ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Apr；21 (4)：301 – 305
http：//www.chinaphar.com

Inhibitory effect of 1-(2, 6-dimethylphenoxy)-2-(3, 4-dimethoxyphenyl-ethylamino) propane hydrochloride on inward rectifier and delayed rectifier K^+ currents in guinea pig ventricular myocytes

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KEY WORDS 1-(2, 6-dimethylphenoxy)-2-(3, 4-dimethoxyphenyl-ethylamino) propane hydrochloride; patch-clamp techniques; myocardium; potassium channels; action potentials

ABSTRACT

AIM: To study the effects of 1-(2,6-dimethylphenoxy)-2-(3,4-dimethoxyphenyl-ethylamino) propane hydrochloride (DDPH) on action potential (AP), inward rectifier K^+ current (I_{K1}), and delayed rectifier K^+ current (I_K) in isolated guinea pig ventricular myocytes. **METHODS**: Whole cell patch-clamp recording techniques. **RESULTS**: DDPH 0.1 – 100 $\mu mol \cdot L^{-1}$ decreased 50 % duration of action potential (APD_{50}) concentration-dependently. APD_{50} was shortened from (493 ± 58) to (262 ± 38) ms ($n = 7$ cells from 5 guinea pigs, $P < 0.01$) by DDPH 10 $\mu mol \cdot L^{-1}$. However, 90 % duration of action potential (APD_{90}) was increased by DDPH (> 1 $\mu mol \cdot L^{-1}$). At high concentration (> 10 $\mu mol \cdot L^{-1}$) DDPH decreased resting membrane potential (RP) and amplitude of action potential (APA). DDPH inhibited tail current of I_K ($I_{K\cdot tail}$) concentration-dependently, 46 % at 10 $\mu mol \cdot L^{-1}$ and 78 % at 100 $\mu mol \cdot L^{-1}$. EC_{50} for DDPH inhibiting I_K was 13.3 (11.6 – 16.7) $\mu mol \cdot L^{-1}$. DDPH also blocked I_{K1}. DDPH at high concentration (> 10 $\mu mol \cdot L^{-1}$) shifted the reverse potential of I_{K1} positively. All the effects of DDPH were reversible after washout. **CONCLUSION**: DDPH blocked both I_{K1} and I_K current in guinea pig ventricular myocytes.

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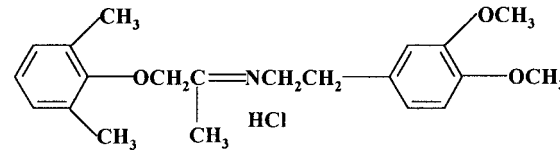
Received 1999-05-11 Accepted 1999-09-05

INTRODUCTION

1-(2,6-Dimethylphenoxy)-2-(3,4-dimethoxyphenyl-ethyl-amino) propane hydrochloride (DDPH), a synthesized compound, has a chemical structure similar to mexiletine and verapamil[1]. Our previous study showed that DDPH could prevent arrhythmia induced by ischemia-reperfusion. In anesthetized cats and rats, pretreatment with DDPH decreased the number of ventricular premature beats and the duration of ventricular tachycardia and ventricular fibrillation during reperfusion[2]. DDPH is also effective in experimental hypertension. Such effects were thought to be due to its weak α_1-adrenoceptor antagonism and calcium blockade[3]. Early studies on membrane current in single ventricular cell of rats showed that DDPH had inhibitory effects on Na^+, Ca^{2+} current, and DDPH 30 $\mu mol \cdot L^{-1}$ suppressed the magnitude of the current[4]. To further investigate the mechanisms of anti-arrhythmic effects of DDPH, the present study was to observe the effects of DDPH on action potential (AP), inward rectifier K^+ current (I_{K1}), and delayed rectifier K^+ current (I_K) in single ventricular cell of guinea pig.



1-(2,6-Dimethylphenoxy)-2-(3,4-dimethoxyphenyl-ethylamino) propane hydrochloride (DDPH, $C_{21}H_{30}O_3NCl$)

MATERIALS AND METHODS

Preparation of ventricular myocytes Single ventricular myocytes from guinea pigs (♂, 270 g ± *s* 35 g, grade Ⅱ, Certification № 19-050, provided by

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Apr；21 (4)
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Shanghai Experimental Animal Center, Chinese Academy of Sciences) were prepared by enzymatic dissociation[5]. Briefly, the heart was rinsed in a cold oxygenated Ca^{2+}-free Tyrode's solution. The aorta was cannulated and the heart was perfused on a Langendorff apparatus at 37 ℃. A perfusion with Ca^{2+}-free Tyrode's solution for 5 min was followed by low Ca^{2+} (50 μmol·L^{-1}) Tyrode's solution containing 0.03 % collagenase and 1 % bovine serum albumin (BSA) for 5 min. The ventricles were cut, minced, and gently triturated with a pipette in the low-Ca^{2+} Tyrode's solution containing 1 % BSA at 37 ℃ for 10 min. The cells were filtered through 200-μm nylon mesh, then resuspended in the Tyrode's solution in which the Ca^{2+} concentration was gradually increased to 1 μmol·L^{-1}. Only the cells with rod shape and clear cross striation were used for experiments.

Potential and current recording Myocytes were placed in a 500 μL chamber on the stage of an inverted microscope (Olympus CK2). The chamber was continuously superfused (2 mL·min^{-1}) with extracellular solution. Membrane currents and AP were recorded using the whole-cell patch-clamp techniques with a patch-clamp amplifier (CEZ 2300, Nihon Kohden, Japan). Patch electrodes were pulled with a vertical puller (PB-7, Narishige, Tokyo, Japan) and had a resistance of 2 – 3 MΩ when filled with electrode internal solution. After gigaseal formed and patch ruptured, AP was recorded in current clamp mode and currents were recorded in voltage clamp mode. Experimental protocols, data acquisition and storage were accomplished with pClamp 5.6 (Axon Instrument, USA) running on a personal computer. All experiments were conducted at 22 – 25 ℃.

Chemicals and solutions DDPH (obtained from China Pharmaceutical University), was a white powder (mp 167 – 168 ℃) of > 99 % purity and was dissolved in distilled water. BSA, collagenase type Ⅱ, taurine, HEPES, egtazic acid, Na_2ATP, and K_2ATP were products of Sigma. 3-(*N*-Morpholino)-propanesulfonic acid (MOPS) was purchased from Shanghai SIBAS Biotech Co.

The composition of the Ca^{2+}-free Tyrode's solution was (mmol·L^{-1}): NaCl 100, KCl 10, NaH_2PO_4 1.2, $MgSO_4$ 5.0, glucose 20, taurine 10, MOPS 10; pH was adjusted with KOH to 7.2.

Extracellular solution for AP recording was composed of (mmol·L^{-1}): NaCl 137, KCl 5.4, $MgCl_2$ 1.0, $CaCl_2$ 1.8, HEPES 10, glucose 20; pH was adjusted with KOH to 7.4. Extracellular solution for recording K^+ current contained (mmol·L^{-1}): choline chloride 137, KCl 5.4, $MgCl_2$ 1.0, HEPES 10, glucose 10; pH was adjusted with KOH to 7.4. The electrode solution for recording AP contained (mmol·L^{-1}): KCl 140, $MgCl_2$ 2.0, egtazic acid 2.0, HEPES 5.0, Na_2ATP 4.0; pH was adjusted with KOH to 7.4 and the electrode solution for recording K^+ current contained (mmol·L^{-1}): KCl 140, $MgCl_2$ 0.5, egtazic acid 10, HEPES 10, K_2ATP 5.0; pH was adjusted with KOH to 7.4.

Statistics Data were expressed as $\bar{x} \pm s$ and compared with the *t*-test.

RESULTS

Action potential AP in guinea pig ventricular myocytes was evoked by a step current pulse of 90 pA, 10 ms duration at the frequency of 0.5 Hz. DDPH (0.1 – 100 μmol·L^{-1}) decreased 50 % duration of action potential (APD_{50}) concentration-dependently. APD_{50} was shortened from (493 ± 58) to (262 ± 38) ms (n = 7 cells from 5 guinea pigs, $P < 0.01$) by DDPH 10 μmol·L^{-1}. DDPH 100 μmol·L^{-1} decreased APD_{50} to (87 ± 9) ms (n = 7, $P < 0.01$). However, DDPH (> 1 μmol·L^{-1}) increased 90 % duration of action potential (APD_{90}). APD_{90} was prolonged from (664 ± 48) to (937 ± 71) ms (n = 7, $P < 0.01$) and (982 ± 56) ms (n = 7 $P < 0.01$) by DDPH 10 μmol·L^{-1} and 100 μmol·L^{-1}, respectively. At high concentration (> 10 μmol·L^{-1}), DDPH decreased resting membrane potential (RP) and amplitude of action potential (APA). All such effects were reversible after wash out (Tab 1).

Tab 1. Effects of DDPH on action potential in single ventricular cell. n = 7 cells from 5 guniea pigs. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

DDPH/ μmol·L^{-1}	APA/mV	RP/mV	APD_{50}/ms	APD_{90}/ms
0	150 ± 11	78 ± 5	493 ± 58	664 ± 48
0.1	141 ± 6^a	74 ± 6^a	416 ± 59^c	619 ± 43^b
1.0	131 ± 6^a	66 ± 5^a	355 ± 47^c	814 ± 68^b
10.0	116 ± 12^b	50 ± 9^a	262 ± 38^c	937 ± 71^c
100.0	94 ± 12^c	34 ± 9^c	87 ± 9^c	982 ± 56^c
Wash out	149 ± 8^a	76 ± 7^a	479 ± 36^a	790 ± 65^a

Delayed rectifier potassium current Tail current of I_K ($I_{K\cdot tail}$) was used to represent I_K. $I_{K\cdot tail}$ in guinea pig ventricular myocytes was obtained by a depolarizing step pulse from the holding potential (E_h) of

−40 mV to 30 mV at the frequency of 0.1 Hz. The step pulse duration was 5 s. When recording membrane current, Na^+ was substituted by choline chloride and Ca^{2+} was reduced to 0 mmol·L^{-1} in extracellular solution to reduce the influence of I_{Na} and I_{Ca}. DDPH inhibited $I_{K\cdot tail}$ concentration-dependently, 46 % [from (293 ± 35) to (158 ± 34) pA, $n = 7$ cells from 5 guinea pigs, $P < 0.05$] at 10 μmol·L^{-1} and 78 % [from (293 ± 35) to (63 ± 11) pA, $P < 0.01$] at 100 μmol·L^{-1}, respectively. EC_{50} for DDPH inhibiting $I_{K\cdot tail}$ was 13.3 (11.6 − 16.7) μmol·L^{-1}. All such effects of DDPH were reversible after washout [from (63 ± 11) to (156 ± 29) pA] (Fig 1).

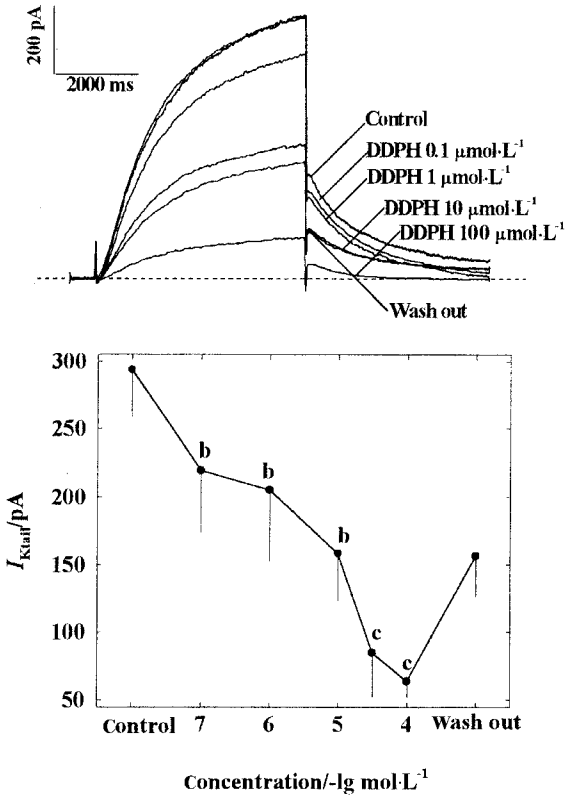

Fig 1. Inhibitory effects of DDPH on I_{Ktail}. $n = 7$ cells from 5 guinea pigs. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* control.

I-V relationship was determined with 5 s steps between −30 to +50 mV from holding potential of −40 mV. I_{Kstep} was defined as a time-dependent component to exclude the contamination of nonspecific currents, which was measured from initial activation to the current at the end of steps. DDPH (0.1 − 100 μmol·L^{-1}) decreased I_{Kstep} and $I_{K\cdot tail}$ at test potential, and the effects were reversible after washout (Fig 2).

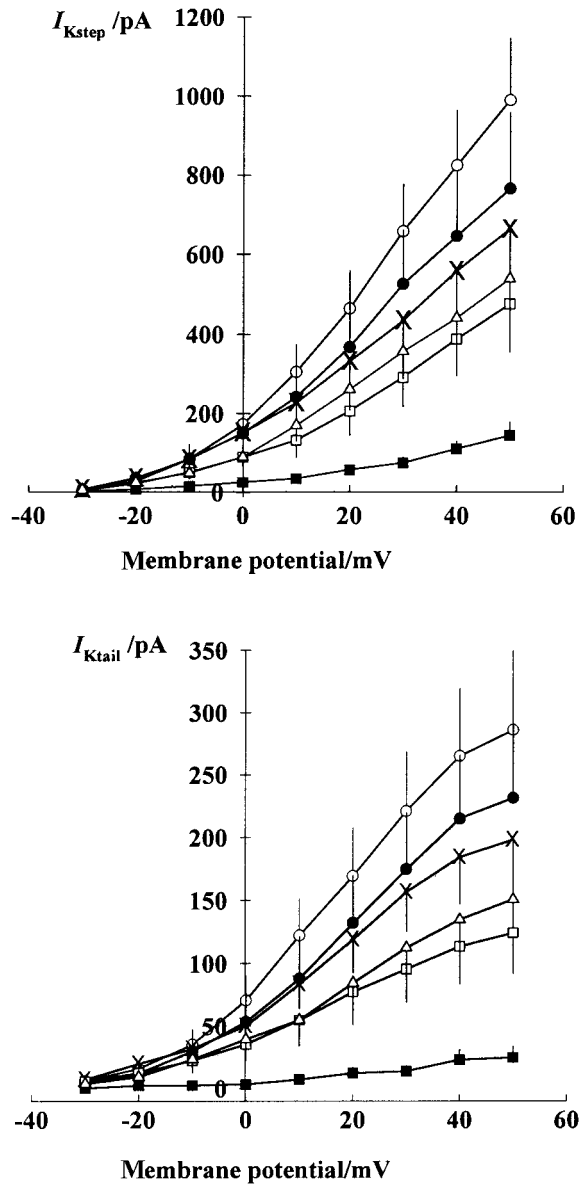

Fig 2. Effects of DDPH on *I-V* relationship of I_{Kstep} and I_{Ktail} in ventricular cell of guinea pig. $n = 5$ cells from 5 guinea pigs. $\bar{x} \pm s$. ○ Control, ● DDPH 0.1 μmol·L^{-1}, × DDPH 1 μmol·L^{-1}, □ DDPH 10 μmol·L^{-1}, ■ DDPH 100 μmol·L^{-1}, △ wash out.

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 potential between −100 and +30 mV with step 10 mV. Addition of DDPH 1 μmol·L^{-1} induced a remarkable depression of I_{K1}. The inward currents of I_{K1}, at E_t (test potential) −100 mV and −90 mV, were depressed by 43 % (from −1556 pA ± 347 pA to −881 pA ± 281 pA, $P < 0.01$) and 49 % (from −975 pA ± 250 pA to −501 pA ± 168 pA, $P <$

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Apr；21 (4)
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0.01, $n = 5$ cells from 5 guinea pigs) of the control. DDPH 10 μmol·L^{-1} had a more remarkable blocking effect on I_{K1}. The inward currents of I_{K1} at E_t of −100 mV and −90 mV were depressed by 59 % (from −1556 pA ± 347 pA to −631 pA ± 234 pA, $P < 0.01$) and 67 % (from −975 pA ± 250 pA to −319 pA ± 140 pA, $P < 0.01$), respectively. The reverse potential of I_{K1} was not affected by low concentration of DDPH (< 1 μmol·L^{-1}) significantly, while at high concentration (> 10 μmol·L^{-1}), DDPH shifted reverse potential positively, from −70 mV to −60 and −40 mV, respectively. All changes were reversible after the drug was washed out (Fig 3).

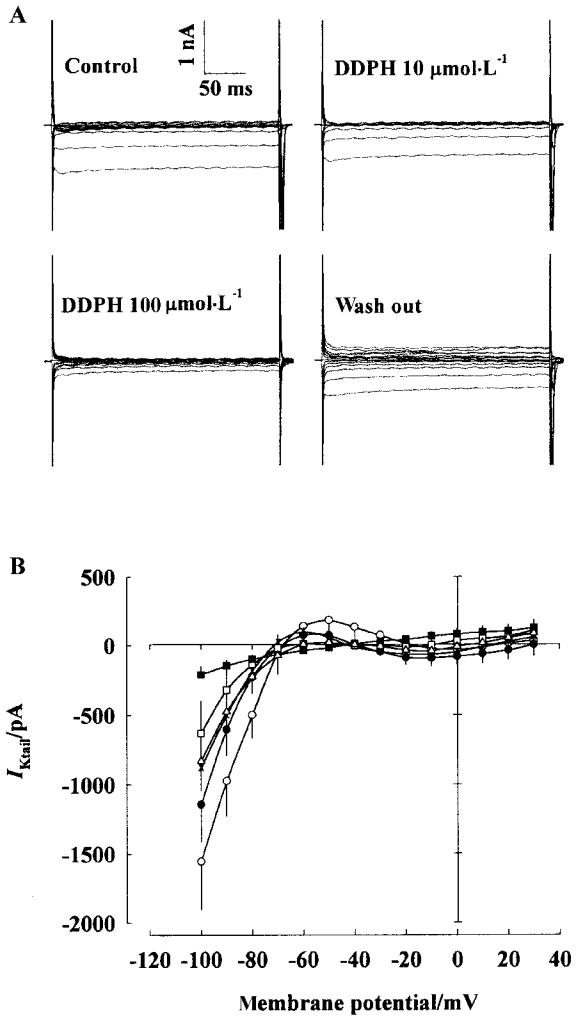

Fig 3. A) Effects of DDPH on inward rectifier potassium currents I_{K1} in single ventricular cell of guinea pig. B) Effects of DDPH on *I-V* relationship of I_{K1} in ventricular cell of guinea pig. $n = 5$ cells from five guinea pigs. $\bar{x} \pm s$. ○ Control, ● DDPH 0.1 μmol·L^{-1}, × DDPH 1 μmol·L^{-1}, □ DDPH 10 μmol·L^{-1}, ■ DDPH 100 μmol·L^{-1}, △ wash out.

DISCUSSION

In our present experiment, it was observed that DDPH had different effects on duration of action potential in single ventricular cells of guinea pig. Since DDPH was synthesized according to the structure of mexiletine (Ib anti-arrhythmic agent) and verapamil (calcium antagonist), so it was supposed to bear the pharmacological properties of these two chemicals. Previous studies revealed that DDPH could depress Na^+ and Ca^{2+} current[4]. The blockade of Na^+ and Ca^{2+} currents by DDPH could support our findings of DDPH shortening APD_{50} and decreasing APA, due to suppression of Ca^{2+} and Na^+ currents, respectively. But such ionic mechanisms can not interpret the prolongation of APD_{90} by DDPH as seen in our results. Such effects of DDPH on APD_{50} and APD_{90} were also found in ventricle papillary muscle preparation of guinea pigs (unpublished data). Such findings indicated that DDPH could affect the potassium current during the depolarization. It was revealed that mexiletine not only affects Na^+ channels, but also modulates K^+ channels. Recently Mitcheson and Hancox reported that mexiletine 30 and 100 μmol·L^{-1} inhibited I_K by 34.3 % ± 5.8 %, and 52.7 % ± 6.1 % in single isolated rabbit atrioventricular nodal myocytes respectively[6]. Similar results were also found with verapamil[7]. In the present study, we demonstrated the inhibitory effects of DDPH on I_K and I_{K1} in single ventricular myocytes of guinea pig, and the inhibitory effects of DDPH on I_K were seen to be more potent than those on I_{K1}. Delayed rectifier potassium channel in cardiac myocytes is thought to play an important role in influencing the shape and duration of the action potential[8]. Class Ⅲ antiarrhythmic agents are thought to be more potent than other drugs in treating arrhythmia due to their selective blockade of I_K[9]. Besides I_K, I_{K1} is very important in maintaining normal RP, and also contributes to the depolarization of action potential[10]. Functional role of blockade of I_{K1} on antiarrhythmic treatment has been previously discussed[11]. Rees and Curtis have reported that I_{K1} blocker, RP 58866, effectively prevented the occurrence of ischemia-induced ventricular fibrillation[12]. The inhibitory effect of DDPH on I_{K1} and I_K may be one of ionic basis of preventing experimental arrhythmia, besides its calcium channel blocking effects. However the action of DDPH on cardiomyocytes is complicated because of its complex structural properties. Such ionic mechanisms warrant further investigation.

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1-(2,6-二甲基苯氧基)-2-(3,4-二甲氧基苯乙氨基)丙烷盐酸盐抑制豚鼠心室肌细胞内向整流和延迟整流钾电流

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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；膜片箝技术；心肌；钾通道；动作电位

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)对心室肌细胞动作电位(AP)、内向整流钾通道电流(I_{K1})及延迟整流钾通道电流(I_K)的影响. 方法：全细胞膜片箝技术. 结果：DDPH 10，100 $\mu mol \cdot L^{-1}$使豚鼠心室肌细胞 AP 时程 APD_{50}明显缩短；但 DDPH (>1 $\mu mol \cdot L^{-1}$) 延长 APD_{90}. DDPH 浓度依赖性地抑制 I_K 尾电流 ($I_{K\cdot tail}$)，EC_{50} 为 13.3 (11.6－16.7) $\mu mol \cdot L^{-1}$. DDPH (>1.0 $\mu mol \cdot L^{-1}$) 明显抑制 I_{K1}；同时，DDPH 使 I_{K1}翻转电位向正电位方向移动. 结论：DDPH 对豚鼠心室肌细胞 I_{K1}和 I_K 具有明显的抑制作用.

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