173-176

BIBI ID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1994 Mar; 15 (2): 173—176

人参二醇组与三醇组皂甙对大鼠心室肌细胞钙通道阻滞作用的单通道分析

R971.9

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Single channel analysis on calcium channel blockade action of panaxadiol and panaxatriol saponins on cultured rat ventricular myocytes

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ABSTRACT Wistar rat ventricular myocytes were isolated. Panaxadiol saponins 1500 $\mu g \cdot ml^{-1}$, panaxatriol saponins 300 $\mu g \cdot ml^{-1}$, verapamil 37.5 $\mu g \cdot ml^{-1}$, or BAY k 8644 5 $\mu mol \cdot L^{-1}$ were added into the bath solution separately. The single channel activities of L, T, and B type calcium channels were recorded before and after the administration, using voltage patch - clamp technique in cell - attached configuration. The calcium channel blockade effect of these 2 groups of ginsenosides was authenticated verified. The mechanism existed in the decrease in both the open time and the open - state probability of the calcium channel.

KEY WORDS ginseng; saponins; cultured cells; myocardium; patch clamp; verapamil; BAY k 8644; electrophysiology

摘要 分离 Wistar 大鼠乳鼠的心室肌细胞. 向培养基中分别加入人参二醇组皂甙1500 $\mu g \cdot ml^{-1}$，人参三醇组皂甙300 $\mu g \cdot ml^{-1}$，维拉帕米37.5 $\mu g \cdot ml^{-1}$或 BAY k 8644 5 $\mu mol \cdot L^{-1}$，用斑片钳的连细胞电压钳法，记录加药前后 L，T，B 三型钙通道的单通道活动. 确证了这两组人参皂甙的钙通道阻滞作用. 其作用机制在于使钙通道的开放时间缩短与开放概率减少.

关键词 人参；皂甙；培养的细胞；心肌；斑片钳；维拉帕米；BAY k 8644；电生理学

Received 1992-01-14 Accepted 1993-04-21

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钱永佑教授等首先在培养的大鼠乳鼠心肌细胞上记录出 L (long lasting and large in unitary conductance)型单钙通道活动[1]. Nilius 等在分离的豚鼠心肌细胞上记录出 T (transient)型单钙通道活动[2]. Rosenberg 等在掺入到人工脂质双层的牛心肌细胞膜小泡上，发现了一种自发性发放的 B (backgroud)型钙通道[3]. 本实验在培养的 Wistar 大鼠乳鼠心肌细胞上，以这三型钙通道的单通道活动为指标，分析人参二醇组皂甙(panaxadiolsaponins，PDS)和人参三醇组皂甙(panaxatriol saponins，PTS)对钙通道的阻滞作用及其机制.

MATERIALS AND METHODS

药品 人参皂甙由本校有机化学教研室自吉林人参(*Panax ginseng* C A Meyer)茎和叶中提取. 高效液相色谱和薄层扫描测定结果：PDS 纯度为92%，含皂甙单体 Rb_1，Rb_2，Rb_3，Rc，Rd. PTS 纯度为90%，含 Rg_1，Rg_2，Rf，Rf_{11}，Re，Rh_1. Dulbecco's modified Eagle medium (DMEM, Life Technologies Inc. USA)、BAY k 8644 (Calbiochem Co, USA).

心肌细胞的分离与培养 取 Wistar 大鼠乳鼠心尖，用含有0.1%胰蛋白酶、0.1%牛血清白蛋白的无钙、镁 Hanks' 液分离心肌细胞. 置于含有80% DMEM 与20%小牛血清的培养基内，在5% CO_2 + 95%空气的孵箱中培养，2 h 后做贴壁分离. 于培养21

—18 h 期间进行实验记录.

钙单通道活动记录 用斑片钳的连细胞电压钳法，记录 L，T，B 三型钙通道的 Ba^{2+} 流. 浸浴液：天门冬酸钾140，EGTA 10，HEPES 10 mmol·L^{-1}，pH 7.4，电极充灌液：$BaCl_2$ 110，HEPES 10 mmol·L^{-1}. 斑片钳放大器(Dagan 8800，USA)与示波器(VC 10 Kohden，Japan)的低通滤波器截止频率设置1 kHz. 微电极与细胞膜之间的封接电阻大于10 GΩ.

数据采集处理与统计 在每次记录中，连续采样50条原始曲线. 删除原始曲线中阶跃命令的电容伪迹，并修平基线后，用 Gauss 曲线拟合采样点电流序列密度直方图的方法(Fig 1)，逐条自采样曲线测出流过 L 与 B 型钙通道的 Ba^{2+} 流幅值后，算出应用每种药物前、后的均值. T 型钙通道的 Ba^{2+} 流幅值按标准电流刻度，自修整过的采样曲线上，用目测法直接读数. 用指数曲线拟合开放或关闭时间分布直方图的方法(Fig 2)，统计出50次采样中，三型钙通道的平均开放与关闭时间. 将50条采样曲线中，通道开放总时间除以总采样时间得出开放概率.

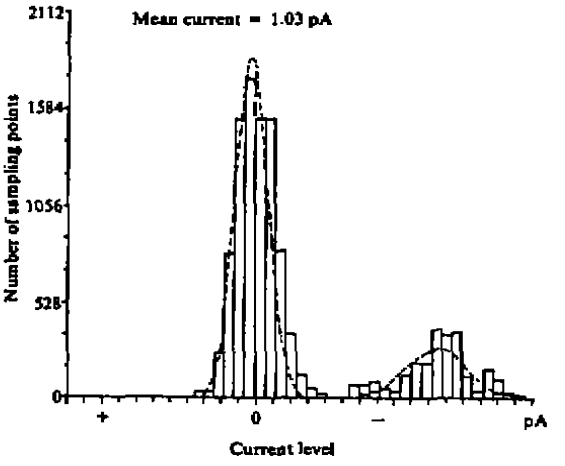

Fig 1. An example of fitting the current sequent density histograms with Gauss curve to get the amplitude of Ba^{2+} current (I_{Ba}). Horizontal line: $\bar{x}$ of I_{Ba} (distance between 2 Gauss curve peaks).

RESULTS

向培养基中分别加入PDS 1500 μg·ml^{-1}，PTS 300 μg·ml^{-1}，维拉帕米37.5 μg·ml^{-1}

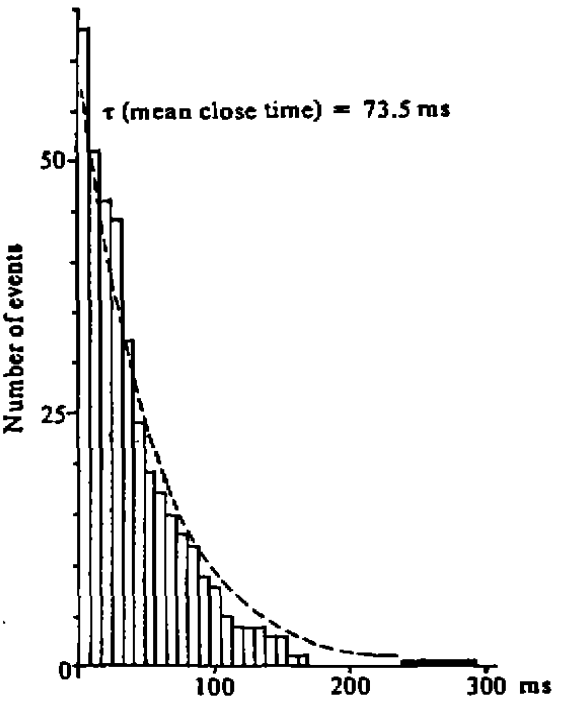

Fig 2. An example of exponentially fitting the close time distribution histograms to get the mean of open time.

或 BAY k 8644 5 μmol·L^{-1}记录加药前后 L，T，B 三型钙通道的单通道活动. 由−50 mV 保持电压阶跃至+10 mV，诱发 L 型钙通道活动. 由−70 mV 阶跃至−10 mV，诱发 T 型钙通道活动. 在−60 mV 保持电压下，记录 B 型钙通道的自发性单通道活动. 在每一种药物的作用下，记录5个心肌细胞膜片上的三型单钙通道活动. PDS，PTS 分别使 L 型钙通道的开放概率下降44 %、75 %；开放时间缩短30 %、44 %；关闭时间延长33 %、53 %. PDS，PTS 分别使 T 型钙通道的开放概率下降35 %、74 %；开放时间缩短15 %、38 %；关闭时间延长23 %、55 %. PDS，PTS 分别使 B 型钙通道的开放概率下降61 %、85 %；开放时间缩短29 %、45 %；关闭时间延长46 %、71 %. PDS，

PTS 对流过三型钙通道 Ba^{2+} 离子流幅值均无明显影响. 这些作用与维拉帕米相似、与 BAY k 8644相反. Tab 1分别为四种药物应用前后，三型钙通道的开放时间、关闭时间、Ba^{2+} 流幅值与开放概率. Fig 3为修整过的三型钙通道活动的原始记录曲线.

DISCUSSION

本实验用连细胞斑片钳技术，在培养的大鼠心肌细胞上，直接记录出人参组甙 PDS 和 PTS 对钙通道的阻滞作用. PTS 300 $\mu g \cdot ml^{-1}$ 和 PDS 1500 $\mu g \cdot ml^{-1}$ 的作用相近，说明 PTS 比 PDS 的钙通道阻滞作用更强.

维拉帕米和 PDS、PTS 的作用相似、与 BAY k 8644相反，进一步证明这两组人参皂甙都有钙通道阻滞作用.

对三型钙通道的开放时间、关闭时间、开放概率，以及流过钙通道的钡流幅值的分析结果表明 PDS、PTS 对钙通道的阻滞作用机制在于使钙通道的开放时间缩短与开放概率减少. 本实验结果与以心肌细胞动作电位与自发性搏动为指标的观察结果[4,5]一致.

Tab 1. Open time, close time, Ba^{2+} current amplitude (I_{Ba}) and open-state probability (P_{open}) of L, T, B type calcium channels ($\bar{x} \pm s$). $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control; PDS 1500 $\mu g \cdot ml^{-1}$; PTS 300 $\mu g \cdot ml^{-1}$; Ver 37.5 $\mu g \cdot ml^{-1}$; BAY = BAY k 8644 5 $\mu mol \cdot L^{-1}$.

		Open time/ms	Close time/ms	I_{Ba}/pA	$10^{-1} \times P_{Open}$
L	Control	1.43±0.28	139±30	1.60±0.51	32±8
	PDS	3.10±0.48[c]	185±50	1.45±0.29	18±80[b]
	Control	1.44±0.66	100±38	1.16±0.17	17±18
	PTS	2.18±0.17[c]	212±19[c]	1.58±0.23	12±10[b]
	Control	4.15±0.30	125±35	1.53±0.49	35±13
	Ver	2.12±0.25[c]	190±19[c]	1.02±0.19	13±2.0[c]
	Control	3.24±0.21	153±30	1.62±0.59	21±1.0
	BAY	4.52±0.39[c]	111±9.0[c]	1.55±0.71	10±5.0[c]
T	Control	1.95±0.04	97±24	0.72±0.06	20±5.0
	PDS	1.66±0.08[c]	126±24[c]	0.76±0.05	13±3.0[c]
	Control	2.58±0.51	80±21	0.68±0.08	34±15
	PTS	1.60±0.09[c]	178±27[c]	0.76±0.06	9.0±2.0[b]
	Control	2.07±0.10	65±10	0.76±0.01	32±6.0
	Ver	1.51±0.27[c]	189±21[c]	0.66±0.13	8.0±2.0[c]
	Control	1.89±0.15	116±14	0.65±0.05	16±3.0
	BAY	2.32±0.03[c]	50±12[c]	0.66±0.05	16±9.0[c]
B	Control	4.32±0.58	52±15	1.26±0.21	83±8.0
	PDS	3.09±0.24[c]	96±20[b]	1.15±0.08	32±6.0[b]
	Control	4.14±1.11	42±15	1.37±0.16	101±11
	PTS	2.27±0.36[c]	147±28[c]	1.25±0.21	15±1.0[c]
	Control	5.71±0.50	33±14	1.20±0.09	160±59
	Ver	2.40±0.20[c]	147±46[c]	1.36±0.02	17±17[b]
	Control	2.95±0.33	88±15	1.41±0.22	33±9.0
	BAY	4.74±0.42[c]	35±12[c]	1.40±0.18	119±98[b]

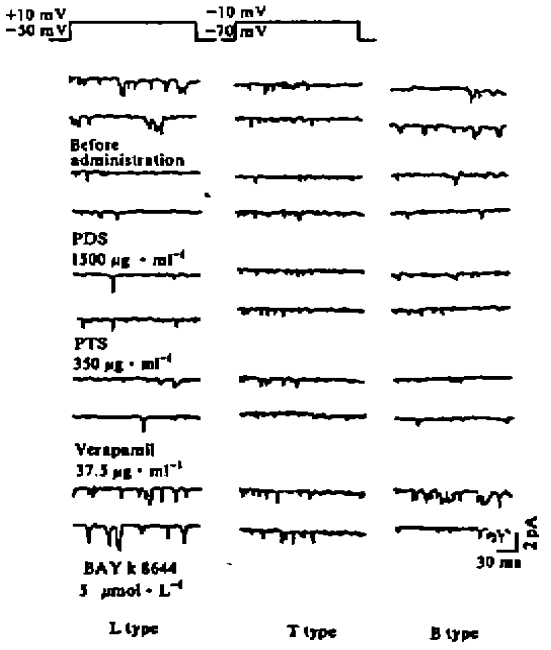

Fig 3. Single channel current recordings of L, T, and B type calcium channels.

REFERENCES

1 Reuter H, Stevens CF, Tsien RW, Yellen G. Properties of single calcium channels in cardiac cell culture. Nature 1982; **297**: 501—4.

2 Nilius B, Hess P, Lansman JB, Tsien RW. A novel type of cardiac calcium channel in ventricular cells. Nature 1985; **316**: 443—6.

3 Rosenberg RL, Coronado R, Messner G. Cardiac calcium channels in planar lipid bilayers. L-Type channels and calcium-permeable channels open at negative membrane potentials. J Gen Physiol 1988; **92**: 27—54.

4 Zhong GG, Yue G, Shao CJ, Ma XY, Xu JD, Jiang Y. Dual-dimensional effect of panaxadiol saponin (PDS) on action potential of cultured cardiac cells. J Bethune Univ Med Sci 1990; **16**: 417—21.

5 Jiang Y, Zhong GG, Yue G, Zhang WJ. Ca^{2+} channel blockade effect of panaxadiol saponins and panaxatriol saponins on cultured cardiac cells. Chin J Chin Mat Med 1992; **17**: 172—3.

176-180 21.

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1994 Mar; 15 (2): 176—180

3,15-二乙酰苯甲酰乌头原碱镇痛的作用部位[1]

R971.1

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Sites of analgesic action of 3,15-diacetylbenzoylaconine[1]

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Received 1993-09-16 Accepted 1993-05-06
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ABSTRACT In the rat hot plate test, vocalization induced by electric stimulation, tail flick test, and the mouse acetic acid writhing test, 3, 15-diacetylbenzoylaconine (DABA) ip exhibited a dose-dependent analgesic activity. Intrathecally (ith) administered DABA (527, 1186 $\mu g \cdot kg^{-1}$) had no analgesic action. Microinjection of DABA 35—75 $\mu g \cdot kg^{-1}$ or 20 μg into the cerebral ventricle (icv) or the periaqueductal gray (PAG) exerted a remarkable analgesic activity, which was abolished after bilateral lesions of locus coeruleus (LC). Microinjection of DABA (20 μg) into LC failed to produce apparent analgesic action. These