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电休克对大鼠空间记忆及海马 p-ERK 活性的影响 *

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摘要 目的:观察电休克对大鼠空间记忆和海马磷酸化细胞外调节蛋白激酶(p-ERK)活性的影响。**方法:**大鼠随机分为电休克组和伪电休克组,每组 12 只。电休克组每天给予电痉挛刺激,伪电休克组每天给予假电痉挛刺激,共 10 天;第 11 天用水迷宫检测各组大鼠的空间学习记忆,然后每组大鼠再随机分为两组,每组 6 只。一组于学习后 1 小时处死取海马用 Western blot 法检测 p-ERK 活性,另一组于 48 小时后行水迷宫空间位置探寻实验检测大鼠的存储记忆。**结果:**电休克组的潜伏期显著长于伪电休克组($P<0.01$)。电休克组在隐匿平台周围区域 / 相反区域的搜寻时间无显著性差异($P>0.05$);伪电休克组在隐匿平台周围区域 / 相反区域的搜寻时间有显著性差异($P<0.05$)。电休克组海马 p-ERK 活性较伪电休克组显著下降($P<0.01$)。**结论:**电休克可导致大鼠显著空间记忆障碍,海马 p-ERK 活性的降低可能是其机制之一。

关键词:电休克;空间记忆;磷酸化细胞外调节蛋白激酶

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The Effects of ECT on Spatial Memory and p-ERK of Rats*

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ABSTRACT Objective: To investigate the effects of Electroshock therapy (ECT) on spatial memory and phosphorylated extracellular-signal related kinase (p-ERK) of rats. **Methods:** Rats were randomized into ECT group and sham ECT group. Each group consisted of 12 rats. ECT group received true ECT and sham group received sham ECT. The true ECT or sham ECT was administered once each day, ten days in a whole. At the 11th day the rats were trained and tested in Morris water maze localization navigation experiment. Then each group was randomized into two groups again and each group had 6 rats. One group was decapitated 1 hour after training. Their hippocampus was taken quickly for detecting p-ERK expression by western blot. The other group received probe tests of Morris water maze 48 hours after training. **Results:** The latency of ECT group was significantly longer than that of sham ECT group ($P<0.01$). There was no significant difference in the search time around the hidden platform and the time in the opposite area in ECT group ($P>0.05$). But there was significant difference between the search time around the hidden platform and the time in the opposite area in sham ECT group ($P<0.05$). The p-ERK1/2 level of ECT group was significantly lower than that of sham ECT group ($P<0.01$). **Conclusions:** ECT can result in significant amnesia for spatial memory. P-ERK may play an important role in the development of spatial memory impairment caused by ECT.

Key words: Electroshock therapy; Spatial memory; Phosphorylated extracellular-signal related kinase

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前言

电休克治疗(Electroshock therapy, ECT),又称电痉挛治疗(Electroconvulsive therapy),是用短暂的电刺激作用于脑部引起短暂的癫痫样放电,从而治疗疾病的一种精神科物理治疗方法。电休克治疗是迄今为止最有效的抗抑郁治疗方法,当前在临床上常用于药物抵抗的抑郁患者及有自杀观念的重度抑郁患者[1-3]。改良电休克治疗(Modified Electroconvulsive Therapy, MECT)是先注射适量的肌肉松弛剂,然后再通电,从而达到无抽搐发作。改良电休克较普通电休克副反应更小,对年老体弱和伴有其它躯体疾病的患者均可酌情施治。但接受电休克治疗的患者,即使是改良电休克治疗仍常伴有认知功能副反应,最多见的是顺行性和逆行性遗忘,严重阻碍了电休克治疗的应用[4,5]。本实验通过观察电休克对大鼠空间记忆的影响及海马磷酸化细胞外调节蛋白激酶(phosphorylated extracellular-signal related kinase, p-ERK)活性的变化,以探索电休克治疗所致记忆障碍的分子机制。

1 材料和方法

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1.1 材料及仪器

Morris 水迷宫记录分析系统购自荷兰 Noldus 公司。电痉挛刺激仪(YA-7 型)购自陕西医疗器械公司。辣根过氧化物酶标记的羊抗兔二抗购自华美生物公司,一抗(兔抗鼠 ERK2 多克隆抗体、兔抗人 p-ERK 多克隆抗体)购自美国 SANTA CRUZ 公司。

1.2 实验分组

Sprague-Dawley 大鼠购自武汉大学医学院动物实验中心,共 24 只,为清洁级健康成年雄性大鼠,体重 180 g-250 g。实验前一周,给予动物自然光照,室温 20-25 ℃,自由摄食和饮水。大鼠随机分为电休克组和伪电休克组(随机数字表法),每组 12 只。

1.3 电休克大鼠模型的制备和对照的设立

轻轻抓取固定大鼠,电休克组给予 50 mA,0.5 秒强度的电休克,伪电休克组大鼠给予伪电休克刺激,即只做刺激动作但不通电。以后每天重复该操作,共 10 天。

1.4 Morris 水迷宫实验检测大鼠空间记忆

在实验的第 11 天,各组大鼠进行水迷宫定位航行实验,检测大鼠的空间学习记忆能力。水迷宫为圆形水池,池壁黑色,底面直径 183 cm,高度 58 cm,在水池边缘做上记号,将水池分为四个象限;站台为黑色圆形柱状体,直径 20 cm,高 25 cm,将站台置于某一象限的中间。向水池里注入清水,水温 24± 2 ℃,水中加入墨汁,使池水成为不透明的黑色,水平面高出站台 2 cm,使大鼠不能看见水面下的站台。从四个不同象限池壁的中点将大鼠面向池壁随机放入水中。计算机采集大鼠找到站台的时间,即为隐匿平台逃逸潜伏期,潜伏期反映大鼠学习记忆能力。以 60 秒为时限,若大鼠在 60 秒内仍未找到站台,则由实验人员引导其找到站台,该大鼠的潜伏期则记为 60 秒。所有大鼠均训练 2 轮,每轮之间休息 2 分钟;每轮大鼠训练 5 次,每次训练后让大鼠在站台上休息 20 秒,然后再进行下一次训练。

所有大鼠两轮实验完成后,再将各组大鼠随机分为 p-ERK 活性检测组和空间位置探寻实验组,每组 6 只大鼠。p-ERK 活性检测组大鼠于定位航行实验结束后 1 小时处死,快速取海马,Western blot 检测 p-ERK 活性;另一组大鼠于 48 小时后行水迷宫空间位置探寻实验。探寻实验的方法:移除水中的站台,随机取某一象限池壁的中点为入水点,记录大鼠在 90 秒内游泳时的运动轨迹和相应参数。空间位置探寻实验检测大鼠存储记忆,其主要参数为在原站台周围区域的搜寻时间和大鼠在原站台相反区域的搜寻时间。

1.5 Western blot 检测 p-ERK 活性

麻醉大鼠,然后断头,将大鼠的头部放于冰盘上,分离海马组织,用锡箔纸包裹编号,迅速置入 -80 ℃冰箱储存备用。检测时取出大鼠海马组织,加入脑组织裂解液,冰上匀浆,用低温超高速离心机 4 ℃,12000 rpm,离心 10 分钟,取上清即为总蛋白,进行蛋白定量,放于 -80 ℃冰箱储存待用。制备分离胶,室温放置 30 min,无水乙醇冲洗,滤纸吸净残留液体,灌注浓缩胶,约 30 min 后浓缩胶凝固,用双蒸水及无水乙醇清洗加样孔,然后将胶放入电泳槽中,用微量加样器向加样孔中缓慢加入样品,SDS-PAGE 电泳分离样品。电泳结束后,剥下有目的蛋白的分离胶,用去离子水漂洗,盖于滤纸上,然后将硝酸纤维膜盖于胶上,最后硝酸纤维膜上盖两张滤纸,排除气泡进行电转移。转完后将膜移至含有丽春红染液的平皿中染色,染色完成后将膜用 TBS 浸湿,移至含 5 %脱脂奶粉的 TBS-T 平皿中,平放在摇床上,室温下摇动封闭 2 h。加入 1:200 的一抗溶液(兔抗鼠 ERK2 多克隆抗体,兔抗人 p-ERK 多克隆抗体),4 ℃孵育过夜后,弃去一抗溶液,加入 1:50 的二抗稀释液,室温下反应 1 小时。然后在暗室加入 DAB 底物液显色,待看到明显的蛋白发光带,将膜移至保鲜膜上包好,放至 X 光片上曝光,然后显影、定影、晾干。用凝胶图象处理系统分析各样本灰度值,p-ERK 与 ERK2 蛋白表达水平灰度值的比为 P-ERK 的相对表达水平,也即 p-ERK 的活性。

1.6 统计学处理

实验数据以均数± 标准差($\bar{x} \pm s$)表示,应用 SPSS 11.5 统计软件进行处理。两样本均数比较用 t 检验,组内比较使用配对 t 检验。P<0.05 认为差异有统计学意义。

2 结果

2.1 大鼠游泳速度

电休克组和伪电休克组的游泳速度无显著性差异(P>0.05),表明电休克不影响大鼠运动能力(见表 1)。

表 1 大鼠游泳速度及定位航行实验潜伏期的比较($\bar{x} \pm s$,n=12)

Table 1 Comparison of swimming speed and latency of Morris water maze localization navigation experiment ($\bar{x} \pm s$, n=12)

	Sham ECT group	ECT group	t	P
Swimming speed (m/s)	0.27± 0.05	0.31± 0.06	1.148	P>0.05
Latency of the first cycle (s)	15.22± 2.23	36.10± 5.02	3.743	P<0.01
Latency of the second cycle (s)	9.08± 1.25	30.28± 4.16	4.014	P<0.01

2.2 大鼠隐匿平台逃逸潜伏期

在第一轮定位航行实验及第二轮定位航行实验中,电休克组的潜伏期均显著长于伪电休克对照组(P< 0.01)(见表 1)。

2.3 大鼠空间位置探寻实验搜寻时间

电休克组在隐匿平台周围区域 / 相反区域的搜寻时间无显著性差异(P>> 0.05)。伪电休克对照组在隐匿平台周围区域的搜寻时间显著长于其在隐匿平台相反区域的搜寻时间(P< 0.05)(见表 2)。

2.4 大鼠海马 p-ERK 活性的变化

ERK 包括两个高度同源的亚类 ERK1 和 ERK2,p-ERK 为 ERK 的磷酸化形式,也是 ERK 的活化形式。Western blot 检测显示 p-ERK 蛋白的表达可见双条带,分别对应 p-ERK1、

表 2 大鼠空间位置探寻实验搜寻时间的比较($\bar{x}\pm s$,n=6)

Table 2 Comparison of search time of Morris water maze probe trains ($\bar{x}\pm s$, n=6)

	Search time around hidden platform (s)	Search time of in opposite area of hidden platform (s)	t	P
sham ECT group	15.23± 3.28	6.08± 1.72	3.263	P<0.05
ECT group	11.22± 2.76	9.87± 2.03	1.023	P>0.05

p-ERK2 两种异构体。电休克组大鼠海马 p-ERK 活性显著低于伪电休克组大鼠(P< 0.01),ERK2 则无显著性变化(P> 0.05)(见图 1)。

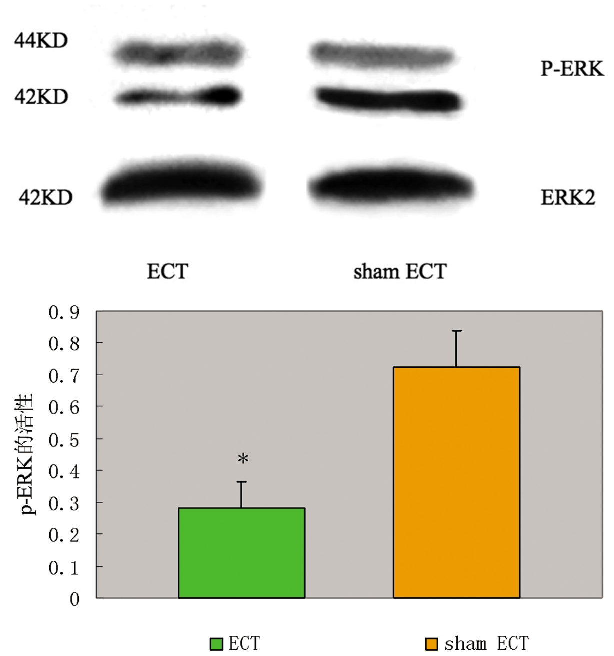

图 1 大鼠海马 p-ERK 活性的变化

Fig.1 p-ERK activity of rat hippocampus

注:ECT 表示电休克组,sham ECT 表示伪电休克对照组。与伪电休克组比较,*P<0.01。

Note: ECT means ECT group; sham ECT means sham ECT group.*P<0.01, compared with sham ECT group.

3 讨论

Morris 水迷宫实验可以研究动物对空间定位任务的信息采集和记忆能力[5],该任务的完成与海马、纹状体、基底前脑、小脑、新皮质等脑区的结构及功能密切相关 [6-9],因此我们采用 Morris 水迷宫实验来研究电休克对动物空间记忆的影响。本实验中电休克组与伪电休克组大鼠的游泳速度无明显差异,表明电痉挛刺激不影响运动能力。电休克组的潜伏期显著长于伪电休克组(P< 0.01),表明电痉挛刺激导致了大鼠显著的学习记忆障碍。伪电休克组大鼠在隐匿平台周围区域的搜寻时间显著长于其在隐匿平台相反区域的搜寻时间(P< 0.05),显示该组大鼠形成了稳定的空间记忆,表现出对学习训练中隐匿平台位置的偏好。而电休克组大鼠在隐匿平台周围区域的搜寻时间与其在隐匿平台相反区域的搜寻时间无显著性差异(P>0.05),表明电休克组大鼠无明显的位置偏好,提示电痉挛刺激对大鼠空间记忆的保持造成破坏。这与 Andrade C,Yao ZH 等的研究结果一致[10,11]。

丝裂素活化蛋白激酶 (mitogen-activated protein kinase, MAPK)超家族是已知信号转导通路中最主要的分子之一,至少包括 ERK1/2、JNK/SAPK、p38、ERK5、ERK7 和 MOK 六个亚类[12,13]。MAPKs 的非磷酸化形式广泛地分布于中枢神经系统各部位[14,15],激活多种 G 蛋白偶联受体导致 MAPK 信号通路的转导[16,17]。研究已证实神经元 MAPK 级联在突触可塑性和记忆形成中具有重要作用 [15,18]。细胞外调节蛋白激酶(Extracellular-signal Related Kinase,ERK1/2) 是目前研究最多的一种 MAPK,p-ERK 为 ERK1/2 的磷酸化形式,也是 ERK 的活化形式。ERK 信号通路与长时程动作单位(long-term potentiation, LTP)的形成以及记忆功能有重要联系。Kelleher 等利用转基因技术研究突触可塑性的调控机制,结果发现,ERK 直接参与树突蛋白合成的调控,而树突蛋白的合成是 LTP 形成和记忆功能所必需的[19]。Satoh Y 等则研究了 ERK 基因敲除鼠的学习记忆功能,结果发现 ERK 基因敲除鼠在迷宫实验中发生明显的学习记忆障碍[20],进一步证明 ERK 信号通路在突触可塑性和记忆形成中具有重要作用。本研究结果发现学习记忆后 1 小时,电休克组海马 p-ERK 活性较伪电休克对照组显著下降(P< 0.01),提示电休克降低大鼠海马 ERK 的活性可能是电休克治疗导致空间记忆障碍的分子机制之一。但其确切机理尚有待进一步研究。

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