

· 基础研究 ·

正中神经电刺激对脑外伤昏迷大鼠前额叶皮质 H1 受体表达的影响

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【摘要】 目的 观察正中神经电刺激对脑外伤昏迷大鼠前额叶皮质 H1 受体表达的影响。**方法** 采用随机数字表法将 72 只 SD 大鼠分为空白对照组、假刺激组、刺激组及拮抗剂组。采用经典自由落体撞击法将假刺激组、刺激组及拮抗剂组大鼠制成脑外伤昏迷模型,刺激组大鼠于制模结束后给予正中神经电刺激,拮抗剂组于制模结束后向侧室注射 OXR1 拮抗剂并给予正中神经电刺激,假刺激组实验操作与刺激组相同,但干预期间电流强度为 0。待实验结束 1 h 后采用双盲法评定大鼠意识状态,于实验结束 6 h、12 h 及 24 h 时采用免疫组织化学技术检测各组大鼠前额叶皮质 H1 受体表达情况。**结果** 实验结束 1 h 后空白对照组 18 只大鼠意识状态均为 I 级,刺激组有 13 只大鼠(72.2%)出现翻正反射,拮抗剂组有 9 只(50.0%)、假刺激组有 5 只(27.8%)大鼠出现翻正反射,4 组大鼠意识状态组间差异均具有统计学意义($P<0.05$)。免疫组化检测发现空白对照组、假刺激组、拮抗剂组及刺激组大鼠 H1 受体表达呈现逐渐增强态势($P<0.05$);实验结束 12 h、6 h 及 24 h 时各组大鼠 H1 受体表达在上述时间点呈现逐渐增强趋势,但各相邻时间点间差异无统计学意义($P>0.05$)。**结论** 正中神经电刺激可作为脑外伤后昏迷的一种有效促醒手段,其治疗机制可能与上调前额叶皮质区 H1 受体表达有关,且 Orexin-A 可能在其中发挥调节作用。

【关键词】 H1 受体; Orexin-A; 正中神经电刺激; 脑外伤; 昏迷

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The effects of electrical stimulation on the expression of H1 receptor in the prefrontal cortex of brain injured, comatose rats Du Qing, Feng Zhen. The First Affiliated Hospital of Nanchang University, Nanchang 30006, China
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【Abstract】 Objective To investigate the expression of H1 protein in the prefrontal cortex of comatose rats which have suffered traumatic brain injury (TBI) after electrical stimulation of the median nerve (MNS). **Methods** Seventy-two Sprague-Dawley rats (weighing 250 to 300 g) were randomly divided into a stimulated group (MNS+TBI), an antagonist group (MNS+TBI+OXR1 antagonist), a model group (TBI) and a control group, with 18 rats in each group. Traumatic brain injury was modeled in all of the rats except those of the control group. After the modeling, the stimulated group was given MNS, the antagonist group was provided with MNS and an OXR1 injection, and the model group was given MNS with a current intensity of 0. One hour after the experiment, the consciousness of each rat was evaluated using a double-blind method. Animals were sacrificed at 6, 12 and 24 hours after the intervention and brain tissue was removed. H1 protein expression was examined using immunohistochemistry. **Results** One hour after the experiment, significant differences were observed in the consciousness of the 4 groups, with the 18 rats of the control group on consciousness level one. Thirteen rats in the stimulated group exhibited a righting reflex, compared with 9 in the antagonist group and 5 in the model group. Immunohistochemistry showed that H1 expression was strongest in the stimulated group, followed by the antagonist, control and model groups. The H1 expression was highest at 24 hours after the experiment, followed by that at 6 h and 12 h, but those differences were not statistically significant. **Conclusion** Median nerve electrical stimulation might modulate wakefulness after traumatic brain injury by promoting H1 expression via orexin-A in the prefrontal cortex.

【Key words】 H1 receptor; Orexin-A; Median nerve; Electrical stimulation; Trauma; Brain injury; Coma

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脑外伤昏迷是临床上常见昏迷类型之一,其致残率及死亡率极高而倍受关注。目前正中神经电刺激(median nerve stimulation, MNS)作为一种有效的促进觉醒的方法已得到广泛应用,其作用机制与促进多种神经递质释放有关,但确切机制尚未明确。组胺是一种兴奋性神经递质,包括 H1、H2、H3 及 H4 共 4 种受体,广泛分布于中枢神经系统中^[1],其中 H1 受体高密度表达于涉及睡眠-觉醒功能的脑区,包括皮质(特别是前额叶皮质)、丘脑及边缘系统等^[2]。有文献表明 H1 受体对调控机体觉醒水平具有重要作用^[3]。基于上述背景,本研究拟采用正中神经电刺激治疗外伤性昏迷大鼠,并检测其行为学变化及 H1 受体表达情况,从而探讨正中神经电刺激的相关促醒机制。

材料与方法

一、主要试剂与仪器

抗 H1 抗体(BA1633-1)由武汉博士德有限公司提供,抗 β -actin 单克隆抗体(CW0096)、组织蛋白抽提试剂盒(CWB10)由北京康为世纪生物科技有限公司提供,OXR1 拮抗剂(SB334867)由美国 Tocris Bioscience 公司提供,ZS-BS 数显脑立体定位仪由北京众实迪科技发展有限公司提供,其它重要仪器设备还包括 ES-420 型电刺激仪、5804-R 型低温高速离心机、Mini-PROTEAN 3 型电泳系统、Mini Trans-Blot 转移系统及 RM2015 型切片机等。

二、分组及制模

选取成年无特定病原体级 SD(Sprague-Dawley, SD)大鼠 72 只,体重 250~300 g,由南昌大学实验动物中心协助提供。所有大鼠实验步骤均按照南昌大学动物实验中心规定指南执行,并经过南昌大学伦理委员会批准。实验大鼠均饲养于光照明亮、温度适宜动物房内,期间给予充足食物。采用随机数字表法将上述大鼠分为空白对照组、假刺激组、刺激组及拮抗剂组,每组 18 只。选用经典自由落体撞击法将假刺激组、刺激组及拮抗剂组大鼠制成脑外伤昏迷模型^[4]。具体制模操作如下:实验大鼠经乙醚吸入麻醉后,头顶部消毒,切开头顶部皮肤,钝性分离骨膜、暴露顶骨,在大鼠前凶区域放置一块大小适合的圆形薄铝片。在此区域内将 400 g 圆柱形撞击锤从 40~44 cm 高度沿垂直金属杆自由下落,撞击粘贴于大鼠颅骨上的薄铝片,致其颅骨凹陷性骨折。随后消毒缝合头皮并将大鼠放回笼内,给予保暖措施。采用大鼠经典 6 级意识状态评估法对制模大鼠进行评定,Ⅰ级:大鼠在笼内活动如常;Ⅱ级:大鼠在笼内活动减少;Ⅲ级:大鼠在笼内活动减少并有运动失调表现;Ⅳ级:将大鼠背部朝下放回笼内时能滚动(同时翻正反射存在)但不能站立;Ⅴ级:大

鼠翻正反射消失但肢体对疼痛刺激有回缩反应;Ⅵ级:大鼠对任何刺激均无反应^[5]。如实验大鼠意识状态评级为Ⅴ级或Ⅵ级并且持续 30 min 以上则认为脑外伤昏迷动物模型制模成功。

三、各组大鼠制模后处理

刺激组大鼠待制模结束后,立即用 ES-420 型低频电刺激仪给予正中神经电刺激。在大鼠前肢内侧正中距腕关节 5 mm 处插入 1 根毫针,同时在距腕关节 15 mm 处插入另 1 根毫针作为参照,电刺激参数如下:电刺激频率 30 Hz,电刺激脉宽 0.5 ms,电流强度为 1.0 mA,电刺激时间为 15 min^[6];待刺激结束消毒后返回笼中。假刺激组大鼠电刺激操作同刺激组,但实验期间电流输出强度为 0。拮抗剂组大鼠待制模结束后常规应用抗生素(注射用硫酸庆大霉素)防止术后感染;30 min 后腹腔注射 10% 水合氯醛(按每千克体重 3 ml)进行麻醉,待麻醉剂起效后再次对大鼠头顶部消毒并清晰暴露颅骨,将大鼠头部及四肢以俯卧位形式固定于脑立体定位仪上,在前凶后约 1.0 mm、正中线旁开 1.5 mm 处标记基点,用颅骨钻在标记基点处钻一小孔,采用微量进样器吸入 5 μ l SB334867(将 10 mg/kg 粉末溶解于 60:40 DMSO 中)并垂直注入颅骨表面下方 4.5 mm 处,设置微量泵注射速度为 2.50 μ l/min,注射时间为 2 min,待注射完毕后留针 2~3 min 再退针,随后缝合大鼠头皮、消毒并置于加热垫上,观察 1 h 后给予正中神经电刺激,电刺激方法同刺激组。

四、大鼠意识状态评定及 H1 受体检测

待实验结束 1 h 后采用经典 6 级意识状态评估法(双盲法)再次评定各组大鼠意识状态^[5];于实验结束 6 h、12 h 及 24 h 时每组随机取 6 只大鼠处死,取大鼠前额叶皮质组织,应用免疫组化技术检测 H1 受体表达情况。

五、统计学分析

本研究实验大鼠意识状态分级、H1 受体表达情况均以秩均值表示,采用 SPSS 17.0 版统计学软件包进行 Kruskal-Wallis H 秩和检验, $P<0.05$ 表示差异具有统计学意义。

结 果

一、各组大鼠意识状态分析

于实验完成后 1 h 应用双盲法对各组大鼠进行意识状态评定,发现空白对照组 18 只大鼠在笼内活动如常,其意识状态均为Ⅰ级;假刺激组大鼠造模后出现短暂呼吸暂停、胡须震颤、眼球突出,偶伴四肢抽搐、尿失禁,随后进入昏迷状态;假刺激组 18 只大鼠中意识状态Ⅳ级 5 只,Ⅴ级 7 只,Ⅵ级 6 只;刺激组及拮抗剂组大鼠在给予正中神经电刺激后均出现类似清醒反应,

包括睁眼、前肢抽动、四肢回缩等,其中刺激组 18 只大鼠中意识状态Ⅱ级 1 只,Ⅲ级 7 只,Ⅳ级 5 只,V 级 3 只,Ⅵ级 2 只;拮抗剂组 18 只大鼠中意识状态Ⅲ级 5 只,Ⅳ级 4 只,V 级 3 只,Ⅵ级 6 只。4 组大鼠意识状态评定结果组间差异均具有统计学意义($P<0.05$),具体数据见表 1。

表 1 各组大鼠意识状态分级比较

秩和检验	空白对照组	假刺激组	刺激组	拮抗剂组	总计	<i>P</i> 值
秩均值	9.50	52.75	37.61	46.14	-	0.000
样本数	18	18	18	18	72	

注:4 组大鼠意识状态组间比较,发现组间差异具有统计学意义($P<0.05$)

二、各组大鼠 H1 受体免疫组化检测结果分析

H1 受体在各组大鼠前额叶皮质神经元胞膜上均有表达(见图 1),空白对照组、假刺激组、拮抗剂组及刺激

组 H1 受体表达水平呈递增趋势,组间差异均具有显著性意义($P<0.05$);实验结束后 12 h、6 h、24 h 时各组大鼠 H1 受体表达水平均呈递增趋势,但相邻时间点组内差异均无统计学意义($P>0.05$),具体数据见表 2~3。

表 2 各组大鼠 H1 受体表达比较

秩和检验	空白对照组	假刺激组	刺激组	拮抗剂组	总计	<i>P</i> 值
秩均值	31.39	32.17	48.72	33.72	-	0.011
样本数	18	18	18	18	72	

注:4 组大鼠 H1 受体表达组间比较,发现组间差异具有统计学意义($P<0.05$)

表 3 实验结束后不同时间点各组大鼠 H1 受体表达比较

秩和检验	实验结束 6 h	实验结束 12 h	实验结束 24 h	总计	<i>P</i> 值
秩均值	36.75	34.83	37.92	-	0.838
样本数	24	24	24	72	

注:实验结束后 3 个时间点比较,发现差异无统计学意义($P>0.05$)

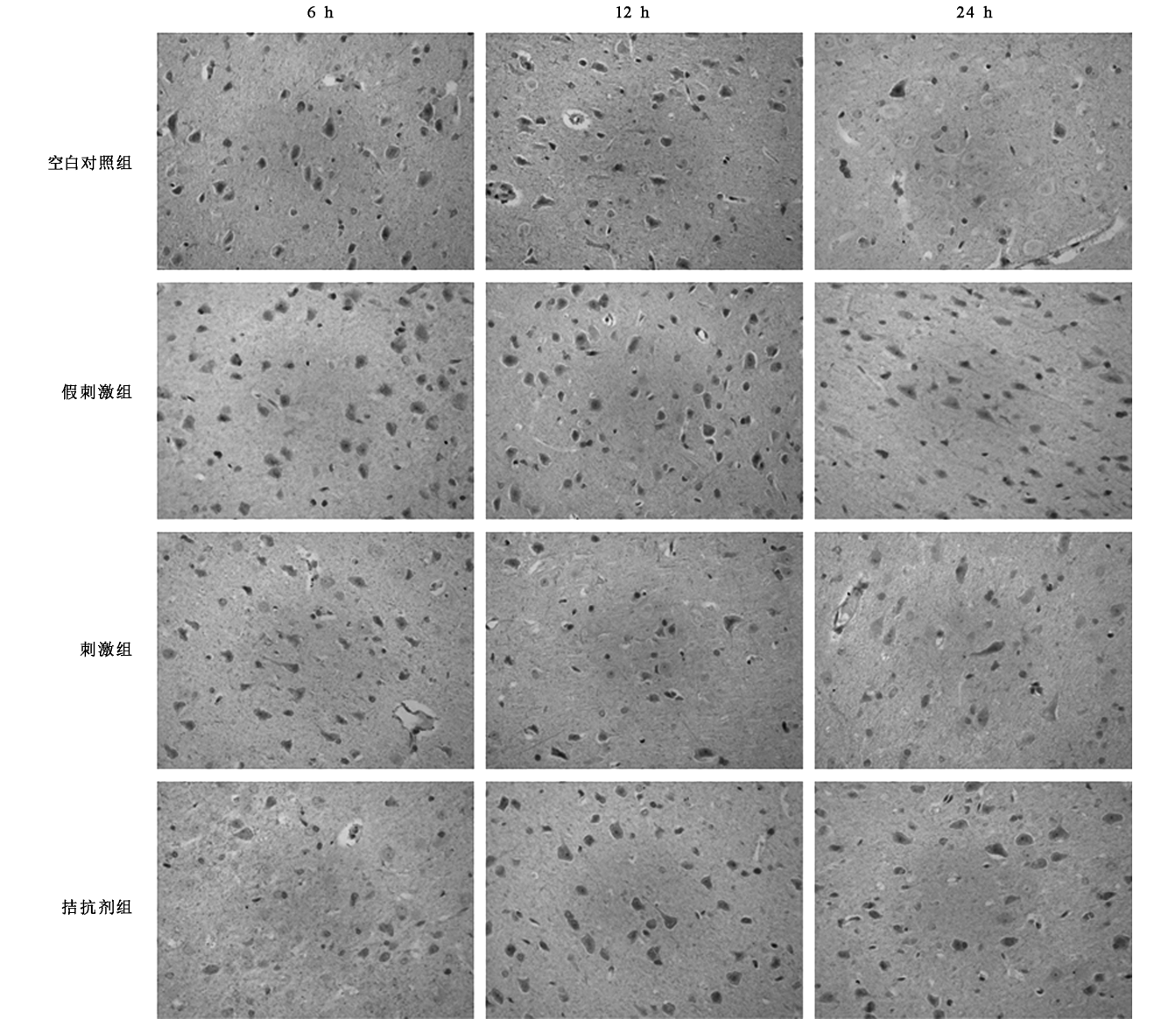


图 1 实验结束后不同时间点各组大鼠前额叶皮质 H1 受体比较(免疫组化染色,×400)

讨 论

昏迷是脑外伤(trumatic brain injury, TBI)严重并发症之一^[7],也是神经康复医学研究领域中的热点及难点。正中神经电刺激由于具有操作简单、创伤小、费用低等优点已成为临床上最常用促醒手段,但其作用机制目前尚未明确。组胺是机体内一种重要的调节觉醒物质,其脑内释放量与机体觉醒水平具有正相关性^[8]。相关研究表明,组胺促醒作用由 H1 受体介导^[9]。Lin 等^[10]发现注射 H1 受体拮抗剂后能促进慢波睡眠,抑制觉醒;Reiner 等^[11]发现 H1 受体拮抗剂能完全阻断皮质神经元去极化,从而降低皮质神经元兴奋性。上述研究均表明 H1 受体是组胺促醒作用的重要调节者。

本研究通过行为学检查发现,实验结束 1 h 后刺激组有 13 只(13/18)大鼠出现翻正反射,假刺激组有 5 只(5/18)大鼠出现翻正反射,组间差异具有统计学意义($P < 0.05$),表明正中神经电刺激可促进脑外伤昏迷大鼠苏醒,与钟颖君等^[12-14]研究结果基本一致。免疫组化染色结果显示刺激组 H1 受体表达显著强于空白对照组及假刺激组,组间差异具有统计学意义($P < 0.05$),提示正中神经电刺激可能通过上调 H1 受体水平促进昏迷大鼠觉醒。假刺激组 H1 受体含量明显高于空白对照组,组间差异具有统计学意义($P < 0.05$),考虑可能与大鼠脑外伤后应激反应有关。上述结果均提示正中神经电刺激治疗脑外伤后昏迷大鼠与上调 H1 受体水平有关。

目前研究发现,在中枢神经系统内,Orexin 神经元特异性投射大多数重要脑区,包括前额叶皮质及组胺能结节乳头体等;相关文献表明组胺神经元高密度投射于前额叶皮质,且参与机体睡眠-觉醒调节^[15]。由此推测,组胺神经元与 Orexin 神经元间似乎有着非同寻常的相互投射关系。Eriksson 等^[16]证实 Orexins 神经元能强烈兴奋结节乳头体,从而发挥促醒作用;Hong 等^[17]也随后证实向侧脑室注入 Orexin-A,能以剂量依赖方式促进前额叶皮质释放组胺;Huang 等^[18]为检测 Orexin-A 对睡眠-觉醒的调节作用是否与 H1 表达有关,发现向侧脑室注射 Orexin-A 能减少 H1 受体基因敲除老鼠觉醒发生次数。本研究向拮抗剂组大鼠侧脑室内注入 OXR1 拮抗剂 SB334867 以阻断 Orexin-A 表达,再给予正中神经电刺激治疗,通过行为学观察发现有 9 只(9/18)大鼠出现意识觉醒状态,仅次于刺激组清醒大鼠数量;免疫组化染色检查显示拮抗剂组大鼠前额叶皮质区 H1 受体表达明显下降,与刺激组间差异具有统计学意义($P < 0.05$),提示 Orexin-A 对 H1 受体表达水平具有调控作用。

综上所述,本研究结果初步证实,正中神经电刺激对脑外伤昏迷大鼠具有促醒作用,激活 H1 受体可能是其重要促醒作用机制之一,Orexin-A 对大鼠 H1 受体表达具有调控作用。

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Acupuncture versus intravenous morphine for acute pain

BACKGROUND AND OBJECTIVE While acupuncture has been introduced to a number of health systems throughout the world, its use in the (ED) is rare. This study evaluated the efficacy and safety of acupuncture compared with that of morphine for adults presenting to the ED with acute pain.

METHODS This prospective, randomized, non-blinded trial included adult patients presenting to the ED of a university hospital in Tunisia with acute pain syndromes. All patients reported a pain intensity of at least 40 on a 100-point visual analogue scale (VAS). In the group randomized to receive acupuncture ($n=150$), protocols were selected from a pool of predetermined acupuncture points for each condition. Those randomized to the morphine group ($n=150$) received IV morphine, titrated from an initial dose of 0.1 mg per kilogram, adding a dose of 0.05 mg per kilogram every five minutes, until reaching sufficient pain relief, to a maximum of 15 mg. The primary outcome measure was pain severity at baseline and five, 10, 20, 30, 45, and 60 minutes.

RESULTS Success, defined as a reduction of at least 50% in pain severity from baseline, was achieved in 92% of the acupuncture group and 78% of the morphine group ($P<0.01$). Pain resolution time averaged 16 minutes in the acupuncture group and 28 minutes in the morphine group ($P<0.01$). Minor adverse events were experienced by 56.6% of those in the morphine group and 2.6% in the acupuncture group.

CONCLUSION This randomized trial of patients presenting with acute pain in the emergency department found that acupuncture could provide better and quicker relief than could IV morphine.

【摘自:Grissa MH, Baccouche H, Boubaker H, et al. Acupuncture versus intravenous morphine in the management of acute pain in the e.d. *Am J Emerg Med*, 2016, 11. 34 (11) : 2112-2116.】

Shockwave therapy versus botox for plantar fasciitis

BACKGROUND AND OBJECTIVE Plantar fasciitis (PF) is very common in the general population, often persisting for many months. As extracorporeal shockwave therapy (ESWT) has been used in the management of tendinopathies and botulinum toxin A (BoNT-A) has been used to treat pain, this study compared the effects of those two interventions for the treatment of PF.

METHODS This open label, prospective, randomized study included patients with PF who had not responded to physiotherapy and electrotherapy. The participants were randomly assigned to receive either ESWT, focused at the area of maximum tenderness for 15 minutes per session, or 100 units of BoNT-A, with injections divided between the insertion of the plantar fascia in the calcaneus and the area of maximal tenderness. The subjects were assessed for pain in the affected foot on a 10-point visual analogue scale (VAS), when taking the first steps in the morning, during daily activity, and while performing exercises. Each patient was also assessed with the Quality of Life Health Status Questionnaire.

RESULTS Data for 72 patients were included in the analysis. The median pain score when taking the first steps in the morning was significantly better in the ESWT group than in the BoNT-A group ($P=0.009$). Better improvement was also noted in the ESWT group than in the BoNT-A group on the Roles and Maudsley Scale of Pain between the first and second visit, as well as in the percentage of patients who noted improvement in pain on at least one of three modalities of VAS pain scores ($P=0.006$ and $P=0.029$, respectively). A regression analysis revealed that ESWT and low body weight were independently associated with improvement in pain.

CONCLUSION This study of patients with recalcitrant plantar fasciitis found that shockwave therapy was superior to botulinum toxin A for reducing pain.

【摘自:Roca B, Mendoza MA, Roca M. Comparison of extracorporeal shockwave therapy with botulinum toxin type a in the treatment of plantar fasciitis. *Disabil Rehabil*, 2016, 10. 38(21) : 2114-2121.】