities induced *in vivo* by ischemia. At the end of the ischemia period, reperfusion was simulated by perfusing the AZ chamber with normal Tyrode's solution for 30 min (the reperfusion period).

During simulated ischemia and reperfusion, several myocardial conduction disturbances and arrhythmias were recorded: (1) myocardial conduction blocks between the two regions; (2) loss of responsiveness in the myocardial tissue, considered when the preparation failed to elicit AP regardless of the compartment stimulated, with a constant stimulation intensity; (3) extra-stimulus-induced repetitive responses, defined as one, two, or a salvo of spontaneous extrasystoles induced by a single extrastimulus; and (4) spontaneous repetitive responses such as sustained activities (fewer than 10 spontaneous APs) independent of the stimulation.

After the 60-min equilibration period, during the simulated ischemia and reperfusion phases, plain bupivacaine diluted in Tyrode's solution at 1 μ M, 5 μ M, or 10 μ M (each $n = 12$), or Tyrode's solution alone ($n = 12$) was perfused in random order in both compartments (NZ and AZ). Thus the electrophysiologic effects of bupivacaine on AP parameters and the incidence of arrhythmias were investigated simultaneously in normal (NZ) and altered (AZ) conditions.

Statistical Analysis

All results were expressed as mean \pm SD. Categorical variables were compared using the chi-square test with Yates correction as appropriate. Multiple comparison of continuous variables was performed by two-way anal-

BUPIVACAINE AND MYOCARDIAL ISCHEMIA-REPERFUSION

Table 1. Evolution of Action Potential Parameters during Simulated Ischemia and Reperfusion (without Drug) in the Two Myocardial Zones

		Initial (n = 12)	Ischemia			Reperfusion	
			5 min	10 min	30 min	10 min	30 min
Resting membrane potential (mV)	AZ	-88 ± 3	-61 ± 7*	-60 ± 4*	-60 ± 4*	-83 ± 11	-91 ± 3
	NZ	-87 ± 4	-86 ± 4	-85 ± 7	-88 ± 2	-86 ± 7	-87 ± 4
Maximal upstroke velocity (V/s)	AZ	248 ± 59	131 ± 78*	73 ± 39*	52 ± 70*	213 ± 49	248 ± 94
	NZ	242 ± 103	239 ± 93	224 ± 111	221 ± 131	218 ± 153	197 ± 115
Action potential amplitude (mV)	AZ	120 ± 7	90 ± 17*	80 ± 16*	78 ± 21*	107 ± 18	119 ± 7
	NZ	115 ± 6	113 ± 7	110 ± 10	111 ± 11	111 ± 9	112 ± 5
Action potential duration 50% (ms)	AZ	125 ± 14	67 ± 31*	41 ± 20*	43 ± 26*	100 ± 24†	127 ± 17
	NZ	127 ± 18	124 ± 25	125 ± 37	126 ± 27	137 ± 21	132 ± 32
Action potential duration 90% (ms)	AZ	151 ± 13	86 ± 27*	56 ± 18*	60 ± 25*	126 ± 17†	155 ± 14
	NZ	151 ± 20	149 ± 25	152 ± 30	152 ± 25	163 ± 24	157 ± 35

AZ = altered zone submitted to simulated ischemic conditions then reperfusion; NZ = normal zone remaining in normoxic conditions during simulated ischemia and reperfusion phases.

Values are mean ± SD; n = 12.

* $P < 0.01$ versus Initial (Dunnett's test).

† $P < 0.05$ versus Initial (Dunnett's test).

ysis of variance followed by comparison with control or initial values using Dunnett's test. Differences were considered significant when $P < 0.05$.

Accounting for losses on impalement during the experiments, data analysis was performed on n = 12 in the control group, n = 11 in the 1 μ M bupivacaine group, n = 10 in the 5 μ M bupivacaine group, and n = 9 in the 10 μ M bupivacaine group.

Results

Ischemia and Reperfusion Effects on Action Potential Parameters

As summarized in table 1, simulated ischemia rapidly induced alterations of AP time course parameters. After 5 min, simulated ischemia induced significant membrane depolarization ($P < 0.01$), V_{\max} and action potential amplitude reduction ($P < 0.01$), and APD₅₀ and APD₉₀ shortening ($P < 0.01$). The maximal electrophysiologic effects occurred and reached a plateau within the first 10 min. In the NZ, all these AP parameters remained unchanged after 30 min and even after 60 min (see Reperfusion - 30 min, in table 1) of superfusion of normal Tyrode's solution. Reperfusion of the AZ was associated with a rapid recovery of the AP parameters. Electrophysiologic effects induced by simulated ischemia were rapidly reversed within 10 min of reperfusion for resting membrane potential, V_{\max} , and action

potential amplitude and within 20 min for APD₅₀ and APD₉₀ (to 114 ± 38 ms and 143 ± 28 ms, respectively).

Bupivacaine Effects on Action Potential Parameters in Normoxic and Ischemic-Reperfused Simulated Conditions

As shown in table 2, there was no significant difference in initial AP parameter values for the four experimental groups. Figure 3 shows the percentage variations of the AP parameters at 10 min of simulated ischemia in NZ and AZ with and without increased bupivacaine concentrations. Because of the high incidence of conduction block leading to inexcitability of the stimulated AZ, the results are shown at 10 min of the ischemic phase, at which time it was possible to measure AP parameters. In NZ, V_{\max} was significantly reduced by bupivacaine at 5 and 10 μ M (respectively, $-26 \pm 27\%$ and $-27 \pm 24\%$ after 30 min, $P < 0.05$ compared with the control group). Unlike APD₉₀, which decreased in NZ in the presence of bupivacaine during the simulated ischemic period (fig. 3), resting membrane potential, action potential amplitude, and APD₅₀ remained unchanged. In AZ, the V_{\max} decrease induced by ischemia was worsened only in the presence of 10 μ M bupivacaine ($-89 \pm 15\%$ after 10 min compared with a decrease of $-69 \pm 15\%$ after 10 min of ischemia with no drug, $P < 0.05$). The time course of recovery of resting membrane potential, action potential amplitude, APD₅₀, and APD₉₀ during reperfusion

Table 2. Initial Values of Action Potential Parameters in Each of the Two Myocardial Zones, for Control Group and before Administration of Bupivacaine

		Control (no drug) (n = 12)	Bupivacaine		
			1 μ M (n = 11)	5 μ M (n = 10)	10 μ M (n = 9)
Resting membrane potential (mV)	AZ	-88 \pm 3	-92 \pm 2	-90 \pm 7	-87 \pm 6
	NZ	-87 \pm 4	-86 \pm 6	-84 \pm 4	-83 \pm 8
Maximal upstroke velocity (V/s)	AZ	248 \pm 59	269 \pm 62	264 \pm 79	309 \pm 105
	NZ	242 \pm 103	265 \pm 110	318 \pm 67	317 \pm 81
Action potential amplitude (mV)	AZ	120 \pm 7	118 \pm 4	117 \pm 9	114 \pm 8
	NZ	115 \pm 6	113 \pm 6	112 \pm 7	113 \pm 6
Action potential duration 50% (ms)	AZ	125 \pm 14	136 \pm 23	123 \pm 19	121 \pm 21
	NZ	127 \pm 18	137 \pm 20	126 \pm 23	131 \pm 17
Action potential duration 90% (ms)	AZ	151 \pm 13	165 \pm 25	158 \pm 27	150 \pm 24
	NZ	151 \pm 20	160 \pm 20	156 \pm 29	157 \pm 17

AZ = compartment becoming the altered zone during simulated ischemia and reperfusion phases; NZ = compartment remaining in normoxic conditions (normal zone) during simulated ischemia and reperfusion phases.

Values are mean \pm SD.

in the AZ was similar in the control and treated groups and reached initial values, with the exception of V_{\max} , which remained significantly depressed after 30 min of reperfusion in the presence of 5 and 10 μ M bupivacaine (respectively, 234 \pm 60 V/s and 200 \pm 63 V/s after 30 min of reperfusion compared with 264 \pm 79 V/s and 309 \pm 105 V/s as initial values, $P < 0.01$). In NZ, the APD₉₀ shortening, measured during exposure to the altered conditions in the presence of 1, 5, and 10 μ M bupivacaine, was reversed during the reperfusion phase (respectively, APD₉₀, 166 \pm 20 ms, 153 \pm 21 ms, and 152 \pm 20 ms after 30 min of the reperfusion phase compared with 160 \pm 20 ms, 156 \pm 29 ms, and 157 \pm 17 ms as initial values).

Bupivacaine Effects on the Ischemia-Reperfusion-induced Conduction Disturbances

As illustrated in figure 4, conduction changes observed during simulated ischemia in the presence of bupivacaine were characterized by the occurrence of unidirectional conduction block in AZ (fig. 4A, 4B) and loss of responsiveness of the AZ to stimulation (fig. 4C, 4D), followed by their removal during reperfusion (fig. 4E, 4F).

During simulated ischemia, the mean occurrence time of conduction block was evaluated in the presence of 1, 5, and 10 μ M bupivacaine. Conduction blocks occurred after 17 \pm 6 min in the control group, 14 \pm 7 min (NS) in the presence of 1 μ M bupivacaine, and after 9 \pm 3 min ($P < 0.05$) with both 5 μ M and 10 μ M bupivacaine.

Further, as shown in figure 5, 1, 5, and 10 μ M bupivacaine enhanced the incidence of loss of responsiveness to stimulation in the AZ. At the end of the ischemic period in the control group, 17% of preparations were unexcitable in their AZ (control), whereas the incidence of responsiveness loss reached 55% in the 1 μ M bupivacaine group and 100% in the presence of 5 and 10 μ M bupivacaine. As shown in figure 6, reperfusion of the AZ induced a rapid recovery of responsiveness that was similar in the four experimental groups.

Bupivacaine Effects on the Incidence of Arrhythmias during Simulated Ischemia-Reperfusion

As figure 7 shows, two types of arrhythmias occurred during simulated ischemia and reperfusion: repetitive responses induced by an extrastimulus (fig. 7A) and spontaneous repetitive responses (fig. 7B). During simulated ischemia, superfusion with bupivacaine (5 and 10 μ M) drastically suppressed extrastimulus-induced and spontaneous repetitive responses (fig. 5). However, in the presence of the lowest concentration of bupivacaine (1 μ M), the number of preparations exhibiting spontaneous repetitive responses was significantly higher (8 of 11) than in the control group (3 of 12; $P = 0.02$). During reperfusion, the extrastimulus-induced arrhythmias observed in the control group were inhibited by the concentrations of bupivacaine, and the spontaneous arrhythmias were inhibited by the two highest bupivacaine concentrations (fig. 6).

BUPIVACAINE AND MYOCARDIAL ISCHEMIA-REPERFUSION

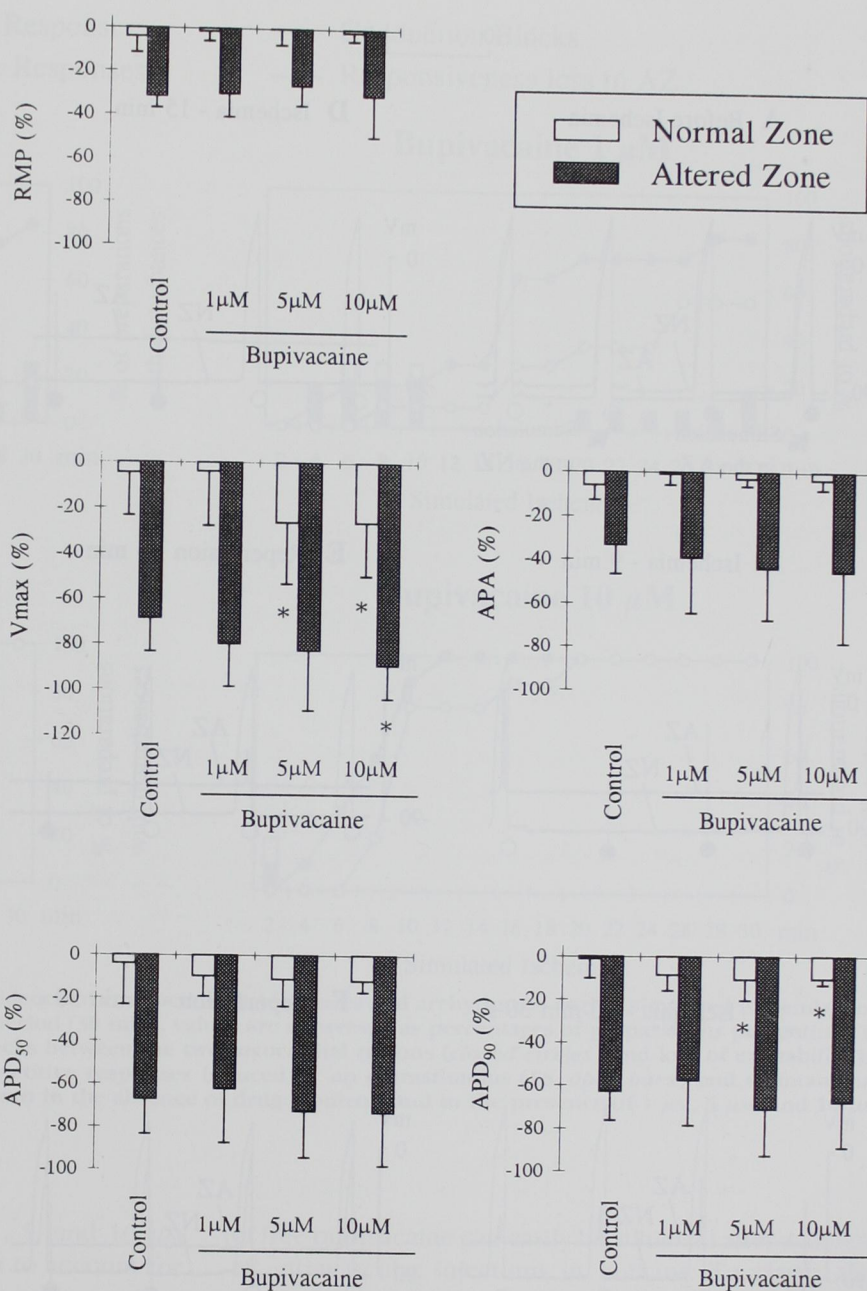


Fig. 3. Effects of bupivacaine on action potential parameters: RMP, V_{max} , APA, APD₅₀, and APD₉₀, measured concomitantly in normoxic and altered (ischemic) conditions. Values are expressed as mean \pm SD of variation percentage measured at 10 min of simulated ischemia. In each compartment (normal and altered zone), values obtained with 1 μ M, 5 μ M, and 10 μ M bupivacaine were compared with control (no drug) using a Dunnett's test (* P < 0.05). RMP, resting membrane potential; V_{max} , maximal upstroke velocity of action potential; APA, action potential amplitude; APD₅₀ and APD₉₀, action potential duration measured at 50% and 90% of repolarization, respectively.

Discussion

This study had several results: (1) Ischemic conditions enhanced the V_{max} decrease induced by bupivacaine (10 μ M), (2) bupivacaine (5 and 10 μ M) shortened the mean time to the occurrence of conduction block during ischemia, (3) bupivacaine cardiotoxicity was enhanced by ischemia, resulting in loss of excitability of the ischemic myocardium, (4) 5 and 10 μ M bupivacaine

decreased the incidence of ischemia-induced arrhythmias, whereas 1 μ M of the drug enhanced them, and (5) 1, 5, and 10 μ M bupivacaine decreased the occurrence of reperfusion-induced arrhythmias.

To simulate ischemic conditions, we used a modified Tyrode's solution and combined acidosis, hyperkalemia, hypoxia, and the lack of substrates because, as previously described in detail,²³⁻²⁶ the association of these components can reproduce the electrical alter-

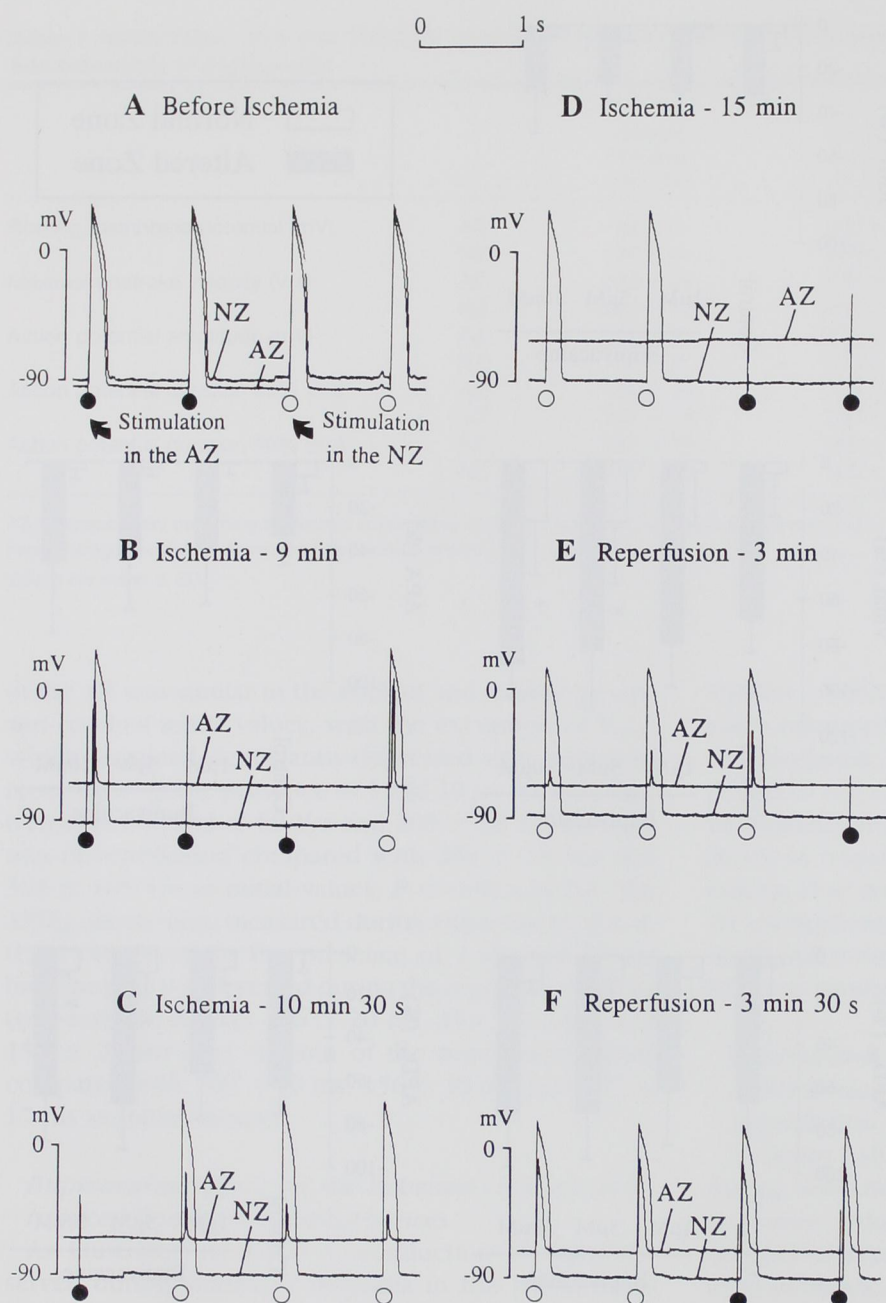


Fig. 4. Representative recordings of conduction block occurrences leading to a responsiveness loss of the myocardial tissue, induced by ischemia and suppressed by reperfusion in the presence of 10 μ M bupivacaine. Action potentials (AP) occurring in the normal zone (NZ) and the altered zone (AZ) were recorded during ischemia and during reperfusion. The stimulation was applied in either NZ (open circle) or AZ (closed circle) on stimulus artifacts. Before initiation of the simulated ischemia (A), two APs were recorded, one in each zone, in response to the stimulation applied in either one or the other compartment. Nine minutes after the onset of the simulated ischemic period (B), signal conduction from NZ to AZ was maintained, whereas the preparation began to fail to respond when stimulated in AZ. More specifically, panel B illustrates the moment when the ventricular myocardium lost its responsiveness to stimulation applied in AZ: The last response elicited by stimulation in AZ (first AP couplet) is followed by the absence of responsiveness to stimuli continuously applied to the same AZ. Responsiveness in AZ appeared only when the preparation was stimulated in NZ (last couplet of AP in panel B). One minute and 30 s later (i.e., 10 min and 30 s after the onset of simulated ischemia, panel C), the AZ still responded to the stimulation applied in NZ, with a decreasing response amplitude. After 15 min of exposure to ischemic conditions (D), the AZ became unexcitable, regardless of the compartment stimulated. Reperfusion led to a recovery of AZ responsiveness, first by removal of the conduction block from NZ to AZ (3 min after the onset of reperfusion phase, panel E), and second by responsiveness recovery of the AZ when stimulated (30 s later; i.e., after 3 min and 30 s of the reperfusion phase, panel F).

ations on cardiac APs observed in more complex *in vivo* animal models during acute myocardial ischemia.¹⁸ The electrical modifications measured in this study, namely resting membrane depolarization, decrease in AP amplitude and V_{max} , AP shortening, and lengthening of myocardial conduction times, are comparable to those found in other *in vitro* studies using similar ischemic-like solutions^{18,26,27} and also to *in vivo* investiga-

tions during coronary artery occlusion.²⁸ Further, the reliability of the double-bath technique, as demonstrated by the constancy of the AP parameters in the NZ adjacent to the AZ submitted to simulated ischemia, made it possible to investigate the electrophysiologic effects of bupivacaine simultaneously in normoxic and ischemic-reperfused myocardium, as might occur *in vivo* during ischemia.

BUPIVACAINE AND MYOCARDIAL ISCHEMIA-REPERFUSION

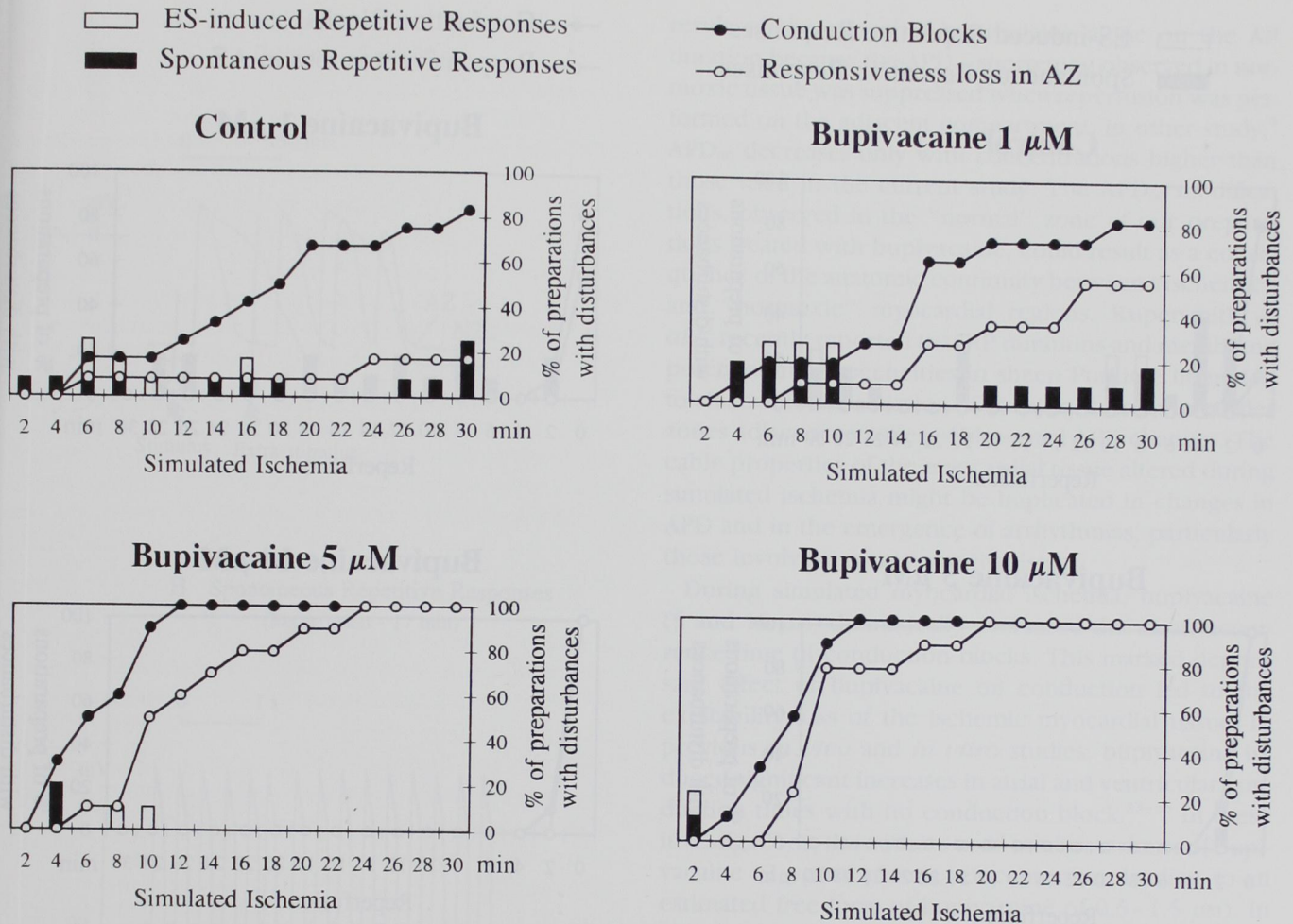


Fig. 5. The effects of bupivacaine on the incidence of conduction disturbances and arrhythmias during simulated ischemia. For each time interval (2 min) of the ischemic period (30 min), values are expressed as percentages of preparations presenting (1) conduction disturbances, or conduction blocks between the two myocardial regions (closed circles) and loss of excitability in altered zone (AZ, open circles); and (2) repetitive responses induced by an extrastimulus (ES, open bars) and spontaneous bupivacaine (closed bars). Patterns are shown in the absence of drug (control) and in the presence of 1 μ M, 5 μ M, and 10 μ M bupivacaine.

The bupivacaine concentrations of 1, 5, and 10 μ M used in our *in vitro* study were chosen to account for clinically observed bupivacaine plasma concentrations and drug concentrations used during *in vivo* investigations. After intravenous injection of bupivacaine in awake, unanesthetized sheep, the clinical toxicity was observed with a whole-blood concentration that ranged from 3–11 μ g/ml.⁶ Assuming a blood-to-plasma concentration ratio of 0.73,²⁹ this should correspond to a plasma dose of 4–15 μ g/ml. Because bupivacaine is 66–88% bound to plasma proteins for this concentration range,³⁰ the free form of the drug should be approximately 0.5–5 μ g/ml (1.5–15 μ M). These concentrations

of free bupivacaine can easily be achieved after accidental intravascular injections in humans. To clarify the mechanisms implicated in cardiotoxic effects of an ischemia-bupivacaine interaction, we used 5 and 10 μ M of the drug, which corresponds to the clinically relevant range for toxicity, and 1 μ M, which is slightly less than the range of toxic doses (1.5–15 μ M).

In normoxic conditions, our results showed a significant ($P < 0.05$) slowing of V_{max} induced by 5 and 10 μ M bupivacaine but not by the 1- μ M dose. These effects can be explained by bupivacaine's ability to block sodium channels, particularly in their inactivated state, as reported by Clarkson and Hondeghem.^{9,31} In our *in*

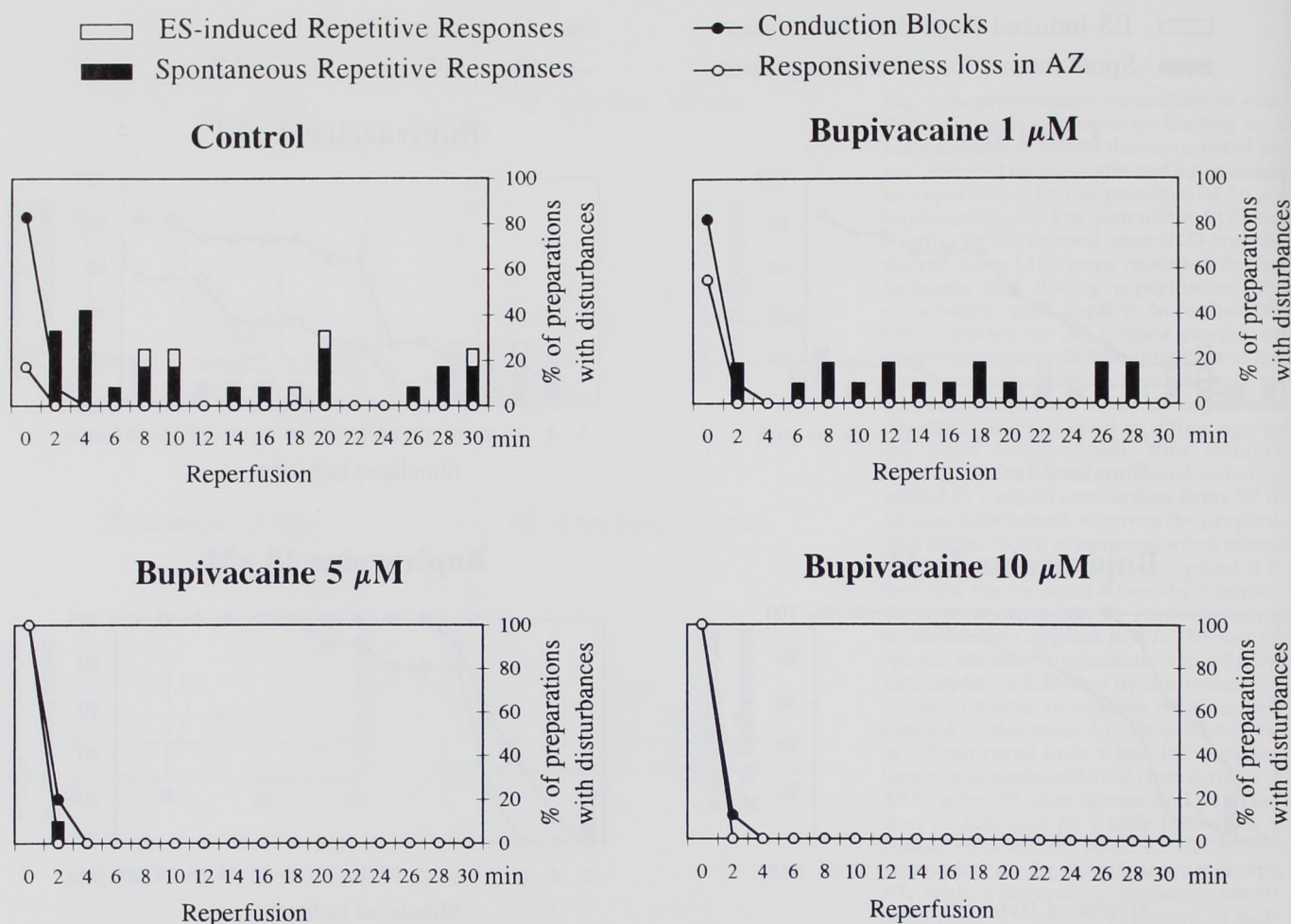


Fig. 6. Effects of bupivacaine on the incidence of conduction disturbances and arrhythmias during simulated reperfusion. For each time interval (2 min) of the reperfusion period (30 min), values are expressed as percentages of preparations presenting (1) conduction disturbances, or conduction blocks between the two myocardial regions (closed circles) and loss of excitability in altered zone (AZ, open circles); and (2) repetitive responses induced by an extrastimulus (ES, open bars) and spontaneous arrhythmias (closed bars). Patterns are shown in the absence of drug (control) and in the presence of 1 μ M, 5 μ M, and 10 μ M bupivacaine.

vitro model of myocardial ischemia, bupivacaine affected V_{\max} only slightly in AZ during simulated ischemia compared with its effects under normoxic conditions. A likely explanation might be the ischemic conditions used, which mimicked acute myocardial ischemia and already dramatically depressed V_{\max} , even in the absence of bupivacaine. Thus 10 μ M bupivacaine only significantly worsened the V_{\max} decrease induced by simulated ischemia. These effects of the ischemia-bupivacaine interaction on V_{\max} may be due to voltage-dependent inactivation of sodium channels at depolarized membrane potentials. Using a single sucrose gap voltage-clamp technique in guinea pig ventricular muscle,

Clarkson and Hondeghem⁹ clearly showed that 1 μ g/ml bupivacaine (3.5 μ M) shifted the voltage dependence of V_{\max} availability toward hyperpolarized potentials by 10.7 ± 2.6 mV ($P < 0.01$). This shift of sodium channel availability, when combined with ischemia-induced depolarization, may substantially increase the fraction of sodium channels in the inactivated state, and it probably explains the larger reduction of V_{\max} with ischemia in the presence of bupivacaine.

We also observed a shortening of APD_{90} on normoxic tissue in preparation treated with bupivacaine while ischemia was simulated on the adjacent myocardial zone. It seems unlikely that this APD_{90} decrease was a

BUPIVACAINE AND MYOCARDIAL ISCHEMIA-REPERFUSION

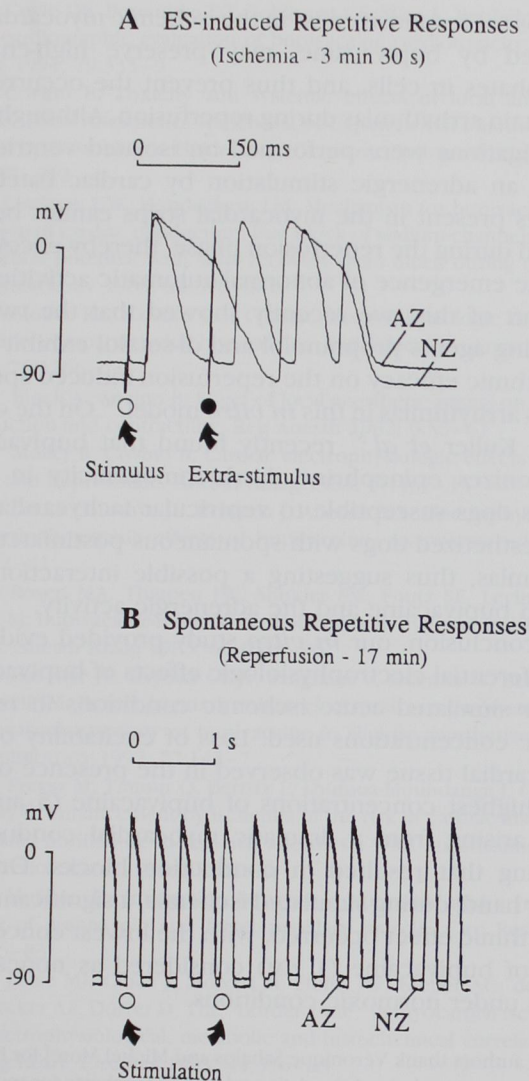


Fig. 7. Representative arrhythmia recordings illustrating repetitive responses induced by (A) an extrastimulus (ES) and (B) spontaneous arrhythmias. Traces show action potentials (AP) recorded simultaneously in normal zone (NZ) and altered zone (AZ). In panel A, a single ES (closed circle), applied 115 ms after the stimulus (open circle) in NZ, induced one response in AZ and NZ and two additional extrasystoles in AZ. The ES-induced arrhythmias might be a result of reentry movements: The ES applied in the NZ elicited a response first in AZ, probably caused by the refractory period in NZ. The signal then propagated in NZ, which in turn reexcited the AZ (first abnormal extrasystole). Considering the action potential duration dispersion between both regions, out of its refractory period the AZ would be reexcited by the depolarization maintained in the NZ (second abnormal extrasystole in the AZ). In panel B, note that stimulation was stopped just after the onset of arrhythmia, although sustained spontaneous activity persisted. These spontaneous arrhythmias probably can be attributed to abnormal automatic activities (see the discussion for more details).

result of the action of bupivacaine alone on the AP duration because the APD_{90} shortening observed in normoxic tissue was suppressed when reperfusion was performed on the adjacent compartment; in other study,⁹ APD_{90} decreases only with concentrations higher than those used in the current study. The APD_{90} modifications, observed in the "normal" zone of our preparations treated with bupivacaine, could result as a consequence of the anatomic continuity between "ischemic" and "normoxic" myocardial regions. Kupersmith *et al.*³² recently reported that AP durations and membrane potential inhomogeneities in sheep Purkinje fibers led to electronic transmission of an injury current to border zones adjacent to zones of abnormal APD changes. The cable properties of the myocardial tissue altered during simulated ischemia might be implicated in changes in APD and in the emergence of arrhythmias, particularly those involving reentry mechanisms.

During simulated myocardial ischemia, bupivacaine (5 and 10 μM) dramatically decreased the mean occurrence time of conduction blocks. This marked depressant effect of bupivacaine on conduction led to the excitability loss of the ischemic myocardial tissue. In previous *in vivo* and *in vitro* studies, bupivacaine induced significant increases in atrial and ventricular conduction times with no conduction block.³³⁻³⁵ In these investigations, the authors used moderate doses of bupivacaine (2 $\mu\text{g}/\text{ml}$ plasma concentration, leading to an estimated free form of bupivacaine of 0.5–1.5 μM). In these studies, investigations were performed in healthy animals or isolated hearts, whereas in our *in vitro* model we studied the cardiotoxic effects of bupivacaine during simulated ischemia. When compared with results obtained on healthy myocardium, our findings suggest that myocardial ischemia reinforces certain cardiotoxic effects of bupivacaine at a "nontoxic" 1 μM concentration. However, care should be taken when extrapolating these results to the clinical setting.

During ischemia, bupivacaine decreased the incidence of extrastimulus-induced arrhythmias at all three concentrations and spontaneous arrhythmias at 5 and 10 μM bupivacaine. On awake or anesthetized animals, hypoxia and acidosis increased the likelihood that bupivacaine would induce arrhythmias.^{14,15} The antiarrhythmic effects of 5 and 10 μM bupivacaine observed in our model of acute ischemia¹⁸ differ from these latter findings. This might be explained primarily by the different *in vivo* and *in vitro* models and the type of ischemic conditions used, as Rosen *et al.* and Heavner *et al.*

studied bupivacaine cardiotoxicity in acidotic-hypoxic¹⁴ and hypoxic¹⁵ conditions, respectively.

In addition to these reasons, we also hypothesized that there are differences in the mechanisms that may underlie the occurrence of arrhythmias. As previously discussed,²³ in our *in vitro* model of ischemic-reperfused myocardium, repetitive responses induced by an extrastimulus are likely a result of reentrant mechanisms between normal and ischemic myocardium. First, the representative arrhythmia induced by an extrastimulus illustrated in figure 7A suggests reentry. Second, it is well established that, to occur, reentry movements require a site of unidirectional block and slow retrograde conduction. Thus, at all three concentrations, bupivacaine, which reduced the mean occurrence time to the onset of myocardial conduction blocks and led to the loss of excitability in the ischemic myocardium, might block pathways involved in reentry movements and impair reexcitation in healthy tissue. Spontaneous repetitive responses observed in our model are probably not related to early and delayed depolarizations, which were not observed in our experiments, but they may also be based on reentry or merely be associated with abnormal automaticity, perhaps induced by injury current occurring between myocardial zones with different electrical properties.³² The loss of excitability induced in all experiments by 5 and 10 μM bupivacaine might explain their antiarrhythmic effects, thus inhibiting the emergence of spontaneous arrhythmias. The promoting effect of 1 μM bupivacaine on ischemia-induced spontaneous arrhythmias was accompanied by loss of ischemic tissue excitability in only 55% of preparations compared with 100% of preparations treated with 5 and 10 μM of the drug. Although in normoxic conditions, De la Coussaye *et al.*¹³ used epicardial mapping to show that bupivacaine prolongs longitudinal and transverse conduction velocity and facilitates induction of reentrant ventricular arrhythmias in isolated rabbit hearts. All these results suggest that, in our ischemic conditions, myocardial conduction with 1 μM bupivacaine was not yet completely blocked, as it is in the presence of 5 or 10 μM of the drug, but was sufficiently slowed to allow the emergence of spontaneous arrhythmias.

The mechanisms involved in arrhythmias that occur during reperfusion and were nearly prevented by bupivacaine in our experimental model are not yet well defined but might involve depletion of high-energy phosphates, sodium, or calcium overload and implication of reactive oxygen species. It cannot be ruled out

that loss of responsiveness in the ischemic myocardium, induced by bupivacaine, may preserve high-energy phosphates in cells, and thus prevent the occurrence of certain arrhythmias during reperfusion. Although our investigations were performed on isolated ventricular walls, an adrenergic stimulation by cardiac catecholamines present in the myocardial strips cannot be excluded during the reperfusion phase, thereby encouraging the emergence of abnormal automatic activities. In support of this, we recently showed that the two β -blocking agents propranolol and dl-sotalol exhibit antiarrhythmic efficacy on the reperfusion-induced spontaneous arrhythmias in this *in vitro* model.³⁶ On the other hand, Kulier *et al.*³⁷ recently found that bupivacaine antagonizes epinephrine dysrhythmogenicity in conscious dogs susceptible to ventricular tachycardia and in anesthetized dogs with spontaneous postinfarct dysrhythmias, thus suggesting a possible interaction between bupivacaine and the adrenergic activity.

In conclusion, our *in vitro* study provided evidence of differential electrophysiologic effects of bupivacaine under simulated acute ischemic conditions in regard to the concentrations used. Loss of excitability of the myocardial tissue was observed in the presence of the two highest concentrations of bupivacaine (5 and 10 μM), arising from a dramatic myocardial conduction slowing that resulted in conduction blocks. On the other hand, during simulated ischemia, a significant proarrhythmic effect occurred, with the lowest concentration of bupivacaine (1 μM) considered as noncardiotoxic under normoxic conditions.

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BUPIVACAINE AND MYOCARDIAL ISCHEMIA-REPERFUSION

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