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Effects of metallothionein on action potentials of anoxic and reoxygenated papillary muscles of guinea pigs

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KEY WORDS metallothionein; papillary muscles; action potentials; arrhythmia

AIM: To study anti-arrhythmic effects of metallothionein (MT). **METHODS**: Standard microelectrode technique was used to study the effects of MT on action potentials (AP) in anoxic and reoxygenated papillary muscles of guinea pigs. **RESULTS**: MT (0.02 mmol·L^{-1}) had no effects on AP of the normal papillary muscles; when the muscles were exposed to ischemic solution without MT, there was a marked shortening of action potential duration (APD) at 20 %, 50 %, and 90 % of repolarization (APD_{20}, APD_{50}, APD_{90}) from 82 ± 7 to 37 ± 7, 131 ± 35 to 63 ± 11, and 167 ± 12 to 100 ± 19 ms, respectively, ($P < 0.01$); and an obvious reduction of resting potential (RP), action potential amplitude (APA), and the maximal upstroke velocity of phase 0 (V_{max}) from −92 ± 9 to −63 ± 12 mV, 135 ± 13 to 80 ± 8 mV, and 286 ± 55 to 164 ± 42 V·s^{-1}, respectively ($P < 0.01$). However, in the presence of MT, the AP parameters (RP, APA, and V_{max}) changed from −63 ± 2 to −82 ± 1 mV, 80 ± 8 to 104 ± 25 mV, and 164 ± 42 to 237 ± 43 V·s^{-1}, respectively, ($P < 0.01$), except that APD_{20}, APD_{50}, and APD_{90} shortened further from 37 ± 7 to 12 ± 3, 63 ± 11 to 28 ± 7, and 100 ± 19 to 82 ± 11 mV, respectively ($P < 0.01$). MT decreased the incidence of automaticity during reoxygenation from 91 % to 33 %. **CONCLUSION**: MT possesses a calcium regulatory property.

Metallothionein (MT), a low molecular weight, and cysteine-rich protein, has profound cytoprotective effects such as scavenging free radicals[1], reducing intracellular calcium overload[2], and stabilizing cellular membrane[3], demonstrating that it possesses a calcium regulatory property. But its role on cellular electrophysiology of anoxic and reoxygenated ventricle muscles has not been known. This study was to explore the effects of MT on action potentials of anoxic and reoxygenated papillary muscles of guinea pigs.

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MATERIALS AND METHODS

Materials Guinea pigs (♂, $n = 11$), weighing 315 - 445 g were decapitated. The hearts were placed in Tyrode's solution at 4 ℃. The papillary muscles ($n = 19$) were dissected from the right ventricles (each of 8 right ventricles was dissected 2 papillary muscles; each of 3 right ventricles was dissected a papillary muscle) and were pinned to a 1.5 mL tissue chamber and continuously perfused with the modified Tyrode's solution gassed with 95 % O_2 + 5 % CO_2. Tyrode's solution was composed of NaCl 129, KCl 3, $NaHCO_3$ 20, NaH_2PO_4 0.9, $CaCl_2$ 2.7, $MgSO_4$ 0.5, and glucose 5.5 mmol·L^{-1} at pH 7.2. To stimulate the ischemic conditions of high K^+ and low pH (pH = 6.8), ischemic solution contained KCl 10, NaCl 123 mmol·L^{-1}, no glucose, the remaining ingredients being the same as those of Tyrode's solution, equilibrated with 95 % N_2 + 5 % CO_2.

AP measurement With standard microelectrode technique[4], the muscles were stimulated with pulse of 1 ms duration at 2-fold diastolic threshold by a bipolar electrode. The rate of stimulation was 0.5 Hz during the equilibration period. The stimuli were delivered by an electronic stimulator (PES-4 model, China). The transmembrane action potential (AP) was recorded by a standard glass microelectrode filled with KCl 3 mol·L^{-1}, which had a tip resistance of 10 - 30 MΩ, coupled to a high input impedance amplifier (MEZ-8300 Nihon Kohden). All the AP parameters, including the resting potential (RP), the action potential amplitude (APA), the action potential duration at 20 %, 50 %, and 90 % of repolarization (APD_{20}, APD_{50}, and APD_{90}) as well as the maximal upstroke velocity of phase 0 (V_{max}) were measured by computer.

Protocol The study was taken on a model of ischemic and reperfusion injury[5]. Guinea pigs were divided into 6 groups: (1) control (C); (2) MT; (3) anoxia (I); (4) reoxygenated (R); (5) anoxia + MT (I + MT); (6) reoxygenated + MT (R + MT). After muscles were equilibrated in Tyrode's solution for 1 h, normal AP was recorded as C group; then the samples were superfused by ischemic solution for

20 min, and AP was recorded in 20 min as I group; subsequently, Tyrode's solution was perfused, AP was recorded in 20 min. After AP was recovered to normal values, muscles were perfused by ischemic solution and MT as I + MT group; ischemic solution was replaced by Tyrode's solution and MT as R + MT group. In the whole process of AP recording, microelectrode was placed in a single cell. Before muscle was exposed to MT, MT was dissolved in Tyrode's and ischemic solution (50 mL), respectively, at the final concentration of 0.02 mmol·L^{-1}. Zn-MT, m 6.5 kDa, was from Research Institute of Science and Technology, Beijing.

Statistical analysis Data were expressed as $\bar{x} \pm s$ and analyzed with ANOVA and chi-square test.

RESULTS

Effects of MT on AP of normal papillary muscles After exposure to MT, AP of normal papillary muscles has no obvious change (APD_{20}, APD_{50}, APD_{90}, RP, APA, and V_{max}), compared with control ($P > 0.05$) (Tab 1).

Changes of AP of anoxic and reoxygenated papillary muscles After perfusion of ischemic solution for 20 min, there was a marked shortening of APD_{20}, APD_{50}, APD_{90}, and APA; RP and V_{max} were profoundly decreased, compared with control ($P < 0.01$) (Tab 1, Fig 1).

There was a progressive increase in all AP parameters during reoxygenation of papillary muscles (Tab 1).

Effets of MT on AP of anoxic and reoxygenated papillary muscles MT shortened APD_{20}, APD_{50}, and APD_{90}, and increased RP, APA, and V_{max} in anoxic papillary muscles, compared with the anoxic group without MT ($P < 0.01$). MT had no obvious effect on all AP parameters of reoxygenated papillary muscles (Tab 1).

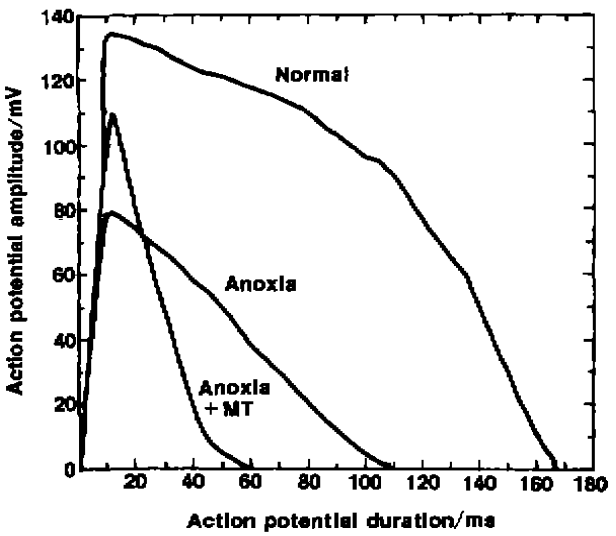

Fig 1. Action potential of treated papillary muscles of guinea pigs.

There was no difference of recovery time of AP of reoxygenated papillary muscles after anoxia with and without MT (1.3 ± 0.5 min *vs* 1.1 ± 0.4 min, $P > 0.05$).

MT decreased the incidence of automaticity of papillary muscles during reoxygenation.

Automaticity occurred differently in anoxia and anoxia + MT groups and 91 % of reoxygenated papillary muscles produced automaticity and 33 % of reoxygenated papillary muscles exposed to MT produced automaticity.

Time to onset of automaticity had no difference, but cumulative duration had marked difference in reoxygenated papillary muscles with and without MT ($P < 0.01$) (Tab 2).

DISCUSSION

MT is an endogenous inducible protein[1]. In

Tab 1. Effects of metallothionein (MT) on action potentials in 20-min anoxic (I) and reoxygenated (R) papillary muscles of guinea pigs. n = 19 muscles of 11 guinea pigs. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control. $^dP > 0.05$, $^eP < 0.05$, $^fP < 0.01$ *vs* anoxia group.

Group	APD_{20}/ms	APD_{50}/ms	APD_{90}/ms	RP/mV	APA/mV	V_{max}/V·s^{-1}
Control	82 ± 7^f	131 ± 35^f	167 ± 12^f	-92 ± 9^f	135 ± 13^f	286 ± 55^f
MT	80 ± 12^{af}	100 ± 35^{af}	145 ± 23^{af}	-90 ± 7^{af}	127 ± 19^{af}	275 ± 60^{af}
I	37 ± 7^c	63 ± 11^c	100 ± 19^c	-63 ± 12^c	80 ± 8^c	164 ± 42^c
R	78 ± 6^{af}	115 ± 31^{af}	153 ± 40^{af}	-91.3 ± 1.08^{af}	124 ± 18^{af}	253 ± 47^{af}
I + MT	12 ± 3^{af}	28 ± 7^{af}	57 ± 17^{af}	-82 ± 11^{bf}	104 ± 25^{bf}	237 ± 43^{bf}
R + MT	89 ± 11^{af}	128 ± 31^{af}	173 ± 30^{af}	-92 ± 11^{af}	129 ± 16^{af}	235 ± 67^{af}

Tab 2. Effects of metallothionein (MT) on duration and incidence of reoxygenated (R) automaticity following 20-min anoxia in papillary muscles of guinea pigs. $n=6$ muscles of 5 guinea pigs. $\bar{x} \pm s$. $^cP<0.01$ *vs* reoxygenated group.

	R+MT	R
Time to onset/min	4.8±1.7	4.2±1.0
Cumulation duration/min	1.7±0.6[c]	6.4±2.6
Incidence of automaticity	33 %[c]	91 %

physiological conditions, MT has no significant roles, but in stresses (eg, ischemia, trauma, inflammation, etc), MT plays important roles such as scavenging oxygen-derived free radicals, inhibiting intracellular calcium overload, and stabilizing cellular membrane so as to provide physiological homeostasis. Therefore, there is a great significance for the research of MT functions in pathological states.

This study found that APD of anoxic papillary muscles exposed to MT shortened more markedly than that of anoxic papillary muscles without MT. It has demonstrated that MT, a resembling calcium channel antagonist, inhibited calcium influx. We speculated that the role of MT might be attributed to inhibiting calcium influx. Calcium overloading is one of important mechanisms that cause myocardial reperfusion injury[6]. So, MT might act as one of myocardial protective factors.

MT has cytoprotective roles[7]. In this study, we examined that MT decreased the automaticity of reoxygenated papillary muscles, this effect may be related to shortening APD and decreasing effective refractory period.

REFERENCES

1 Sato M, Bremner I. Oxygen free radicals and metallothionein. Free Radical Biol Med 1993; 44: 325-37.

2 Li ZP, Tang CS, Su JY, Ceng S. Effects of metallothionein on Na^+-Ca^{2+} exchange in rat heart-derived cells and protein recombinant liposome. J Beijing Med Univ 1989; 21: 104.

3 Li SM, Hu DY, Tang CS, Tang XN, Liu Y, Li DP, *et al*. Protective effects of metallothionein on isopreterenol-induced myocardial injury in rats. Chin Pharmacol Bull 1996; 12: 332-4.

4 Satoh H. Electrophysiological actions of taurine on spontaneously beating rabbit sino-atrial nodal cells. Jpn J Pharmacol 1995; 67: 29-34.

5 Cheng S, Liu Z, Wang ZM, Jia HY, Li ZP, Tang CS. The effect of metallothionein on postischemic reperfusion injury of isolated rat hearts — a study of electron microscopic morphometry. J Beijing Med Univ 1989; 21: 101-3.

6 Jeroudi MO, Hartley CJ, Bolli R. Myocardial reperfusion injury: role of oxygen radicals and potential therapy with antioxidants. Am J Cardiol 1994; 73: 2B-7B.

7 Mimura T, Tsujikawa K, Yasuda N, Nakajima H, Haruyama M, Ohmura T, *et al*. Suppression of gastric ulcer induced by stress and HCl-ethanol by intravenously administered metallothionein-Ⅱ. Biochem Biophys Res Comm 1988; 151: 725-8.

(145-147)

金属硫蛋白对缺氧和复氧豚鼠乳头状肌动作电位的影响

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关键词 金属硫蛋白; 乳头状肌; 动作电位; 心律失常

目的: 研究金属硫蛋白 (MT) 对缺氧和复氧豚鼠乳头状肌动作电位的影响. **方法**: 利用标准的玻璃微电极技术, 将标本暴露于缺氧液(低氧, 高钾, 无糖, pH 6.8)和 MT. **结果**: MT(0.02 mmol·L^{-1}) 对正常豚鼠乳头状肌的动作电位无影响; 单纯将标本暴露于缺氧液, 明显使 APD_{20}, APD_{50}和 APD_{90}缩短, 增高 RP 水平, 降低 APA 及 V_{max}; 缺氧液加入 MT (0.02 mmol·L^{-1}) 后, 则使缺氧期间的 RP, APA 和 V_{max}的改变恢复正常, 但 APD 进一步缩短; 同时发现 MT 减少复氧期间导致的自律性的发生率. **结论**: MT 具有钙调节剂的特征.