Effects of Ropivacaine on Action Potential Configuration and Ion Currents in Isolated Canine Ventricular Cardiomyocytes

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Background: Despite the widespread clinical application of ropivacaine, there is little information on the cellular cardiac effects of the drug. In the current study, therefore, the concentration-dependent effects of ropivacaine on action potential morphology and the underlying ion currents were studied and compared with those of bupivacaine in isolated canine ventricular cardiomyocytes.

Methods: Action potentials were recorded from the enzymatically dispersed cells using sharp microelectrodes. Conventional patch clamp and action potential voltage clamp arrangements were used to study the effects of ropivacaine on transmembrane ion currents.

Results: Ropivacaine induced concentration- and frequency-dependent changes in action potential configuration, including shortening of the action potentials, reduction of their amplitude and maximum velocity of depolarization, suppression of early repolarization, and depression of plateau. Reduction in maximum velocity of depolarization was characterized with an EC_{50} value of 81 ± 7 μM at 1 Hz. Qualitatively similar results were obtained with bupivacaine ($EC_{50} = 47 \pm 3$ μM). Under voltage clamp conditions, a variety of ion currents were blocked by ropivacaine: L-type calcium current ($EC_{50} = 263 \pm 67$ μM), transient outward current ($EC_{50} = 384 \pm 75$ μM), inward rectifier potassium current ($EC_{50} = 372 \pm 35$ μM), rapid delayed rectifier potassium current ($EC_{50} = 303 \pm 47$ μM), and slow delayed rectifier potassium current ($EC_{50} = 106 \pm 18$ μM).

Conclusions: Ropivacaine, similarly to bupivacaine, can modify cardiac action potentials and the underlying ion currents at concentrations higher than the usual therapeutic range. However, in cases of overdose, cardiac complications may be anticipated both during and after anesthesia due to the blockade of various ion currents.

ROPIVACAINE is a local anesthetic extensively applied in regional anesthesia. Its structure is similar to that of bupivacaine (1-propyl homolog), but it is believed to be less cardiotoxic than bupivacaine.[1] Despite its widespread clinical use, little is known about the effect of ropivacaine on native cardiac ion channels responsible for generation of the ventricular action potential. Ropivacaine was shown to block sodium (I_{Na}) and L-type calcium (I_{Ca}) currents in guinea pig ventricular myocytes without affecting the delayed rectifier (I_K) and inward rectifier (I_{K1}) potassium currents.[2] On the other hand, several cloned K^+ channels, including Kv1.5, Kv4.3, human *ether-a-go-go*–related gene, and Kv7.1, were effectively blocked by ropivacaine.[3-8] Consistent with K^+ channel blockade, ropivacaine was found to induce early afterdepolarizations in rabbit Purkinje fiber and ventricular preparations[9]; furthermore, cases of ventricular fibrillation and cardiac arrest were reported during its clinical application.[10-16] To obtain data predictive regarding the electrophysiologic actions of ropivacaine in the human heart, we studied the concentration-dependent effects of the drug on the action potential morphology and the underlying ion currents in isolated canine ventricular cardiomyocytes. The canine myocytes were chosen because their electrophysiologic properties are believed to be most similar to those of humans regarding the distribution and kinetics of transmembrane ion currents.[17,18] We found that ropivacaine can modify cardiac action potentials and the underlying ion currents at concentrations higher than the typical therapeutic range. However, in cases of accidental overdose, cardiac complications may be anticipated during the anesthesia as well as the early postoperative period.

Materials and Methods

Isolation of Single Canine Ventricular Myocytes

Adult mongrel dogs of either sex were anesthetized with intravenous injections of 10 mg/kg ketamine hydrochloride (Calypsol; Richter Gedeon Rt., Budapest, Hungary) plus 1 mg/kg xylazine hydrochloride (CP-Xilazin; CP-Pharma, Burgdorf, Germany) according to a protocol approved by the Animal Care Committee of the University of Debrecen (Debrecen, Hungary) conforming to the guidelines laid out in the *Guide for the Care and Use of Laboratory Animals*. The hearts were quickly removed and placed in Tyrode solution. Single myocytes were obtained by enzymatic dispersion using the segment perfusion technique.[17] Briefly, a wedge-shaped section of the ventricular wall supplied by the left anterior descending coronary artery was dissected, cannulated, and perfused with oxygenated Tyrode solution containing 144 mM NaCl, 5.6 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 5 mM HEPES, and 11 mM dextrose at pH = 7.4. Perfusion was maintained until the removal of blood from the

coronary system and then switched to a nominally Ca^{2+}-free Joklik solution (Minimum Essential Medium Eagle Joklik Modification; Sigma-Aldrich Co., St. Louis, MO) for 5 min. This was followed by 30 min of perfusion with Joklik solution supplemented with 1 mg/ml collagenase (Type II; Worthington Biochemical Co., Lakewood, NJ) and 0.2% bovine serum albumin (Fraction V; Sigma-Aldrich Co.) containing 50 μM Ca^{2+}. Portions of the left ventricular wall were cut into small pieces, and the cell suspension, obtained at the end of the procedure predominantly from the midmyocardial region of the left ventricle, was washed with Joklik solution. Finally, the Ca^{2+} concentration was gradually restored to 2.5 mM. The cells were stored in Minimum Essential Medium Eagle until use.

Recording of Action Potentials

All electrophysiologic measurements were performed at 37°C. The rod-shaped viable cells, showing clear striation, were sedimented in a lucid chamber allowing continuous superfusion with oxygenated Tyrode solution. Transmembrane potentials were recorded using 3 M KCl–filled sharp glass microelectrodes having a tip resistance between 20 and 40 MΩ. These electrodes were connected to the input of an Axoclamp-2B amplifier (Axon Instruments Inc., Foster City, CA). The cells were paced through the recording electrode at steady cycle length of 1 s using 1-ms-wide rectangular current pulses with 120% threshold amplitude. Before each experiment, action potentials were recorded for 15 min before drug application to allow the cells to equilibrate. The experiment was continued only if action potential parameters remained stable during this period of time. Because the cytosol was not dialyzed, time-dependent changes in action potential duration were negligible under these experimental conditions.

Concentration-dependent effects of ropivacaine and bupivacaine were determined in a cumulative manner by applying increasing concentrations of the drug starting from 1 μM in action potential measurements and 10 μM in the voltage clamp experiments. Each concentration was superfused for 3 min. This incubation period was sufficient to develop the steady state drug effect. When performing frequency-dependent measurements, the cycle length was set to 5 s, and after equilibration for at least 5 min, the cycle length was continuously varied to the shorter values. Action potentials were digitized at 200 kHz using Digidata 1200 A/D card (Axon Instruments Inc.) and stored for later analysis.

Conventional Voltage Clamp

The cells were superfused with oxygenated Tyrode solution. Suction pipettes, fabricated from borosilicate glass, had a tip resistance of 2 MΩ after filling with pipette solution containing 100 mM K-aspartate, 45 mM KCl, 1 mM $MgCl_2$, 5 mM HEPES, 10 mM EGTA, and 3 mM

K-ATP or, alternatively, 110 mM KCl, 40 mM KOH, 10 mM HEPES, 10 mM EGTA, 20 mM TEACl, and 3 mM K-ATP when measuring potassium or calcium currents, respectively (pH = 7.2 in both cases). Membrane currents were recorded with the Axopatch-2B amplifier (Axon Instruments Inc.) using the whole cell configuration of the patch clamp technique.[19] After establishing a high (1- to 10-GΩ) resistance seal by gentle suction, the cell membrane beneath the tip of the electrode was disrupted by further suction or by applying 1.5-V electrical pulses for 1–5 ms. The series resistance was typically 4–8 MΩ before compensation (usually 50–80%). Experiments were discarded when the series resistance was substantially increasing during the measurement. Outputs from the clamp amplifier were digitized at 100 kHz under software control (pClamp 9.0; Axon Instruments Inc.). These records were analyzed using Clampfit 9.0 software (Axon Instruments Inc.). Ion currents were normalized to cell capacitance, determined in each cell using short hyperpolarizing pulses from −10 mV to −20 mV. The experimental protocol for each measurement is described where pertinent in the Results section. No attempt was made to measure I_{Na} directly because this current cannot be resolved under physiologic experimental conditions due to its extremely large density resulting in inadequate voltage control.

Action Potential Voltage Clamp

After gigaseal formation, action potentials were recorded in current clamp mode from the myocytes superfused with Tyrode solution. The pipette solution was identical to that used for potassium current measurement under conventional voltage clamp conditions. The cells were continuously paced through the recording electrode at steady stimulation frequency of 1 Hz so a 1- to 2-ms gap between the stimulus artifact and the upstroke of the action potential could occur. Ten subsequent action potentials were recorded from each cell, which were digitized and averaged. This averaged signal was delivered to the same cell at the identical frequency as command voltage after switching the amplifier to voltage clamp mode. The current trace obtained under these conditions is a horizontal line positioned at the zero level except for the capacitive transient. When blocking an ion current pharmacologically, the blocked current has to be delivered by the amplifier to maintain the original action potential. For example, when repolarizing K current was prevented from flowing near the end of the action potential, an additional negative current had to be applied. This current, therefore, appeared in the original record as an inward current, which was inverted to appear as an outward deflection on the presented record consistent with the voltage clamp convention for an outward current. Ropivacaine and bupivacaine were applied in a cumulative manner (from 10 to 1,000 μM concentration). The profile of the ion current

blocked was determined by subtracting the predrug curve from the postdrug one. This procedure resulted in composite current profiles containing three distinct current peaks after reverting the polarity: an early outward for I_{to}, an inward for I_{Ca}, and a late outward for $I_{Kr} + I_{K1}$.[20,21] Unfortunately, I_{to} and I_{Ca} could not be fully separated because of their partial temporal coincidence. This may result in underestimation of both I_{to} and I_{Ca} when they are simultaneously blocked. Because of the poor voltage control during the action potential upstroke, I_{Na} was buried in the capacitive transient. Therefore, I_{Na} transients are not shown in the figures; they are considered as artifacts and are removed from the presented records.

Statistics

Results are expressed as mean $\pm$ SEM. Statistical significance of differences was evaluated using one-way analysis of variance followed by the Dunnett test. Differences were considered significant when P was less than 0.05. Concentration–response curves were obtained by fitting data to the Hill equation using Microsoft Origin 6.0 software (Redmond, WA). Half-effective concentrations (EC_{50} values) and Hill coefficients were determined from these Hill fits. Action potential parameters, such as the maximum velocity of depolarization measured during the action potential upstroke (V_{max}), action potential duration measured at 50% and 90% level of repolarization (APD_{50} and APD_{90}, respectively), and amplitude of phase 1 repolarization measured as a difference between the overshoot potential and the plateau potential during the notch, were determined in off-line mode using Clampfit 9.0 software (Axon Instruments Inc.).

Drugs

Ropivacaine (Naropin ampoules, 20 ml, 7.5 mg/ml) and bupivacaine (Marcaine ampoules, 20 ml, 5 mg/ml) were purchased from AstraZeneca AB (Söderstalje, Sweden) and were freshly diluted with Tyrode solution to final concentration on the day of experiment. Other drugs were obtained from Sigma-Aldrich Co.

Results

Effect of Ropivacaine on Action Potential Configuration

Ropivacaine treatment caused concentration-dependent changes in action potential morphology in canine ventricular myocytes, paced at a constant frequency of 1 Hz, including reduction of V_{max}, shortening of action potential duration, reduction of the amplitude of phase 1 repolarization, and depression of the plateau (fig. 1). From these effects, reduction of phase 1 repolarization and V_{max} was statistically significant from 10 μM, and shortening of action potentials was significant from 30

μM. Fitting the V_{max} data to the Hill equation, an EC_{50} of 81 $\pm$ 7 μM and a Hill coefficient of 1.02 $\pm$ 0.08 were obtained in the average of six myocytes studied. Suppression of V_{max} and phase 1 repolarization was fully reversible within the 10-min period of washout. Interestingly, APD_{90} showed a marked rebound phenomenon: It increased above the normal level after superfusion with ropivacaine-free Tyrode solution. Action potential amplitude was decreased by 100 μM ropivacaine from 114.6 $\pm$ 2.2 mV to 100.8 $\pm$ 2.8 mV ($P < 0.05$, n = 6), which returned to 113.8 $\pm$ 2.9 mV after 10 min of washout. Although ropivacaine did not induce statistically significant changes in the resting membrane potential of the cells, a tendency of depolarization was observed at large concentrations (above 100 μM).

Frequency-dependent Properties

Both reduction of V_{max} and shortening of action potentials by ropivacaine were frequency dependent in the six myocytes studied (figs. 2A and B). Reduction of V_{max} was more prominent at faster pacing rates (normal frequency dependence), which is due to the use-dependent action of ropivacaine on Na channels. The APD shortening effect showed the properties of reverse rate-dependent action, as it became more pronounced with lengthening the pacing cycle length, and was absent at cycle lengths shorter than 1,000 ms. This reverse rate-dependent action is a general feature of several ion channel blocking agents, including antiarrhythmic drugs and local anesthetics, and is attributable to the smaller net outward current flowing during the plateau of the longer action potential observed at longer cycle lengths. Therefore, the shortening effect of ropivacaine is likely due to inhibition of I_{Ca} and window I_{Na}, changes that may have major influence on APD at longer cycle lengths.

Restitution kinetics of V_{max} and action potential duration were also determined. In these experiments, the myocytes were paced using a train of 20 basic stimuli delivered at a basic cycle length of 1,000 ms. Each train was followed by a single extra stimulus applied with successively longer coupling intervals. The train of basic stimuli was reinitiated after the delivery of the extra stimulus. In this way, each 20th basic action potential was followed by a single extra action potential occurring at gradually increasing diastolic intervals. The diastolic interval was defined as the time from APD_{90} of the last basic action potential of the train to the upstroke of the extra action potential. Recovery curves were generated by plotting the V_{max} or APD of each extra action potential against the respective diastolic interval (figs. 2C and D). In addition to the 37 $\pm$ 1% of tonic block measured after the longest diastolic interval of 5,000 ms, a marked rate-dependent block also became evident on shortening the diastolic interval. The offset kinetics of this rate-dependent block was estimated by fitting the data to a single exponential yielding an offset time constant of 340 $\pm$ 40
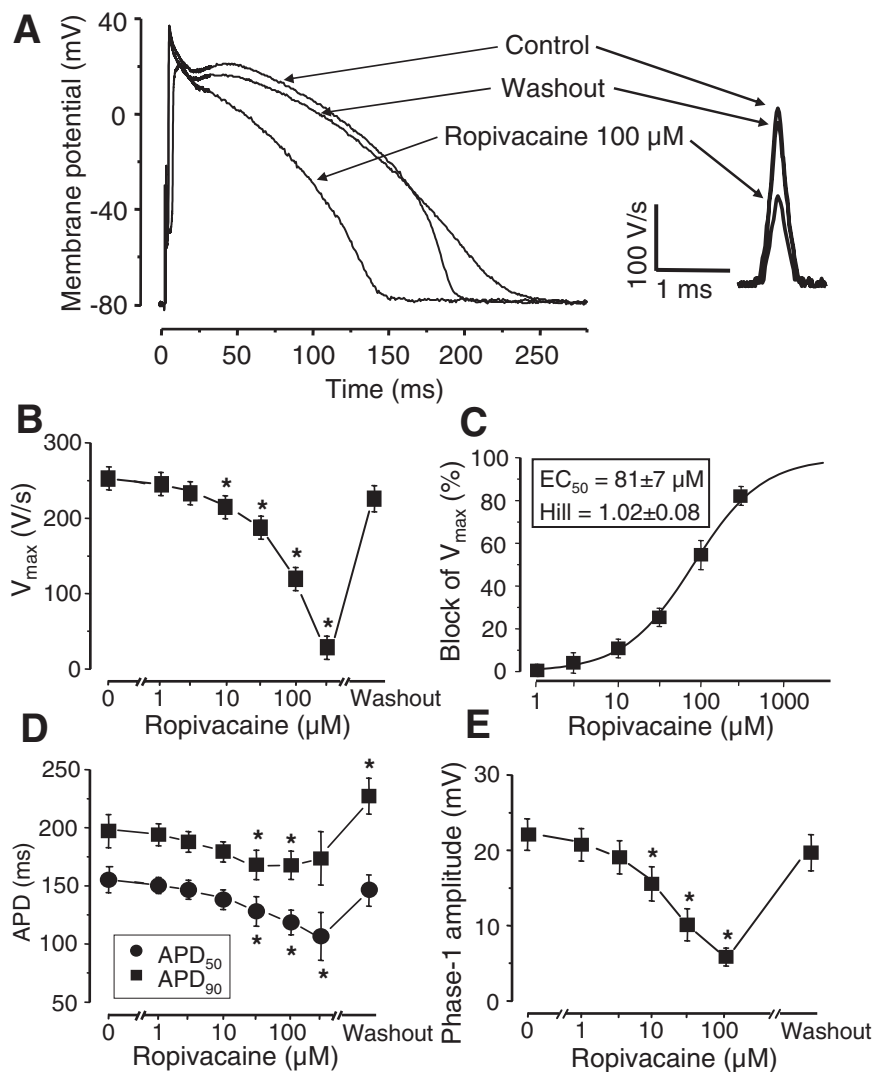
Fig. 1. (*A*) Effect of 100 μM ropivacaine on action potential configuration at a constant pacing frequency of 1 Hz. The first time-derivative of action potential upstroke is shown in the *inset*. (*B*, *D*, and *E*) Cumulative concentration-dependent effects of ropivacaine on the maximum velocity of depolarization (V_{max}), action potential duration measured at 50% (APD_{50}) and 90% (APD_{90}) level of repolarization, and phase 1 amplitude, respectively. Each concentration of ropivacaine was superfused for 3 min; the washout lasted for 10 min. V_{max} data derived from the experiments of *B* and fitted to the Hill equation are shown in *C*. *Symbols* and *bars* represent mean ± SEM values obtained in six myocytes. * Significant ($P < 0.05$) change from control.

ms (n = 6, fig. 2C). Curves describing the APD–diastolic interval relation in the presence of 100 μM ropivacaine were flat, and in contrast to the control situation, where APD increased with increasing diastolic interval, APD_{50} values actually decreased in the presence of ropivacaine when the diastolic interval was increased (fig. 2D).

Effect of Ropivacaine on Cardiac Ion Currents Measured by Conventional Voltage Clamp

In these experiments, performed under conventional voltage clamp conditions, cumulative concentration-dependent drug effects were studied between 10 and 1,000 μM, increasing the concentration of ropivacaine in steps of half decade. The results are summarized in figure 3.

L-type calcium current (I_{Ca}) was recorded at +5 mV using 200-ms-long depolarizations arising from the holding potential of −40 mV. At this holding potential, I_{Na} is inactivated, which minimizes the distortion of I_{Ca}. Tyrode solution was supplemented with 3 mM 4-aminopyridine, 1 μM E 4031, and 30 μM chromanol 293B to block K^+ currents. Ropivacaine blocked I_{Ca} in a concentration-

dependent manner (figs. 3A and B). An EC_{50} value of 263 ± 67 μM and a Hill coefficient of 1.27 ± 0.15 were obtained when fitting the results to the Hill equation (n = 5).

The transient outward current (I_{to}) was activated by depolarization to +50 mV arising from the holding potential of −80 mV and having duration of 200 ms. Before each test pulse a short (5-ms) depolarization to −40 mV was applied to inactivate Na^+ current, whereas Ca^{2+} current was blocked by 1 μM nisoldipine. The blocking effect of ropivacaine was characterized with an EC_{50} of 384 ± 75 μM and a Hill coefficient of 0.89 ± 0.15 in the six myocytes studied (figs. 3C and D).

The inward rectifier K^+ current (I_{K1}) was studied by applying hyperpolarizing pulses to −135 mV from the holding potential of −80 mV. The steady state current was determined after 400 ms. I_{K1} was also blocked by ropivacaine with an EC_{50} of 372 ± 35 μM and a Hill coefficient of 1.3 ± 0.13 in five myocytes (figs. 3E and F).

The rapid component of the delayed rectifier K^+ current (I_{Kr}) was activated by 1-s-long depolarizing pulses to +40 mV arising from the holding potential of −80 mV.
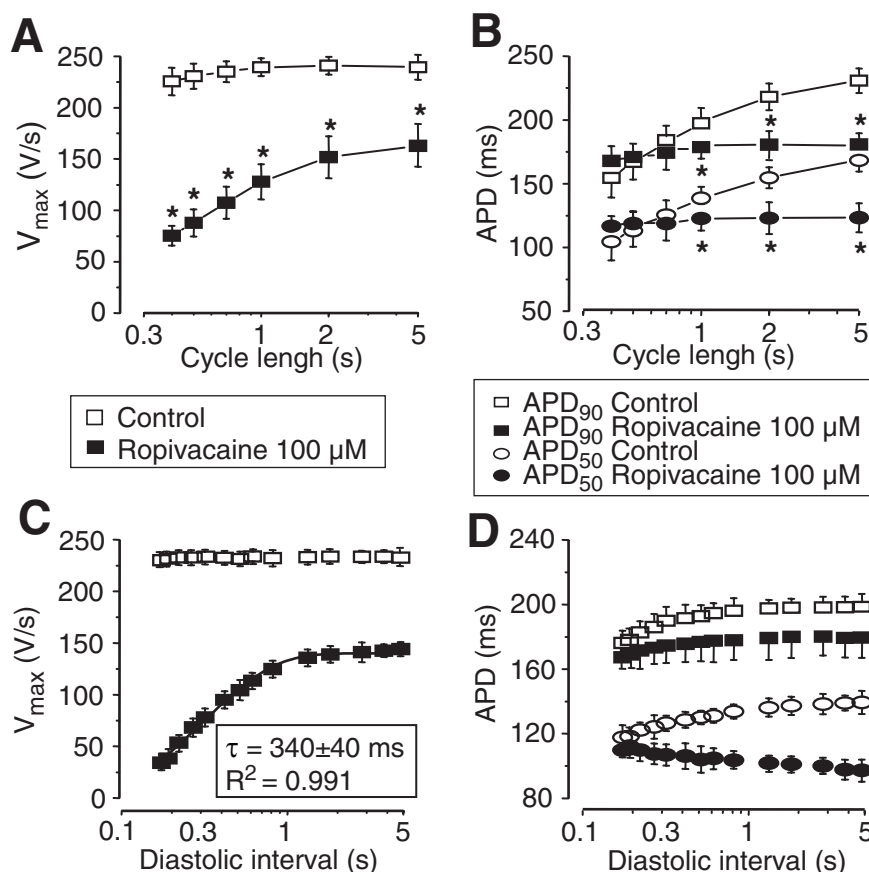
Fig. 2. (*A* and *B*) Steady state frequency-dependent effects of 100 μM ropivacaine on maximum velocity of depolarization (V_{max}) and action potential duration (APD) (n = 5). (*C* and *D*) Restitution kinetics of V_{max} and APD in the absence and presence of 100 μM ropivacaine (n = 6). The *solid line* in *C* was obtained by exponential fitting to determine the dissociation time constant of ropivacaine from the sodium channel. *Significant (*P* < 0.05) change from control.

I_{Kr} was assessed as tail current amplitudes recorded after repolarization to −30 mV. I_{Ca} and I_{Ks} were suppressed by 1 μM nisoldipine and 30 μM chromanol 293B, respectively. As shown in figures 3G and H, the amplitudes of the I_{Kr} current tails were progressively decreased by increasing ropivacaine concentration. The EC_{50} value and Hill coefficient were estimated as 303 ± 47 μM and 0.88 ± 0.09, respectively, in the average of five cells.

The slow component of the delayed rectifier K$^+$ current (I_{Ks}) was also evaluated as tail currents shown in figures 3I and J. The current was activated by 3-s-long depolarization to +50 mV, and the amplitude of tail currents was determined at the holding potential of −40 mV after repolarization. I_{Ca} was inhibited by 1 μM nisoldipine, and I_{Kr} was blocked by 1 μM E 4031. The EC_{50} was 106 ± 18 μM and the Hill coefficient was 1.13 ± 0.05 in the five myocytes examined.

Reversibility of the ropivacaine-induced suppression of ion currents showed marked diversity (fig. 4). Blockade of I_{to} was fully reverted within the initial 3 min of washout. Inhibition of I_{Ca} was not fully reversible; however, the current was restored to 80% of the initial predrug level after 10 min of washout. In contrast, I_{Kr}, I_{Ks}, and particularly I_{K1} displayed slow and only partial reversion within the 10-min washout period.

Effect of Ropivacaine on Ion Currents under Action Potential Clamp Conditions

The profile of an ion current may be markedly different when compared under conventional voltage clamp and action potential clamp conditions.[21] An advantage of the action potential clamp technique is that the effect of any drug on the *net* membrane current can be recorded, thus allowing the monitoring of drug effects simultaneously on more than one ion current. Furthermore, this technique enables the recording of true current profiles flowing during an actual cardiac action potential. Of course, in the case of a drug acting on more than one ion current, such as ropivacaine is, a series of peaks can be detected on the current trace, each of them corresponding to the fingerprint of an individual ion current or a mixture of them.[20] Accordingly, the early outward current peak, shown in figure 5, arises when I_{to} is suppressed, whereas the inward deflection indicates a blockade of I_{Ca}. The late outward current peak, coincident with the terminal repolarization of the action potential, is composed of I_{K1} plus I_{Kr} in a ratio of 3:1.[20] Ropivacaine significantly blocked I_{to}, I_{Ca}, and the late current peak, containing both I_{K1} and I_{Kr}, in the four myocytes studied. Inhibition of these currents increased with increasing the concentration of ropivacaine up to 1,000 μM. Ropivacaine blocked I_{to} with the highest sensitivity under action potential clamp conditions (fig. 5B). Suppression of the inward and the late outward current

A I_{Ca}

B

EC$_{50}$ = 263±67 µM
Hill = 1.27±0.15
n = 5

C I_{to}

D

EC$_{50}$ = 384±75 µM
Hill = 0.89±0.15
n = 6

E I_{K1}

F

EC$_{50}$ = 372±35 µM
Hill = 1.3±0.13
n = 5

G I_{Kr}

H

EC$_{50}$ = 303±47 µM
Hill = 0.88±0.1
n = 5

I I_{Ks}

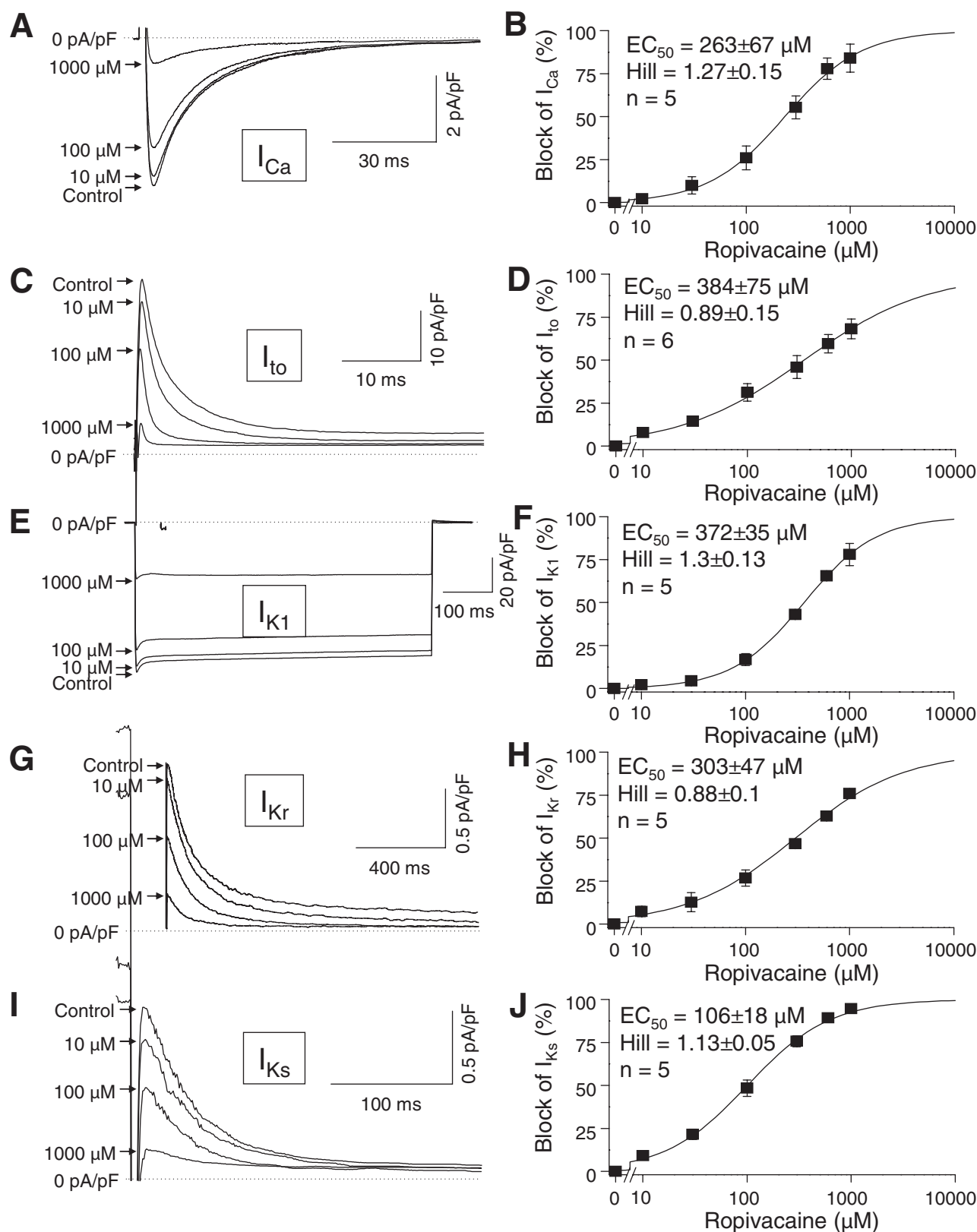**J**

EC$_{50}$ = 106±18 µM
Hill = 1.13±0.05
n = 5

Fig. 3. Concentration-dependent effects of ropivacaine (from 10 to 1,000 µM) on ion currents measured under conventional voltage clamp conditions. (*A, C, E, G,* and *I*) Superimposed current traces recorded before and after superfusion with 10, 100, and 1,000 µM ropivacaine. I$_{Ca}$, I$_{to}$, and I$_{K1}$ were recorded during the test pulse, whereas in the case of I$_{Kr}$ and I$_{Ks}$, the tail currents, obtained upon repolarization, are depicted. Current values were normalized to the cell capacitance. (*B, D, F, H,* and *J*) Results fitted to the Hill equation. n indicates the number of cells studied. I$_{Ca}$ = L-type calcium current; I$_{K1}$ = inward rectifier potassium current; I$_{Kr}$ = rapid delayed rectifier potassium current; I$_{Ks}$ = slow delayed rectifier potassium current; I$_{to}$ = transient outward current.
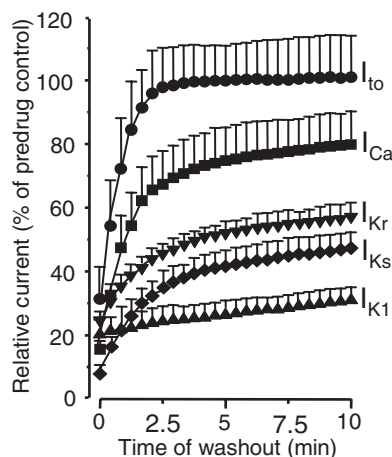
Fig. 4. Reversibility of the inhibitory effect of ropivacaine on the ion currents shown in figure 3. Current amplitudes were normalized to the predrug control and plotted as a function of the washout time. I_{Ca} = L-type calcium current; I_{K1} = inward rectifier potassium current; I_{Kr} = rapid delayed rectifier potassium current; I_{Ks} = slow delayed rectifier potassium current; I_{to} = transient outward current.

peak increased symmetrically with increasing concentrations of ropivacaine (fig. 5C).

Effect of Bupivacaine on Action Potential Configuration and Transmembrane Ion Currents

Bupivacaine—similarly to ropivacaine—reversibly decreased action potential amplitude and V_{max}, shortened action potential duration, reduced the amplitude of phase 1 repolarization, and depressed the plateau in a concentration-dependent manner (figs. 6A–E). All these actions of bupivacaine were stronger than those of ropivacaine, because the inhibition of V_{max} and phase 1 amplitude was significant from the concentration of 3 μM, and the shortening of APD_{50} from 10 μM ($P < 0.05$, n = 8). Accordingly, EC_{50} estimated for V_{max} block was 47 ± 3 μM. In contrast to ropivacaine, bupivacaine did not shorten APD_{90} significantly; however, the rebound effect on action potential duration (*i.e.*, lengthening of APD after washing out the drug) was evident with bupivacaine as well. Similar to results obtained with ropivacaine, the blocking effect of bupivacaine on V_{max} was rate dependent, whereas the shortening effect on APD_{50} showed reverse rate-dependent properties in the five myocytes examined (figs. 6F and G). Shortening of APD_{90} was not statistically significant at any of the applied frequencies. The blocking profile of bupivacaine on ion currents studied under action potential clamp conditions in canine ventricular myocytes (n = 4) was also similar to that obtained with ropivacaine, except for the more pronounced inhibition seen at nonsaturating drug concentrations (fig. 7).

Discussion

This is the first study to analyze the effects of ropivacaine on ion currents in canine ventricular cardiomyo-
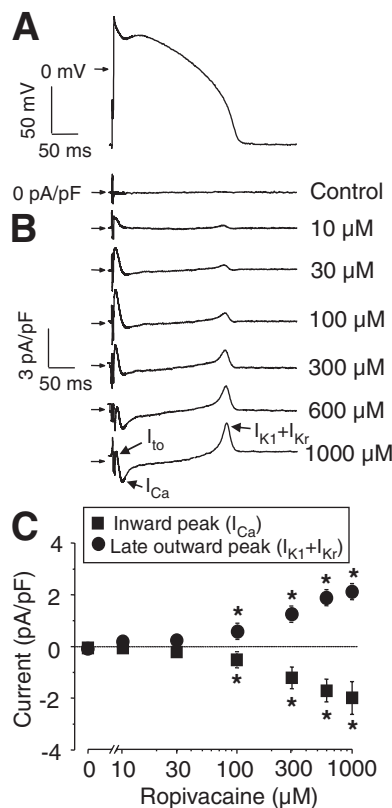

Fig. 5. Effects of ropivacaine on ion currents under action potential voltage clamp conditions. Representative records of a command signal (*A*) and the underlying current traces obtained in the presence of increasing concentrations of ropivacaine (*B*). Upward and downward deflections from the zero line (*horizontal arrows*) indicate the outward and inward currents, respectively, blocked by ropivacaine. I_{Na} was buried in the capacitive transient; both are removed from the presented records. (*C*) Suppressive effects of ropivacaine on the inward (representing I_{Ca}) and late outward (composed of I_{K1} + I_{Kr}) current peaks. *Symbols* and *bars* represent mean ± SEM values obtained in four myocytes. * Significant (*P* < 0.05) change from control.

cytes. The results revealed that ropivacaine—similarly to bupivacaine—suppressed several ion currents in a concentration-dependent manner with the concomitant alterations of action potential morphology. These changes observed in the configuration of the action potential can be deduced from suppression of the various ion currents. Reduction of V_{max} and action potential amplitude are clearly consequences of inhibition of I_{Na}. Because V_{max} is an indicator of I_{Na} density and is believed to be linearly related to I_{Na},[22] I_{Na} is the current that was most effectively blocked by ropivacaine, considering the 81 μM EC_{50} value obtained for V_{max} block at the frequency of 1 Hz. This is in a good agreement with voltage clamp data indicating that 50 and 100 μM ropivacaine caused 33.3% and 62.5% block of I_{Na}, respectively, at room temperature in guinea pig ventricular myocytes.[2] However, when comparing this EC_{50} to the 41 μM obtained with bupivacaine, the effect of ropivacaine on I_{Na} seems to be weaker than that of bupivacaine. A similar conclusion was drawn from V_{max} measurements in multicellu-
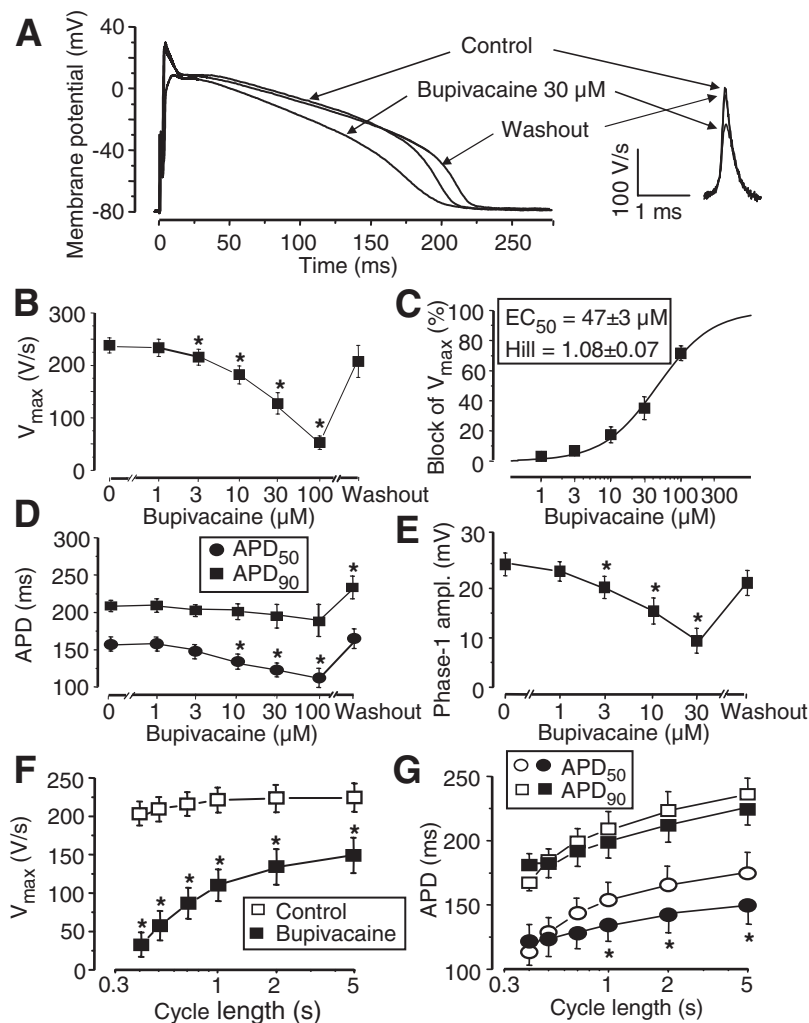
Fig. 6. (*A*) Effect of 30 μM bupivacaine on action potential configuration at a constant pacing frequency of 1 Hz. (*B*, *D*, and *E*) Cumulative concentration-dependent effects of bupivacaine on maximum velocity of depolarization (V_{max}), action potential duration (APD), and phase 1 amplitude, respectively. The experimental protocol was similar to that applied in the ropivacaine studies. V_{max} data, fitted to the Hill equation, are shown in *C*. *Symbols* and *bars* represent mean $\pm$ SEM values obtained in eight myocytes. * Significant (*P* < 0.05) change from control. Steady state frequency-dependent effects of 30 μM bupivacaine on V_{max} and action potential duration are presented in *F* and *G*, respectively (n = 5).

lar guinea pig ventricular preparations.[23] Suppression of the window I_{Na}, together with the inhibition of I_{Ca}, likely contributed to the ropivacaine-induced shortening of action potentials and depression of their plateau. Finally, the reduction of the amplitude of early (phase 1) repolarization may be related to the inhibition of both I_{to} and I_{Na}. Interestingly, the blocking effect of ropivacaine on I_{to} seemed to be more pronounced under action potential clamp conditions compared with the conventional voltage clamp experiments. This discrepancy can probably be explained by differences in the applied voltage protocols. In general, the inhibitory effects of ropivacaine on canine ventricular ion currents are qualitatively similar to those observed in guinea pig ventricular myocytes,[2] except for the suppression of I_{K1}, which was prominent in our study, whereas it was virtually absent in guinea pigs. The reason for this interspecies difference is unclear; however, it underlines the importance of experiments performed in human or canine ventricular myocardium. It is interesting that the human *ether-a-go-go*–related gene current seems to be more sensitive to ropivacaine than the canine I_{Kr}.[5,6]

The clinical relevance of the present electrophysiologic data can be evaluated only when comparing the concentrations used in our experiments to the ropivacaine plasma levels measured in patients during anesthesia. The peak plasma level in ropivacaine anesthesia may reach 2.6–2.9 mg/l, corresponding to a concentration of 10–12 μM.[24,25] Although the lowest ropivacaine concentration that caused statistical significant effects on V_{max} and phase 1 repolarization was 10 μM in our study, ropivacaine is not likely to markedly alter cardiac electrogenesis at plasma levels typically obtained during neuraxial or regional anesthesia. But what can be anticipated in cases of ropivacaine overdose or intoxication caused by accidental intravenous injection of the drug? Under such extraordinary conditions, much higher plasma levels may occur: 40 μM if calculated with 14 l of total extracellular fluid volume, or 180 μM if calculated with 3 l of plasma volume (after intravenous injection of an ampoule containing 20 ml ropivacaine, 7.5 mg/ml). Although the EC_{50} values obtained with ropivacaine for I_{Ca}, I_{Kr}, I_{K1}, and I_{to} were found within a relatively narrow range of 250–400 μM, suppression of inward currents
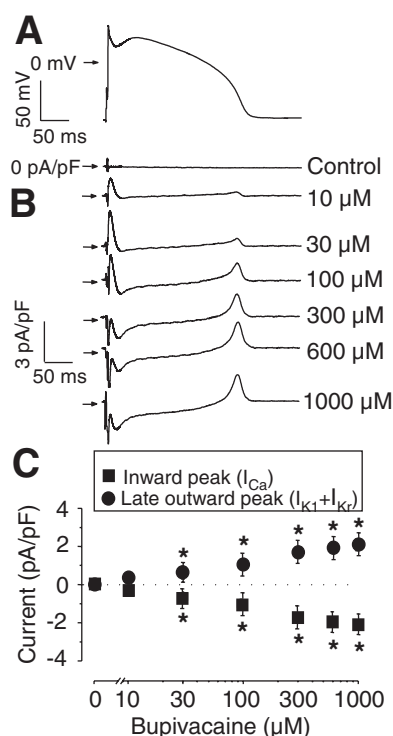
Fig. 7. Effects of bupivacaine on ion currents under action potential voltage clamp conditions. Representative records of a command signal (*A*) and the underlying current traces obtained in the presence of increasing concentrations of bupivacaine (*B*). (*C*) Suppressive effects of ropivacaine on the inward and late outward current peaks. *Symbols* and *bars* represent mean ± SEM values obtained in four myocytes. * Significant (*P* < 0.05) change from control.

during the action potential plateau seems to be dominant, as indicated by the APD shortening observed at cycle lengths of 1,000 ms or longer. This may account for the flat APD–frequency and APD–diastolic interval relations observed with ropivacaine. In contrast to ropivacaine, bupivacaine did not lengthen APD_{90} significantly at any of the pacing frequencies; furthermore, its shortening effect on APD_{50} was less pronounced than that observed with ropivacaine. This difference may suggest that the blocking effects of bupivacaine on the inward and outward currents are well balanced compared with those of ropivacaine.

Surprisingly, washing out of ropivacaine (and also bupivacaine) resulted in a prolongation of action potential duration beyond its control value. This rebound effect can be explained by the differences in kinetics of washout from the various ion channels, as was demonstrated with ropivacaine. The ropivacaine-induced inhibition of V_{max} (indicator of I_{Na}) was rapidly and fully reversible, and 80% of I_{Ca} was also restored within 10 min. In contrast, currents responsible for repolarization (I_{Kr}, I_{Ks}, and more importantly I_{K1}) showed only slow and incomplete recovery during the 10-min period of washout. The reason for this variance in reversibility is unknown; it is probably due to differences in intramolecular localization of the binding sites. It is noteworthy, however, that

the molecular structure of Kir channels (responsible for I_{K1}) is basically different from those of other ion channels. As a consequence of the sustained block of outward currents seen upon washout, action potential duration is likely increased throughout the period of drug elimination after anesthesia. This carries an extra proarrhythmic risk for patients having the inherited or acquired form of long QT syndrome. All of these patients have reduced repolarization reserve due to relative deficiency of outward compared with inward currents, and therefore they are more susceptible to further drug-induced APD lengthening, which may result in development of early afterdepolarizations with the threat of torsade de pointes–type ventricular arrhytmias.[26] Although I_{Ks} has little influence on repolarization at normal heart rates,[27,28] it is an important component of the repolarization reserve.[29,30] Because of the relatively high I_{Ks} blocking potency of ropivacaine (EC_{50} = 106 μM), patients having normal APD at rest with already decreased repolarization reserve may also have an increased risk for arrhythmias.[31,32] Finally, because the contribution of an ion current to the action potential is not uniform within the ventricular wall, the transient asymmetrical blockade of ion channels may further increase the incidence of arrhythmias due to the increased dispersion of repolarization. Indeed, ventricular fibrillation and cardiac arrest were reported in anesthesia induced by ropivacaine[10–16] similarly to cases of bupivacaine-induced anesthesia.[33–36] Consistently with this, our cellular electrophysiologic analysis did not reveal marked qualitative differences between the cardiac actions of ropivacaine and bupivacaine. Bupivacaine is a more potent blocker of ion channels than ropivacaine; however, its therapeutic concentration is also proportionally lower.[37] In conclusion, the present results suggest that ropivacaine and bupivacaine are not expected to induce cardiac complications when applied regularly in the normal population; however, under extraordinary conditions (such as accidental intravenous injection, cases of overdose, or patients susceptible to arrhythmias), special care has to be taken not only during anesthesia but in the postoperative recovery period as well.

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