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Inhibitory effect of melittin on Na^+, K^+-ATPase from guinea pig myocardial mitochondria[1]

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KEY WORDS melittin; bee venoms; Na^+, K^+-exchanging ATPase; myocardium; mitochondria

ABSTRACT

AIM: To investigate the effect of melittin (Mel) of Na^+, K^+-ATPase activity and it's kinetic mode of action on guinea pig myocardial mitochondria. **METHODS**: Effect of Mel on heart mitochondrial Na^+, K^+-ATPase activity was determined with colorimetry method. **RESULTS**: Mel inhibited Na^+, K^+-ATPase in a concentration and time dependent manner, IC_{50} was 2.60 μmol/L. Kinetic studies of interaction between Mel and K^+, Na^+, ATP revealed that inhibitory effect of Mel was competitive with K^+, but not with Na^+ and ATP. **CONCLUSION**: Mel polypeptide potently inhibits Na^+, K^+-ATPase, possibly by binding to the K^+ site.

INTRODUCTION

The Na^+, K^+-ATPase is the enzyme responsible for the primary active transport of Na^+ and K^+ in all animal cells. By maintaining the normal distribution of these ions across the plasma membrane, Na^+, K^+-ATPase plays a principal role in the regulation of membrane potential, cell ion content, and cell volume in various kinds of cells including myocytes[1]. A failure of Na^+, K^+-ATPase activity could precipitate depolarization, cell swelling and interference with the cellular transport of calcium and hydrogen ions and excitatory amino acids[2]. So exploration of Na^+, K^+-ATPase inhibitors will be useful in the study on both physiologic and pathologic processes of cells.

Melittin (Mel), the major component of bee venom, is a water-soluble, amphiphilic 26 amino acid bioactive polypeptide, having been found to have an active interaction with cell membranes[3], and effects on the electrical properties of excitable tissues[4]. Recent works in this laboratory suggested that Mel increased the I_k and I_{Na} as well as stimulated Na^+-Ca^{2+} exchange in ventricular myocytes of guinea pigs[5, 6]. Mel has also been found to inhibit and interact with the H^+, K^+-ATPase as well as Na^+, K^+-ATPase in nervous synapsis, brain, kidney and gland[7-10], however, their action on Na^+, K^+-ATPase of heart so far has not been reported.

In the present studies, we examined the effect of Mel on heart mitochondria Na^+, K^+-ATPase activity and it's kinetic mode of action on Na^+, K^+-ATPase by changing the concentrations of K^+, Na^+, and ATP.

MATERIALS AND METHODS

Drugs and reagents Melittin (Mel) was purchased from Sigma Co (USA). Ouabain was purchased from Merck (Germany). Na_2ATP, Tween-20 were from Sino-American Biotechnology Co. Other solvents and reagents were of AR grades produced by Shanghai Chemical Plant.

Preparation of mitochondria[11] Guinea pigs of either sex weighing 200 - 300 g were used, sacrificed by decapitation and hearts were rapidly isolated. Whole ventricular muscle of the heart was homogenized with five volumes of ice cold Tris-HCl buffer (pH 7.2 - 7.4), scraped and centrifuged at 1000 × *g* for 20 min, then the supernatant was centrifuged again at 15 000 × *g* for 15 min. The resulting sediment was diluted to 1 - 2 g/L with buffer and stored at - 70 ℃ until used for Na^+, K^+-ATPase assay. All preparative procedures were performed at 4 ℃. The protein content was determined by Coomassie brilliant blue method.

Assay of the Na^+, K^+-ATPase Myocardial mitochondria ATPase activity was assayed from the amount of inorganic phosphate (Pi) released from ATP by col-

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orimetry method[12]. The enzyme preparation (1 mL aliquots containing 1 – 2 mg protein) was incubated with an indicated concentration of the test drugs and with 3 mL related compound [in mmol/L: imidazole 25, $MgCl_2$ 4, presence or absence of NaCl 130, presence or absence of KCl 20, (absence of both Na^+ and K^+ activity was Mg^{2+}-ATPase)] at 37 ℃ for 10 min. The enzymic activity was then initiated by adding 2 mmol/L ATP. Further incubation at 37 ℃ for 20 min was carried out and reaction was stopped by 24 % 0.3 mL sodium citrate. The tubes were left at room temperature for 30 min and the absorbance was measured at 660 nm with 722 spectrophotometer. Enzyme active unit was expressed as mitochondria mmol Pi per gram homogenate protein per hour. Na^+, K^+-ATPase activity was calculated as the difference between the total and Mg^{2+}-dependent ATPase activity.

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

RESULTS

Inhibitory effect of Mel on Na^+, K^+-ATPase As shown in Tab 1, Mel inhibited Na^+, K^+-ATPase in a concentration-dependent manner. Mel 10 μmol/L was more active than ouabain 10 μmol/L [12 ± 4 mmol·h^{-1}·g^{-1}(protein)] in inhibition Na^+, K^+-ATPase. The IC_{50} values calculated by logit software was 2.60 μmol/L, the 95% confidence interval was 1.35 – 4.99.

Tab 1. The inhibitory effect of melittin on Na^+, K^+-ATPase activity. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* control.

Concentration/ μmol·L^{-1}	*n*	Na^+, K^+-ATPase activity/ mmol·h^{-1}·g^{-1}(protein)
0	8	17.1 ± 5.0
1	6	11.4 ± 2.8^b
3	5	10.4 ± 2.6^b
5	5	6.2 ± 1.6^c
8	6	3.4 ± 1.0^c
10	6	2.8 ± 0.8^c

Time course of the inhibitory effect of Mel on Na^+, K^+-ATPase The activity of Na^+, K^+-ATPase increased within first 20 min and then reached steady state. Mel 3 μmol/L lowered the time course curve (Fig 1), suggesting Mel inhibited the activity of Na^+, K^+-ATPase.

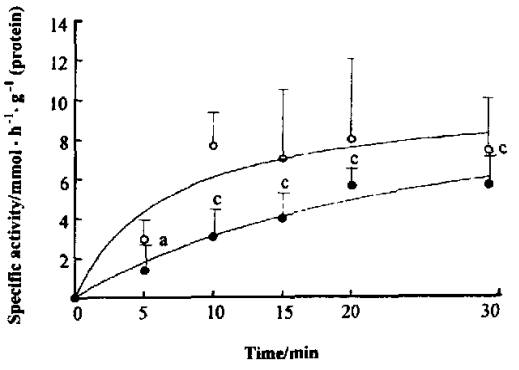

Fig 1. Time course of Na^+, K^+-ATPase activity in the absence and presence of Mel. (○) control. (●) Mel 3 μmol/L. $n = 5$. $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* control.

Influence of Mel and ouabain on the double reciprocal plot of Na^+, K^+-ATPase activity against K^+ Keeping Na^+ and ATP concentration unchanged, and by changing K^+ concentration over a range of 10 – 40 μmol/L, the activity of Na^+, K^+-ATPase was measured. The results showed as double-reciprocal plot. After addition of Mel or ouabain, shifting upward of the curves indicated that drugs exerted inhibitory action on the enzyme. The lines after addition of different drugs all intercept at one point on the vertical axis, suggesting that Mel inhibited Na^+, K^+-ATPase in a competitive manner with K^+ (Fig 2). V_{max} and K_m for potassium-enzyme complex from the double-reciprocal plot was calculated. V_{max} for control, Mel 3 μmol/L, and ouabain 10

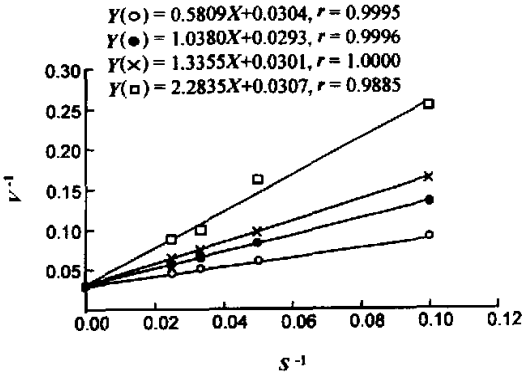

Fig 2. Double-reciprocal plot of Na^+, K^+-ATPase activity against K^+. (○) Control. (●) Ouabain 10 μmol/L. (×) Mel 3 μmol/L. (□) Mel 5 μmol/L. $n = 5 – 6$. K^+ concentration (S, mmol/L).

μmol/L were 32.89, 33.22, and 34.12 mmol·h^{-1}·g^{-1} (protein), and K_m were 19.11, 44.37, and 35.42 mmol/L respectively.

Influence of Mel on the double reciprocal plot of Na^+, K^+-ATPase activity against Na^+

Keeping K^+ and ATP concentration unchanged, by changing Na^+ concentration over a range of 60 – 240 mmol/L, the activity of Na^+, K^+-ATPase was measured and analyzed as above. V_{max} for control and Mel 3 μmol/L were 53.19 and 42.02 mmol·h^{-1}·g^{-1} (protein), and K_m were 343.11 and 339.23 mmol/L respectively. The result revealed that Mel inhibited Na^+, K^+-ATPase in a noncompetitive manner with Na^+ (Fig 3).

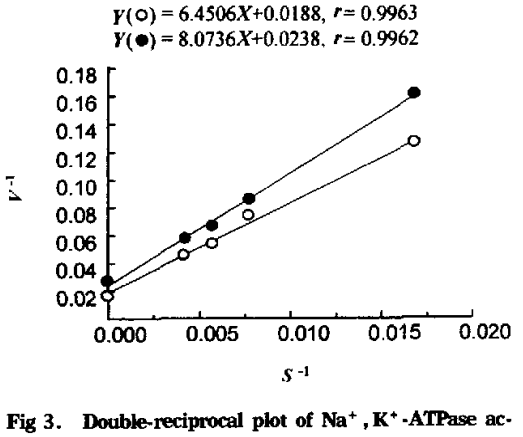

Fig 3. Double-reciprocal plot of Na^+, K^+-ATPase activity against Na^+. (○) Control. (●) Mel 3 μmol/L. n = 5 – 6. Na^+ concentration (S, mmol/L).

Influence of Mel on the double reciprocal plot of Na^+, K^+-ATPase activity against Na^+

Keeping Na^+ and K^+ concentration unchanged, by changing ATP concentration over a range of 1 – 3 mmol/L, the activity of Na^+, K^+-ATPase was measured and analyzed as above. V_{max} for control and Mel 3 μmol/L were 96.15 and 55.25 mmol·h^{-1}·g^{-1}(protein) respectively, and K_m were 12.01 and 8.71 mmol/L respectively. The result revealed that Mel inhibited Na^+, K^+-ATPase in a noncompetitive manner with ATP (Fig 4).

DISCUSSION

Mel inhibited Na^+, K^+-ATPase from guinea pig heart mitochondria in a concentration and time dependent manner. The range of doses are narrow confirming the findings in our previous studies[5,6,13].

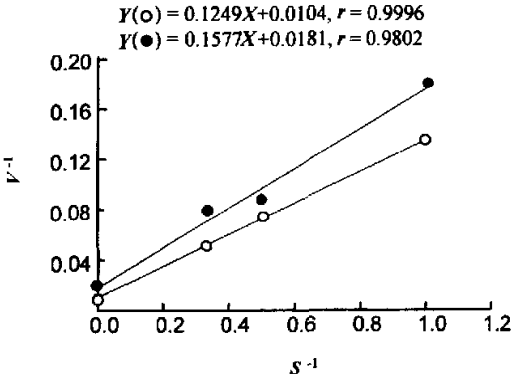

Fig 4. Double-reciprocal plot of Na^+, K^+-ATPase activity against ATP. (○) Control. (●) Mel 3 μmol/L. n = 5. ATP concentration (S, mmol/L).

The IC_{50} for the inhibitory effect of Mel on Na^+, K^+-ATPase from guinea pigs heart mitochondria is 2.60 μmol/L. Although it is slightly different from that of lamb, kidney (1.2 μmol/L)[9], and rat synaptic membrane (0.13 or 2.0 μmol/L)[7,10], yet this variation remains within μmol level. Nevertheless, the IC_{50} for the inhibitory effect of Mel on H^+, K^+-ATPase from different source are quite different. For example, that from the hog gastric mucosa is 1.3 μmol/L[14] but from the bovine-heart mitochondria is 5 mmol/L[15].

Our study revealed that Mel like ouabain, inhibited the enzyme competitively with K^+, and noncompetitively with Na^+ and ATP. This result is consistent with that of Cuppoletti[9] from lamb kidney and Chen[7] from rat synaptic membrane who suggested that Mel probably like ouabain acted at a site closely related to the K^+ binding site in the external surface of the enzyme.

Although the modes of action of Mel and ouabain on Na^+, K^+-ATPase are similar in many aspects, yet there exist some discrepancies. As an amphiphilic peptide, Mel might takes the lipid bilayer of the membrane as another important target and it's hydrophobic residues may insert the bilayer producing a conformational change, with the result of inhibition of activity of Na^+, K^+-ATPase[16], other than by the action located on the catalytic subunit of the enzymes as ouabain.

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蜂毒肽对豚鼠心肌线粒体 Na^+,K^+-ATP 酶的影响[1]

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关键词 蜂毒肽；蜂毒液类；Na^+,K^+-交换 ATP 酶；心肌；线粒体

目的：研究蜂毒肽对豚鼠心肌线粒体 Na^+,K^+-ATP 酶活性的影响. **方法**：应用光电比色法测定 Na^+,K^+-ATP 酶活性. 以 Line-weaver-burk 双倒数作图法分析酶动力学参数. **结果**：蜂毒肽抑制心肌 Na^+,K^+-ATP 酶表现为浓度和时间依赖性，IC_{50}为 2.60 μmol/L. 酶动力学研究表明，蜂毒肽对 Na^+,K^+-ATP 酶有抑制作用，对 K^+表现为竞争性结合，对 Na^+及 ATP 表现为非竞争性结合. **结论**：蜂毒肽对心肌 Na^+,K^+-ATP 酶有抑制作用，其抑制机制主要是与 K^+竞争结合酶外侧的 K^+结合位点.

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