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小剂量氯胺酮抑制慢性神经痛大鼠脊髓背角 P2X₄受体表达

周双琼¹, 鄢建勤^{2*}, 石学银¹, 袁红斌¹, 谢乐斯³

1. 第二军医大学长征医院麻醉科, 上海 200433

2. 中南大学湘雅医院麻醉科, 长沙 410078

3. 中南大学湘雅医学院人体解剖与神经生物学系, 长沙 410013

[摘要] **目的:** 观察小剂量氯胺酮腹腔注射对慢性坐骨神经收缩损伤 (CCI) 大鼠的镇痛效应及其对大鼠脊髓背角 P2X₄受体表达的影响, 探讨其可能的镇痛机制。 **方法:** 24只SD大鼠随机分为假手术组、CCI组及氯胺酮治疗组 ($n=8$)。CCI组及氯胺酮治疗组大鼠均制备慢性坐骨神经痛 CCI模型; 术后3d测定热缩足反射潜伏期 (thermal withdrawal latency, TWL) 确定痛觉过敏形成后, 氯胺酮治疗组大鼠腹腔注射小剂量氯胺酮 ($10 \text{ mg} \cdot \text{kg}^{-1}$), CCI组腹腔注射等量的生理盐水, 给药至术后7d。假手术组大鼠单纯坐骨神经暴露, 不用肠线结扎, 也不给药治疗。分别于术前1d、术后1、3、7d用热辐射法测定TWL; 术后7d用免疫组织化学法检测大鼠腰段脊髓 P2X₄受体的表达。 **结果:** 术前1d, 3组大鼠TWL无统计学差异; 假手术组术后术侧TWL轻度下降, 但与术前相比无统计学差异。与术前、CCI组及假手术组相比, 氯胺酮治疗组术后3d始TWL呈进行性下降, 以术后7d为基 ($P<0.05$); 术后7d氯胺酮治疗组TWL较CCI组显著升高 ($P<0.05$), 但仍低于假手术组 ($P<0.05$)。与假手术组相比, CCI组及氯胺酮治疗组大鼠术侧脊髓背角 P2X₄受体表达显著增加 ($P<0.01$); 氯胺酮治疗组 P2X₄受体表达明显少于CCI组 ($P<0.05$)。 **结论:** 小剂量氯胺酮腹腔注射可部分缓解慢性神经痛大鼠的痛觉过敏症状, 可能部分与其直接或者间接抑制脊髓背角 P2X₄受体表达有关。

[关键词] 神经病理痛; P2X₄受体; 氯胺酮; 小神经胶质细胞

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Low-dose ketamine inhibits expression of P2X₄ receptor in spinal dorsal horn of rats with chronic neuropathic pain

ZHOU Shuang-qiong¹, YAN Jian-qin^{2*}, SHI Xue-yin¹, YUAN Hong-bin¹, XIE Le-si³

1. Department of Anesthesiology, Changzheng Hospital, Second Military Medical University, Shanghai 200003, China

2. Department of Anesthesiology, Xiangya Hospital, Central South University, Changsha 410078

3. Department of Anatomy and Neurobiology, Xiangya School of Medicine, Central South University, Changsha 410013

[ABSTRACT] **Objective:** To observe the effect intraperitoneal injection of low dose ketamine on thermal hyperalgesia and expression of P2X₄ receptor in spinal dorsal horn of rats with chronic constriction injury (CCI) of sciatic nerve, and to explore the potential role of P2X₄ receptor in the neuropathic pain. **Methods:** Totally 24 Sprague-Dawley rats were randomly divided into 3 groups ($n=8$): group S (sham group), group C: CCI + normal saline; and group K: CCI + ketamine ($10 \text{ mg} \cdot \text{kg}^{-1}$). Rat CCI model was used in the latter 2 groups. Three days after operation the thermal withdrawal latency (TWL) was determined to confirm the thermal hyperalgesia. Rats in group K were given low dose of ketamine ($10 \text{ mg} \cdot \text{kg}^{-1}$) and those in group C were given the same volume of normal saline for 7 days after operation. Animals in group S only had sciatic nerve exposed, with no ligation or drugs. TWL was determined 1 day before and 1, 3, 7 days after the operation. The expression of P2X₄ receptor was assessed 7 days after the operation using immunohistochemistry. **Results:** The TWL values were similar between the 3 groups before operation. The value in group S was slightly decreased after operation compared with before operation. Compared with the pre-operation, group S, and group C, the TWL value of group K began to gradually increase 3 days after operation till day 7 after operation ($P<0.05$). On day 7 after operation, the TWL value was significantly higher than group C ($P<0.05$), but was still lower than that in group S ($P<0.05$). Immunohistochemistry showed that the expression of P2X₄ receptor in group C, K were significantly higher than that of group S ($P<0.01$), and the expression in group K was significantly lower than that in group C ($P<0.05$). **Conclusion:** Intraperitoneal injection of ketamine can partly relieve the thermal hyperalgesia in rats with CCI of

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[作者简介] 周双琼, 硕士, 助教、住院医师. E-mail: zsq628628@yahoo.com.cn

* 通讯作者 (Corresponding author). Tel: 0731-4327411, E-mail: yanjq480@sina.com

sciatic nerve, which might be related to the inhibition of P2X₄ receptor expression in the spinal dorsal horn.

[KEY WORDS] neuropathic pain; P2X₄ receptor; ketamine; microglia

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神经病理性疼痛(neuropathic pain)是由外伤、炎症或其他疾病等引起神经损伤或病变所致的一种难治的慢性疼痛综合征,其病理生理学特点是痛觉的反应性增高,主要表现为痛觉过敏(hyperalgesia)和超敏反应(allodynia)^[1-2]。此类疼痛发生机制不明,难以对症处理,目前常用的强效麻醉性镇痛药很难奏效^[3]。小胶质细胞在神经病理性疼痛中发挥重要作用,激活的小胶质细胞可通过释放各种生物活性物质、细胞因子及神经递质诱导神经病理性疼痛^[4-5]。P2X₄受体是P2嘌呤受体的一种,其选择性表达于激活的小胶质细胞,对激活小胶质细胞及维持其活化状态起重要作用,参与了神经性疼痛及外周炎症性疼痛的发病过程,是神经病理性疼痛治疗的新靶点,但目前缺乏特异性的P2X₄受体拮抗剂^[6-8]。N-甲基-D-天冬氨酸(N-methyl-D-aspartate, NMDA)受体拮抗剂可以抑制小胶质细胞的激活^[9-10],但其确切机制不很清楚。为此,本研究应用小剂量氯胺酮(NMDA受体拮抗剂)作用于坐骨神经收缩损伤(神经病理性疼痛模型)大鼠,观察其镇痛效果及对大鼠脊髓组织上P2X₄受体表达的影响,探讨其可能的作用机制。

1 材料和方法

1.1 主要材料及试剂 盐酸氯胺酮注射液(江苏恒瑞药业股份有限公司);兔抗P2X₄多克隆抗体(Sigma,美国);SABC免疫组化染色试剂盒(武汉博士德生物工程有限公司);CTY-1型热痛阈测试仪(第四军医大学生理学教研室研制)。

1.2 动物分组及处理 24只纯种清洁级SD大鼠(雌雄不限),体质量160~220g,由中南大学湘雅医学院动物实验中心提供。大鼠随机分为3组($n=8$):假手术组、坐骨神经结扎组(CCI组)及氯胺酮治疗组。CCI组及氯胺酮治疗组均采用Bennett等^[11]的方法制备慢性坐骨神经痛模型,坐骨神经结扎术后第3日测定热刺激缩足反射潜伏期(thermal withdrawal latency, TWL)确定热痛觉过敏形成,证实模型制备成功后,氯胺酮治疗组大鼠腹腔注射小剂量氯胺酮($10\text{ mg}\cdot\text{kg}^{-1}$),CCI组腹腔注射等量的生理盐水,给药至术后第7日。假手术组大鼠单纯坐骨神经暴露,不用肠线结扎,也不给药治疗。

1.3 各项指标的观察 一般情况观察:动物术后单

笼饲养,注意观察体位、走路姿态、有无自噬行为、后肢持重等情况。热痛阈的测定:术前1d,术后1、3、7d进行热痛阈测试,采用热辐射抬足法,测定TWL作为热痛阈值,连续测定5次,每次间隔5min,去掉最大和最小值,取平均值。术前1d的热痛阈作为基础热痛阈值;术后每次均在腹腔给药2h后测定热痛阈。

1.4 免疫组织化学法测定脊髓组织P2X₄受体的表达 3组大鼠分别于术后7d灌注固定后取动物腰段脊髓浸泡在4%多聚甲醛中固定24h,常规脱水、浸蜡、包埋制作石蜡切片,厚度5 μm 。免疫组化SABC法进行反应:正常山羊血清封闭,P2X₄抗体(1:100, Sigma公司)4 $^{\circ}\text{C}$ 冰箱过夜,羊抗鼠IgG、辣根过氧化物酶标记的三抗中孵育,然后DAB显色,苏木精复染,分化,返蓝,梯度乙醇脱水,透明,烤片,封片。阴性对照:以正常大鼠血清代替一抗,其余步骤按上述进行。每例动物取5张腰段脊髓背角(其中1张用于阴性对照染色),每一张切片于光学显微镜下进行光镜观察;并于高倍镜($\times 400$)下在脊髓背角相同部位随机选取1个完整视野,计数免疫反应阳性细胞数。免疫组化以细胞出现棕黄色颗粒者为阳性。

1.5 统计学处理 采用SPSS 13.0统计软件进行统计学分析,数据以 $\bar{x}\pm s$ 表示,组间各指标均数采用单因素方差分析,均数间两两比较采用SNK- q 检验,以 $\alpha=0.05$ (双尾)为检验水准, $P<0.05$ 为差异有统计学意义。所有数据均进行正态性检验和方差齐性检验。

2 结果

2.1 一般情况观察 所有大鼠术后无1例出现感染和自残现象。假手术组大鼠术后未出现术侧爪内收和运动障碍,坐骨神经结扎大鼠术后第3日始,可见大鼠患足外翻,足趾背屈,呈现防御姿势,运动时明显跛行;站立或者坐下时,经常抬起患肢,使之处于保护性体位。

2.2 各组大鼠热痛阈值的变化 术前1d,3组大鼠的TWL无统计学差异;假手术组大鼠术后术侧TWL轻度下降,但与术前相比无统计学差异。CCI组及氯胺酮治疗组大鼠术后第3日始,TWL进行性降低,明显低于术前及假手术组,差异具有统计学意义($P<0.05$),术后7d最为显著;其中CCI组TWL

降低更为明显,明显低于氯胺酮治疗组,差异具有统计学意义($P < 0.05$)。具体结果见表1。

表1 各组大鼠热痛阈值的变化

Tab 1 Comparison of TWL values of rats in each group

Group	Pre-operation	Time after operation <i>t</i> /d		
		1	3	7
Sham	7.885±0.484	7.423±0.732	7.545 ±0.678	7.534±0.636
CCI	7.901±0.589	7.740±0.487 4	5.087 5±0.515 7 *	2.968±0.293 1 *
CCI+ketamine	7.661±0.945 7	7.467±0.958	4.991 3±0.541 1 *	3.690±0.071 3 *△

* $P < 0.05$ vs Sham group; △ $P < 0.05$ vs CCI group

2.3 各组大鼠脊髓组织 P2X₄ 阳性细胞的分布
CCI组及氯胺酮治疗组大鼠 P2X₄ 受体免疫阳性产物密集表达于脊髓背角,阳性细胞胞体较小,阳性表

达产物不仅见于胞膜上,还分布于胞质,着色较深,呈棕褐色;而假手术组大鼠脊髓背角少有 P2X₄ 受体阳性细胞的表达(图1)。

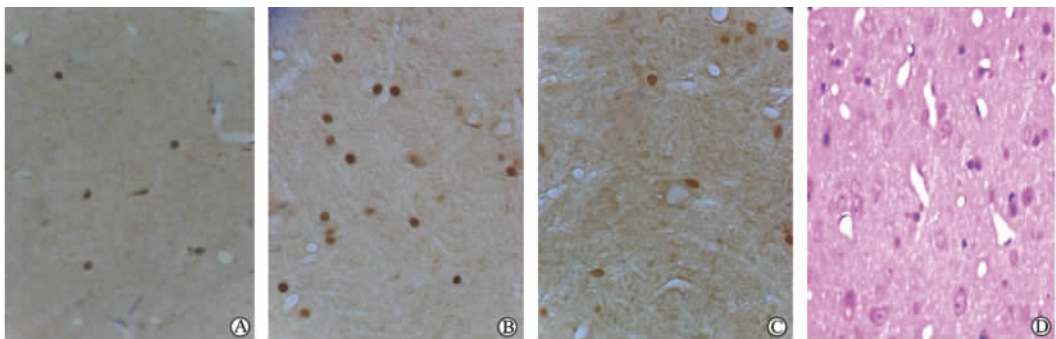


图1 各组大鼠免疫组化染色(A~C)及 H-E 染色结果(D)

Fig 1 Immunohistochemistry results of each group(A-C) and H-E result(D)

A: Sham group; B: CCI model group; C: Ketamine treatment group; D: H-E staining of CCI model group. Original magnification: ×400

2.4 各组大鼠脊髓背角 P2X₄ 阳性细胞数目的变化
与假手术组(3.63±1.59)大鼠相比,CCI组及氯胺酮治疗组大鼠术侧脊髓背角的 P2X₄ 受体表达显著增加(20.25±4.59、14.25±2.38, $P < 0.01$);与 CCI组相比,氯胺酮治疗组大鼠脊髓背角 P2X₄ 受体表达明显减少($P < 0.05$)。大多数 P2X₄ 受体阳性细胞呈现激活的小胶质细胞的形态。

3 讨论

CCI模型是经典的研究慢性神经病理性疼痛的模型^[11],其异常疼痛行为包括痛觉过敏、痛觉异常和自发痛(spontaneous pain)^[12]。本研究中大鼠坐骨神经结扎术后3d开始出现患足外翻,足趾背屈,呈现防御姿势,运动时明显跛行;同时大鼠结扎侧热痛阈下降,出现热痛觉过敏,表明 CCI模型制备成功^[11]。多种伤害性刺激均可引起小胶质细胞释放 ATP,激活 P2X 受体,诱导神经病理性疼痛的发生^[13-14]。P2X₄ 受体是介导 ATP 功能的主要受体种类之一,主要表达于活化的小胶质细胞,对激活小胶

质细胞及维持其活化状态起重要作用^[15-19],但具体机制仍不清楚。NMDA 受体激活被认为是伤害性刺激后中枢敏化的关键所在和持续性疼痛产生与维持的关键因素^[9-10]。NMDA 受体在小胶质细胞活化中发挥较重要的作用,激活的小胶质细胞膜表面可表达 NMDA 受体^[20];外周神经损伤时,脊髓背角神经元释放兴奋性氨基酸,特别是过度释放的谷氨酸^[21],可作用于 NMDA 受体而激活小胶质细胞。NMDA 受体抑制剂可阻断 NMDA 受体的过度兴奋及过多的上传冲动,在抑制了脊髓小胶质细胞活化的同时,也抑制了上传冲动对脊髓背角初级感觉神经元的激动^[9-10]。

氯胺酮是 NMDA 受体非特异拮抗剂,其镇痛机制十分复杂,可能通过多种机制阻断 EAA 对 NMDA 受体的激活,抑制 Ca²⁺ 离子通道开放,促使 Ca²⁺ 内流减少,从而发挥镇痛作用^[22];并且氯胺酮的代谢产物甲基氯胺酮也是 NMDA 受体的非竞争性拮抗剂,它与氯胺酮相配合可能会更完全地阻断 NMDA 受体^[23]。Glu/NMDA 受体系统可能参与了

脊髓组织 ATP 与 P2X 受体对伤害性信息的传递和痛过敏的发生^[24]。激活的 P2X 受体引起中枢性谷氨酸的释放, 释放的谷氨酸可进一步诱发脊髓背角 NMDA 受体介导的突触可塑性变化, 导致热痛觉过敏的持续存在。

本研究结果表明, 腹腔内注射小剂量氯胺酮后, CCI 损伤大鼠的热痛觉过敏明显缓解, 而且脊髓背角的 P2X₄ 受体表达也相应减少。痛觉过敏的有效缓解可能是由于氯胺酮直接抑制了谷氨酸盐对 NMDA 受体的过度兴奋, 从而抑制疼痛信号的转导而缓解了神经病理性疼痛。这可能通过抑制突触前膜释放的谷氨酸盐对 NMDA 受体的过度兴奋, 以及通过抑制伤害性刺激引起的谷氨酸对小胶质细胞表面 NMDA 受体的过度兴奋两方面途径来实现。并且, 由于其抑制了小胶质细胞的激活, 从而使选择性表达于小胶质细胞表面的 P2X₄ 受体表达减少。

综上所述, 本研究结果表明小剂量氯胺酮腹腔注射可以部分缓解神经病理性疼痛大鼠的痛觉过敏, 能够有效降低损伤脊髓背角细胞表面 P2X₄ 受体的表达, 其镇痛作用部分是通过直接或间接减少 P2X₄ 受体表达来实现。

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