

DDPH inhibited L-type calcium current and sodium current in a single ventricular myocyte of guinea pig¹

HU Xiao-Dong, QIAN Jia-Qing² (Department of Pharmacology, Tongji Medical College of Huazhong University of Science and Technology, Wuhan 430030, China)

KEY WORDS 1-(2, 6-dimethylphenoxy)-2-(3, 4-dimethoxyphenyl-ethylamino) propane hydrochloride; patch-clamp techniques; myocardium; calcium channels; sodium channels; verapamil; mexiletine

ABSTRACT

AIM: To investigate the effects of 1-(2, 6-dimethylphenoxy)-2-(3, 4-dimethoxyphenyl-ethylamino) propane hydrochloride (DDPH) on L-type calcium current (I_{Ca}) and sodium current (I_{Na}), and to compare its inhibitory potency with verapamil and mexiletine. **METHODS:** Whole-cell patch clamp technique was used to record I_{Ca} and I_{Na} in a single ventricular myocytes of guinea pig. **RESULTS:** (1) DDPH (3-300 $\mu\text{mol}\cdot\text{L}^{-1}$) decreased I_{Ca} at 0 mV in a concentration-dependent manner with an IC_{50} value of 28.5 $\mu\text{mol}\cdot\text{L}^{-1}$ (95 % confidence limits: 14.3-42.7 $\mu\text{mol}\cdot\text{L}^{-1}$, $n=8$ cells from 8 guinea pigs). Verapamil (0.3-30 $\mu\text{mol}\cdot\text{L}^{-1}$) reduced I_{Ca} with an IC_{50} value of 1.8 $\mu\text{mol}\cdot\text{L}^{-1}$ (95 % confidence limits: 1.3-2.3 $\mu\text{mol}\cdot\text{L}^{-1}$, $n=6$ cells from 6 guinea pigs). Mexiletine 100 $\mu\text{mol}\cdot\text{L}^{-1}$ did not affect I_{Ca} ($n=5$ cells from 5 guinea pigs, $P>0.05$). The degree of use-dependent blocking effect of DDPH 30 $\mu\text{mol/L}$ on I_{Ca} was 58 % \pm 13 % ($n=5$ cells from 5 guinea pigs, $P<0.01$) at 1 Hz and 76 % \pm 11 % ($n=5$ cells from 5 guinea pigs, $P<0.01$) at 3 Hz. (2) DDPH (20-320 $\mu\text{mol}\cdot\text{L}^{-1}$) could also block I_{Na} in a concentration-dependent manner with an IC_{50} value of 89.0 $\mu\text{mol}\cdot\text{L}^{-1}$ (95 % confidence limits: 68.7-109.3 $\mu\text{mol}\cdot\text{L}^{-1}$, $n=9$ cells from 9 guinea pigs). The IC_{50} value of mexiletine was 32.2 $\mu\text{mol}\cdot\text{L}^{-1}$ (95 % confidence limits: 11.7-52.7 $\mu\text{mol}\cdot\text{L}^{-1}$, $n=5$ cells from 5 guinea pigs). Ver-

apamil at the concentration of 10 $\mu\text{mol}\cdot\text{L}^{-1}$ did not affect I_{Na} ($n=5$ cells from 5 guinea pigs, $P>0.05$). The blocking effect of DDPH 80 $\mu\text{mol/L}$ on I_{Na} was non use-dependent. **CONCLUSION:** DDPH exhibited inhibitory effects on both I_{Ca} and I_{Na} , but its inhibitory effect on I_{Ca} was weaker than verapamil, and on I_{Na} was weaker than mexiletine.

INTRODUCTION

1-(2, 6-Dimethylphenoxy)-2-(3, 4-dimethoxyphenyl-ethylamino) propane hydrochloride (DDPH), is a new compound with chemical structure partly similar to that of verapamil and mexiletine^[1]. Our previous work demonstrated that DDPH could prevent arrhythmias induced by ischemia-reperfusion^[2]. DDPH also possessed antihypertensive effect, α_1 -adrenoceptor blocking effect and calcium antagonistic effect^[3]. In the present study, we used whole-cell patch clamp technique to observe the effects of DDPH on I_{Ca} and I_{Na} in a single ventricular myocyte of guinea pigs and to compare its inhibitory potency with verapamil and mexiletine.

MATERIALS AND METHODS

Solutions and drugs DDPH was from China Pharmaceutical University (Nanjing). The stock solution (4 mmol $\cdot\text{L}^{-1}$) was prepared with distilled water and the pH value was adjusted to 7.0 with NaOH. Tyrode's solution contained (mmol $\cdot\text{L}^{-1}$) NaCl 135, KCl 5.4, CaCl₂ 1.8, MgCl₂ 1.0, NaH₂PO₄ 0.33, glucose 10, and HEPES (Sigma) 10, with pH value 7.4. The ventricular myocyte dissociation solution was Ca²⁺-free Tyrode's solution containing collagenase (type II, Sigma) 0.25 g $\cdot\text{L}^{-1}$, protease E (Sigma) 0.25 g $\cdot\text{L}^{-1}$, and bovine serum albumin (BSA) 0.25 g $\cdot\text{L}^{-1}$. The intracellular solution consisted of (mmol $\cdot\text{L}^{-1}$) CsCl 120, NaCl 10, CaCl₂ 1.0, Mg-ATP 5.0, egtazic acid (Sigma) 11, and HEPES 10, adjusted to pH 7.3 with CsOH. The extra-

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²Correspondence to Prof QIAN Jia-Qing.

Phn 86-27-8369-3634. E-mail qianjq_tjmc@yahoo.com

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cellular solution used to record I_{Na} contained ($\text{mmol} \cdot \text{L}^{-1}$) choline chloride 100, NaCl 40, KCl 5.4, CaCl_2 1.0, MgCl_2 1.0, NaH_2PO_4 0.33, glucose 10, and HEPES 10, with the pH value 7.4.

Cell isolation Ventricular myocytes were obtained from the hearts of adult guinea pigs provided by the Medical Experimental Animal Center of Tongji Medical College of Huazhong University of Science and Technology (Grade II, Certificate No 19-025), using enzymatic dissociation method similar to that previously described⁽⁴⁾.

Electric recordings Whole-cell recordings were performed using an EPC 9 patch-clamp amplifier (HEKA, Germany). Myocytes were placed into a 1.5-mL chamber mounted on the stage of an inverted microscope (IX70, Olympus) and were superfused with Tyrode's solution. After a gigaseal (seal resistance 2–50 G Ω) formation, the membrane was ruptured with gentle suction to obtain whole-cell voltage-clamp configuration. Thereafter, capacitance and series resistance compensations were optimized, usually 50%–80% compensation was obtained. The current was filtered at 2.9 kHz and then stored on hard disk for subsequent analysis. Data acquisition and command potentials were controlled with a commercial software program (pCLAMP 6.0). The pipette resistance was 2 to 4 M Ω when the pipette was filled with the intracellular solution and immersed into Tyrode's solution. To record I_{Na} , the temperature was maintained at 19 °C and the pipette resistance was kept less than 1 M Ω for better voltage control⁽⁵⁾.

Statistic analysis Values were expressed as $\bar{x} \pm s$. Statistic significance was determined by a unpaired *t*-test. The Marquardt-Levenberg method of nonlinear regression analysis was used to fit the concentration-response curves and to calculate the IC_{50} value according to the equation, $I = I_{\text{max}} / (\text{IC}_{50} / [C] + 1)$.

RESULTS

L-type calcium current (I_{Ca}) Recordings were obtained 5 min after the rupturing of the membrane. The peak whole-cell I_{Ca} was measured during a 200-ms depolarizing pulse to 0 mV after a 100-ms period at a holding potential of -40 mV at 0.1 Hz. DDPH (3–300 $\mu\text{mol} \cdot \text{L}^{-1}$) blocked the I_{Ca} in a concentration-dependent manner with an IC_{50} value of 28.5 $\mu\text{mol} \cdot \text{L}^{-1}$ (95% confidence limits: 14.3–42.7 $\mu\text{mol} \cdot \text{L}^{-1}$, $n = 8$ cells from 8 guinea pigs) (Fig 1). Verapamil had a similar

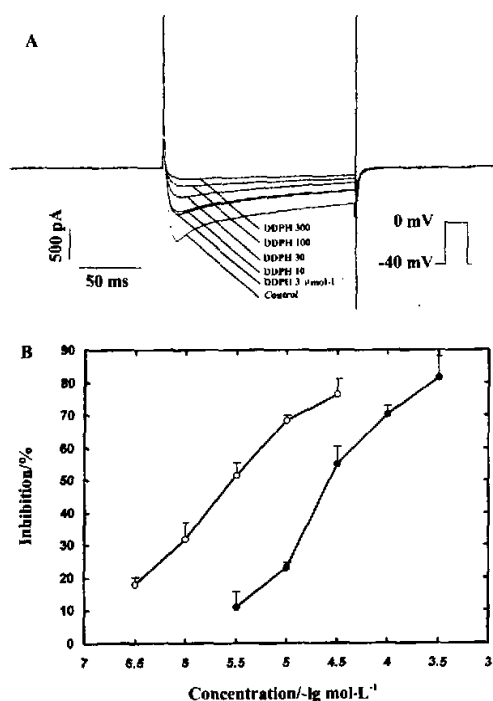


Fig 1. Whole-cell I_{Ca} in guinea pig ventricular myocytes. A) I_{Ca} traces before and after exposure to different concentrations of DDPH 3–300 $\mu\text{mol} \cdot \text{L}^{-1}$ ($n = 8$). B) Percent change of I_{Ca} after exposure to verapamil (○) ($n = 6$) and DDPH (●) ($n = 8$). $\bar{x} \pm s$

but stronger blocking effect on the I_{Ca} , with an IC_{50} value of 1.8 $\mu\text{mol} \cdot \text{L}^{-1}$ (95% confidence limits: 1.3–2.3 $\mu\text{mol} \cdot \text{L}^{-1}$, $n = 6$ cells from 6 guinea pigs). Our experiment showed that mexiletine, a class I_b antiarrhythmic agent, 100 $\mu\text{mol} \cdot \text{L}^{-1}$ reduced the I_{Ca} from -549 pA \pm 156 pA to -399 pA \pm 176 pA ($n = 5$ cells from 5 guinea pigs, $P > 0.05$). When the myocytes were voltage-clamped from -40 mV to different membrane potentials ranging from -40 mV to +50 mV in 10-mV steps at 0.1 Hz, the voltage-dependent I_{Ca} was recorded. DDPH at the concentration of 30 $\mu\text{mol} \cdot \text{L}^{-1}$ decreased peak I_{Ca} with no shift of the maximum activation potential of about 0 mV, where the peak I_{Ca} decreased from -758 pA \pm 222 pA to -404 pA \pm 149 pA ($n = 6$ cells from 6 guinea pigs, $P < 0.01$) (Fig 2). Verapamil had use-dependent blocking effect on I_{Ca} ⁽⁶⁾. To test whether DDPH also possessed the use-dependent characteristics in its action, we elicited the I_{Ca} with 10 repetitive clamp steps from -40 mV to 0 mV for 200 ms at 1.0 and 3.0 Hz pacing

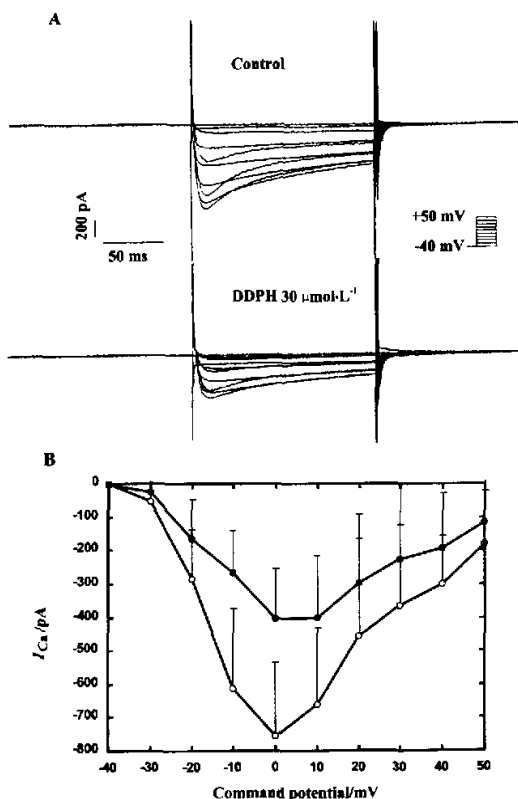


Fig 2. Voltage-dependent block of I_{Ca} in guinea pig ventricular myocytes by DDPH. A) I_{Ca} current traces before and after DDPH 30 $\mu\text{mol}\cdot\text{L}^{-1}$ treatment. B) Current-Voltage relationship curve of I_{Ca} . ○: control. ●: DDPH 30 $\mu\text{mol}\cdot\text{L}^{-1}$. ($n = 6$ cells from 6 guinea pigs. $\bar{x} \pm s$).

rates. The reduction of I_{Ca} at the first pulse after DDPH was defined as the tonic block. The use-dependent block was determined by the reduction of I_{Ca} at the end of the 10th pulse, where the reduction of I_{Ca} reached steady state. The tonic block of DDPH 30 $\mu\text{mol}\cdot\text{L}^{-1}$ at 1 Hz and 3 Hz was 56% \pm 13% and 62% \pm 14% ($n = 5$ cells from 5 guinea pigs), respectively. After exposure to DDPH 30 $\mu\text{mol}\cdot\text{L}^{-1}$, the degree of use-dependent block was 58% \pm 13% ($P < 0.01$) at 1 Hz, and 76% \pm 11% ($P < 0.01$) at 3 Hz. The blocking effect at 3 Hz was stronger than that at 1 Hz ($P < 0.05$) (Fig 3A). To further elaborate the use-dependence, the average beat-to-beat reduction of I_{Ca} (I/I_{1st}) during the 10-pulse train is shown in Fig 3B. In presence of DDPH 30 $\mu\text{mol}\cdot\text{L}^{-1}$, the reduction at the 10th pulse was greater at 3 Hz

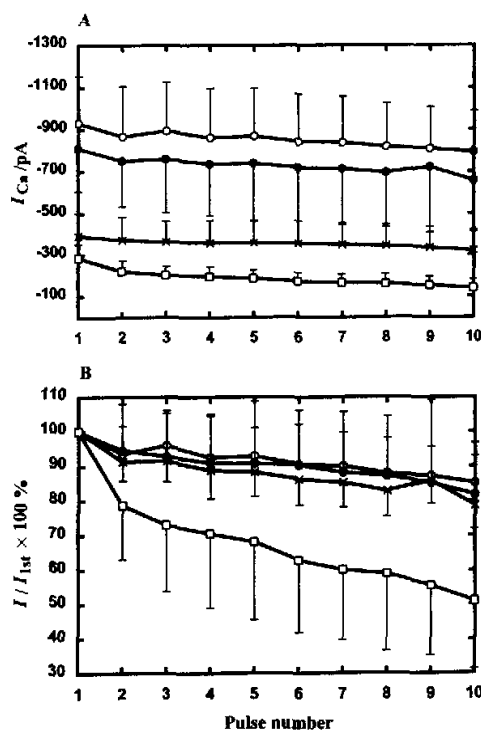


Fig 3. Frequency-dependent blocking curve of I_{Ca} . A) I_{Ca} current at 1 Hz (○), 3 Hz (●) before drug and at 1 Hz (×), 3 Hz (□) after DDPH 30 $\mu\text{mol}\cdot\text{L}^{-1}$. B) The average beat-to-beat reduction of I_{Ca} (I/I_{1st}) during the 10-pulse train. $n = 5$ cells from 5 guinea pigs. $\bar{x} \pm s$.

than that at 1 Hz ($n = 5$ cells from 5 guinea pigs, $P < 0.05$), while the reduction at the 10th pulse on drug-free condition showed no difference between 1 Hz and 3 Hz ($P > 0.05$).

Sodium current (I_{Na}) To study the effect of DDPH on I_{Na} , we activated the whole-cell I_{Na} with a 200-ms depolarization from a holding potential of -80 mV to -30 mV at 0.1 Hz. DDPH (20 - 320 $\mu\text{mol}\cdot\text{L}^{-1}$) blocked the I_{Na} in a concentration-dependent manner, with an IC_{50} value of 89.0 $\mu\text{mol}\cdot\text{L}^{-1}$ (95% confidence limits: 68.7 - 109.3 $\mu\text{mol}\cdot\text{L}^{-1}$, $n = 9$ cells from 9 guinea pigs) (Fig 4). The IC_{50} value of mexiletine was 32.2 $\mu\text{mol}\cdot\text{L}^{-1}$ (95% confidence limits: 11.7 - 52.7 $\mu\text{mol}\cdot\text{L}^{-1}$, $n = 5$ cells from 5 guinea pigs) (Fig 4). Verapamil 10 $\mu\text{mol}\cdot\text{L}^{-1}$ reduced I_{Na} from -9.6 nA \pm 3.4 nA to -9.2 nA \pm 3.3 nA ($n = 5$ cells from 5 guinea pigs, $P > 0.05$). Current-voltage relationship of

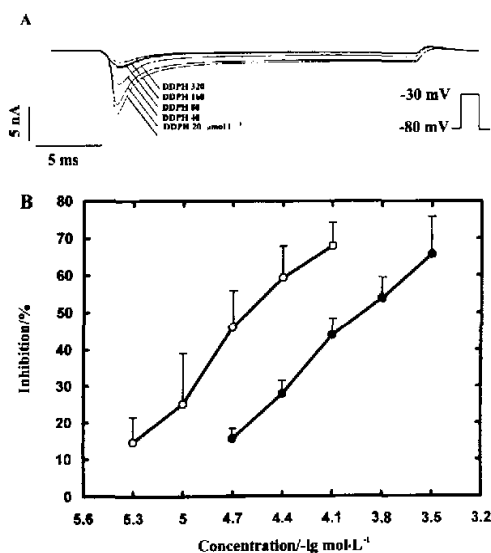


Fig 4. Whole-cell I_{Na} in guinea pig ventricular myocytes. A) I_{Na} traces before and after exposure to different concentrations of DDPH (20 – 320 $\mu\text{mol} \cdot \text{L}^{-1}$). B) Percent change of I_{Na} after mexiletine (○) ($n = 5$) and DDPH (●). $n = 9$. $\bar{x} \pm s$.

I_{Na} was generated by applying depolarizing pulses from -70 mV to $+40 \text{ mV}$ for 200 ms with a 10-mV increment. DDPH $80 \mu\text{mol} \cdot \text{L}^{-1}$ decreased the I_{Na} at -30 mV from $-7.3 \text{ nA} \pm 1.7 \text{ nA}$ to $-2.9 \text{ nA} \pm 2.0 \text{ nA}$ ($n = 6$ cells from 6 guinea pigs, $P < 0.01$) (Fig 5).

To test use-dependent blocking effect on I_{Na} of DDPH, we evoked I_{Na} with 10 repetitive pulses from -80 mV to -30 mV for 200 ms at two different frequencies; 1.0 and 3.0 Hz. The interval between the trains lasted 2 min. In the presence of DDPH $80 \mu\text{mol} \cdot \text{L}^{-1}$, there was a reduction of I_{Na} during the first pulse (decrease by $46\% \pm 31\%$ at 1 Hz and $52\% \pm 22\%$ at 3 Hz, $n = 5$ cells from 5 guinea pigs, $P < 0.05$). But the I_{Na} at the 10th pulse of the train had no difference between 1 Hz and 3 Hz, suggesting that DDPH could not block I_{Na} in a use-dependent manner.

DISCUSSION

The major findings in our experiment were that DDPH could block both I_{Ca} and I_{Na} in guinea pig ventricular myocytes. Compared with verapamil and mexiletine, although the inhibitory potency of DDPH on I_{Ca} and

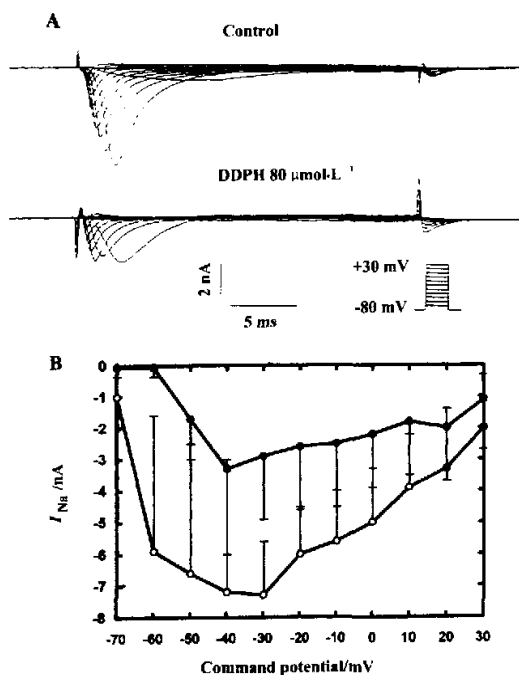


Fig 5. Voltage-dependent block of I_{Na} in guinea pig ventricular myocytes by DDPH. A) I_{Na} traces before and after DDPH $80 \mu\text{mol} \cdot \text{L}^{-1}$. B) Current-voltage relationship curve of I_{Na} . ○: control. ●: DDPH $80 \mu\text{mol} \cdot \text{L}^{-1}$. $n = 6$. $\bar{x} \pm s$.

I_{Na} was weaker than that of verapamil and mexiletine, it had a striking characteristics of blocking effects on multiple ion channel currents including I_K and I_{K1} ⁽⁷⁾. This property is now considered very important in the treatment of cardiac arrhythmias. It is suggested that an antiarrhythmic agent affecting multiple ion channels in a diseased heart may be favorable for cardiac arrhythmias and may hopefully reduce the incidence of proarrhythmias⁽⁸⁾. The inhibitory effects of DDPH on I_{Ca} , I_{Na} , I_K , and I_{K1} indicated that it bore the pharmacologic properties of both verapamil and mexiletine. However, DDPH blocked multiple ion channel currents with different potencies. In our experiment, the IC_{50} value of I_{Na} was $89.0 \mu\text{mol} \cdot \text{L}^{-1}$, much larger than that of I_{Ca} ($28.5 \mu\text{mol} \cdot \text{L}^{-1}$). Zhong *et al* reported that DDPH inhibited I_K with an IC_{50} value of 13.3 (11.6 – 16.7) $\mu\text{mol} \cdot \text{L}^{-1}$ and the inhibitory effects of DDPH on I_K was more potent than those of I_{K1} . It is certain that DDPH inhibited I_{Na} more weakly than I_K and I_{Ca} . These results were supported by stan-

dard microelectrode studies⁽⁹⁾ showing that lower concentrations of DDPH altered the action potential duration without any changes of APA, V_{max} , or RP, while larger concentrations of DDPH shortened APD_{50} , prolonged APD_{90} , and decreased APA or slowed V_{max} .

Since the chemical structure and the effects of DDPH are complicated, some studies such as to observe the effects and mechanism of its action in a diseased heart are needed for further investigation to elucidate its mechanism of antiarrhythmic action.

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DDPH 抑制豚鼠单个心室肌细胞 L-钙电流和钠电流¹

胡晓东, 钱家庆²

(华中科技大学同济医学院药理教研室, 武汉 430030, 中国)

关键词 1-(2, 6-二甲基苯氧基)-2-(3, 4-二甲氧基苯乙氨基)丙烷盐酸盐; 膜片钳技术; 心肌; 钙通道; 钠通道; 维拉帕米; 美西律

目的: 研究 1-(2, 6-二甲基苯氧基)-2-(3, 4-二甲氧基苯乙氨基)丙烷盐酸盐 (DDPH) 对豚鼠心室肌细胞 L-型钙电流和钠电流的作用。 **方法:** 全细胞膜片钳技术。 **结果:** (1) DDPH ($3-300 \mu\text{mol}\cdot\text{L}^{-1}$) 浓度依赖性地抑制 L-型钙电流, IC_{50} 为 $28.5 \mu\text{mol}\cdot\text{L}^{-1}$ (95% 可信限: $14.3-42.7 \mu\text{mol}\cdot\text{L}^{-1}$)。 维拉帕米 $0.3-30 \mu\text{mol/L}$ 浓度依赖性地抑制钙电流, IC_{50} 为 $1.8 \mu\text{mol}\cdot\text{L}^{-1}$ (95% 可信限: $1.3-2.3 \mu\text{mol}\cdot\text{L}^{-1}$)。 美西律 $100 \mu\text{mol}\cdot\text{L}^{-1}$ 对钙电流无影响。 DDPH $30 \mu\text{mol}\cdot\text{L}^{-1}$ 使用依赖性阻滞钙电流, 1 Hz 时抑制率为 $58\% \pm 13\%$ ($n=5, P<0.01$), 3 Hz 时为 $76\% \pm 11\%$ ($n=5, P<0.01$)。 (2) DDPH ($20-320 \mu\text{mol}\cdot\text{L}^{-1}$) 浓度依赖性地抑制钠电流, IC_{50} 为 $89.0 \mu\text{mol}\cdot\text{L}^{-1}$ (95% 可信限: $68.7-109.3 \mu\text{mol}\cdot\text{L}^{-1}$)。 美西律抑制钠电流的 IC_{50} 为 $32.2 \mu\text{mol}\cdot\text{L}^{-1}$ (95% 可信限: $11.7-52.7 \mu\text{mol}\cdot\text{L}^{-1}$)。 维拉帕米 $10 \mu\text{mol}\cdot\text{L}^{-1}$ 对钠电流无影响 ($P>0.05$)。 DDPH $80 \mu\text{mol}\cdot\text{L}^{-1}$ 对钠电流无使用依赖性阻滞。 **结论:** DDPH 抑制豚鼠心室肌细胞 L-型钙电流和钠电流, 但抑制钙电流的作用弱于维拉帕米, 抑制钠电流的作用弱于美西律。

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