ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Mar；21 (3)：221 – 224
http：//www.chinaphar.com

Biphasic manner of melittin on isolated guinea pig atria[1]

YANG Shen[2]，LIU Jun-E，ZHANG Ao-Zhen，JIANG Ming-Hua
(*Department of Pharmacology，School of Pharmacy，Shanghai Medical University，Shanghai 200032，China*)

KEY WORDS melittin；bee venoms；verapamil；guinea pigs；atrial function

ABSTRACT

AIM：To investigate the effect of melittin (Mel) on isolated guinea pig atria. METHODS：The effect of Mel on the contraction and heart rate of isolated guinea pig atria at different concentrations was determined. RESULTS：Mel at a lower concentration (0.1 – 0.8 $\mu mol \cdot L^{-1}$) enhanced the contraction of left atria in a concentration-dependent manner；but at a higher concentration (1.6 – 12.8 $\mu mol \cdot L^{-1}$) it exerted an inhibitory effect. At 0.1 – 30 $\mu mol \cdot L^{-1}$ it was found to increase heart rate of right atria. In addition，verapamil (Ver) 0.3 $\mu mol \cdot L^{-1}$ was found to depress the effect of Mel. CONCLUSION：Mel possesses a biphasic effect on left atria and a positive chronotropic effect on right atria. Its mechanism might be related with Ca^{2+} channel.

INTRODUCTION

Melittin (Mel)，the major toxin of bee venom，is an amphipathic polypeptide containing 26 amino acids[1]. It is used as a pharmacological tool to understand cell membrane function[2]，and is known to affect the cardiovascular system[3]. Whilst Mel was observed to cause a dose-dependent biphasic alteration in rat heart adenylate cyclase activity[4]，we report here a study of the effects of Mel on guinea pig atria showing its biphasic manner，and its possible mechanism of action.

MATERIALS AND METHODS

Preparation of isolated atria from guinea pig

Guinea pigs of either sex weighing 300 – 400 g were stunned by a sharp blow on the head. The heart was quickly removed，and both left and right atria were dissected free in Tyrode's solution containing the following composition ($mmol \cdot L^{-1}$)：NaCl 137，KCl 5.4，$MgCl_2$ 1.05，$CaCl_2$ 1.8，$NaHCO_3$ 12，NaH_2PO_4 0.43，Glucose 10 (pH 7.3 – 7.4). Both atria were vertically suspended in different organ baths containing 20 mL of the above solution at 30 ℃ and 36 ℃，respectively and bubbled continuously with a mixture of 95 % O_2 + 5 % CO_2. A 45-min equilibration period was allowed before control measurements were made. Resting tension was adjusted at 1.0 g and tension developed was monitored continuously with a force-displacement transducer (made by Shanghai Medical University) and an auto-equilibration recording instrument (Model XWT-204). The left atria located between two platinum electrodes was electric-field stimulated at 1 Hz with square wave pulses of 3 ms duration at a voltage approximately 20 % higher than the threshold voltage.

Agents Melittin (Mel) (purchased from Sigma Co) was kindly provided by Dr Garrasquer G. Mel gives a single peak by HPLC and SDS-PAGE，and it is soluble in water[5,6]. Verapamil hydrochloride was obtained from He Feng Pharmaceutical Co，Shanghai.

Mel solution was freshly prepared for each experiment，it was dissolved and diluted in Tyrode's solution before injecting into the organ bath to obtain the final concentration. Cumulative concentration-effect curves of Mel were determined by stepwise increase of drug concentration in Tyrode's solution added at 5-min intervals. The results were presented as percent of the initial contraction amplitude of left atria or the initial spontaneous heart rate of right atria before drug administration.

Statistics *t*-test with paired and unpaired observations were performed to determine the level of significance when applicable.

RESULTS

Concentration-effect curves of Mel on left atria

[1] Supported by the National Natural Science Foundation of China，№ 39770885.
[2] Correspondence to Dr YANG Shen.
Phn 86-21-6404-1900，ext 2557. E-mail mhjiang@shmu.edu.cn
Received 1999-07-23 Accepted 1999-10-15

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Mar；21 (3)
E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629

Preliminary experiments were carried out in 5 isolated working preparations for stability tests, the atrial function parameters remained constantly stable at the end of the 60 min.

Mel altered the contractile amplitude of left atria in a dose-dependent biphasic manner. At lower concentration (0.1 – 0.8 μmol·L^{-1}), it enhanced the contractile amplitude significantly, but at higher concentration (1.6 – 12.8 μmol·L^{-1}) it progressively converted into an inhibitory action. In two out of five atria (12.8 μmol·L^{-1} group) a contracture was observed (Fig 1).

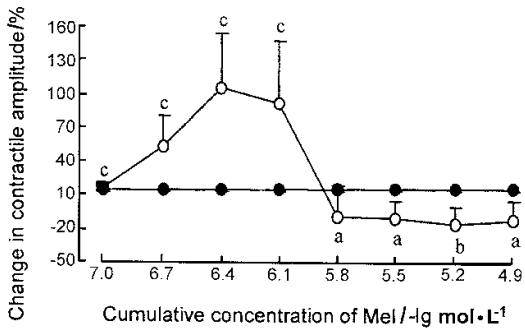

Fig 1. Effect of Mel on contraction of guinea pig left atria. Mel (○, n = 9 animals), control (●, n = 5 animals). $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

Time-course of the action of Mel on left atria

Mel 0.4 μmol·L^{-1} and 3 μmol·L^{-1} defined as the time *vs* change in the contractile amplitude were examined. It was shown that significant contractile effects of Mel on the tension were developed in the first min. Mel 0.4 μmol·L^{-1} increased the contraction to the maximum within 10 min, followed by a slight decrease until over 60 min. The maximal enhancing effect was 114 % ± 50 % ($P < 0.01$) at 10 min. However, the maximal enhancing effect of Mel 3 μmol·L^{-1} appeared at 1 min, which was 70 % ± 20 % ($P < 0.01$) and lasted for only 3 min, then it gradually converted into inhibition and lasted for over 20 min. The maximal depression was 55 % ± 18 % ($P < 0.01$) at 10 min. This result indicates that the time-course effect of Mel 3 μmol·L^{-1} appears to be biphasic. (Fig 2)

The effects of both concentrations were accompanied with an incompletely increased diastolic tone 3 min after administration, which implies that Mel might be affecting Ca^{2+} outflow across the cell membrane.

Mel 0.4 μmol·L^{-1} was easily reversed by washing with Tyrode's solution. However, Mel 3 μmol·L^{-1} could only be incompletely washed out.

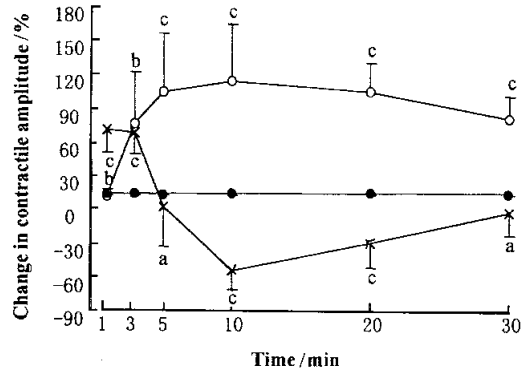

Fig 2. Time-course of action of Mel 0.4 **μmol·L^{-1}** (○, n = 5 animals), control (●, n = 5 animals), Mel 3 μmol·L^{-1} (×, n = 8 animals) on the contractile amplitude of guinea pig left atria. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

Effect of Mel on right atria Mel 0.1 – 30 μmol·L^{-1} showed concentration-dependent positive chronotropic actions on right atria (Tab 1).

Tab 1. Positive chronotropic action of Mel on guinea pig right atria. n = 7 animals. $\bar{x} \pm s$. $^cP < 0.01$ *vs* normal rate.

Concentration/μmol·L^{-1}	Increase in heart rate/%
0.1	2.6 ± 1.6^c
0.3	9.1 ± 6.1^c
1	27.0 ± 7.5^c
3	26.8 ± 7.7^c
10	33.9 ± 8.4^c
30	41.7 ± 5.1^c

However, there was a negative ionatropic tendency in right atrial amplitude without statistical significance except for Mel 10 μmol·L^{-1} (– 35 % ± 19 %, $P < 0.01$, data not shown).

Time-course of the chronotropic action of Mel 1 μmol·L^{-1} on right atria The significant positive chronotropic action started at 3 min after administration of Mel 1 μmol·L^{-1}. The maximal effect with an increasing rate of 24 % ± 5 % ($P < 0.01$) was attained at 5 min without the biphasic phenomenon. This positive chronotropic actions remained continually over 60 min (Tab 2).

Influence of verapamil (Ver) on the effect of Mel in left atria Eight left atria preparations were exposed to Ver 0.3 μmol·L^{-1}, a calcium channel blocker, for 20 min, then cumulative concentration effect of Mel

Tab 2. Time-course of the positive chronotropic action of Mel 1 μmol·L^{-1} on guinea pig right atria. $n = 7$ animals. $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* normal rate.

Time/min	Increase in heart rate/%
1	4.6 ± 3.9^a
3	22.5 ± 7.3^c
5	23.7 ± 5.4^c
10	19.3 ± 5.1^c
20	19.3 ± 4.6^c
30	18.2 ± 4.4^c
40	18.0 ± 5.2^c
50	17.6 ± 4.8^c
60	16.7 ± 5.6^c

was obtained as above. The results showed that Ver depressed cumulative concentration effect of Mel resulting in a biphasic curve similar to the control one (Fig 3).

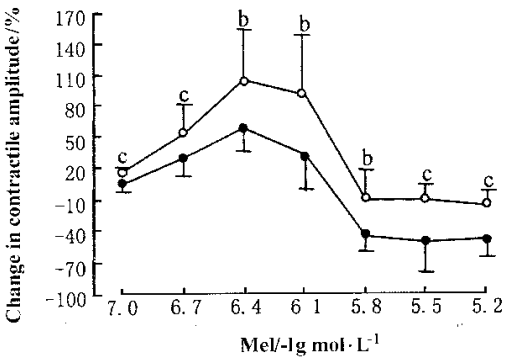

Fig 3. Effect of Mel in the absence (○, $n = 9$ animals) and presence (●, $n = 8$ animals) of Ver on the contractile amplitude of guinea pig left atria. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* presence of Ver.

DISCUSSION

The results in this study demonstrate that the effect of Mel on isolated guinea pig atria was concentration-dependent and time-dependent. It exerted a positive chronotropic action on right atria and a pronounced influence on the electrically driven left atria in a biphasic manner. At lower concentration, it enhanced the contractile amplitude, whereas at higher concentration, it inhibited the contractile amplitude. This biphasic effect of Mel is similar to its actions on erythrocyte membranous fluidity[7], and heart adenylate cyclase activity of the rat[4] but it differs from the report which says that Mel occurs in rat atrium[8].

Time-course observation on the effect of single dose of Mel has so far not been reported in literature. Our results show that single dose of Mel 3 μmol·L^{-1} also exerted a biphasic effect in time course on the left atria. This phenomenon indicates that the inhibitory action of Mel might be the result of overstimulation.

Mel *in vitro* has a narrow dose range affecting the left atria contraction. If it exceeds this range, contractures appear. These results conform to the findings that Mel might be a powerful cardiotoxin *in vitro*[3]. The optimal Mel concentration for the activation of guinea pig left atria probably occurred at 0.4 μmol·L^{-1} and 3 μmol·L^{-1}; and for the activation of right atria, occurred at 1 μmol·L^{-1} to 30 μmol·L^{-1}.

In the presence of Ver, a Ca^{2+} channel blocker, the concentration-effect curve of Mel was depressed implying that Mel might have a characteristic of calcium channel action unlike to that reported in cultured mouse cardiac myocytes[9]. As calcium homeostasis mediated by Na^+-K^+-ATPase, Na^+-Ca^{2+} exchange system other than Ca^{2+} channels played fundamental role in regulating cardiac excitation and contraction, further studies are being carried out in our laboratory.

Exposure to single dose 3 μmol·L^{-1} of Mel significantly enhanced the contractile amplitude in left atria at first 3 min, but after cumulative-dose exposure, this response was lost (− log 5.8 to 5.5 μmol·L^{-1}, about 1.6 to 3.2 μmol·L^{-1} in Fig 1). Thus, the phenomenon of tachyphylaxis might have occurred. This phenomenon is also noted in blood pressure[3].

ACKNOWLEDGMENT To Dr Gaspar Carrasquer (University of Louisville, USA) for the supply of melittin. To Dr LI Le for technical help.

REFERENCES

1 Habermann E. Bee and wasp venoms. Science 1972；177：314－22.
2 Yang S, Garrasquer G. Effect of melittin on ion transport across cell membranes. Acta Pharmacol Sin 1997；18：3－5.
3 Marsh NA, Whaler BC. The effects of honey bee (apis melliferal) venom and two of its constituents, melittin and phospholipase A_2, on the cardiovascular system of the rat. Toxicon 1980；18：427－35.
4 Lad PL, Shier TW. Effect of melittin-induced membrane alterations on rat heart adenylate cyclase activity. Arch Biochem Biophys 1980；204：418－24.
5 Garrasquer G, Yang S, Schwartz M, Dinno MA. Effect of melittin on the apical membrane Na^+ and Cl^- conductances of frog cornea epithelium. Can J Physiol Pharmacol 1997；75：199－204.

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Mar；21 (3)
E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629

6 Cuppoletti J，Abbott AJ. Interaction of melittin with the ($Na^+ + K^+$) ATPase：evidence for a melittin-induced conformational change. Arch Biochem Biophys 1990；283：249－57.
7 Hegner D，Schummer U，Schnepel GH. The interaction of a lytic peptide，melittin，with spin-labeled membranes. Biochim Biophys Acta 1973；291：15－22.
8 You YH，Huang ZQ，LI CC. Effect of melittin on isolated rat atrium. Acta Pharmacol Sin 1997；18：87－9.
9 Okamoto T，Isoda H，Kubota N，Takahata K，Takahashi T，Kishi T，*et al*. Melittin cardiotoxicity in cultured mouse cardiac myocytes and its correlation with calcium overload. Toxicol Appl Pharmacol 1995；133：150－63.

蜂毒肽对离体豚鼠心房的双向作用[1]

杨　申[2]，刘俊娥，张敖珍，江明华　（上海医科大学药学院药理教研室，上海 200032，中国）

关键词　蜂毒肽；蜂毒液；维拉帕米；豚鼠；心房功能

目的：研究蜂毒肽（Mel）对离体豚鼠心房的作用. **方法**：累积浓度法测定 Mel 给药前后左房收缩幅度及右房频率，观察量效曲线及时效曲线. 观察钙离子通道阻滞剂 Ver 对 Mel 作用的影响. **结果**：低浓度 Mel（0.1－0.8 $\mu mol \cdot L^{-1}$）对左心房产生正性肌力作用；高浓度 Mel（1.6－12.8 $\mu mol \cdot L^{-1}$）产生负性肌力作用. Mel 对右心房产生正性频率作用. Mel 的双向作用可被 Ver 0.3 $\mu mol \cdot L^{-1}$ 压低. **结论**：Mel 对离体豚鼠左心房收缩具双向作用，对右心房具正性频率作用. Mel 对左心房收缩的双向作用可被 Ver 压低，提示 Mel 对心房的作用可能通过电压依赖性钙通道.

（责任编辑　朱倩蓉）