Effect of melittin on potassium currents and action potential in ventricular myocytes of guinea pig[1]

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KEY WORDS melittin; patch-clamp techniques; action potentials; potassium channels; myocardium

ABSTRACT

AIM: To examine the effects of melittin (Mel), the major component of bee venom, on delayed rectifier K^+ current (I_k), inward rectifier K^+ current (I_{k1}) and action potential (AP) in guinea pig ventricular myocytes. **METHODS**: I_k, I_{k1}, and AP were recorded using the whole-cell patch-clamp technique. **RESULTS**: The action potential duration (APD) was shortened by Mel in a concentration-dependent manner. Mel 0.05, 0.1, 0.2 $\mu mol \cdot L^{-1}$ shortened APD_{50} from (520 ± 55) to (459 ± 91) ($n = 5$, $P > 0.05$), (385 ± 102) ($n = 5$, $P < 0.01$), and (281 ± 81) ms ($n = 5$, $P < 0.01$), respectively; and APD_{90} from (613 ± 96) to (536 ± 93) ($n = 5$, $P > 0.05$), (467 ± 96) ms ($n = 5$, $P < 0.01$), and (354 ± 95) ms ($n = 5$, $P < 0.01$), respectively. Mel increased the amplitude of I_k also in a concentration-dependent manner. Mel 0.05, 0.1, 0.2 $\mu mol \cdot L^{-1}$ increased I_k from (295 ± 109) to (371 ± 142) ($n = 5$, $P < 0.05$), (467 ± 180) ($n = 5$, $P < 0.05$), (552 ± 248) pA ($n = 5$, $P < 0.05$), respectively at testing potential of +40 mV. But no significant effect of Mel on I_{k1} was observed at these concentrations. **CONCLUSION**: Mel significantly increased I_k in a concentration-dependent manner which contributed to shortening of APD.

INTRODUCTION

Melittin (Mel), a 26-amino acid polypeptide, is derived from bee venom and accounts for half of its dry weight. It is characterized as an amphiphilic peptide and considered as an ideal tool to study the protein-lipids and protein-protein interactions in membranes[1].

Though Mel plays an important role in the biophysical area, its pharmacological activities are less intensively studied, which is partly due to its "ubiquitous pharmacological effects"[2]. Recently the effect of Mel on ion transport across cell membrane has drawn much attention[3,4,5]. However only Okamoto and his colleagues performed a study on cardiac myocytes[6] and no detailed report regarding the electrophysiological effect of Mel on heart is available. Moreover, no ion currents were directly recorded with patch-clamp techniques. Thus, we intend to perform research with this technique to provide adequate analysis of the effect of Mel.

Myocardial potassium currents function to control the resting potential, the action potential duration (APD), refractoriness and automaticity. In ventricles the main outward potassium currents are I_k, I_{k1}, and the transient outward current (I_{to}), among which I_{to} is not found in guinea pig. I_k affects the late plateau phase to initiate the rapid final repolarization of action potential (AP). I_{k1} is important in setting the resting potential and in controlling myocardial cell excitability[7]. In the present study we investigate the effect of Mel on potassium current and AP in ventricular myocytes of guinea pigs.

MATERIALS AND METHODS

Preparation of ventricular myocytes Single ventricular myocytes were isolated from adult guinea pigs, (315 ± 24) g, by enzymatic disaggregation[8]. After stunning the guinea pig the heart was rapidly put into oxygenated calcium-free Tyrode's solution. The aorta was cannulated and the heart was perfused on Langendorff apparatus at 37 ℃. Following perfusion with calcium-free Tyrode's solution for about 5 min, the low calcium (50 $\mu mol \cdot L^{-1}$) Tyrode's solution containing

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Received 1999-09-28 Accepted 2000-02-24

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Sep; 21 (9)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

0.03 % type-Ⅱ collagenase and 1 % bovine serum albumin (BSA) was used for about 4 min. The ventricles were chopped, minced, and gently agitated to obtain myocytes. Cells were filtered through a 200-μm nylon mesh and the calcium concentration was gradually increased.

Whole-cell patch-clamp recording The cells were put in a 1 mL pool on the stage of inverted microscope (Nikon, Japan). Only cells with a rod-shape and clear striations were used in experiments. The pool was perfused with test solution at a rate of 1.5 mL·min^{-1}. Microelectrodes were pulled with a microelectrodes puller (Narrishage, Japan) and had a resistance of 3 - 4 MΩ when filled with pipette solution. Ag-AgCl electrode was used as the reference electrode. After gigaseal was formed and the membrane was ruptured, AP was recorded in mode of current clamp and potassium current in mode of voltage clamp. Axopatch 200 A amplifier (Axon Instrument, USA) was employed and pclamp 6.0 software (Axon Instrument, USA) was used to produce protocols, acquire and process data. Serial concentrations of Mel solutions were prepared and administered by perfusion for at least 1 min to assure the drug in the pool reached the anticipated concentrations.

Chemicals and solution Mel, type-Ⅱ collagenase , Na_2ATP, K_2ATP were purchased from Sigma. TEA-Cl and HEPES were Merck products. 3-(*N*-morpholino) propanesulfonic acid (MOPS), BSA were purchased from Sino-American Biotechnology Company. Other reagents in AR grades were products of Shanghai Chemical Plant.

The calcium-free solution contained: NaCl 100, KCl 10, NaH_2PO_4 1.2, $MgSO_4$ 5.0, glucose 20, taurine 10, MOPS 10 mmol·L^{-1}; pH was adjusted with KOH to 7.2. Test solution of AP recording was composed of : NaCl 137, KCl 5.4, $MgCl_2$ 1.0, $CaCl_2$ 1.8, HEPES 10, glucose 10 mmol·L^{-1}; pH was adjusted with NaOH to 7.3. The pipette solution of AP recording was: KCl 140, $MgCl_2$ 2, egtazic acid 2, HEPES 5, Na_2ATP 4 mmol·L^{-1}; pH was adjusted with KOH to 7.2. The test solution of potassium current recording was composed of : NaCl 137, KCl 5.4, $MgCl_2$ 1, HEPES10, glucose 10 mmol·L^{-1}; pH was adjusted with NaOH to 7.2. The pipette solution of potassium current recording was: KCl 140, $MgCl_2$ 0.5, egtazic acid 10, HEPES 10, K_2ATP 5 mmol·L^{-1}; pH was adjusted with KOH to 7.2.

Statistics The data are expressed as $\bar{x} \pm s$ and analyzed with paired-*t* test.

RESULTS

Effect of Mel on AP In the mode of current clamp, the AP was elicited by a step current pulse of 900 pA for 15 ms at the frequency of 0.2 Hz. Mel shortened the APD in a concentration-dependent manner (Fig 1). Mel 0.05, 0.1, 0.2 μmol·L^{-1} shortened APD_{50} from (520±55) to (459±91) ($n=5$, $P>0.05$), (385±102) ($n=5$, $P<0.01$), and (281±81) ms ($n=5$, $P<0.01$), respectively; and APD_{90} from (613±96) to (536±93) ($n=5$, $P>0.05$), (467±96) ($n=5$, $P<0.01$), and (354±95) ms ($n=5$, $P<0.01$), respectively. But it did not change the action potential amplitude (APA), resting potential (RP) and the maximum rate of depolarization phase (V_{max}) significantly at the tested concentration (Tab 1). The effect of Mel was hardly reversed by washout.

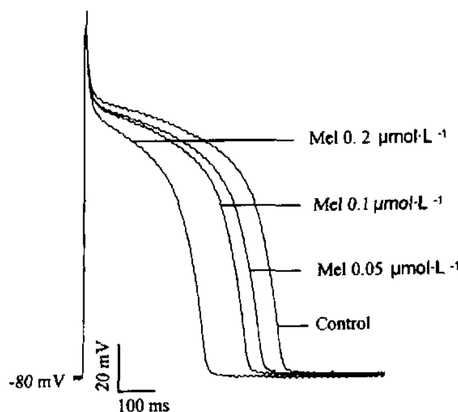

Fig 1. Effect of melittin on action potential in guinea pig ventricular cells.

Tab 1. Effect of Mel on resting potential, action potential amplitude, and V_{max}. $\bar{x} \pm s$. $^aP>0.05$ *vs* control.

Concentration μmol·L^{-1}	RP (mV) $n=4$	APA (mV) $n=5$	V_{max} $n=4$
Control	-77±11	136±15	189±38
0.05	-78±10^a	134±22^a	210±54^a
0.1	-76±9^a	133±19^a	184±48^a
0.2	-77±10^a	132±24^a	180±62^a

Effect of Mel on I_k When the membrane was depolarized from the holding potential of - 40 mV to

+80 mV for 5 s with step 10 mV at the frequency of 0.2 Hz, the current-voltage relationship of I_k was obtained (Fig 2). Mel 0.05, 0.1, 0.2 μmol·L^{-1} increased I_k from (295 ± 109) to (371 ± 142) (n = 5, P < 0.05), (467 ± 180) (n = 5, P < 0.05), (552 ± 248) pA (n = 5, P < 0.05) respectively at the testing potential of +40 mV (Fig 3).

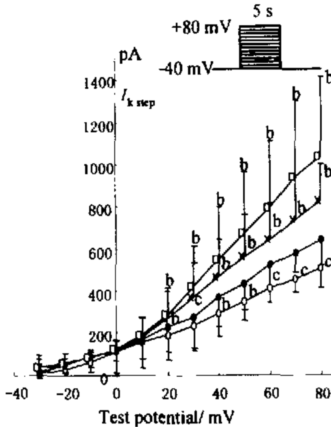

Fig 2. Effect of Mel on current-voltage relationship of I_k in guinea pig ventricular myocytes. (○) Control, (●) Mel 0.05 μmol·L^{-1}, (×) 0.1 μmol·L^{-1}, (□) 0.2 μmol·L^{-1}. n = 5 from 4 guinea pigs. $x \pm s$. ^bP < 0.05, ^cP < 0.01 *vs* control.

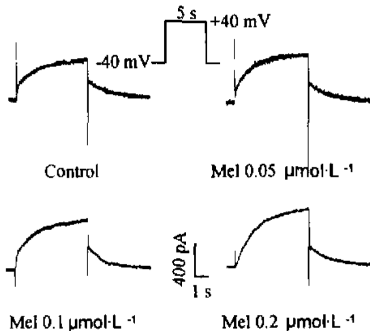

Fig 3. Effect of Mel on delayed rectifier potassium current (I_k) in guinea pig ventricular myocytes.

Effect of Mel on I_k I_{k1} was recorded when the cells were hyperpolarized from the holding potential of −40 mV to −100 mV for 300 ms, followed by the depolarization in steps of 10 mV to +50 mV[9] at frequency of 0.2 Hz. Though Mel had a trend to increase I_{k1}, especially at high concentration, the difference was not statistically significant (Fig 4).

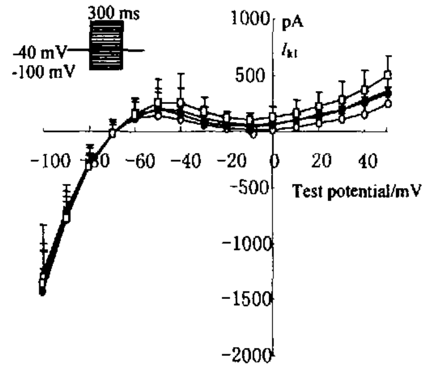

Fig 4. Effect of Mel on inward rectifier current (I_{k1}) in guinea pig ventricular myocytes. (○) Control, (●) Mel 0.05 μmol/L, (×) 0.1 μmol/L, (□) 0.2 μmol/L. n = 5 from 3 guinea pigs. $\bar{x} \pm s$.

DISCUSSION

Several components of animal venom such as margatoxin and charybdotoxin have been proved to be selective blockers of delayed rectifier potassium current. But opener of delayed rectifier potassium currents has not so far been reported in literature[10]. Our results indicated that Mel produced a dose-dependent irreversable increase in I_k in ventricular myocytes of guinea pig but had no significant effect on I_{k1}. Though the effect of Mel on I_{to} is unknown, it appears to be a candidate for selective opener of I_k. Thus it may be developed into a helpful tool drug in laboratory.

It should be also noticed that the result of increasing I_k was in accordance with that of shortening of APD. It is well known that inward L-type calcium current and outward potassium currents are mainly involved in determining the APD. Okamoto and his colleagues have reported that Mel has no effect on calcium channel[6] as observed by fluorescent method. On basis of these results, we concluded that the increase in I_k might play an important role in shortening of APD caused by Mel.

Mel has a narrow effective dose-range suggesting that it is a potent cardiotoxin[11]. If it exceeds this range, some toxic effects might be observed. When we tried a relatively higher concentration (0.5 μmol·L^{-1}) some toxicity such as raised RP and arrhythmia appeared. But the concentration causing the toxicity is variable for individual cells, and this phenomenon has been also noticed by others[12].

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Sep; 21 (9)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

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蜂毒肽对豚鼠心室肌细胞钾电流和动作电位的影响[1]

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关键词 蜂毒肽；膜片箝技术；动作电位；钾通道；心肌

目的：研究蜂毒肽(Melittin，Mel)对豚鼠心室肌细胞钾电流和动作电位的影响． **方法**：全细胞膜片箝记录． **结果**：蜂毒肽可呈浓度依赖性促进延迟整流钾电流(I_k)，在测定电压为 40 mv 时，0.05，0.1，0.2 μmol·L^{-1}蜂毒肽分别使 I_k从(295 ± 109)增大到(371 ± 142) ($n=5$ $P<0.05$)，(467 ± 180) ($n=5$，$P<0.05$)，(552 ± 248) pA ($n=5$，$P<0.05$)． 但药物在三个浓度时对内向整流钾电流(I_{kl})均无显著影响． 蜂毒肽 0.05，0.1，0.2 μmol·L^{-1}分别使动作电位 APD_{50}由(520 ± 55)减小到(459 ± 91) ($n=5$，$P>0.05$)，(385 ± 102) ($n=5$，$P<0.01$)，(281 ± 81) ms ($n=5$，$P<0.01$)，使 APD_{90}由(613 ± 96)减小到(536 ± 93) ($n=5$，$P>0.05$)，(467 ± 96) ($n=5$，$P<0.01$)，(354 ± 95) ms ($n=5$，$P<0.01$)． **结论**：蜂毒肽促进延迟整流钾电流，缩短动作电位时程．

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