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地塞米松对培养大鼠搏动心肌细胞感染 Cocksackie B-2 病毒作用的电生理观察

袁卫龙、陈灏珠、杨英珍、杨学义、金佩英、林佑善 (上海医科大学中山医院,

上海市心血管病研究所, 上海 200032, 中国)

周泰生 (中国科学院上海生理研究所, 上海 200031, 中国)

Influence of dexamethasone on electrical activities in cultured rat beating myocardial cells infected with Cocksackie B-2 virus

YUAN Wei-Long, CHEN Hao-Zhu, YANG Ying-Zhen, YANG Xue-Yi, LIN You-Shan, JIN Pei-Ying (Shanghai Institute of Cardiovascular Diseases, Zhong-Shan Hospital, Shanghai Medical University, Shanghai 200032, China), ZHOU Tai-Sheng (Shanghai Institute of Physiology, Chinese Academy of Sciences, Shanghai 200031, China)

ABSTRACT The effects of dexamethasone (Dex) on electrical activities in cultured rat beating myocardial cells infected with 100 TCID₅₀ Cocksackie B-2 virus (CB₂V) was evaluated by conventional intracellular microelectrode technique. The frequency began to increase, the beating % decreased, and multiform arrhythmias were shown in the infected group 24 h post-challenge. Meanwhile, the cytopathic effect (CPE) appeared rapidly from 1+ to 3+. In the infected and Dex-treated group, the beating % was higher and the arrhythmias and CPE were less than in the infected group at the same intervals. The numbers of non-beating cells increased parallel to the incubation time in the infected group. Decreases of maximal diastolic potential (MDP), maximal upstroke rate (V_{max}), overshoot (OS) and action potential amplitude (APA), and abbreviation of action potential

duration (APD₅₀ and APD₁₀₀) in infected and Dex-treated group were less than those in control group during 24-96 h post-challenge. Premature beats, tachycardia, bradycardia and fibrillation occurred in the early stages after infection. It is surmised that steroids can probably save the lives of patients with severe myocarditis if Dex was supplemented.

KEY WORDS dexamethasone; cultured cells; Cocksackie B viruses; action potentials; myocardium

摘要 Cocksackie B-2 病毒感染培养搏动心肌细胞加地塞米松后 24-96 h 能使感染心肌细胞停搏%, 节律, 最大舒张电位负值, 动作电位振幅和超射, 最大上升速率, 动作电位时程等参数异常大部分有所改善, 提示重症病毒性心肌炎患者宜在发病早期应用较大剂量肾上腺皮质激素, 结合一般支持疗法, 可能有挽救生命的意义。

关键词 地塞米松; 培养细胞; 柯萨奇 B 病毒; 动作电位; 心肌

重症心律失常常为急性病毒性心肌炎的猝死因素, 对于这类患者是否使用肾上腺皮质激素的问题, 临床上至今存有不同的看法^(1,2)。实验室研究也未能得出一致的结论⁽³⁾。我们曾发现地塞米松 (dexamethasone, Dex) 能使 Cocksackie B-2 病毒 (CB₂V) 感染培养搏动大鼠心肌细胞的搏动% 增多, 细胞病变 (CPE) 及

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超微结构损害减轻,病毒滴度降低,天冬氨酸氨基转移酶释放减少⁽⁴⁾。在此基础上,我们应用常规细胞内微电极技术观察了 Dex 对培养大鼠搏动心肌细胞感染 CB₂V 后电活动改变的影响,可能更有助于决定今后在重症病毒性心肌炎患者中的激素应用问题。

MATERIALS AND METHODS

出生 1-3 d 的 Sprague-Dawley 大鼠心室肌,用 0.1% 胰蛋白酶溶液分次消化细胞,细胞制备和培养按本所常规方法进行⁽⁵⁾,生长液用含 20% 小牛血清的 MEM Eagle's 液, CB₂V (ATCCVR-29) 在 HEP-2 细胞(人喉癌细胞)中传代 3 次,在大鼠心肌细胞中测 50% 组织感染率(TCID-50),地塞米松 5 mg/ml(上海信谊制药厂,以双蒸水为溶媒)。

细胞悬液贴壁 2h 以去除部分类内皮细胞后,将未贴壁的心肌细胞分装为 $4 \times 10^6 / (5 \text{ ml} \cdot \text{瓶})$ 37℃ 孵育 24 h 后心肌细胞以 80-100 bpm 规则地搏动,弃上清液,病毒组和病毒加 Dex 组每瓶细胞各加 2 ml 内含 100 个 TCID-50 的 CB₂V 生长液,正常对照组各加 2 ml 不含 CB₂V 的生长液,37℃ 孵育 1 h 后,病毒组和正常对照组分别加生长液至每瓶总量为 5 ml。病毒加 Dex 组每瓶加 Dex 125 μg (25 μl),然后加生长液至每瓶总量达 5 ml,置 37℃ 孵育,每天在倒置相差显微镜下观察细胞搏动频率,节律,搏动%和 CPE, CPE 判别标准同前文^(5,6)。在感染病毒后 24, 48, 72 和 96 h 分别取样作电生理学研究。

电位测定 以不加小牛血清的 Eagle's 生长液作循环灌流,流速 3 ml/min,恒温 36-37℃, pH 7.0-7.2,灌流液充以 95% O₂+5% CO₂,流量 0.5 L/min。玻璃微电极尖端直径小于 0.5 μm,充灌 KCl 3 mol/L,阻抗 50 MΩ 左右,从培养瓶中取出长有心肌细胞的盖玻片,放入微浴槽内,玻璃微电极以 45 度角刺入细胞,整个操作都在倒置显微镜下进

行,玻璃微电极在心肌细胞内稳定 10-15 min,搏动频率及各项电活动参数趋于稳定后摄影记录。

RESULTS

细胞搏动%及 CPE 在倒置显微镜下,接种病毒前细胞以 80-100 bpm 自发性搏动,感染病毒后 24h 搏动频率增快,无 CPE, 48h 后搏动幅度变弱,时而心肌细胞搏动不规则,出现 CPE, 72h 后 CPE++~+++,搏动快慢波动增大,强弱不均,约 2/3 细胞停搏, 96h 后成片细胞停搏,视野中仅有少数幸存细胞呈不规则蠕动, CPE+++。病毒加 Dex 组心肌细胞搏动频率波动不大, 48 h 后,搏动%明显高于病毒组,心肌细胞偶有不规则搏动, CPE 亦较轻(+~++),二组的搏动幅度无明显差别。96 h 后,病毒加 Dex 组心肌细胞搏动%和频率均高于病毒组(Tab 1),但光镜下搏动微弱,出现节律失常, CPE 明显加重,正常对照组心肌细胞在整个观察期间呈同步节奏性搏动,无节律失常,无 CPE。

电活动变化 (Tab 1) 病毒组细胞的电活动变化在形态学改变之前出现,感染病毒 24 h 后电位形态上即能记录到搏动频率和节律失常,表现为二、三联律和短阵快速搏动,振幅和形态多变,细胞对玻璃微电极和机械刺激敏感,细胞膜脆弱易被刺穿,微电极尖端易被细胞碎片堵塞,微电极难以稳定在细胞内,随感染时间的延长,上述变化更趋明显。

从感染病毒 24h 开始,最大舒张电位(MDP)负值降低,最大上升速率(V_{\max})减慢,动作电位振幅(APA)和超射(OS)缩小,动作电位时程(APD)缩短,随着感染时间的延长,各项电参数进一步减低,停搏细胞数逐日增加,感染病毒 96 h 后,约 70% 细胞停搏,电活动消失,少数幸存细胞的各项电活动参数均明显低于正常。

病毒加 Dex 组心肌细胞在实验过程中搏

Tab 1. Effects of dexamethasone on electrical activities and beating % in coxsackie virus B-2 infected rat heart cells in culture. Uninfected control (A): 24-96 h, n=20; infected control (B): 24 h, n=25, 48h, n=47, 72 h, n=22, 96 h, n=23; Infected Dex-treated (C): 24h, n=27, 48 h, n=26, 72 h, n=32, 96 h, n=23. $\bar{x} \pm SD$. *P>0.05, **P<0.05, *P<0.01 vs A. +P>0.05, **P<0.05, ***P<0.01 vs C.**

Parameter	Group	24 h	48 h	72 h	96 h
MDP (-mV)	A	59 ± 16	56 ± 11	51 ± 8	50 ± 6
	B	33 ± 9*** +	30 ± 10*** +	30 ± 10*** +	25 ± 10*** +
	C	47 ± 6***	43 ± 9***	40 ± 9***	46 ± 12*
APA (mV)	A	75 ± 10	75 ± 13	69 ± 10	71 ± 12
	B	39 ± 13*** +	36 ± 15*** +	35 ± 11*** +	24 ± 9*** +
	C	61 ± 14**	50 ± 14**	47 ± 9**	50 ± 15*
OS (mV)	A	16 ± 11	19 ± 9	18 ± 9	21 ± 12
	B	8 ± 9*** +	6 ± 8*** +	6 ± 6*** +	2 ± 4*** +
	C	14 ± 9*	8 ± 8**	6 ± 7**	4 ± 6**
V _{max} (V/s)	A	12 ± 3	11 ± 3	12 ± 3	11 ± 4
	B	2.9 ± 2*** +	2.1 ± 2*** +	2.1 ± 1*** +	1.4 ± 1*** +
	C	8 ± 3**	5 ± 3**	4 ± 2**	4 ± 3**
APD ₅₀ (ms)	A	82 ± 12	76 ± 11	79 ± 11	81 ± 11
	B	77 ± 24*	66 ± 18** +	61 ± 18*** +	57 ± 12*** +
	C	78 ± 10*	72 ± 8*	78 ± 10*	72 ± 9**
APD ₁₀₀ (ms)	A	188 ± 21	178 ± 14	178 ± 25	173 ± 28
	B	161 ± 41*** +	133 ± 34*** +	132 ± 25*** +	130 ± 37*** +
	C	145 ± 21***	143 ± 19**	158 ± 30**	138 ± 26**
bpm	A	118 ± 55	162 ± 42	148 ± 46	166 ± 52
	B	151 ± 47*** +	163 ± 65* +	141 ± 68* +	116 ± 61*** +
	C	104 ± 36*	131 ± 50**	125 ± 50**	134 ± 46**
Beating(%)	A	98 ± 4	98 ± 9	94 ± 11	90 ± 11
	B	76 ± 20*** +	61 ± 20*** +	38 ± 25*** +	28 ± 19*** +
	C	75 ± 12***	74 ± 11***	61 ± 15***	50 ± 0***

MDP: maximal diastolic potential; APA: action potential amplitude; OS: overshoot; V_{max}: maximal upstroke rate; APD: action potential duration.

动频率和节律较为恒定, 电位幅度和形状大致正常, 心肌细胞对微电极穿刺的反应和微电极稳定在细胞内的时间与正常对照组相仿, 在感染病毒 24h 后, 各项电活动参数大多数低于正常对照组, 但显然高于病毒组(Fig 1). 从感染病毒 24-96h, 病毒加 Dex 组心肌细胞电活动参数逐渐降低, 但其间差异无统计学意义. 96h 后, CPE 明显加重, 成片细胞死亡, 电活动消失. 正常对照组心肌细胞在实验期间电活动参数稳定, 正常心肌细胞加 Dex 后的电活动参数与正常对照组细胞相仿(图略). 本文中各组细胞数均为 10 次实验的累计数. 总实验共用 200 只大鼠心脏, 各组 50 瓶细胞.

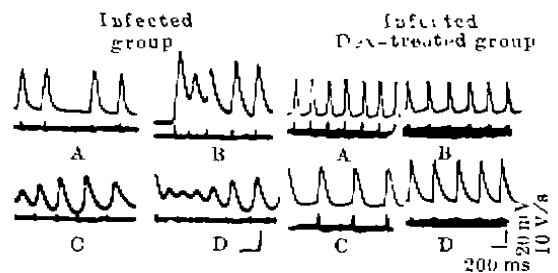


Fig 1. Action potentials (upper tracing) and maximal upstroke rate (lower tracing) of cultured rat beating heart cells in infected group and infected, dexamethasone treated group. A) 24 h; B) 48 h; C) 72 h; D) 96 h.

DISCUSSION

前文已述及心肌细胞感染病毒后能记录到明显电生理参数异常及心律改变⁽⁷⁾，与临床上急性病毒性心肌炎患者中出现的心律失常相似。本实验用地塞米松后能改善 CB₂V 感染培养心肌细胞的心律失常及电生理参数异常，此与激素有稳定细胞膜完整性，使细胞膜流动性和通透性维持正常有关，所用 Dex 剂量是在前文⁽⁴⁾用不同剂量 Dex 实验下所取得的最佳浓度-效应而定。我们在实验中发现所用 Dex 剂量 > 125 μg / (4 × 10⁶ 细胞 · 5 ml)，尤其是 400 μg / (4 × 10⁶ 细胞 · 5 ml) 时，在光镜下对感染 48-72 h 的心肌细胞仍有相似保护作用，但心肌细胞浆内出现空泡，在地塞米松对照组中也有同样毒性反应。小于此剂量，则对感染早期的心肌细胞保护作用较差，用此剂量在实验中未见到对正常心肌细胞电活动有所影响。

目前临床上对病毒性心肌炎尚缺乏特效治疗方法⁽⁸⁾，按本文及前文⁽⁴⁾结果，地塞米松的早期较大剂量应用可望减少心肌组织坏死和致命性心律失常发生，结合一般支持疗法，可能有挽救生命的意义。

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五味子乙素和五味子酚对药物代谢 II 相酶及雌二醇代谢的影响

卢 华、刘耕陶¹ (中国医学科学院药物研究所, 北京 100050, 中国)

Effects of schizandrin B and schisanhenol on drug metabolizing-phase II enzymes and estradiol metabolism

LU Hua, LIU Geng-Tao¹ (Department of Pharmacology, Institute of Materia Medica, Chinese

Academy of Medical Sciences, Beijing 100050, China)

ABSTRACT Intragastric gavage of schizandrin B (Sin B) and schisanhenol (Sal) 200 mg/kg once daily for 3 d significantly increased liver glutathione-S-transferase (GSH-S-T) and microsomal cytochrome P-450 in mice and rats. Sin B and Sal antagonized the increase of uterus weight induced by sc estradiol in

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¹To whom correspondence should be addressed.