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Effects of melittin on isolated papillary muscles of guinea pig[1]

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KEY WORDS melitten; papillary muscles; myocardial contraction; neurologic refractory period; action potentials

ABSTRACT

AIM: To investigate the effect of melittin (Mel) on papillary muscles of guinea pigs. **METHODS**: Contraction of papillary muscles were examined by conventional method and action potentials (AP) were recorded by standard glass microelectrode technique. **RESULTS**: Mel (0.5, 3 μmol/L) significantly increased the contractility of guinea pig papillary muscles while 5 μmol/L exerted dual action with a transient decrease followed by an increase of the contractility. Mel shortened the functional refractory period (FRP) at concentrations of 0.5, 3, and 5 μmol/L and increased the automaticity induced by adrenaline (Adr) at 3 and 5 μmol/L. Mel shifted the duration-intensity curve upward at 3 μmol/L. It shortened the action potential duration (APD) of fast action potential (FAP), decreased the action potential amplitude (APA) and resting potential (RP) at 0.5 and 3 μmol/L. As to slow action potential (SAP), Mel 0.8 μmol/L shortened APD_{20} and APD_{50}, and decreased APA and RP. **CONCLUSION**: Mel increased the contractility and automaticity of papillary muscles, shortened the FRP, decreased the excitability, shortened the APD, and decreased APA and RP of AP.

INTRODUCTION

Melittin (Mel), an amphipathic polypeptide containing 26 amino acids, comprises about 50 % of the dry weight of bee venom[1]. It has numerous effects on the electrical properties of excitable tissues[2]. It was reported that Mel was cardiotoxic *in vitro*, causing paralysis of rat isolated heart[3] and inducing apparent dysfunction of cultured cardiac myocytes[4]. However, basic physiological properties of Mel on cardiac tissues have not been clarified. In the present study, we observed the influence of Mel on the contractility, automaticity, functional refractory period, excitability, and action potentials of guinea pig papillary muscles.

MATERIALS AND METHODS

Drugs Melittin (Mel, Sigma Co); adrenaline (Adr, Shanghai 10th Pharmaceutical Factory, China); isoprenaline (Shanghai 14th Pharmaceutic Factory); verapamil (Shanghai Hefeng Pharmaceutical Co).

Stock solution of Mel was diluted in Tyrode's solution to obtain the final concentration.

Methods Papillary muscles from the right ventricle of guniea pigs (300 g ± 50 g, ♀ + ♂, supplied by the Experimental Animal Center of Fudan University, Grade Ⅱ) were suspended in a 10-mL bath containing Tyrode's solution kept at 30 ℃ and aerated with 95 % O_2/5 % CO_2. The composition (in mmol/L) of the medium was: NaCl 137, KCl 5.4, $MgCl_2$ 1.05, $CaCl_2$ 1.8, $NaHCO_3$ 12, NaH_2PO_3 0.43, and glucose 10. Resting tension was adjusted to 0.5 g. Equilibrium was maintained for 60 min before the start of the experiment. The papillary muscles were stimulated at a rate of 1 Hz with a stimulator (SEN-3201 Nihon Konden, Japan). Developed tension was recorded with a force-displacement transducer (made by Shanghai Medical University, China) connected to an auto-equilibration recording instrument (XWT-204 Shanghai).

For action potentials (AP), the papillary muscle was pinned in a 1 mL-chamber and perfused with modified Tryode's solution (34 ℃) containing NaCl 154, KCl 4, $MgCl_2$ 1, $CaCl_2$ 2, Hepes 5, glucose 5.5 mmol/L. The solution flowed through the chamber at a rate of 8 mL/min. The preparation was stimulated at 1 Hz, 3 ms, 1.5 times threshold. AP was recorded with glass microelectrode filled with KCl 3 mmol/L (resistance of 10 - 30 MΩ) and photographed from a storage oscilloscope (VC-

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10 Nihon Konden, Japan).

For slow action potentials (SAP), high K^+ (18 mmol/L) Tyrode's solution was prepared by equimolar substitution of KCl for NaCl to inactivate the fast Na^+ channels and isoprenaline 0.1 μmol/L was added to elicit SAP.

Statistics Statistical analysis was made using paired *t*-test.

RESULTS

Contractility Mel 0.1, 0.3, 1, 3, and 10 μmol/L were added cumulatively to the bath at 5-min intervals ($n=5$). Mel 0.1 μmol/L enhanced the contractile amplitude by 6.4 % ± 5.9 % ($P>0.05$); at 0.3, 1, and 3 μmol/L, it significantly enhanced the amplitude by 29.7 % ± 13.2 % ($P<0.01$), 27.7 % ± 9.7 % ($P<0.05$), and 25.6 % ± 14.4 % ($P<0.05$) respectively. However, at 10 μmol/L, it did not change the contractile amplitude (the control value of amplitude was 0.32 g ± 0.08 g).

Time-course for single dose of Mel 0.5, 3, and 5 μmol/L was examined. At concentrations of 0.5 and 3 μmol/L, maximal enhancing effects were attained at 5 min, followed by a slight decrease until 40 min. The maximal effects were 77.3 % ± 14.5 % and 58.4 % ± 3.0 % respectively, however, Mel 5 μmol/L produced a biphasic action, an initial transient decrease followed by an increase, it reached the maximal effect at 10 min by 112.3 % ± 38.5 % ($P<0.01$). The action of Mel 0.5 μmol/L was easily reversed by washing, while Mel 3 and 5 μmol/L could only be incompletely washed out. An example of time-response curve for Mel 0.5 and 5 μmol/L were shown in Fig 1.

Automaticity We examined the effects of Mel on automaticity by testing the change of the threshold concentration of Adr to induce the automatic activity of papillary muscles after Mel administration[5]. Mel 3 and 5 μmol/L increased the automaticity by lowering the threshold concentration of Adr (μmol/L) from 26.40 ± 0.01 and 23.40 ± 0.01 to 7.60 ± 0.01 ($P<0.01$, $n=6$) and 7.40 ± 0.01 ($P<0.01$, $n=6$) respectively. Mel 0.5 μmol/L had no effect on automaticity ($P>0.05$, $n=7$).

Functional refractory period (FRP) FRP of papillary muscle[6] was examined before and after Mel administration. Mel 0.5, 3, and 5 μmol/L shortened the FRP(ms) from 266 ± 10, 243 ± 20, and 249 ± 28 to 240 ± 16 ($P<0.01$, $n=6$), 204 ± 12 ($P<0.01$, $n=6$), and 233 ± 26 ($P<0.01$, $n=6$) respectively.

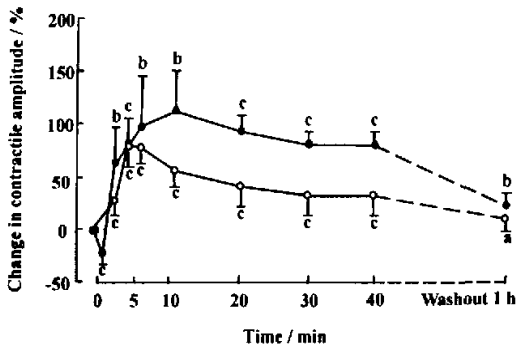

Fig 1. Time-course of action of Mel 0.5 μmol/L (○, $n=6$), 5 μmol/L (●, $n=5$) on the contractilily of guinea pig papillary muscles. $\bar{x} \pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control. The control value of contractile amplitude were (0.35 ± 0.08) g.

Excitability Effects of Mel on the excitability were defined by examining the shifting of duration-intensity curve[7] (Fig 2). Mel 3 μmol/L decreased the excitability by shifting upward the duration-intensity curve, while Mel 0.5 and 5 μmol/L did not alter it.

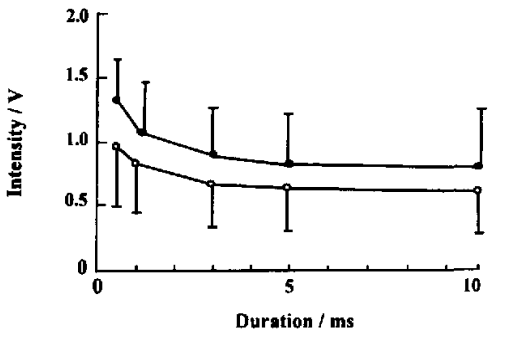

Fig 2. Effects of Mel 3 μmol/L on intensity-duration curve of guinea pig papillary muscles. $n=7$. $\bar{x} \pm s$. (○, control; ●, Mel).

Fast action potential (FAP) After 3 – 5 min of perfusion with Tyrode's solution containing Mel 3 μmol/L, action potential duration (APD_{20}, APD_{50}, APD_{90}) began to shorten, while action potential amplitude (APA) and resting potential (RP) were decreased. Maximal effect on APD reached at 10 – 20 min, lasting for 60 min and partially recovered by washing; while the action of decreasing APA and RP only lasted for 30 min (Tab 1). The time-course of Mel 0.5 μmol/L on FAP

was also observed, similar results to that on contractility were obtained.

Slow action potential (SAP) Since SAP could not be evoked after administration of Mel at concentrations $\geqslant 1$ μmol/L, It was examined at Mel 0.8 μmol/L (Tab 2). The results showed that it decreased RP and APA of SAP. The action occurred at 3 or 5 min respectively, reached steady state at 20 min and lasted for at least 40 min. Effects of shortening APD_{20}, APD_{50} occurred at 10 min and lasted for only 10 – 15 min, the effect of APD_{90} did not change.

DISCUSSION

The present study showed that Mel affected the basic physiologic properties of guinea pig papillary muscles, enhanced contractility and automaticity, shortened FRP, and depressed excitability. The enhancing effects of Mel on the contraction of cardiac muscles were also observed on isolated guinea pig atria in our previous study [8].

Result of the present work revealed that in guinea pig papillary muscles, Mel decreased APA and RP of FAP and SAP, as well as shortened APD. It had been suggested by Morad and Tung that the depolarization of myocardial AP is mainly dependent on Na^+ influx (on FAP) or Ca^{2+} influx (on SAP), while its repolarization relates to both Ca^{2+} influx and K^+ outflow[9]. Recently our laboratory had demonstrated by whole cell path-clamp technique that Mel significantly increased I_k which may contribute to the shortening of APD, increased I_{Na} and stimulated Na^+-Ca^{2+} exchange, but did not influence L-type calcium current in ventricular myocytes of guinea pigs[10,11]. Taken all over, it might be suggested that Mel possesses action of Na^+, K^+ and Na^+-Ca^{2+} change transmembrane activities in guinea pigs myocardium.

Tab 1. Effects of Mel 3 μmol/L on the fast AP of guinea pig papillary muscles. $n = 6$. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* 0 min.

Time/min	APA/mV	APD_{20}/ms	APD_{50}/ms	APD_{90}/ms	RP/mV
0	122 ± 8	116 ± 20	180 ± 15	210 ± 17	− 84 ± 7
3	97 ± 17[a]	67 ± 24[c]	135 ± 21[c]	174 ± 17[c]	− 72 ± 9[c]
5	89 ± 14[c]	52 ± 11[c]	94 ± 44[c]	154 ± 16[c]	− 69 ± 6[c]
10	88 ± 14[c]	53 ± 12[c]	115 ± 12[c]	152 ± 13[c]	− 68 ± 7[c]
20	91 ± 8[c]	48 ± 15[c]	117 ± 15[c]	154 ± 18[c]	− 69 ± 8[c]
30	100 ± 8[b]	56 ± 18[c]	124 ± 17[c]	165 ± 18[c]	− 78 ± 9[a]
40	112 ± 6[a]	75 ± 21[c]	144 ± 28[b]	187 ± 26[b]	− 83 ± 11[a]
50	114 ± 4[a]	80 ± 21[c]	153 ± 27[b]	193 ± 27[b]	− 82 ± 9[a]
60	117 ± 3[a]	82 ± 20[c]	153 ± 27[b]	196 ± 24[a]	− 83 ± 9[a]
Washout	120 ± 2[a]	91 ± 21[b]	165 ± 22[b]	201 ± 28[a]	− 84 ± 7[a]

Tab 2. Effects of Mel 0.8 μmol/L on the slow AP of guinea pig papillary muscles. $n = 6$. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* 0 min.

Time/min	APA/mV	APD_{20}/ms	APD_{50}/ms	APD_{90}/ms	RP/mV
0	91 ± 5	94 ± 12	160 ± 20	179 ± 20	− 51 ± 4
3	88 ± 4[c]	91 ± 15[a]	158 ± 20[a]	177 ± 19[a]	− 50 ± 3[a]
5	86 ± 5[c]	88 ± 16[a]	156 ± 19[a]	177 ± 20[a]	− 48 ± 3[c]
10	81 ± 5[c]	85 ± 12[b]	153 ± 18[b]	174 ± 20[a]	− 47 ± 3[c]
15	81 ± 4[c]	86 ± 11[b]	153 ± 19[b]	174 ± 21[a]	− 47 ± 3[c]
20	79 ± 7[c]	86.7 ± 1.2[b]	157 ± 18[a]	178 ± 21[a]	− 45 ± 2[c]
25	78 ± 8[c]	87.7 ± 1.9[b]	159 ± 19[a]	180 ± 20[a]	− 45 ± 3[c]
30	78 ± 9[c]	92 ± 4[a]	161 ± 20[a]	181 ± 20[a]	− 45 ± 4[c]
40	79 ± 8[c]	95.3 ± 2.3[a]	164 ± 19[a]	184 ± 20[a]	− 46 ± 6[b]

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蜂毒肽对离体豚鼠乳头状肌的影响[1]

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关键词　蜂毒肽；乳头状肌；心肌收缩；神经性不应期；动作电位

目的：研究蜂毒肽(Mel)对离体豚鼠乳头状肌的影响。**方法**：观察 Mel 对豚鼠乳头状肌基本生理特性的影响，并应用标准玻璃微电极技术记录快反应动作电位(FAP)和慢反应动作电位(SAP)。**结果**：Mel (0.5, 3 μmol/L)显著增强乳头状肌收缩力，Mel (5 μmol/L)在增强收缩力之前可出现短暂的抑制作用。Mel (0.5, 3, 5 μmol/L)可显著缩短功能性不应期，在一定浓度下(Mel 3, 5 μmol/L)可升高肾上腺素诱发的自律性，使时间-兴奋曲线上移(Mel 3 μmol/L)。Mel (0.3, 5 μmol/L)可显著缩短 FAP 的动作电位时程(APD)，减小动作电位幅度(APA)和静息电位(RP)。Mel 0.8 μmol/L 可减小 SAP 的 APA 和 RP，仅缩短 APD_{20}和 APD_{50}，对 APD_{90}无影响。**结论**：Mel 增强豚鼠乳头状肌收缩性和自律性，缩短不应期，降低兴奋性，缩短 APD，减小 APA 和 RP。

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