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四氢巴马汀对豚鼠心室肌细胞跨膜内向 Ca^{2+} 电流的抑制作用

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关键词 四氢巴马汀；心肌；钙通道；膜片箝技术；培养的细胞

目的：研究四氢巴马汀（THP）对豚鼠心室肌细胞膜 Ca^{2+} 通道的作用 方法：分离豚鼠心室肌细胞，利用膜片箝技术观察、记录心室肌细胞膜跨膜内向钙电流（I_{Ca}） 结果：THP 灌流 5 min 后明显抑制豚鼠心室肌细胞内向 I_{Ca}，其作用呈现剂量-效应关系，并具有频率依赖性。0.1，1，10 μmol·L^{-1} 的 THP 使 I_{Ca} 从 1.15 ± 0.22，0.91 ± 0.18，1.60 ± 0.42 nA（control，n = 5）分别降低到 0.90 ± 0.21（P < 0.01），0.56 ± 0.21（P < 0.01），0.83 ± 0.21（P < 0.05）nA，抑制率分别为 22 %，38 % 和 48 %，THP 的作用易于洗脱。峰值电压为 0 mV，此时 THP 作用最明显，THP 对较高频率刺激产生的电流抑制作用较强，2 Hz 时的抑制率高于 0.1 Hz 时的抑制率（P < 0.01） 结论：THP 具有 Ca^{2+} 通道阻滞作用。

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Effect of sodium glycyrrhetinate on neonatal rat myocardial cells

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KEY WORDS glycyrrhetinate; myocardium; cultured cells; cyclic AMP; calcium; oxygen

AIM: To investigate the effect of sodium glycyrrhetinate (SG) on neonatal rat myocardial cells. **METHODS**: The neonatal rat myocardial cells were cultured *in vitro*. Radioimmunoassay and fluorimetry were used to determine cAMP and [Ca^{2+}], respectively. **RESULTS**: The beating rate of myocardial cells was depressed by SG 0.4 mmol·L^{-1} at 5, 10, and 15 min, from 73 ± 9 min^{-1} to 62 ± 5, 59 ± 7, and 56 ± 6 min^{-1}, respectively. SG 0.1 and 0.2 mmol·L^{-1} showed above similar results at 15 min and 10, 15 min, respectively. When the myocardial cells were incubated with SG 0.2 and 0.4 mmol·L^{-1} at 37 ℃ for 10 and 15 min, the concentration of cAMP and [Ca^{2+}] were reduced. cAMP contents in SG 0.2 mmol·L^{-1} treated group at 10 and 15 min were lower than

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control group (1.09 ± 0.18 *vs* 1.65 ± 0.48 pmol per vial, $P < 0.05$; 1.12 ± 0.35 *vs* 1.72 ± 0.49 pmol per vial, $P < 0.01$), and so was $[Ca^{2+}]$ (30 ± 4 nmol·L^{-1} *vs* 41 ± 6 nmol·L^{-1}, $P < 0.05$ and 28 ± 6 nmol·L^{-1} *vs* 38 ± 7 nmol·L^{-1}, $P < 0.01$). SG 0.1 and 0.2 nmol·L^{-1} increased the pO_2 change rate (87 % ± 5 %, 75 % ± 4 % *vs* 54 % ± 3 %, $P < 0.01$) in suspension fluid of myocardial cells, but SG 0.4 mmol·L^{-1} decreased it (31% ± 2 % *vs* 54 % ± 3 %, $P < 0.01$). **CONCLUSION**: SG protects myocardium or treats ischemic cardiac disease.

Glycyrrhetinate, the hydrolysate of glycyrrhezine, had pharmacological actions of anti-inflammation, scavenging oxygen free radicals, and immunomodulating function[1,2]. Sodium glycyrrhetinate (SG) decreased the heart rate of anesthetized rat[3]. In this experiment, its effects on myocardial cells were observed.

MATERIALS

SG (colorless powder, purity: 98.5 %), made by Ningxia Institute of Chemical Industry, was dissolved in 10 % Me_2SO (Sigma Chemical Co). Medium DMEM was made by Gibco. The cyclic [^{3}H]AMP box was purchased from Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences. Fura 2-AM (light yellow powder, AR grade), synthesized by Institute of Materia Medica, Chinese Academy of Medical Sciences, was dissolved in Me_2SO. Wistar rats were inbred in Research Centre of Experimental Animals, Chinese Academy of Medical Sciences, and were fed to bear neonatal rat.

METHODS AND RESULTS

Myocardial cell cultures[4] Heart ventricles from 2 - 5 d old rat were digested with 0.06 % trypsine. The suspension of myocardial cells was purified[5]. Myocardial cells were dispersed and cultured on DMEM containing 15 % fetal bovine serum at 37 ℃ with 95 % O_2 + 5 % CO_2.

Effect of SG on beating rate After myocardial cells were cultured under standard condition for 5 - 10 d, the culture flasks which contained SG 0.1, 0.2, and 0.4 mmol·L^{-1} and 10 % Me_2SO 2 mL·L^{-1} (all of final concentrations) were placed under a microscope. At 37.0 ± 0.2 ℃, SG 0.4 mmol·L^{-1} decreased the beating rates at 5, 10, and 15 min. SG 0.1 and 0.2 mmol·L^{-1} showed similar effects at 15 min and 10, 15 min, respectively (Tab 1).

Effect of SG on cAMP Having been cultured for 5 - 10 d, myocardial cells were incubated with SG and 10 % Me_2SO at 37 ℃ for 10 and 15 min. The medium was thrown away and cAMP was extracted with $HClO_4$[6] and measured by radioimmunoassay. SG 0.2 and 0.4 mmol·L^{-1} decreased the cAMP concentration in myocardial cells ($P < 0.05$) (Tab 1).

Effect of SG on $[Ca^{2+}]_i$ The myocardial cells prepared were loaded with Fura 2-AM[7], and were treated with SG and 10 % Me_2SO at 37 ℃ for 10 or 15 min. The Ca^{2+} concentration was determined fluorimetrically[8]. It was shown that SG 0.2 and 0.4 mmol·L^{-1} reduced the intracellular free Ca^{2+} concentration ($P < 0.01$ or 0.05). (Tab 1)

Effect of SG on pO_2[9] SG 0.1 and 0.2 mmol·L^{-1} increased the rate of pO_2 changing ($P < 0.01$), but SG 0.4 mmol·L^{-1} decreased it ($P < 0.01$). (Tab 1)

DISCUSSION

SG concentration-dependently decreased the

Tab 1. Effects of sodium glycyrrhetinate on neonatal rat myocardial cells. n = numbers of culture flasks. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

SG/ mmol·L^{-1}	Number of cell beats per minute						cAMP/pmol per vial		$[Ca^{2+}]_i$/nmol·L^{-1}		pO_2 change/ %
	0	1	3	5	10	15	10	15	10	15 min	
0	71 ± 7 (6)	73 ± 7	70 ± 11	72 ± 7	73 ± 8	74 ± 10	1.65 ± 0.48 (6)	1.72 ± 0.49 (5)	41 ± 6 (5)	38 ± 7 (5)	54 ± 3 (5)
0.4	73 ± 9 (5)^a	74 ± 7^a	68 ± 9^a	62 ± 5^b	59 ± 7^c	56 ± 6^c	0.92 ± 0.32 (5)^b	1.08 ± 0.28 (5)^b	20 ± 4 (5)^c	22 ± 5 (5)^c	31 ± 2 (6)^c
0.2	74 ± 6 (5)^a	71 ± 7^a	70 ± 6^a	67 ± 6^a	62 ± 3^b	60 ± 4^b	1.09 ± 0.18 (5)^b	1.12 ± 0.35 (5)^b	30 ± 4 (5)^c	28 ± 6 (5)^b	75 ± 4 (5)^c
0.1	75 ± 9 (5)^a	73 ± 10^a	71 ± 4^a	68 ± 4^a	66 ± 7^a	61 ± 5^b	1.29 ± 0.42 (5)^a	1.26 ± 0.41 (5)^b	36 ± 5 (5)^a	30 ± 7 (5)^a	87 ± 5 (5)^c

beating rate of myocardial cells, which is related to the decrease of intracellular cAMP and Ca^{2+} concentration[10]. Influence of SG in high or low concentratoin on the oxygen consumption of myocardial cells was different. It suggests that SG in low concentration improve the catabolic metabolism, like its structural amaglucocortid[11]. The effect of SG on decreasing the rate of pO_2 changing in high concentration may be related to the sharp decrease of beating rate. Above all, it is considered that SG may be used to protect myocardium or treat ischemic cardiac disease.

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甘草次酸钠对乳鼠心肌细胞的影响

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关键词 甘草次酸; 心肌; 培养的细胞; 腺苷环磷酸; 钙; 氧

目的: 研究甘草次酸钠(SG)对乳鼠心肌细胞的作用. **方法**: 体外培养乳鼠心肌细胞, 用放射免疫法和荧光法分别测定 cAMP 和[Ca^{2+}]$_i$. **结果**: SG 0.4 mmol·L^{-1}于 5, 10 和 15 min 使心肌细胞搏动频率由 73±9 min^{-1}分别降至 62±5, 59±7 和 56±6 min^{-1}. SG 0.1 和 0.2 mmol·L^{-1}分别在 15 和 10, 15 min 呈现以上相似的结果. SG 0.2 和 0.4 mmol·L^{-1}与心肌细胞 37 ℃ 共孵 10, 15 min, cAMP 浓度和[Ca^{2+}]$_i$ 均被降低. SG 0.2 mmol·L^{-1}处理组的 cAMP 含量低于对照组(1.09±0.18, 1.12±0.35 pmol per vial, $P<0.05$); [Ca^{2+}]$_i$ 的变化也类似(30±4 nmol·L^{-1} $P<0.01$, 28±6 nmol·L^{-1} $P<0.05$). SG 0.1 和 0.2 mmol·L^{-1}提高心肌细胞悬液氧分压变化率(87 %±5 %, 75 %±4 %, $P<0.01$), 但 SG 0.4 mmol·L^{-1}降低氧分压变化率(31 %±2 %, $P<0.01$). **结论**: SG 可保护心肌或治疗缺血性心脏病.