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蝎毒对培养小鼠心肌细胞钠通道的阻滞作用

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Sodium channel blocking effect of scorpion venom on cultured mouse myocardiocytes

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ABSTRACT Myocardiocytes of mice were cultured. Action potentials were recorded with microelectrodes inside the cells. Scorpion venom from *Buthus martensii* Karsch 3.75 or 7.5 $\mu g \cdot ml^{-1}$ decreased the duration of action potential and all of the depolarization concerned parameters of myocardiocytes. The V_{max}, TP, APA behaved apparently in a dosage-dependent way. Restoration happened after washing out. Tetrodotoxin 2.5 $\mu g \cdot ml^{-1}$ acted in a similar way. Nimodipine 3.0 $\mu g \cdot ml^{-1}$ led to a decrease in action potential duration. $BaCl_2$ 0.1 $mmol \cdot L^{-1}$ elongated the action potential duration, while decreased the parameters concerned with depolarization. These results indicate that the scorpion venom has Na^+ channel blocking action.

KEY WORDS scorpion venoms; tetrodotoxin; nimodipine; *Buthus martensii*; action potentials; myocardium; cultured cells

摘要 培养小鼠心肌细胞，引导其动作电位。东亚钳蝎粗毒3.75或7.5 $\mu g \cdot ml^{-1}$使心肌细胞动作电位的时程与除极有关参数全部减小。V_{max}，TP，APA呈显著的剂量依赖性。洗脱后恢复。河豚毒2.5 $\mu g \cdot ml^{-1}$效果相似。尼莫地平3.0 $\mu g \cdot ml^{-1}$使动作电位时程缩短。$BaCl_2$ 0.1 $mmol \cdot L^{-1}$使时程延长、除极有关参数减小，表明蝎毒有钠通道阻滞作用。

关键词 蝎毒类；河豚毒素；尼莫地平；东亚钳蝎；动作电位；心肌；培养的细胞

细胞培养

心室肌细胞在培养过程中，由快反应非自律细胞转化为慢反应自律细胞[1]。小鼠心肌细胞的转变较慢，培养4—6 d仍有60%的快反应非自律细胞[2]。这些细胞可被驱动，产生快反应心肌细胞动作电位，是研究钠通道活动的理想模型。有报道，蝎毒能延缓钠通道的失活[3]，产生可被河豚毒阻断的异常钠电流，改变钠通道激活过程的电压依赖性[4]，与受体结合促进钠通道的激活[5]，激活钠通道使多巴胺释放[6]以及通过不同受体既影响激活又影响失活过程[7]等。本实验以培养的小鼠快反应心肌细胞动作电位为指标，探讨蝎毒对钠通道的药理作用。

MATERIALS AND METHODS

东亚钳蝎粗毒（Scorpion venom from *Buthus martensii* Karsch）由本校有机化学教研室制备。在严格统一的条件下，自蝎尾取新鲜毒液，用蒸馏水提取水溶性成分，离心、冷冻干燥后，获得白色粉末。其可溶部分为蝎毒的活性组分，不溶部分无生理活性。蝎毒是一类由20—80个氨基酸组成的多肽，对温度和pH稳定[8]。尼莫地平（天津市中央制药厂）。河豚毒（Sigma）。Dubecco's modified Eagle medium (DMEM, Life Technologies Inc, USA)。

心肌细胞培养 取出生24—48 h的昆明种小鼠全心室，剪成1 mm^3小块，然后用0.1%胰蛋白酶加机械搅拌的方法分离心肌细胞。将心肌细胞置入培养瓶（Greiner Labortechnik Inc, Germany），在36.5℃，5% CO_2培养。培养基内含80% DMEM与本实验室自制的20%小牛血清。

动作电位记录 于培养的d 4，将培养瓶放在倒置显微镜的恒温套内，保持pH 7.2，36.5℃的细胞外环境。用微电极记录心肌细胞动作电位，经微机连机分

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析以下电参数:动作电位幅值(APA)、超射(OS)、阈电位(TP)、最大舒张电位(MDP)、最大除极速度(V_{max})、复极10%，50%，90%水平的动作电位时程(APD_{10}，APD_{50}，APD_{90})及动作电位发放频率(APF).

RESULTS

于培养 d 4—5，自搏动群落的心肌细胞内引导动作电位. 按最大除极速度大于80 $V \cdot s^{-1}$的标准判定为快反应心肌细胞后，向培养基中加入蝎毒3.75 $\mu g \cdot ml^{-1}$，使动作电位的 APA，OS，TP，MDP，V_{max}，APD_{10}，APD_{50}，APD_{90}比加药前显著变小；APF 显著加快($P < 0.01$). 在此基础上，再次加入蝎毒3.75 $\mu g \cdot ml^{-1}$，将培养基中的蝎毒浓度升至7.5 $\mu g \cdot ml^{-1}$，APA，TP，V_{max}的变化比低浓度组更加显著. 其他各项指标，除APD_{50}外均有类似趋势，但两浓度组间的差异无统计学意义. 用新鲜培养基洗脱蝎毒后，各项指标均立即恢复. 钙通道阻滞剂尼莫地平3.0 $\mu g \cdot ml^{-1}$，仅使快反应心肌细胞动作电位的时程显著缩短，但对与除极化有关的各项参数无明显影响. 钾通道阻滞剂 $BaCl_2$ 0.1 $mmol \cdot L^{-1}$能使除极化有关参数减小，但使动作电位时程延长而不是缩短. 钠通道阻滞剂河豚毒2.5 $\mu g \cdot ml^{-1}$与蝎毒完全一致，使动作电位的时程及除极化有关参数全面减小 (Tab 1, Fig 1, Fig 2).

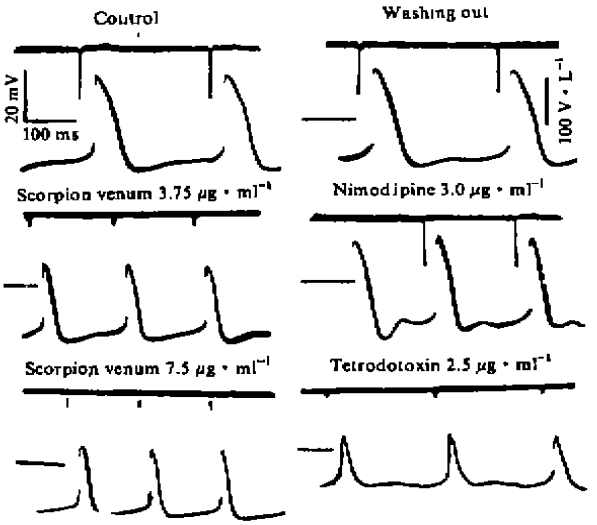

Fig 1. Influence of scorpion, nimodipine and tetrodotoxin on action potentials of cultured myocardiocytes. Upper traceing — V/dt; Lower tracing — action potential.

为了排除频率对时程的影响，对 APD_{10}，APD_{50}，APD_{90}三项参数又进行了协方差(covariance)检验，证明蝎毒本身可使动作电位的时程缩短.

为了排除心肌细胞动作电位的抑制是由于蝎毒的毒性作用所致，以自发性搏动为指标观察了蝎毒的时效关系. 在向培养基中加入蝎毒7.5 $\mu g \cdot ml^{-1}$之前有114个搏动群落，加药后5 min，1，2，20 h 的搏动群落数分别为115，121，124与122个. 可见在蝎毒作用下，心肌

Tab 1. Influence of scorpion venom on action potentials of cultured mouse myocardiocytes. $n = 30$, $\bar{x} \pm s$. $^{*}P > 0.05$, $^{**}P < 0.05$, $^{***}P < 0.01$ *vs* control; $^{\dagger}P > 0.05$, $^{\dagger\dagger}P < 0.05$, $^{\dagger\dagger\dagger}P < 0.01$ *vs* control (covariance); scorpion venom: scorpion$_1$ 3.75 $\mu g \cdot ml^{-1}$, scorpion$_2$ 7.5 $\mu g \cdot ml^{-1}$; nimodipine: 3.0 $\mu g \cdot ml^{-1}$; tetrodotoxin: 2.5 $\mu g \cdot ml^{-1}$.

	APA/mV	OS/mV	MDP/mV	TP/mV	V_{max}/$V \cdot s^{-1}$	APD_{10}/ms	APD_{50}/ms	APD_{90}/ms	APF/bpm
Control	87±5	32±3	55±4	37±5	98±14	13±1	62±12	138±10	224±45
Scorpion$_1$	64±8***	22±5***	42±5***	31±7***	55±12***	11±4***†††	46±11***†††	90±17***†††	253±37**
Wash Out	87±6*	30±3*	56±6*	35±6*	96±22*	12±4*†	66±11*†	122±30**†††	218±37*
Scorpion$_2$	58±8***	20±5***	38±10***	28±1***	49±11***	10±4***††	49±17***†††	88±30***†††	272±69***
Wash Out	85±6*	30±4*	55±5*	42±7*	100±19*	11±2***†††	53±17***††	116±33***†††	233±68*
Nimodipine	84±6*	28±8*	56±14*	37±12*	97±16*	10±3***†††	46±5***†††	85±20***†††	249±47**
Tetrodotoxin	46±3***	12±5***	34±7***	16±4***	8±3***	7±2***†††	45±6***†††	82±11***†††	139±20***

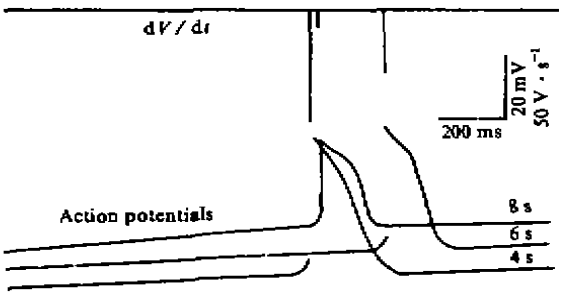

Fig 2. Influence of $BaCl_2$ on action potentials of cultured myocardiocytes. Three continuous sweepings of oscilloscope were overlapped to show the membrane depolarization induced by $BaCl_2$. Only one action potential with its derivative was shown in each sweeping.

细胞除搏动群落数稍有增多外、长达20 h 后仍搏动良好. 表明向培养基中加入蝎毒后，跨膜电活动的即时性改变不是蝎毒毒性所致.

DISCUSSION

本实验用最大除极速度大于80 $V \cdot s^{-1}$的典型快反应心肌细胞动作电位[2]，其除极化主要靠钠内流. 故 V_{max}等除极有关电参数的抑制，提示蝎毒有钠通道阻滞作用. 动作电位时程缩短，可能是因蝎毒刺激培养心肌细胞对钙的摄取[9]，$[Ca^{2+}]_i$ 增多，继之使钙通道失活[10]与钾通道激活[11]所致. 钙通道阻滞剂尼莫地平选择性抑制与钙内流密切相关的动作电位时程，而不影响与钠内流有关的除极参数，从另一角度支持蝎毒抑制除极有关参数是由于钠通道的阻滞. Fig 1直接显示 $BaCl_2$阻滞钾通道所致的膜除极化[12]，在几秒钟之内使钠通道失活，导致除极有关参数的减小. 钠通道阻滞剂河豚毒对动作电位的影响与蝎毒相似，不仅进一步支持蝎毒对钠通道有阻滞作用，而且从用药浓度看出，蝎毒作用强度约为河豚毒的1/4.

洗脱蝎毒后心肌细胞动作电位的抑制立即恢复、在高浓度蝎毒的持续作用下，群落维持自发性搏动20 h 无衰减等实验所见，证明上述结果不是蝎毒的毒性反应. 至于蝎毒使心肌细胞动作电位的发放频率加快，以及向培养基中加蝎毒后，搏动群落数似有增多趋势，则可能因蝎毒刺激钙的摄取，$[Ca^{2+}]_i$ 增多既可使心肌细胞的舒张期除极速度加快[13]，又可通过兴奋收缩耦联始动收缩[14].

上述结果表明蝎毒有钠通道阻滞作用. 值得在此基础上进一步提纯，可望形成一种新的阻断钠通道的工具药.

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奥昔非君对肾上腺素加冰水诱致大鼠高血粘度和心肌坏死的作用

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Effects of oxyfedrine on high blood viscosity and myocardial necrosis induced by epinephrine and ice water stress in rats

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ABSTRACT Acute high blood viscosity (HBV) and myocardial necrosis was established by epinephrine (Epi) and ice water stress in rats. Effects of iv oxyfedrine (Oxy) on HBV, plasma viscosity (PV), hematocrit, erythrocyte electrophoretic time (EET), and fibrinogenic viscosity (FV) were studied in model. Results showed that Oxy 1 mg·kg^{-1} iv markedly decreased the arterial and venous blood HBV at shear rates of 700 s^{-1} and 70 s^{-1}, respectively ($P<0.01$). There were significant differences in the alleviation of HBV among 3 groups (Oxy 0.01, 0.1, and 1 mg·kg^{-1} iv). The above doses markedly decreased the HBV, PV, and FV, and shortened the EET. Effects of iv Oxy on the myocardial necrosis rat model were scrutinized under the light and electron microscopes. Oxy iv 1 mg·kg^{-1}·d^{-1}×1, 3, and 5 d prevented or mitigated the occurrence and development of myocardial necrosis. The structure of heart mitochondria and myofibrils were clearly discernible. This action may be related to the alleviation of HBV by Oxy.

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KEY WORDS oxyfedrine; epinephrine; blood viscosity; stress; myocardium; necrosis; heart mitochondria; myofibrils

摘要 iv 奥昔非君(oxyfedrine, Oxy)可量一效相关地降低 Epi 加冰水诱致大鼠高血粘模型的 HBV, PV, FV 和红细胞比容, 缩短 EET. Oxy (1 mg·kg^{-1}·d^{-1}) 单次或连续 iv, 电镜下显现有保护心肌线粒体及肌原纤维的作用, 显著减轻 Epi 加冰水应激造成的大鼠急性心肌损伤和坏死. 结果提示 Oxy 治疗冠心病、心绞痛和心肌坏死等的依据.

关键词 奥昔非君; 肾上腺素; 血液粘度; 应激; 心肌; 坏死; 心脏线粒体; 肌原纤维

心肌坏死

奥昔非君(oxyfedrine, Oxy)属麻黄碱类衍生物, 为冠脉扩张药. 对循环血液等方面实验研究虽有报道[1-3], 但对实验性高血粘度(high blood viscosity, HBV)和心肌坏死(myocardial necrosis)有无作用未见报道. 本文以肾上腺素(epinephrine, Epi)加冰水制备大鼠高血粘和心肌坏死模型, 研究 Oxy 的作用, 为其防治疾病寻求理论基础.

MATERIALS AND METHODS

Oxy, 内蒙古赤峰制药厂产品. Epi (盐酸肾上腺素), 杭州制药厂产品. 肝素钠, 上海生物化学制药厂