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东亚钳蝎蝎毒二、三级提取物对快反应心肌细胞动作电位的影响

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Influence of the 2nd and 3rd grade abstracts from scorpion venom of *Buthus martensii* Karsch on action potentials of fast response myocardiocytes

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AIM: To screen the active components of sodium channel blockade action from *Buthus martensii* Karsch (BMK) scorpion venom. **METHODS**: Myocardiocytes of mice were cultured. Action potentials of fast response myocardiocytes were recorded. The effects of 22 contents of BMK scorpion venom in a concentration of 3 mg·L^{-1} were tested and compared with that of TTX 2.5 mg·L^{-1}, nimodipine (Nim) 3 mg·L^{-1} and $BaCl_2$ 24.4 mg·L^{-1}. **RESULTS**: BMK-1 and other 19 contents significantly decreased depolarization parameters V_{max}, APA, OS, and MDP, which was similar to that of TTX and different from that of Nim and $BaCl_2$. **CONCLUSION**: The variations in the action potentials of fast response myocardiolcytes indicated that 20 contents of BMK scorpion venom had sodium channel blockade action.

KEY WORDS cultured cells; myocardium; action potentials; scorpion venoms

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目的：从东亚钳蝎蝎毒(BMK)中筛选有钠通道阻滞作用的活性成分. 方法：培养小鼠心肌细胞，记录快反应动作电位，观察22种BMK蝎毒提取物3 mg·L⁻¹对动作电位的影响，并与河豚毒2.5 mg·L⁻¹、尼莫地平3 mg·L⁻¹、氯化钡24.4 mg·L⁻¹进行对照. 结果：BMK-1及其它19种组分使除级化参数 V_{max}，APA，OS 和 MDP 减小. 其作用与河豚毒相似，而与尼莫地平和氯化钡不同. 结论：快反应心肌细胞动作电位的改变表明，BMK 蝎毒中的20种组分有钠通道阻滞作用.

关键词　培养的细胞；心肌；动作电位；蝎毒

我们在培养的小鼠心肌细胞上，观察到东亚钳蝎粗毒可使快反应心肌细胞动作电位的时程与除级有关参数全部减小[1]. 除极参数减小提示其钠通道阻滞作用，而动作电位时程的改变则既可能是继发于除极参数的改变，也可能与钾通道[2]、钙通道[3]的活动，以及肌质网对钙的摄取[4]有关，为进一步分析蝎毒的作用及其机制以及筛选选择性作用于钠通道的蝎毒组分，本实验对10种蝎毒二级提取物与12种三级提取物对快反应心肌细胞动作电位的影响进行了观察比较.

MATERIALS AND METHODS

药品与试剂　东亚钳蝎(BMK，*Buthus martensii* Karsch)蝎毒由本校有机化学教研室提取. 将粗毒通过 Sephadex G 10柱除盐后，经 CM Sephadex C 50离子交换层析得到13个蛋白峰，每个单峰为一种电泳纯的二级提取物，依次命名为 BMK-1，BMK-2，··· BMK-13. 其中 BMK-2，BMK-9，BMK-11 再经 Sephadex G 50凝胶过滤分别提到4、5、3个峰，每个单峰为一种电泳纯的三级提取物，命名为 BMK-2-(1)，BMK-2-(2)等，培养基、尼莫地平、河豚毒、小牛血清等药品试剂同前[1].

心肌细胞培养与动作电位记录　同前[1]，数据用分组 *t* 检验进行统计处理.

RESULTS

于培养4－5d 后，用微电极自搏动群落的心肌细胞内，引导动作电位. 按最大除极速度(V_{max}) ＞80V·s⁻¹的标准判定快反应心肌细胞[5]. 分别向培养基中加入每一种蝎毒二，三级提取物，剂量均为3 mg·L⁻¹. 记录加药前后的心肌细胞动作电位，经微机连机分析5项电参数：动作电位幅值(APA)，超射(OS)，最大舒张电位(MDP)，V_{max}，复极50%水平的动作电位波宽(APD_{50}). 结果与 TTX 2.5 mg·L⁻¹，Nim 3 mg·L⁻¹，$BaCl_2$ 24.4 mg·L⁻¹进行对照(Tab 1).

DISCUSSION

BMK 粗毒及其20种二级与三级提取物均使直接反应钠内流的除极参数显著减小，而且与 TTX 的效应相同，表明 BMK 蝎毒的大部组分都有钠通道阻滞作用. 这与其它蝎种的蝎毒能使钠通道失活的报道一致[6].

蝎毒二级提取物 BMK-6能使动作电位的除极有关参数显著增大，提示它可能有钠通道激活作用. 这与其它蝎种蝎毒能显著增加钠内流[7]、激活钠通道[8]的报道一致.

在22种二、三级提取物中有9种蝎毒组分使动作电位波宽缩短、5种使之延长，提示蝎毒可能对钙通道和钾通道也有影响. 这与其它蝎种蝎毒能激活钙通道[4]和阻滞钾通道[9]等报道吻合. 不过，本实验中单纯阻滞钠通道的 TTX 不仅使除极参数显著减小，而且也使波宽缩短. 此波宽缩短显然并非钾、钙通道活动的变化，而是继发于除极参数的显著变化，所以 BMK 蝎毒对钾、钙通道有无影响还有待进一步研究.

总之，本实验以培养心肌细胞快反应动作电位为指标的结果表明，在22种 BMK 蝎毒二、三级提取物中，BMK-1等20种组分可能有钠通道阻滞作用；BMK-6可能有钠通道激动作用.

Tab 1. Influence of the 2nd and 3rd grade extracts from BMK scorpion venom on action potential parameters of cultured mouse myocardiocytes. n = penetrations, $\bar{x} \pm s$, $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ vs control; BMK series: 3.0 $mg \cdot L^{-1}$; Nim 3.0 $mg \cdot L^{-1}$; TTX: 2.5 $mg \cdot L^{-1}$; $BaCl_2$: 24.4 $mg \cdot L^{-1}$.

	n	APA/mV	OS/mV	MDP/mV	$V_{max}/V \cdot s^{-1}$	APD_{50}/ms
Control	73	76 ± 15	25 ± 7	51 ± 12	93 ± 37	52 ± 12
BMK-1	24	51 ± 7^c	16 ± 4^c	35 ± 7^c	51 ± 14^c	31 ± 13^c
BMK-2	30	53 ± 13^c	14 ± 7^c	38 ± 10^c	45 ± 21^c	47 ± 11^a
BMK-2-(1)	47	60 ± 11^c	19 ± 4^c	41 ± 9^c	64 ± 20^c	47 ± 4^c
BMK-2-(2)	34	56 ± 13^c	17 ± 4^c	39 ± 10^c	49 ± 18^c	45 ± 3^c
BMK-2-(3)	70	79 ± 10^c	25 ± 4^a	54 ± 6^a	77 ± 22^c	39 ± 6^c
BMK-2-(4)	80	70 ± 14^c	21 ± 6^c	49 ± 11^c	89 ± 27^c	51 ± 7^a
BMK-3	31	53 ± 10^c	17 ± 4^c	36 ± 7^c	44 ± 4^c	48 ± 5^a
BMK-4	32	73 ± 16^a	23 ± 7^a	50 ± 11^a	96 ± 39^a	57 ± 7^c
BMK-6	29	85 ± 14^c	27 ± 6^b	57 ± 11^c	122 ± 32^c	45 ± 4^c
BMK-8	35	45 ± 18^c	12 ± 7^c	33 ± 13^c	48 ± 23^c	36 ± 6^c
BMK-9	22	68 ± 14^b	21 ± 4^b	47 ± 9^b	25 ± 27^c	59 ± 12^b
BMK-9-(1)	32	72 ± 21^a	23 ± 8^a	49 ± 13^a	75 ± 35^c	75 ± 13^c
BMK-9-(2)	31	77 ± 16^a	27 ± 8^a	50 ± 10^a	88 ± 31^c	64 ± 2^c
BMK-9-(3)	25	59 ± 14^c	18 ± 4^c	41 ± 11^c	62 ± 15^c	53 ± 25^a
BMK-9-(4)	35	54 ± 10^c	17 ± 5^c	37 ± 9^c	59 ± 26^c	52 ± 11^a
BMK-9-(5)	27	37 ± 7^c	7 ± 3^c	30 ± 4^c	35 ± 9^c	54 ± 11^a
BMK-10	73	44 ± 15^c	12 ± 6^c	32 ± 11^c	46 ± 17^c	40 ± 7^c
BMK-11	32	40 ± 15^c	12 ± 8^c	28 ± 8^c	42 ± 22^c	44 ± 21^b
BMK-11-(1)	24	49 ± 17^c	12 ± 6^c	36 ± 12^c	65 ± 15^c	40 ± 6^c
BMK-11 (2)	27	37 ± 9^c	5 ± 2^c	32 ± 8^c	25 ± 10^c	37 ± 1^c
BMK-11-(3)	26	48 ± 12^c	15 ± 4^c	33 ± 8^c	58 ± 17^c	52 ± 6^a
BMK-12	38	52 ± 13^c	16 ± 5^c	36 ± 10^c	37 ± 13^c	47 ± 8^b
Nim	30	73 ± 6^a	23 ± 8^a	55 ± 14^a	92 ± 16^a	39 ± 5^c
$BaCl_2$	30	63 ± 3^c	21 ± 2^c	42 ± 3^c	56 ± 9^c	188 ± 11^c
TTX	30	46 ± 3^c	11 ± 5^c	34 ± 7^c	8 ± 3^c	45 ± 6^c

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