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人参二醇与三醇组皂甙对正常与黄嘌呤—黄嘌呤氧化酶致损的培养心肌细胞动作电位的影响

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Effects of panaxadiol and panaxatriol saponins on action potentials of normal and xanthine-xanthine oxidase damaged cultured myocardial cells

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ABSTRACT Wistar rat myocardial cells were cultured. PDS 20-80 $\mu g \cdot ml^{-1}$; PTS 1.25-20 $\mu g \cdot ml^{-1}$ dose-dependently decreased their action potential parameters, indicating the possibility of being concerned in the blockage of Ca channel. After the free radical damage was induced by xanthine 0.42 $mmol \cdot L^{-1}$ — xanthine oxidase 5.3 $nmol \cdot L^{-1}$ (X-XO). All the action potential parameters of the cardiac cells decreased without exception. Both PDA and PTS antagonized the electrical appearance of membrane damage induced by X-XO, suggesting that both PDS and PTS protect the myocardial cells from oxidative damage.

KEY WORDS ginseng; saponins; myocardium; cultured cells; action potentials; xanthines; xanthine oxidase

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提要 培养 Wistar 大鼠乳鼠的心肌细胞. PDS 20-80 $\mu g \cdot ml^{-1}$; PTS 1.25-20 $\mu g \cdot ml^{-1}$ 使其动作电位各参数呈剂量依赖性减小，提示可能与钙通道阻滞有关. 用黄嘌呤 0.42 $mmol \cdot L^{-1}$—黄嘌呤氧化酶 5.3 $nmol \cdot L^{-1}$ (X-XO)诱发自由基损伤后，心肌细胞动作电位各参数一致减小，PDS，PTS 均能抵消 X-XO 所致的膜损伤性电位表现，提示 PDS，PTS 对心肌细胞的氧化损伤均具有保护作用.

关键词 人参；皂甙类；心肌；培养的细胞；动作电位；黄嘌呤类；黄嘌呤氧化酶

人参二醇组皂甙(panaxadiol saponins, PDS)与人参三醇组皂甙(panaxatriol saponins, PTS)的作用不同. 比如PDS对中枢神经有抑制作用、PTS对中枢神经有兴奋作用[1]; PDS抗红细胞溶血、PTS促红细胞溶血[2]; PDS减少缺氧心肌对外源性ATP的摄取、PTS增加缺氧心肌对外源性ATP的摄取[3]等. 本实验拟在正常的与黄嘌呤—黄嘌呤氧化酶(X-XO)致损的培养心肌细胞上, 以跨膜电活动为指标, 对这两种人参皂甙的作用进行对比观察.

MATERIALS AND METHODS

心肌细胞培养[4] 培养基为80% DMEM (Dulbecco's Modified Eagle Medium, Life Technologies, INC. USA)与20%小牛血清(NBS, 本实验室自制).

动作电位记录[5] 启开培养瓶之上壁, 胞内引导心肌细胞动作电位, 经微机连机分析以下电参数: 动作电位波幅(APA)、超射(OS)、最大舒张电位(MDP)、阈电位(TP)、最大除极速度(V_{max})、动作电位波宽(APD)以及动作电位发放频率(F).

人参皂甙溶液配制 人参皂甙由白求恩医大化学教研室提取. 经高效液相色谱和薄层扫描法测定, PDS纯度为92%、PTS为90%. PDS含有皂甙单体 R_{b1}, R_{b2}, R_{b3}, R_c, R_d; PTS含 R_{g1}, R_{g2}, R_f, R_e, R_{b1}. 均配成0.8%的水溶液, 经9磅20 min高压后储存. 临用时, 用培养基稀释至所需浓度.

RESULTS

对正常的培养心肌细胞动作电位的影响 实验分PDS与PTS两大组. 每大组内又按皂甙用量的不同, 将心肌细胞分8组培养: 对照组不含人参皂甙, 其它7组分别向培养基中加PDS或PTS 1.25, 2.5, 5, 10, 20, 40, 80 $\mu g \cdot ml^{-1}$.

培养后4 d, 在呈现自发性搏动的群落上引导心肌细胞动作电位. 结果汇于Tab 1. 实

Tab 1. Effects of PDS and PTS on action potentials of cultured rat myocardial cells. $\bar{x} \pm SD$; n: number of penetrations; $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* before; bpm: beats per minute.

Number of penetrations		APA mV	OS mV	MDP mV	TP mV	V_{max} $V \cdot s^{-1}$	APD_{50} ms	F bpm
Before PDS	55	65.6±7.7	23.7±3.7	42.3±6.6	22.6±4.4	10.0±1.5	111±41	96.1±47.0
Before PTS	29	76.3±12.8	26.2±6.8	50.1±9.3	27.7±6.8	13.5±5.8	131±27	139±35
PDS 1.25 μg	52	68.9±7.7*	24.7±5.0*	43.3±5.8*	24.4±4.3*	12.4±4.3***	153±56***	83.0±32.8*
PTS 1.25 μg	32	70.9±13.8*	23.0±4.9***	47.9±9.3*	27.5±7.8*	12.3±2.8*	117±45*	162±68*
PDS 2.5 μg	51	70.1±8.5***	25.4±5.7*	44.7±5.0*	25.8±5.0**	12.6±2.9***	155±57**	92.4±48.2*
PTS 2.5 μg	32	67.9±10.7***	22.2±5.5**	45.6±7.4**	23.1±6.8***	9.2±3.9**	108±44**	119±49*
PDS 5 μg	28	66.2±9.4*	22.3±4.2*	43.1±7.9*	25.7±7.4*	12.9±5.3***	169±30***	73.4±28.5**
PTS 5 μg	31	65.0±7.6***	22.3±2.8***	42.7±4.9***	23.2±10.4*	9.7±2.3***	116±20**	142±30*
PDS 10 μg	53	67.3±9.9*	22.5±4.4*	44.8±8.7*	25.3±6.6*	13.1±3.6*	98.6±24.0*	104±56*
PTS 10 μg	33	57.1±12.1***	18.9±6.9***	38.1±1.9***	21.7±6.0***	8.7±3.1***	81.7±27.7***	200±63***
PDS 20 μg	56	60.4±10.8***	19.5±4.5***	40.9±7.5*	23.7±6.7*	8.8±2.2***	103±28*	118±43**
PTS 20 μg	12	33.3±6.9***	8.6±3.1***	26.5±7.5***	15.3±5.6**	3.4±0.7**	91.8±6.9***	138±17*
PDS 40 μg	29	49.4±8.7***	13.4±6.4***	36.0±4.8***	22.9±10.2*	7.8±2.7***	65.6±19.9***	194±62***
PTS 40 μg	(stop discharging action potential)							
PDS 80 μg	10	46.1±23.1***	12.4±11.1***	33.8±13.3***	23.6±8.5*	6.6±4.7***	77.4±42.3**	159±45***
PTS 80 μg	(stop dischraging action potential)							

验数据经 t 检验处理.

培养心肌细胞动作电位各电参数在低浓度 PDS (1.25–2.5 $\mu g \cdot ml^{-1}$)时一致增大，在高浓度(20–80 $\mu g \cdot ml^{-1}$)时一致减小. 动作电位发放频率在低浓度 PDS 作用下无明显改变，在高浓度 PDS 作用下显著加快. PTS 为 1.25 $\mu g \cdot ml^{-1}$ 时电参数即呈下降趋势. 2.5 $\mu g \cdot ml^{-1}$ 时下降到显著程度. 在浓度倍增至 5，10，20 $\mu g \cdot ml^{-1}$ 的过程中，下降程度越来越显著. 动作电位发放频率在 1.25–10 $\mu g \cdot ml^{-1}$ 的范围内，基本上随 PTS 的浓度加大而加快，PTS 由 10 $\mu g \cdot ml^{-1}$ 增至 20 $\mu g \cdot ml^{-1}$ 时发放频率剧烈下降，在 PTS 浓度为 40–80 $\mu g \cdot ml^{-1}$ 时动作电位发放停止.

为了突出显示低浓度分组皂甙的相反效应，特与各自的对照组比较，算出 PDS，PTS 1.25–2.5 $\mu g \cdot ml^{-1}$ 使电参数增减的百分比值，示于 Fig 1.

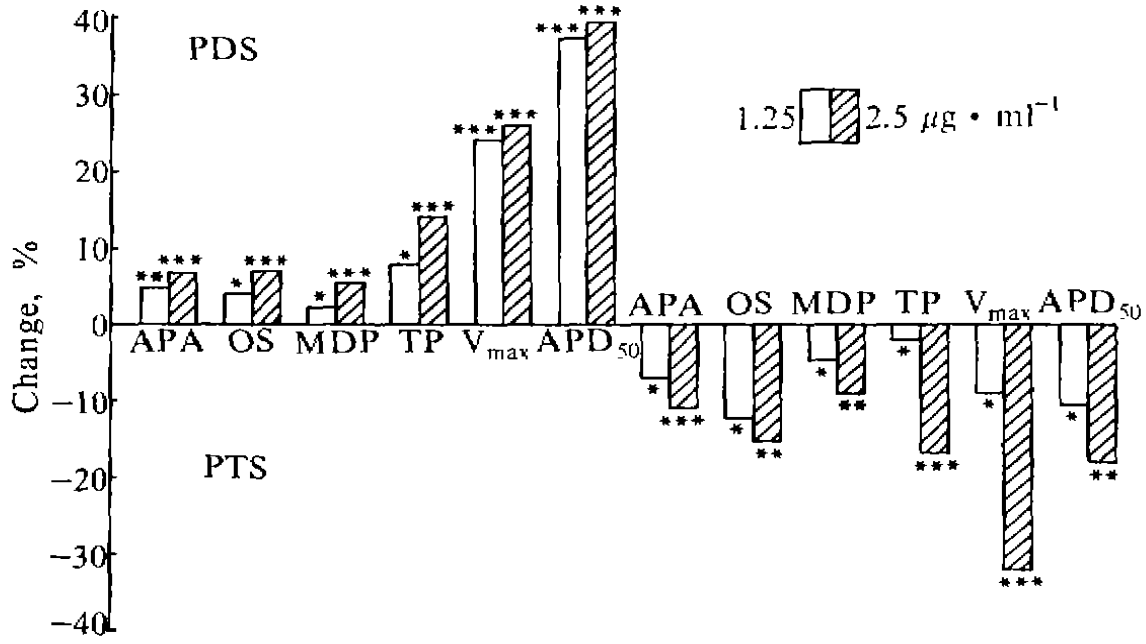

Fig 1. Effects of PDS and PTS on electric parameters of cultured rat myocardial cells. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control.

Tab 2. Effects of X–XO, PDS, and PTS on action potentials of cultured rat myocardial cells. $\bar{x} \pm SD$; $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* X–XO. $^{+}P>0.05$, $^{++}P<0.05$, $^{+++}P<0.01$ *vs* Control. $^{\ddagger}P>0.05$, $^{\ddagger\ddagger}P<0.05$, $^{\ddagger\ddagger\ddagger}P<0.01$ *vs* X–XO+PDS.

Number of penetrations		APA mV	OS mV	MDP mV	TP mV	V_{max} $V \cdot s^{-1}$	APD_{90} ms	F bpm
X–XO	24	56.2±7.8	16.8±4.4	39.4±5.4	23.6±4.9	7.4±2.4	165±54	199±24
Control	25	70.6±14.5***	23.5±4.5***	47±10.5***	28.6±8.5***	23.5±11.5***	191±29**	157±16***
X–XO+PDS	15	72.3±12.8**	23.0±5.8***	49.1±13.2**	33.3±9.7**	20.2±5.8***	198±58*	161±28**
X–XO+PTS	31	62.7±13.4$^{**}_{\ddagger\ddagger}$	19.8±6.7$^{**}_{\ddagger}$	42.7±6.9$^{**}_{\ddagger\ddagger}$	25.0±7.8$^{**}_{\ddagger\ddagger\ddagger}$	10.1±3.9$^{***}_{\ddagger\ddagger\ddagger}$	160±52$^{**}_{\ddagger\ddagger}$	203±63$^{**}_{\ddagger\ddagger}$

对自由基损伤心肌细胞动作电位的影响

心肌细胞分四组进行培养，各组培养基成份为，对照组：80% DMEM + 20% NBS; X-XO 组：80% DMEM + 20% NBS + 0.42 mmol · L^{-1} X + 5.3 nmol · L^{-1} XO; X-XO+PDS 组：80% DMEM + 20% NBS + 0.42 mmol · L^{-1} X + 5.3 nmol · L^{-1} XO + 2.5 μg · ml^{-1} PDS; X-XO+PTS 组：80% DMEM + 20% NBS + 0.42 mmol · L^{-1} X + 5.3 nmol · L^{-1} XO + 2.5 μg · ml^{-1} PTS. 其中，X-XO 于电位记录前 16 h 加入培养基.

于培养 5-8 d，自四组共引导 95 个心肌细胞的动作电位. 各组的电参数汇于 Tab 2.

Fig 2 为典型的动作电位记录.

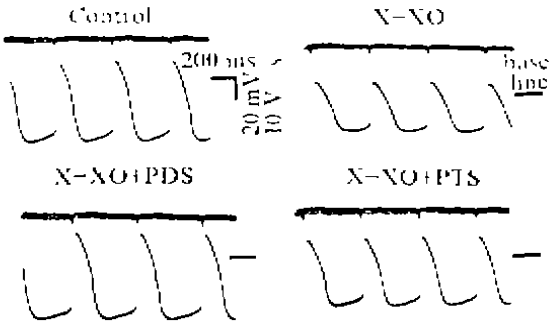

Fig 2. Typical recordings of action potentials. Upper tracing: dV/dt. Lower tracing: action potential.

可以看出，用 X-XO 诱发自由基损伤的心肌细胞，其动作电位的所有电参数均显著低于对照组. 而在含有 PDS 的培养基中加入相同剂量的 X-XO 则动作电位不再减小，所有电参数均与对照组无明显差异. 在 PTS 组的培养基中加入同量的 X-XO 后，动作电位参数虽仍显著低于对照组，但比 X-XO 组则显著增大.

DISCUSSION

本实验用 X-XO 诱发培养心肌细胞的自由基损伤[6,7]，使其动作电位的各参数一致减小，呈典型的膜损伤性电位表现[8]. PDS, PTS 均能反转 X-XO 所致的电参数减小，而 PDS 使参数反转的程度显著大于 PTS. 表明这两种人参皂甙均能对抗自由基损伤、保护心肌细胞膜的完整性，且 PDS 的这一作用显著强于 PTS. 这部分结果与 PDS 能提高超氧化物歧化酶活性、减少缺血心肌超氧阴离子自由基的报道[9]一致.

另外，本实验在正常的培养心肌细胞上看到，低浓度 PDS 使其电参数增大、PTS 与高浓度 PDS 使其电参数减小，而且 PTS 使动作电位发放停止的阈浓度比 PDS 低. 根据培养心肌细胞是慢反应细胞，除心肌细胞膜损伤外，钙通道阻滞亦可导致其慢反应电位的减小[10]，两种人参皂甙可能都有钙通道阻滞作用，且 PTS 的这一作用比 PDS 更强. 这与三七总皂甙与三七皂甙单体 R 有钙通道阻滞作用的报道[11,12]是一致的.

本实验证明 PDS 与 PTS 均有抗自由基损伤作用，且 PDS 比 PTS 的这一作用更强，对临床上更合理地应用人参分组皂苷有一定意义.

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左旋千金藤立定对家兔离体基底动脉、肠系膜动脉和胸主动脉平滑肌的作用

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Effects of *l*-stepholidine on isolated rabbit basilar artery, mesenteric artery, and thoracic aorta

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ABSTRACT *l*-Stepholidine (SPD) has been shown to be effective in treating migraine, but its mechanism is not clear. So the effects of SPD on isolated rabbit basilar artery (BA), mesenteric artery (MA) and thoracic aorta (TA) were studied. The contractions of BA and MA were induced by KCl (10-160 mmol · L^{-1}) and the contraction of TA was caused by 5-HT (0.1-100 μmol · L^{-1}). Ketanserin was used as reference.

SPD (0.1-0.2 mmol · L^{-1}) relaxed the contractions of BA and MA induced by KCl in a non-competitive manner with pD_2' 3.4 ± 0.3 and 4.0 ± 0.3, respectively. SPD had no selectivity in BA and MA. SPD also inhibited the contraction of TA induced by 5-HT with pA_2 9.7 ± 2.0 and pD_2' 5.4 ± 0.6, which showed a dual of both competetive and noncompetetive antagonisms.

These results suggested that SPD had a blockade effect on the calcium channel and 5-HT_2 receptors.

KEY WORDS stepholidine; basilar artery; mesenteric arteries; thoracic aorta; drug dose-response relationship; ketanserin

提要 左旋千金藤立定(SPD)可非竞争性地松弛 KCl 引起的家兔离体基底动脉(BA)和肠系膜动脉(MA)的收缩，pD_2'分别为 3.4 ± 0.3 和 4.0 ± 0.3，提示有较弱的钙拮抗作用. SPD 亦能松弛 5-HT 引起的家兔离体胸主动脉(TA)的收缩，其特点为既有竞争性又有非竞争性的二重拮抗作用，pA_2 = 9.7 ± 2.0; pD_2' = 5.4 ± 0.6，提示 SPD 有 5-HT_2 受体拮抗作用.

关键词 左旋千金藤立定；基底动脉；肠系膜动脉；胸主动脉；药物剂量-效应关系；ketanserin

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