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Effects of ropivacaine on sodium, calcium, and potassium currents in guinea pig ventricular myocytes

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KEY WORDS ropivacaine; patch-clamp techniques; myocardium; sodium channels; calcium channels; potassium channels

ABSTRACT

AIM: To study the effects of ropivacaine (Rop) on sodium current (I_{Na}), L-type calcium current (I_{Ca-L}), inward rectifier potassium current (I_{K1}), and delayed rectifier potassium current (I_K) in isolated guinea pig ventricular myocytes. **METHODS**: Whole cell patch-clamp techniques were used in our experiment. **RESULTS**: At potential of −40 mV, Rop 10, 50, and 100 μmol/L decreased sodium current by 8.3 %, 33.3 %, and 62.5 %, respectively and prolonged the time constant of I_{Na} inactivation by 8.2 %, 24.7 %, and 64.4 %, respectively ($n = 5$ cells from 3 animals, $P < 0.05$). At potential of +10 mV, Rop 50 and 100 μmol/L decreased L-type calcium current by 7.6 % and 22.5 %, and prolonged the slow time constant of I_{Ca-L} inactivation by 15.5 % and 33.0 %, respectively ($n = 5$ cells from 4 animals, $P < 0.05$). Rop 50 and 100 μmol/L did not markedly change the peak current of delayed rectifier potassium current and inward rectifier potassium current ($n = 5$ cells from 3 animals, $P > 0.05$), respectively. **CONCLUSION**: Rop depressed I_{Na} and I_{Ca-L}, which may be related to its cardiotoxic effect.

INTRODUCTION

Ropivacaine (Rop), a new amide local anesthetic agent chemically related to bupivacaine, is a pure sinister enantiomer first used in the clinical practice of anesthesia. Several studies showed that the cardiotoxicity of Rop resulted from the suppression of cardiac conduction and the negative inotropic effect[1~3]. Sodium current is responsible for the conductivity and excitability of myocardiums and calcium current is related to the excitability-contraction coupling. Myocardial potassium channels control the resting potential, the duration of action potential (APD), refractoriness, and so on[4]. APD is determined by the balance between inward currents (including I_{Ca-L}, I_{Na}, and Na^+/Ca^{2+} exchange current, *etc*) and outward currents (potassium currents). *If the potassium channels are blocked, it can prolong* APD and exacerbate block of sodium channels[5]. The present study was to provide adequate basal analysis of the effects of Rop on I_{Na}, I_{Ca-L}, I_K, and I_{K1}. It may help us know the electrophysiologic effect of Rop on the various ionic channels in guinea pig ventricular myocytes and also have a better understanding of the cardiovascular toxicity of Rop.

MATERIALS AND METHODS

Preparation of ventricular myocytes Single ventricular myocytes from adult guinea pigs (196 g ± 27 g, from Experimental Animal Center of Soochow University, Grade Ⅱ, Certificate № 97018) were prepared by enzymatic dissociation method[6]. In brief, cells were dissociated from Langendorff-perfused hearts by a constant-flow perfusion (8 – 10 mL/min) protocol at 37 ℃ as follows: 1) 5 min of Ca^{2+}-free Tyrode's solution; 2) 8 – 10 min of low Ca^{2+} (25 μmol/L) Tyrode's solution containing 0.02 % collagenase type Ⅱ; 3) 5 min of KB medium. The ventricles were chopped, minced, and gently triturated with a pipette in KB medium at 37 ℃ for 5 min. Cells were filtered through a 150-μm nylon mesh, then stored in KB medium at room temperature (22 ℃) for at least 1 h

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before use.

Whole-cell patch-clamp recording Myocytes were placed in a 500-μL chamber on the stage of an inverted microscope. Only rod-shaped cells with clear striation were used for experiments. The chamber was continuously perfused (2 mL/min) with extracellular solution. Patch electrodes were pulled with a two-stage vertical puller (PB-7, Narishige, Japan) and had a resistance of 2 – 4 MΩ when filled with electrode internal solution. After gigaseal formed and the patch ruptured, currents were recorded in a voltage-clamp mode using a patch-clamp amplifier (Dagan 8900, USA). Capacitive transients and series resistance were compensated. Experimental protocols, data acquisition and storage were accomplished with pClamp 5.5 (Axon Instrument, USA) running on a personal computer. All experiments were conducted at 20 – 22 ℃.

Chemicals and solution Ropivacaine (provided by ASTRA corporation), was dissolved in distilled water as stock solution and then diluted in Tyrode's solution to the desired final concentrations. Serial concentrations of Rop solutions were perfused at a rate of 0.5 – 1 mL/min for 10 min to assure the drug in the chamber reached the anticipated concentrations. Collagenase type Ⅱ, taurine, HEPES, egtazic acid, Na_2ATP, and K_2ATP were products of Sigma.

The composition of Tyrode's solution for perfusing the hearts was (mmol/L): NaCl 144, KCl 4.0, $CaCl_2$ 1.8, $MgCl_2$ 0.5, HEPES 5.0, glucose 5.5; pH was adjusted with NaOH to 7.4. The composition of KB medium was (mmol/L): KOH 70, KCl 40, KH_2PO_4 20, glutamic acid 50, $MgCl_2$ 3, taurine 20, egtazic acid 0.5, HEPES 10, glucose 10; pH was adjusted with KOH to 7.4. Extracellular solution for recording I_{Na} contained (mmol/L): Choline-Cl 120, NaCl 25, $MgCl_2$ 1, CsCl 4, HEPES 10, glucose 10; pH was adjusted with CsOH to 7.4. The electrode solution for recording I_{Na} contained (mmol/L): CsCl 120, NaCl 10, Na_2ATP 5, egtazic acid 11, HEPES 10; pH was adjusted with CsOH to 7.4. Extracellular solution for recording $I_{Ca\text{-}L}$ contained (mmol/L): Choline-Cl 120, $CaCl_2$ 2, $MgCl_2$ 2, CsCl 4, HEPES 10, glucose 10; pH was adjusted with CsOH to 7.4. The electrode solution for recording $I_{Ca\text{-}L}$ contained (mmol/L): CsCl 120, $CaCl_2$ 1, $MgCl_2$ 2, Na_2ATP 5, egtazic acid 11, HEPES 10; pH was adjusted with CsOH to 7.4. Extracellular solution for recording I_K contained (mmol/L): Choline-Cl 149, KCl 5.4, $MgCl_2$ 2.8, egtazic acid 0.5, HEPES 5, glucose 5.5; pH was adjusted with LiOH to 7.4. The electrode solution for recording I_K contained (mmol/L): KCl 140, $MgCl_2$ 1, K_2ATP 5, egtazic acid 10, HEPES 5; pH was adjusted with KOH to 7.4. Extracellular solution for recording I_{K1} contained (mmol/L): Choline-Cl 145, KCl 5, $MgCl_2$ 1, egtazic acid 5, HEPES 10, glucose 10; pH was adjusted with KOH to 7.4. The electrode solution for recording I_{K1} contained (mmol/L): KCl 140, $MgCl_2$ 1, K_2ATP 5, egtazic acid 10, HEPES 5; pH was adjusted with KOH to 7.4.

Statistics Data were presented as $\bar{x} \pm s$, paired t-tests were made. The steady-state activation or inactivation curve was fitted with Boltzman equation: $I/I_{max} = 1/\{1 \pm EXP[(V-V_{1/2})/k]$. I is the current, I_{max} is the maximal amplitude of the current; V is the voltage of conditioning pulse, $V_{1/2}$ is the half activation or inactivation voltage and k is the slope factor.

RESULTS

Sodium current I_{Na} in guinea pig ventricular myocytes was elicited by 20 ms depolarization steps from the holding potential (E_h) of – 90 mV to + 30 mV with step 10 mV. When the testing potential was depolarized at – 40 mV, I_{Na} reached its peak. Rop inhibited the peak current of I_{Na} by 8.3 %, 33.3 %, and 62.5 % at 10, 50, and 100 μmol/L, respectively. After washout of Rop, I_{Na} was restored to 62.5 % of the baseline value ($P < 0.01$ *vs* control). Rop prolonged its time constant of inactivation by 8.2 %, 24.7 %, and 64.4 % at 10, 50, and 100 μmol/L, respectively. Rop did not affect its time constant of activation, nor did it change its active potential, peak potential, or reverse potential (Tab 1, Fig 1).

L-type calcium current $I_{Ca\text{-}L}$ was elicited by

Tab 1. Effect of ropivacaine on the parameters of I_{Na}. $n = 5$ cells from 3 guinea pigs. $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* control.

Ropivacaine/ μmol·L^{-1}	Peak current/ nA	Time constant of activation/ ms	Time constant of inactivation/ ms
0	4.8 ± 0.8	1.0 ± 0.3	1.46 ± 0.23
10	4.4 ± 0.7^c	1.1 ± 0.4^a	1.58 ± 0.27^c
50	3.2 ± 0.4^c	1.1 ± 0.4^a	1.82 ± 0.29^c
100	1.8 ± 0.6^c	1.26 ± 0.24^a	2.4 ± 0.3^c
Washout	3.0 ± 0.7^c	–	–

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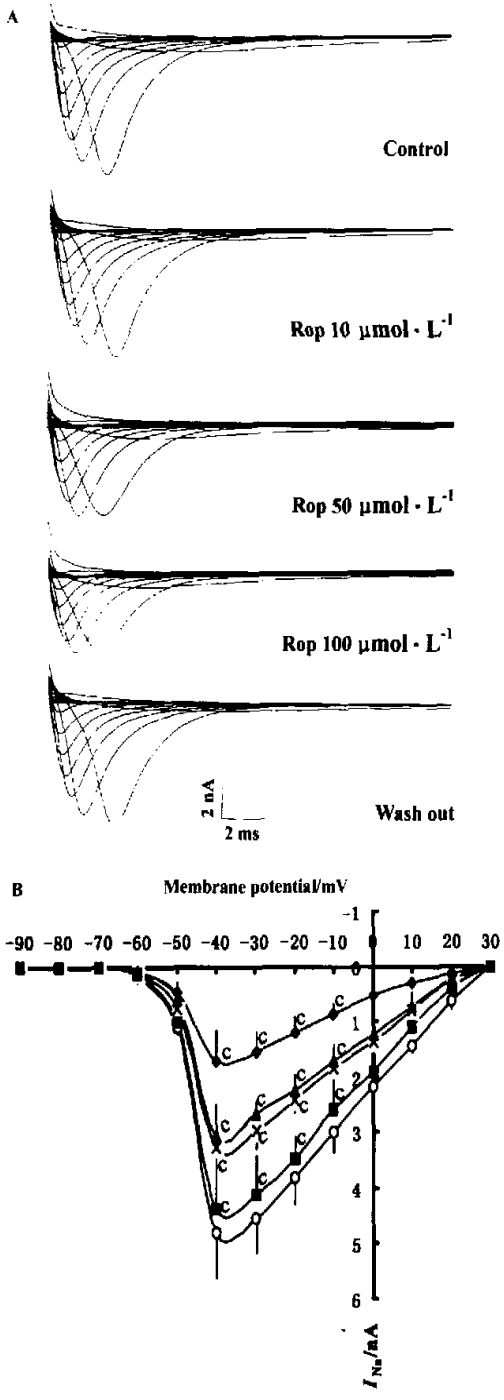

Fig 1. A) Effect of Rop on I_{Na} in single ventricular cell of guinea pig. B) Effect of Rop on I-V relationship of I_{Na} in ventricular cell of guinea pig. $n = 5$ cells from 3 guinea pigs. $x \pm s$. $^cP < 0.01$ *vs* control. ○ Control. ■ Rop 10 μmol/L. × Rop 50 μmol/L. ◆ Rop 100 μmol/L. ▲ Wash out.

200 ms depolarization steps from the holding potential (E_h) of −40 mV to +50 mV with step 10 mV. When the testing potential was depolarized at +10 mV, $I_{Ca\text{-}L}$ reached its peak. Rop inhibited the peak current of $I_{Ca\text{-}L}$ by 7.6 % at 50 μmol/L and 22.5 % at 100 μmol/L (excluding the influence of the natural "run-down" phenomenon). Rop prolonged its slow time constant of inactivation by 15.5 % at 50 μmol/L and 33.0 % at 100 μmol/L. Rop did not prolong its time constant of activation and fast time constant of inactivation, nor did it affect the shape of its current-voltage curve (Tab 2, Fig 2).

Delayed rectifier potassium current The

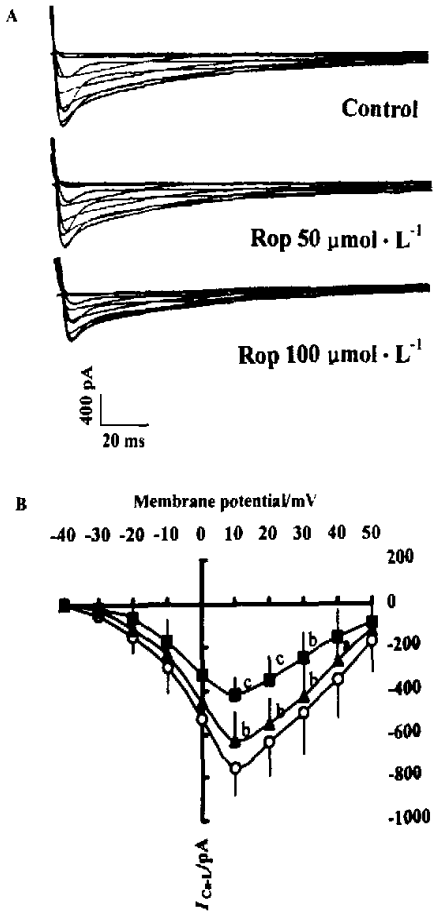

Fig 2. A) Effect of Rop on $I_{Ca\text{-}L}$ in single ventricular cell of guinea pig. B) Effect of Rop on I-V relationship of $I_{Ca\text{-}L}$ in ventricular cell of guinea pig. $n = 5$ cells from 4 guinea pigs. $x \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control. ○ Control. ▲ Rop 50 μmol/L. ■ Rop 100 μmol/L.

Tab 2. Effect of ropivacaine on the parameters of $I_{Ca\text{-}L}$. $n = 5$ cells from 4 guinea pigs. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

Ropivacaine/ μmol·L^{-1}	Peak current/pA	Time constant of activation/ms	Fast time constant of inactivation/ms	Slow time constant of inactivation/ms
0	752 ± 127	1.67 ± 0.05	9.2 ± 0.5	97 ± 7
50	632 ± 138^b	1.66 ± 0.09^a	9.2 ± 1.0^a	112 ± 12^b
100	415 ± 82^c	1.68 ± 0.11^a	9.5 ± 0.7^a	129 ± 18^c

depolarization pulse of 4.5 s duration was used at various potentials from −30 mV to +90 mV with step of 20 mV to evoke outward potassium current (I_K) accompanied by an outward tail current ($I_{K\text{-}tail}$). Rop 50 and 100 μmol/L did not markedly change the peak current of I_K [from (1488 ± 32) to (1479 ± 33) and (1476 ± 36) pA, $n = 5$ cells from 3 guinea pigs, $P > 0.05$] and did not change the peak current of $I_{K\text{-}tail}$ markedly [from (294 ± 20) to (285 ± 22) and (280 ± 23) pA, $n = 5$ cells from 3 guinea pigs, $P > 0.05$], respectively. Rop did not affect the shape of its current-voltage curve.

Inward rectifier potassium current I_{K1} was elicited by a number of step pulses (300 ms) from the E_h of −40 mV to test potentials from −120 mV to 0 mV with step of 10 mV. Rop 50 and 100 μmol/L did not change the peak inward current of I_{K1} [from (4652 ± 527) to (4629 ± 503) and (4589 ± 612) pA, $n = 5$ cells from 3 guinea pigs, $P > 0.05$] and did not change the peak outward current of I_{K1} markedly [from (828 ± 103) to (823 ± 95) and (816 ± 99) pA, $n = 5$ cells from 3 guinea pigs, $P > 0.05$], respectively. Rop did not change the reverse potential of I_{K1}.

DISCUSSION

As we all know, I_{Na} is responsible for the conductivity and excitability of myocardiums. If sodium channels are blocked, it can lead to depression of maximal upstroke velocity of phase 0 depolarization (V_{max}) and amplitude of action potential (APA). Several studies showed that Rop could depress V_{max} and APA[7,8]. The results in our experiment showed that Rop 10, 50, and 100 μmol/L could decrease the peak current of I_{Na} and prolong its time constant of inactivation, but could not prolong the time constant of activation. The only prolongation of its time constant of inactivation meant that Rop acted on the inactivation state of I_{Na}. It suggested that Rop could not quickly let sodium channels restore to the resting state and reactivate, which could produce an inhibitory effect on the conductivity and excitability of myocardiums. It might be related to the cardiotoxicity of Rop.

The "run-down" phenomenon has long been noticed in recording L-type calcium current. In order to distinguish the inhibitory effect caused by drug from the natural "run-down" phenomenon, we compared the drug groups with control group at 10, 20, and 30 min respectively and excluded the influence of the natural "run-down." Our results demonstrated that Rop inhibited $I_{Ca\text{-}L}$ by depressing its peak current and prolonging its slow time constant of inactivation. It suggested that Rop might have negative inotropic effect. The data from our Langendorff model showed that Rop really had negative inotropic effect (data not provided). The inhibitory effect of Rop on $I_{Ca\text{-}L}$ also might be related to the cardiotoxicity of Rop.

Moller's study demonstrated that Rop could prolong the duration of action potential (APD) and induce early after depolarization (EAD) in New Zealand rabbits' Purkinje fiber[8]. I_K is one of the factors that affect APD. Based on Moller's study, Valenzuela showed that it might be related to the inhibitory effect of Rop on human Kv1.5 channels (part of I_K)[9]. But our result showed that Rop had no effect on I_K. The differences may be due to the experiment animal model, the animal species, the used concentrations of drug, and so on. If I_K is blocked, it can prolong APD and exacerbate block of sodium channels[5]. If I_{K1} is blocked, it can affect resting potential. Both of them can increase the cardiotoxicity of drugs. The present results showed that Rop did not modify I_K and I_{K1}, which suggested that Rop had low cardiotoxicity.

In conclusion, Rop depressed I_{Na} and $I_{Ca\text{-}L}$, which may be related to its cardiotoxic effect.

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罗哌卡因对豚鼠心室肌细胞钠电流、钙电流和钾电流的影响

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关键词 罗哌卡因；膜片箝技术；心肌；钠通道；钙通道；钾通道

目的：研究罗哌卡因(Rop)对豚鼠心室肌细胞钠电流(I_{Na})、L-型钙电流(I_{Ca-L})、内向整流钾电流(I_{K1})及延迟整流钾电流(I_K)的影响. **方法**：全细胞膜片箝技术. **结果**：罗哌卡因 10，50 与 100 μmol/L 使 I_{Na} 的峰电流分别减小 8.3 %、33.3 %和 62.5 % ($P<0.01$)，使失活时间常数分别延长 8.2 %、24.7 %和 64.4 % ($P<0.05$)；罗哌卡因 50 与 100 μmol/L 使 I_{Ca-L}的峰电流分别减小 7.6 % 和 22.5 % ($P<0.05$)，使慢失活时间常数分别延长 15.5 % 和 33.0 % ($P<0.01$)；罗哌卡因 50 与 100 μmol/L 对 I_{K1}和 I_K 的峰电流无明显影响. **结论**：罗哌卡因抑制 I_{Na}和 I_{Ca-L}，可能与其心脏毒性作用有关.

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