·基础研究·

瑞芬太尼下调大鼠背根神经节和脊髓背角GIRK2的表达

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摘　要：【目的】观察G蛋白门控内向整流钾离子通道亚单位2(GIRK2)在瑞芬太尼诱导的痛觉过敏大鼠背根神经节和脊髓背角中的表达和分布的变化。【方法】成年雄性SD大鼠尾静脉输注瑞芬太尼4 μg/(kg·min)2 h建立痛觉过敏模型。瑞芬太尼注射后6 h、1 d、3 d和5 d，采用免疫荧光化学法观察GIRK2在瑞芬太尼诱导痛觉过敏大鼠的背根神经节(DRG)和脊髓背角中分布的变化。采用免疫印迹法检测大鼠背根神经节和脊髓背角GIRK2总蛋白和膜蛋白的表达。最后，采用行为学评价瑞芬太尼输注后，鞘内注射GIRK2特异性激动剂ML297对痛阈的影响。【结果】免疫荧光结果显示，在背根神经节和脊髓背角I-II板层，GIRK2主要与IB4阳性的小神经元及其神经纤维共定位，而瑞芬太尼注射后GIRK2表达显著降低。免疫印迹结果显示，静脉输注瑞芬太尼1 d后，与对照组相比，背根神经节GIRK2总蛋白(0.47 ± 0.10 *vs.* 1.01 ± 0.17，$P < 0.001$)和膜蛋白(0.47 ± 0.11 *vs.* 1.06 ± 0.12，$P < 0.001$)表达水平均显著减少；与背根神经节结果相一致的是，脊髓背角GIRK2总蛋白水平(0.52 ± 0.09 *vs.* 1.10 ± 0.08，$P < 0.001$)和膜蛋白表达水平(0.54 ± 0.10 *vs.* 1.01 ± 0.13，$P < 0.001$)也显著降低。行为学结果显示，与生理盐水组相比，在瑞芬太尼处理大鼠，ML297延长热撤足潜伏期的作用显著降低($P<0.001$)。【结论】持续静脉输注瑞芬太尼可能通过诱导大鼠背根神经节和脊髓背角GIRK2表达下调诱发痛觉过敏。

关键词：瑞芬太尼；痛觉过敏；G蛋白门控内向整流钾离子通道亚单位2；背根神经节；脊髓
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Remifentanil Down-regulates GIRK2 Expression in Rat Dorsal Root Ganglion and Spinal Dorsal Horn

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Abstract:【Objective】To observe the changes in the expression and distribution of G protein-gated inwardly rectifying potassium channel subunit 2 (GIRK2) in the dorsal root ganglion (DRG) and spinal cord dorsal horn of rats with remifentanil-induced hyperalgesia.【Methods】Hyperalgesia was induced by intravenous infusion of remifentanil 4 μg/kg/min for 2 h in adult male SD rats. At 6th hour and on days 1, 3 and 5 following remifentanil treatment, we used immunofluorescence to examine the changes in the GIRK2 distribution and expression. Immunoblotting was used to detect GIRK2 expression of the total protein and membrane protein in DRG and spinal dorsal horn of rats. Behavioral testing was applied to evaluate the effect of intrathecal injection of GIRK2-specific agonist ML297 on thermal nociceptive threshold on day 1 after

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remifentanil infusion.【Results】mmunofluorescence results showed that GIRK2 was mainly co-localized with IB4-positive small neurons in DRG and nerve fibers in spinal dorsal horn. GIRK2 expression was significantly downregulated following remifentanil treatment. Immunoblotting results revealed that on day 1 following intravenous infusion of remifentanil, compared with those in the control group, GIRK2 expression levels of the total protein and membrane protein in DRG (0.47 ± 0.10 *vs.* 1.01 ± 0.17, $P < 0.001$; 0.47 ± 0.11 *vs.* 1.06 ± 0.12, $P < 0.001$) and spinal dorsal horn (0.52 ± 0.09 *vs.* 1.10 ± 0.08, $P < 0.001$; 0.54 ± 0.10 *vs.* 1.01 ± 0.13, $P < 0.001$) were all significantly decreased. The behavioral results showed that intrathecal ML297 effect on thermal withdrawal latency was significantly reduced following remifentanil treatment ($P < 0.001$).【Conclusions】Remifentanil might induce hyperalgesia via down-regulating GIRK2 expression in rat DRG and spinal cord dorsal horn.

Key words: remifentanil; hyperalgesia; G protein-gated inwardly rectifying potassium channel subunit 2; dorsal root ganglion; spinal cord

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瑞芬太尼作为μ阿片受体(μ-opioid receptor, MOR)激动剂,由于其具有超短效作用,临床上主要用于全麻诱导、全麻中维持镇痛或辅助麻醉。但临床以及基础研究均报道瑞芬太尼可诱发人或大鼠痛觉过敏[1]。脊髓水平的中枢敏感化被认为是介导瑞芬太尼诱导痛觉过敏的重要原因[2-6],但机制不清。GIRK通道(G protein-gated inwardly rectifying K,GIRK)属于内向整流钾通道家族成员,其在稳定和维持细胞兴奋性中发挥重要作用[7-8]。在生理条件下,抑制性G蛋白偶联受体(G protein-coupled receptors, GPCRs)激活后解离出的Gβγ亚单位复合体激活GIRK,后者通过介导外向钾离子电流,降低细胞兴奋性,实现GPCRs的抑制性作用[9-10]。功能性GIRK通道是GIRK1~4亚基组成的同四聚体或异四聚体,其中GIRK1/2异四聚体已被确定为神经元中主要的GIRK通道。研究表明,阿片类药物可通过外周MOR激活GIRK1/2通道产生镇痛作用[11-18]。有意思的是,最近的研究表明,外周神经损伤导致的背根神经节和脊髓背角GIRK2表达下调与痛觉过敏密切相关[19-20]。然而,阿片类药物持续激活MOR能否通过调控GIRK表达导致痛觉过敏尚未见报道。本研究拟利用瑞芬太尼诱导的痛觉过敏大鼠模型,观察背根神经节和脊髓背角GIRK2表达与分布的变化,同时通过行为学评价特异性激活GIRK对热痛阈影响的变化,为探究瑞芬太尼是否通过调控GIRK诱导痛觉过敏的形成提供新的参考依据。

1 材料与方法

1.1 实验动物

成年雄性SD大鼠,质量200~250 g,购自中山大学实验动物中心(国家级实验动物中心)。动物生产许可证编号:SCXK(粤)2021-0029;动物质量合格证:44008500016931。于室温为25 ℃、湿度为(50 ± 10)%、12 h光照-黑暗循环、安静的环境中单笼饲养,自由摄食和饮水。1%戊巴比妥钠腹腔注射麻醉后,于实验物使用许可证号[SCXK(粤)2017-0081]设施中取材,所有操作均经中山大学医学院实验动物伦理委员会监管和批准实施(审批号:SYSU-IACUC-MED-2020-B0051)。

1.2 尾静脉注射瑞芬太尼

将瑞芬太尼(宜昌人福公司,10A09151,中国)溶解于0.9%生理盐水至终浓度为40 μg/mL,大鼠于3.0%七氟烷(丸石制药有限公司,CN2L9117,日本)麻醉后,电子注射泵(Asena PK,TCL-Ⅲ,英国)尾静脉持续输注4 μg/(kg·min)2 h。

1.3 鞘内注射

大鼠鞘内注射的方法参考叶柳[21]的研究。分别持续尾静脉输注生理盐水和瑞芬太尼1 d后,大鼠吸入异氟醚麻醉后,让大鼠保持俯卧位,定位脊柱L4~L5间隙,备皮,皮肤消毒,先用粗针头垂直脊柱进针,突破皮肤后退出,再用微量注射器于原位椎间隙垂直缓慢进针,当尾巴出现颤动或甩动,则标志成功进入鞘内,此时保持穿刺针位置不变并缓慢注入ML297(ab143564,Abcam,英国)。

1.4　行为学实验

行为学测试参考叶柳等[21]，使用热辐射刺激仪进行撤足潜伏期测试。设置参数：光强30 V，基础光强5 V，自动断电时间30 s。生理盐水和瑞芬太尼输注前1 d测试基础值，生理盐水和瑞芬太尼输注后1 d测试撤足潜伏期，ML297输注5 min后测试撤足潜伏期。每次测试前将大鼠放置在测试环境中适应30 min，每只大鼠重复测量3次，每次间隔10 min，取三次测量的均值为热痛阈值。ML297的抑制效应的计算方式参考Marker等[14]，即抑制效应 $n\Delta$TF latency =（ML297注射后撤足潜伏期－基础值）－ΔTF，其中ΔTF为注射生理盐水后撤足潜伏期/瑞芬太尼注射后撤足潜伏期与基础值差值的平均值。

1.5　免疫荧光

实验大鼠采用质量分数2%戊巴比妥钠，45 mg/kg，麻醉后，经升主动脉灌注预冷的0.9%生理盐水和40 g/L多聚甲醛。取L4~L5脊髓节段和对应的背根神经节置于多聚甲醛中后固定2 h，转入预冷的质量分数30%蔗糖溶液脱水至沉底。组织用OCT包埋进行冰冻切片，片厚20 μm。切片用5%山羊血清在室温下封闭1 h后，与下列一抗与于4 ℃孵育过夜：兔源抗GIRK2多克隆抗体（1∶200，Alomone Labs，#APC-006，以色列）；Isolectin B4（BSI-B4）（Sigma，FITC-conjugated，#L2895，美国）；鼠源抗CGRP（ab81887，Abcam，英国）；鼠源抗NF200（60331-1-1g，proteintech，中国）；抗胶质纤维酸性蛋白（glial fibril-lary acidic protein，GFAP）小鼠单克隆抗体（astrocyte marker，1∶200，Cell Signaling Technology，美国）；鼠源抗CD11b单克隆抗体（microglia marker，1∶100，Millipore，美国）；鼠源抗Synaptotagmin单克隆抗体（Abcam，#ab13259，英国）。次日洗涤后，用相应的AlexaFluor二抗（1∶2 000，Thermo，美国）室温避光孵育1 h。切片于荧光显微镜（Olympus BX63，日本）下拍摄观察。采用ImageJ V 1.8.0软件对免疫荧光进行图像进行分析，阳性率的计算方法参考Lyu[19]，即将神经元中GIRK2阳性神经元的数量除以神经元的数量，计算百分比。

1.6　Western Blot 实验

提取脊髓和背根神经节（dorsal root ganglion，DRG）组织中的匀浆蛋白。根据实验说明手册，用膜蛋白提取试剂盒（Thermo，#89842）分离膜质蛋白和胞浆蛋白。蛋白质样品（30 μg）经SDS-PAGE凝胶分离后转移到PVDF膜上，使用质量分数5%脱脂牛奶封闭1 h后，与下列抗体4 ℃孵育过夜：兔源抗GIRK2（1∶1 000，ab229913，Abcam，英国）。次日洗膜后与相应的二抗于室温孵育1 h，显影曝光。用ImageJ V 1.8.0软件对条带进行灰度分析。

1.7　统计学分析

采用SPSS 25.统计分析软件进行统计分析，实验结果以均数±标准差（$\bar{x} \pm s$）表示。免疫荧光结果采用单因素方差分析比较各组差异。Western Blot结果采用单因素方差分析比较组间及差异。行为学结果采用单因素方差分析比较组间镇痛效应的差异。$P < 0.05$被认为差异具有统计学意义。

2　结　果

2.1　GIRK2在正常大鼠背根神经节中的表达和分布

免疫荧光结果显示，DRG中GIRK2主要与IB4共定位（27.3%），与NF200存在少量共定位（11.6%），而与CGRP几乎不存在共定位（表1和图1），提示DRG GIRK2主要在小无髓鞘非肽能神经元中表达，在中/大有髓鞘初级感觉神经元少量表达，而在小的无髓鞘肽能神经元乎不表达。

表1　背根神经节GIRK2阳性神经元与IB4、NF200和CGRP共定位的百分比的比例

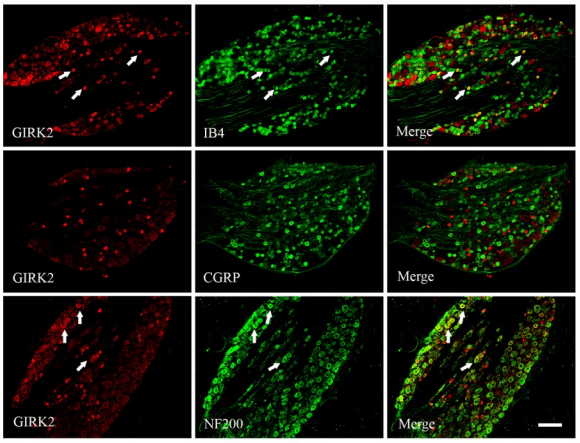
Table 1　Percentage of GIRK2-positive neurons in the dorsal root ganglion co-localized with IB4, NF200 and CGRP

Item	Mean±SD
IB4	27.3 ± 3.3
NF200	11.6 ± 4.2
CGRP	0

IB4：isolectin-B4；NF200：Neurofilament-200；CGRP：calcitonin gene-related peptide.

2.2　瑞芬太尼诱导大鼠背根神经节中GIRK2表达减少

对GIRK2阳性率进行单因素方差分析结果显示差异具有统计学意义（$F = 10.04$，$P < 0.001$），采用Bonferroni法作两两比较，发现与对照组相比，静



Immunofluorescence double-staining results showed colocalization of GIRK2 with IB4 or NF200. There was almost no GIRK2 positive labeling in CGRP-positive cells. The percentage is equal to the number of GIRK2-positive neurons in the neuron divided by the total number of neurons. Scale bar=200 μm.

图1 GIRK2在正常大鼠背根神经节中的分布

Fig. 1 Distribution of GIRK2 in the dorsal root ganglion of normal rats

脉持续输注瑞芬太尼后1 d,DRG小直径非肽能IB4阳性神经元的GIRK2阳性率降低(0.18 ± 0.03 *vs.* 0.27 ± 0.03, $P < 0.001$; 图2A)。对DRG GIRK2总蛋白和膜蛋白印迹相对灰度值进行单因素方差分析结果显示差异具有统计学意义($F = 24.18$, $P < 0.001$; $F = 22.25$, $P < 0.001$),采用Bonferroni法作两两比较,发现与对照组相比,静脉持续输注瑞芬太尼后6 h,DRG GIRK2总蛋白和膜蛋白水平开始降低($P < 0.001$);1 d后GIRK2总蛋白(0.47 ± 0.10 *vs.* 1.01 ± 0.17, $P < 0.001$; 图2B)和膜蛋白(0.47 ± 0.11 *vs.* 1.06 ± 0.12, $P < 0.001$; 图2C)降低到最低水平。瑞芬太尼注射后第5 d,DRG GIRK2蛋白表达恢复到正常水平(图2A)。

2.3 GIRK2在大鼠脊髓背角中的表达和分布

免疫荧光结果显示,GIRK2在脊髓背角表达丰富,且主要分布于脊髓背角的Ⅰ-Ⅱ板层(图3A)。免疫荧光双染结果显示,GIRK2主要与IB4共定位,部分与CGRP共定位,而与NF200几乎不存在共定位(图3A)。此外,脊髓背角的GIRK2与小胶质细胞、星形胶质细胞不存在共定位,而与突触前

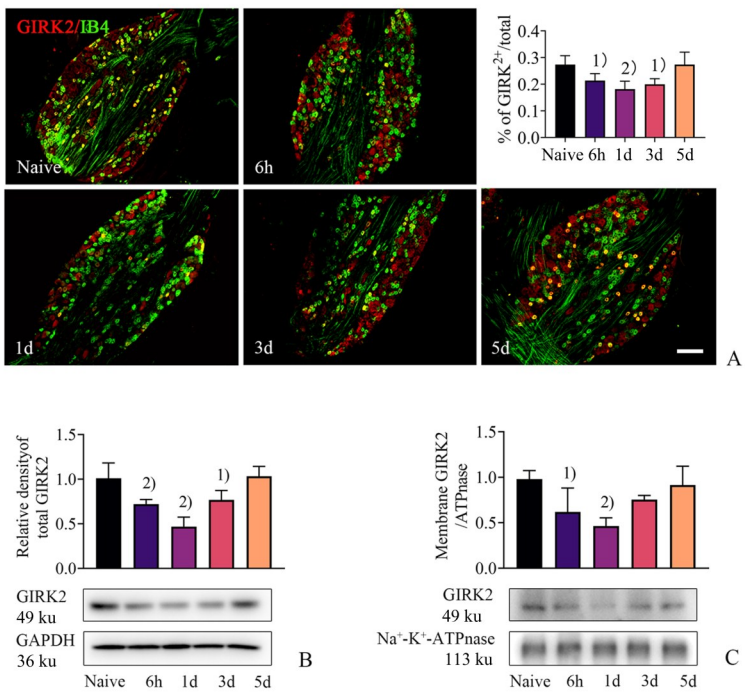
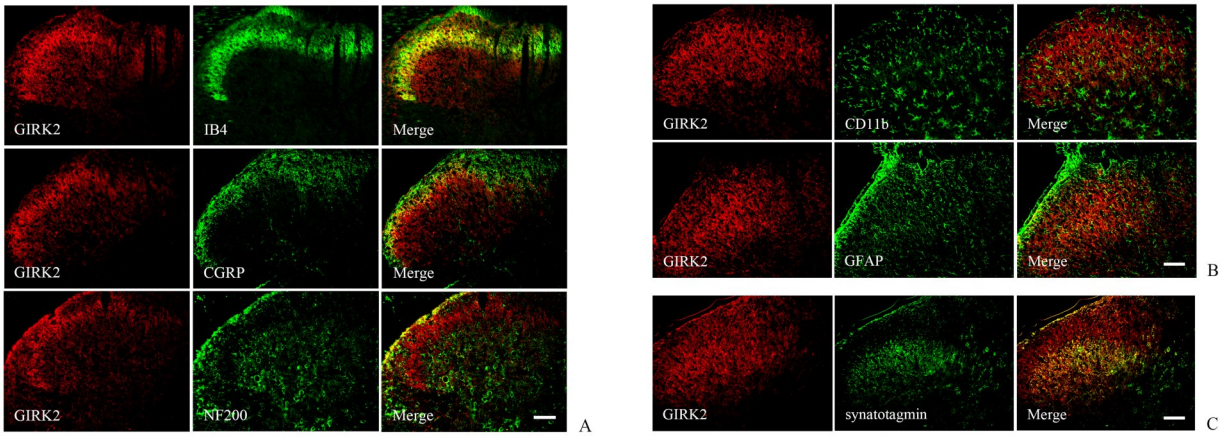


Fig. 2A showed the changes in the proportion of DRG GIRK2-positive neurons in rats after 6 h, 1 d, 3 d, and 5 d of remifentanil treatment, $n = 6$ per group, Scale bar=200 μm. Fig. 2B showed the expression of DRG GIRK2 total protein after 6 h, 1 d, 3 d, and 5 d of remifentanil treatment, $n = 6$ per group. Fig. 2C showed the expression of DRG GIRK2 membrane protein at 6 h, 1 d, 3 d, and 5 d following remifentanil treatment, $n = 6$ per group. 1) $P < 0.05$ and 2) $P < 0.001$ compared with the Naive group.

图2 瑞芬太尼下调大鼠DRG中的GIRK2表达

Fig. 2 Remifentanil downregulates the expression level of GIRK2 in DRG of rats



A. Fig. 3A showed that GIRK2 was co-localized with IB4 and NF200 but not with CGRP. Fig. 3B showed that GIRK2 was not co-localized with GFAP (astrocytic marker) or CD11b (microglial marker). Fig. 3C showed that GIRK2 was co-localized with Synatotagmin (presynaptic marker). Scale bar =100 μm.

图3　GIRK2在正常大鼠脊髓中的分布

Fig. 3　Distribution of GIRK2 in the spinal cord of normal rats

标记物synatotagmin共定位（图3B和C）。以上结果提示，在大鼠脊髓背角浅层，GIRK2主要表达于IB4阳性神经纤维的突触前末梢。

2.4　瑞芬太尼诱导大鼠脊髓背角GIRK2表达减少

对脊髓GIRK2荧光强度进行方差分析结果显示差异具有统计学意义（$F = 55.62$，$P < 0.001$），采用Bonferroni法作两两比较，发现与对照组相比，静脉持续输注瑞芬太尼1 d后，脊髓背角GIRK2的平均荧光强度减弱（13.70 ± 2.05 *vs.* 48.46 ± 4.61，$P < 0.001$；图4A）。对脊髓GIRK2总蛋白和膜蛋白印迹灰度值进行单因素方差分析结果显示差异具有统计学意义（$F = 45.84$，$P < 0.001$；$F = 16.08$，$P < 0.001$），采用Bonferroni法作两两比较，进一步证实，与对照组相比静脉持续输注瑞芬太尼1 d后脊髓背角GIRK2的总蛋白（图4B）和膜蛋白（图4C）水平显著降低（0.52 ± 0.09 *vs.* 1.10 ± 0.08，$P < 0.001$；0.54 ± 0.10 *vs.* 1.01 ± 0.12，$P < 0.001$）。

2.5　瑞芬太尼降低了GIRK2激动剂对热痛的抑制效应

由于阻断或敲除GIRK能够导致痛敏发生，为明确GIRK2的下调能否影响GIRK对热伤害性感受的调控效应，观察大鼠鞘内注射不同剂量（3、10、30和100 μg/10 μL）的GIRK2特异性激动剂ML297对热刺激撤足潜伏期的影响。对各组的抑制效应进行单因素方差分析结果显示差异具有统计学意义（$F = 84.756$，$P < 0.001$），采用Bonferroni法作两两比较，发现与对照组相比，在生理盐水组ML297以剂量依赖的方式延长大鼠的撤足潜伏期（$P < 0.001$），而在瑞芬太尼处理1 d后ML297对撤足潜伏期的延长效应显著降低（$P < 0.001$）。以上结果提示，ML297对热痛的抑制效应的降低可能与瑞芬太尼下调GIRK2表达有关（图5）。

3　讨 论

本研究首次发现：瑞芬太尼显著下调背根神经节和脊髓背角GIRK2总蛋白和膜蛋白表达，且GIRK2的下调主要发生于IB4阳性非肽能神经元和神经纤维。另外，瑞芬太尼输注后，鞘内注射GIRK2特异性激动剂ML297对疼痛的抑制效应显著降低，提示GIRK2的下调可能是瑞芬太尼引起大鼠痛觉过敏的重要原因。

阿片类药物是目前临床治疗疼痛最为常用且有效的药物，但是阿片类药物的使用常伴随痛觉过敏，表现为痛阈下降、痛觉过敏。大量证据显示，G蛋白门控内向整流钾离子通道（GIRK）通过维持神经元膜电位，在调控神经元兴奋性以及神经递质释放过程中发挥关键作用[7, 10, 22-23]。最近研究发现，鞘内注射GIRK特异性激动剂ML297可产生显著的镇痛作用[24]。近年来研究表明，GIRK与痛觉过敏发生密切相关。GIRK2敲除或突变小鼠出现热痛觉过敏[13]，而鞘内注射GIRK特异性抑制剂也能诱导痛敏的发生[14]。新近研究显示，大鼠坐骨神经

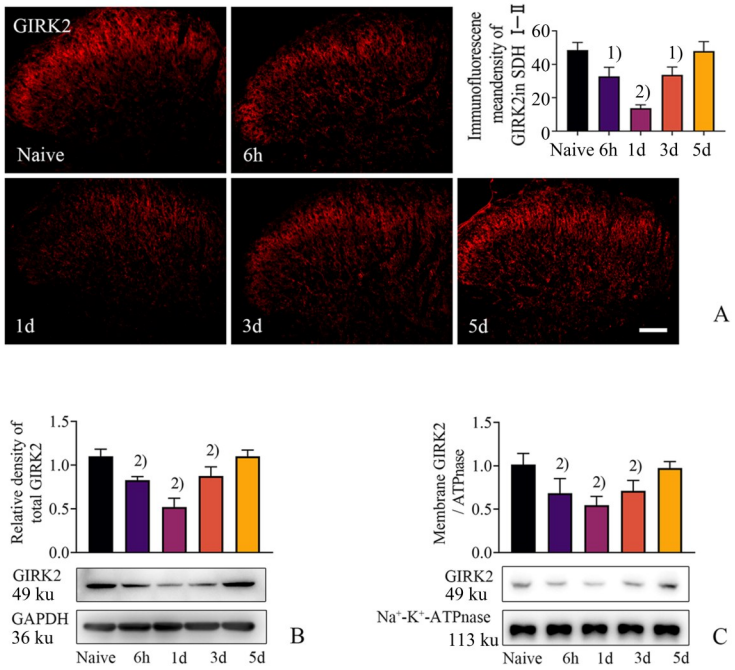
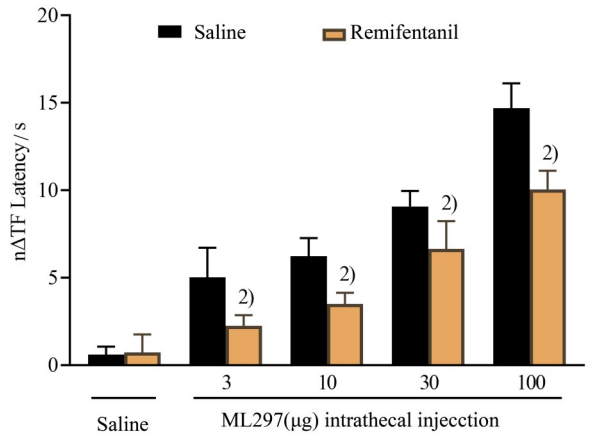


Fig. 4A showed the changes in fluorescence intensity of spinal GIRK2 after 6 h, 1 d, 3 d, and 5 d of remifentanil treatment, n = 6 per group, Scale bars=100 μm. Fig. 4B showed the changes in total protein expression of spinal GIRK2 after 6 h, 1 d, 3 d, and 5 d of remifentanil treatment, n = 6 per group. Fig. 4C showed the changes in membrane protein expression of spinal GIRK2 after 6 h, 1 d, 3 d, and 5 d of remifentanil treatment, n = 6 per group. 1) P <0.05 and 2) P < 0.001 compared with the Naive group.

图4 瑞芬太尼诱导大鼠脊髓表达的GIRK2下调

Fig. 4 Expression of GIRK2 in the spinal cord of rats was downregulated following remifentanil treatment



The results of the behavioral experiment were analyzed using a one-way ANOVA. 2) P < 0.001 compared with the saline-treated group, n = 6 per group.

图5 瑞芬太尼降低鞘内注射GIRK2激动剂ML297产生的抑制效应

Fig. 5 Remifentanil reduces the inhibitory effect of the intrathecal injection of GIRK2 agonist ML297

完全切断后脊髓和DRG的GIRK1/GIRK2的表达降低[19],提示GIRK的下调可能是介导疼痛发生的重要原因。与我们前期研究结果相一致的是,持续静脉输注瑞芬太尼后6 h,大鼠开始出现痛觉过敏,输注后第一天痛觉达到峰值。值得注意的是,在大鼠痛敏发生过程中,在背根神经节和脊髓背角浅层突触前,IB4阳性的小神经元及初级传入神经性纤维末梢表达的GIRK2显著下调。有证据显示,抑制背根神经节IB4阳性感觉神经元的兴奋性突触传递能够减轻痛觉过敏[25]和吗啡耐受的形成[26]。因此,以上研究结果提示IB4阳性的外周伤害性感受器的GIRK2表达下调可能参与了瑞芬太尼诱导的痛敏发生。

目前已知阿片类药物通过激活外周MOR,抑制脊髓背角突触前兴奋性神经递质的释放产生镇痛作用。研究表明,G蛋白耦联受体途径激活的GIRK通道,通过使神经元发生超极化作用,介导了吗啡34%以上的镇痛作用[27]。然而,我们及同行以往研究表明,持续应用阿片类镇痛药会导致耐受[28]和痛觉过敏的形成[29-31],且两种病理生理过程存在共同的信号分子机制[32-34]。近年来研究发现,促进小鼠GIRK表达[15]或阻断GIRK分别会增强和减弱吗啡镇痛作用[16],而中脑导水管周围灰质神经元

GIRK介导的电流降低被认为是吗啡耐受形成的重要原因[35]。由于外周表达的GIRK在抑制痛觉传导通路中的兴奋性突触传递发挥重要作用[15, 36]，且鞘内注射GIRK特异性激动剂ML297可产生显著的镇痛作用[24]，因此本研究进一步验证瑞芬太尼诱导的GIRK2下调是否影响激活GIRK对疼痛的抑制效应。结果显示，鞘内注射ML297显著延长对照组大鼠对热刺激的反应时间。而瑞芬太尼输注后一天，ML297对痛反应的抑制效应显著降低，提示GIRK表达水平降低减弱了其对痛觉传导通路的抑制作用，这可能是导致瑞芬太尼诱导痛敏发生的重要原因。

综上所述，本研究表明瑞芬太尼诱导GIRK2下调，从而导致大鼠痛觉过敏。本研究结果为研究瑞芬太尼引起痛觉过敏的分子机制提供了新的参考依据，也为临床上研发新的更安全有效的镇痛药物提供了新思路。

参考文献

[1] Kim SH, Stoicea N, Soghomonyan S, et al. Remifentanil-acute opioid tolerance and opioid-induced hyperalgesia: a systematic review[J]. Am J Ther, 2015, 22(3): e62-e74.

[2] Horii Y, Matsuda M, Takemura H, et al. Spinal and peripheral mechanisms individually lead to the development of remifentanil-induced hyperalgesia[J]. Neuroscience, 2020, 446: 28-42.

[3] Romero A, Romero-Alejo E, Vasconcelos N, et al. Glial cell activation in the spinal cord and dorsal root ganglia induced by surgery in mice[J]. Eur J Pharmacol, 2013, 702(1-3): 126-134.

[4] Lee M, Silverman SM, Hansen H, et al. A comprehensive review of opioid-induced hyperalgesia[J]. Pain Physician, 2011, 14(2): 145-161.

[5] Rohde DS, Detweiler DJ, Basbaum AI. Spinal cord mechanisms of opioid tolerance and dependence: Fos-like immunoreactivity increases in subpopulations of spinal cord neurons during withdrawal [corrected][J]. Neuroscience, 1996, 72(1): 233-242.

[6] Drdla R, Gassner M, Gingl E, et al. Induction of synaptic long-term potentiation after opioid withdrawal[J]. Science, 2009, 325(5937): 207-210.

[7] Jeremic D, Sanchez-Rodriguez I, Jimenez-Diaz L, et al. Therapeutic potential of targeting G protein-gated inwardly rectifying potassium (GIRK) channels in the central nervous system[J]. Pharmacol Ther, 2021, 223: 107808.

[8] Zhao Y, Gameiro-Ros I, Glaaser IW, et al. Advances in Targeting GIRK Channels in Disease[J]. Trends Pharmacol Sci, 2021, 42(3): 203-215.

[9] Nagi K, Pineyro G. Kir3 channel signaling complexes: focus on opioid receptor signaling[J]. Front Cell Neurosci, 2014, 8: 186.

[10] Kano H, Toyama Y, Imai S, et al. Structural mechanism underlying G protein family-specific regulation of G protein-gated inwardly rectifying potassium channel[J]. Nat Commun, 2019, 10(1): 2008.

[11] Marker CL, Cintora SC, Roman MI, et al. Hyperalgesia and blunted morphine analgesia in G protein-gated potassium channel subunit knockout mice[J]. Neuroreport, 2002, 13(18): 2509-2513.

[12] Torrecilla M, Marker CL, Cintora SC, et al. G-protein-gated potassium channels containing Kir3.2 and Kir3.3 subunits mediate the acute inhibitory effects of opioids on locus ceruleus neurons[J]. J Neurosci, 2002, 22(11): 4328-4334.

[13] Blednov YA, Stoffel M, Alva H, et al. A pervasive mechanism for analgesia: activation of GIRK2 channels[J]. Proc Natl Acad Sci U S A, 2003, 100(1): 277-282.

[14] Marker CL. Spinal G-Protein-Gated K+ Channels formed by GIRK1 and GIRK2 subunits modulate thermal nociception and contribute to morphine analgesia[J]. J Neurosci, 2004, 24(11): 2806-2812.

[15] Nockemann D, Morgane RY, Dominika LY, et al. The K(+) channel GIRK2 is both necessary and sufficient for peripheral opioid-mediated analgesia[J]. EMBO Mol Med, 2013, 5(8): 1263-1277.

[16] Kanbara T, Nakamura A, Shibasaki M, et al. Morphine and oxycodone, but not fentanyl, exhibit antinociceptive effects mediated by G-protein inwardly rectifying potassium (GIRK) channels in an oxaliplatin-induced neuropathy rat model[J]. Neurosci Lett, 2014, 580: 119-124.

[17] Takasu K, Ogawa K, Nakamura A, et al. Enhanced GABA ergic synaptic transmission at VLPAG neurons and potent modulation by oxycodone in a bone cancer

pain model[J]. Br J Pharmacol, 2015, 172(8): 2148-2164.
[18] Marker CL, Lujún R, Loh HH, et al. Spinal G-protein-gated potassium channels contribute in a dose-dependent manner to the analgesic effect of mu- and delta- but not kappa-opioids[J]. J Neurosci, 2005, 25(14): 3551-3559.
[19] Lyu C, Mulder J, Barde S, et al. G protein-gated inwardly rectifying potassium channel subunits 1 and 2 are down-regulated in rat dorsal root ganglion neurons and spinal cord after peripheral axotomy[J]. Mol Pain, 2015, 11: s12915-s12990.
[20] Bagley EE, Chieng BC, Christie MJ, et al. Opioid tolerance in periaqueductal gray neurons isolated from mice chronically treated with morphine[J]. Br J Pharmacol, 2005, 146(1): 68-76.
[21] 叶柳, 杨诗颖, 肖力, 等. 活性氧激活的脊髓星形胶质细胞参与瑞芬太尼诱发的痛觉过敏[J]. 中山大学学报(医学科学版), 2016, 37(4): 548-555.
Ye L, Yang SY, Xiao L, et al. Activation of spinal astrocyte via reactive oxygen species contributes to remifentanil-induced hyperalgesia[J]. J Sun Yat-sen Univ (Med Sci), 2016, 37(4): 548-555.
[22] Ikeda K, Kobayashi T, Kumanishi T, et al. Molecular mechanisms of analgesia induced by opioids and ethanol: is the GIRK channel one of the keys?[J]. Neurosci Res, 2002, 44(2): 121-131.
[23] Dascal N. Signalling via the G protein-activated K+ channels[J]. Cell Signal, 1997, 9(8): 551-573.
[24] Kimura M, Shiokawa H, Karashima Y, et al. Antinociceptive effect of selective G protein-gated inwardly rectifying K+ channel agonist ML297 in the rat spinal cord[J]. PLoS One, 2020, 15(9): e239094.
[25] Joseph EK, Levine JD. Mu and delta opioid receptors on nociceptors attenuate mechanical hyperalgesia in rat[J]. Neuroscience, 2010, 171(1): 344-350.
[26] Ohashi Y, Sakhri FZ, Ikemoto H, et al. Yokukansan inhibits the development of morphine tolerance by regulating presynaptic proteins in DRG neurons[J]. Front Pharmacol, 2022, 13: 862539.
[27] Mitrovic I, Margeta-Mitrovic M, Bader S, et al. Contribution of GIRK2-mediated postsynaptic signaling to opiate and alpha 2-adrenergic analgesia and analgesic sex differences[J]. Proc Natl Acad Sci U S A, 2003, 100(1): 271-276.
[28] 陈宇, 崔宇, 刘卫锋, 等. 吗啡耐受大鼠背根神经节磷酸化 p38 MAPK 的表达[J]. 中山大学学报(医学科学版), 2009, 30(6): 657-661.
Chen Y, Cui Y, Liu WF, et al. Expression of phosphorylated p38 MAPK in the dorsal root ganglion of morphine-tolerant rats[J]. J Sun Yat-sen Univ (Med Sci), 2009, 30(6): 657-661.
[29] 王晓娥, 李琪, 肖力, 等. 脊髓CCL2在瑞芬太尼诱导的大鼠痛觉过敏中的作用机制[J]. 中山大学学报(医学版), 2019, 40(5): 706-714.
Wang XE, Li Q, Xiao L, et al. Mechanism of the role of spinal CCL2 in remifentanil-induced nociceptive hyperalgesia in rats[J]. J Sun Yat-sen Univ (Med Sci), 2019, 40(5): 706-714.
[30] 于萍, 王晓娥, 崔宇, 等. 脊髓PKCε和μ阿片受体参与瑞芬太尼诱导大鼠痛觉过敏[J]. 中山大学学报(医学科学版), 2020, 41(6): 850-857.
Yu P, Wang XE, Cui Y, et al. Spinal PKCε and μ opioid receptors are involved in remifentanil-induced nociceptive hyperalgesia in rats[J]. J Sun Yat-sen Univ (Med Sci), 2020, 41(06): 850-857.
[31] 白雪, 肖力, 叶柳, 等. 背根神经节MMP-9在瑞芬太尼引起的大鼠痛觉过敏中的作用[J]. 中山大学学报(医学科学版), 2015, 36(4): 556-563.
Bai X, Xiao L, Ye L, et al. Role of dorsal root ganglion MMP-9 in remifentanil-induced nociceptive hyperalgesia in rats[J]. J Sun Yat-sen Univ (Med Sci), 2015, 36(4): 556-563.
[32] Corder G, Tawfik VL, Wang D, et al. Loss of μ opioid receptor signaling in nociceptors, but not microglia, abrogates morphine tolerance without disrupting analgesia[J]. Nat Med, 2017, 23(2): 164-173.
[33] Baron R, Hans G, Dickenson AH. Peripheral input and its importance for central sensitization[J]. Ann Neurol, 2013, 74(5): 630-636.
[34] Kliewer A, Schmiedel F, Sianati S, et al. Phosphorylation-deficient G-protein-biased μ-opioid receptors improve analgesia and diminish tolerance but worsen opioid side effects[J]. Nat Commun, 2019, 10(1): 367.
[35] Ingram SL, Macey TA, Fossum EN, et al. Tolerance to repeated morphine administration is associated with increased potency of opioid agonists[J]. Neuropsychopharmacology, 2008, 33(10): 2494-2504.
[36] Chung MK, Cho YS, Bae YC, et al. Peripheral G protein-coupled inwardly rectifying potassium channels are involved in delta-opioid receptor-mediated anti-hyperalgesia in rat masseter muscle[J]. Eur J Pain, 2014, 18(1): 29-38.

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