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研究论文

牛肾上腺髓质22肽削弱完全弗氏佐剂引起的早期痛觉过敏

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摘 要：本研究旨在探讨感觉神经元特异性受体(sensory neuron-specific receptor, SNSR)的内源性激动剂牛肾上腺髓质22 肽(bovine adrenal medulla 22, BAM22)对完全弗氏佐剂(complete Freund's adjuvant, CFA)引起的早期炎性痛的影响。在大鼠后足皮下注射CFA形成炎性痛模型，通过撤足反射、脚肿测定和免疫组织化学等方法观察鞘内给予BAM22对炎性痛的影响。结果显示：在撤足反射实验中，BAM22能剂量依赖地延长CFA炎性鼠撤足反射潜伏期基础阈值，增强抗伤害作用，并降低脚肿程度。10 nmol BAM22作用48 h后，撤足反射潜伏期恢复至正常的83.2%，脚肿仅增加60.0%；24 h时延长撤足反射潜伏期达最大可能作用的33.5%，并持续至少1 h。在免疫组织化学实验中，BAM22能显著降低CFA引起的L3-L5脊髓背角神经元型一氧化氮合酶(neuronal nitric oxide synthase, nNOS)阳性细胞和降钙素基因相关肽(calcitonin gene-related peptide, CGRP)样免疫活性物质的表达，与生理盐水组相比分别下降了25.6% ($P < 0.01$)和25.2% ($P < 0.001$)；BAM22处理组背根神经节(dorsal root ganglion, DRG)中的小型和中型CGRP阳性细胞分别为57.4%和35.2%，明显低于生理盐水组($P < 0.001$)。结果表明，BAM22可能通过SNSR下调nNOS和CGRP的表达来削弱CFA引起的早期热痛觉过敏和恢复抗伤害效力。

关键词：牛肾上腺髓质 22 肽；完全弗氏佐剂；炎性痛；神经型一氧化氮合酶；降钙素基因相关肽
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Bovine adrenal medulla 22 attenuates hyperalgesia in the early phase of complete Freund's adjuvant-induced inflammation in rats

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Abstract: The present study investigated the effects of intrathecal (i.t.) application of bovine adrenal medulla 22 (BAM22), an endogenous opioid peptide potently activating opioid receptors and sensory neuron-specific receptor (SNSR), on a model of complete Freund's adjuvant (CFA)-induced inflammatory pain. Unilateral, but not bilateral, inflammatory pain was induced by intraplantar (i.pl.) injection of CFA in one side, as indicated by the shortened paw withdrawal latency and the increased edema of paw. Paw withdrawal latency test, paw edema determination and immunohistochemistry were used in CFA-induced inflammatory pain model after i.t. administration of BAM22 or saline. It was found that administration of BAM22 dose-dependently attenuated CFA-induced hyperalgesia and edema, and resumed antinociceptive effects against thermal stimulation in behavioral test. In 10 nmol BAM22 group, paw withdrawal latency was resumed to 83.2% of normal, and edema increased only by 60% of normal at 48 h. The potency of BAM22 was 33.5% of maximal possible effect (MPE) at 24 h, and the antinociception persisted for at least 1 h. Furthermore, i.t. treatment of 10 nmol BAM22 evidently decreased the expressions of CFA-evoked neuronal nitric oxide synthase (nNOS)-positive cells and calcitonin gene-related peptide (CGRP)-immunoreactivity positive nerve fibers by 25.6% ($P < 0.01$) and 25.2% ($P < 0.001$) compared with saline group, respectively, at L3-L5 segments of the spinal cord. Small and medium CGRP-positive cells were 57.4% and 35.2% in dorsal

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root ganglion (DRG) in 10 nmol BAM22 group, respectively, which were remarkably lower than those in saline group ($P < 0.001$). The present study suggests that BAM22 relieves CFA-induced thermal hyperalgesia in the early phase and resumes antinociceptive effects through down-regulation of nNOS and CGRP expressions in DRG and spinal cord, which is possibly mediated via SNSR.

Key words: bovine adrenal medulla 22; complete Freund's adjuvant; inflammatory pain; neuronal nitric oxide synthase; calcitonin gene-related peptide

牛肾上腺髓质22肽(bovine adrenal medulla peptide 22, BAM22)于上世纪80年代被发现，广泛分布于大脑、丘脑、脑干和脊髓[1-3]。它是一种含有22个氨基酸的内源性阿片肽，是脑啡肽原(proenkephalin A)自然降解的一种产物[4]。Swain等[5]发现在应激状态下，大鼠血浆中BAM22的水平急剧上升；还有研究发现鞘内注射BAM22能有效地抑制膀胱收缩反射[6]，但其生理功能尚不清楚。

近年来的研究表明，BAM22可能参与了痛觉信息传递的调制过程。研究发现鞘内注射BAM22能显著延长缩足反射的潜伏期[7]，抑制福尔马林引起的疼痛反应[8]，并能抑制福尔马林和伤害性热刺激引起的大鼠腰段脊髓背角c-fos蛋白的表达[9]。此外，在吗啡耐受鼠中，鞘内注射BAM22不仅能抑制伤害性反应，还能部分地逆转吗啡的耐受[7,10]。由此可见，BAM22在伤害性信息传递中可能起着重要的作用，但BAM22在炎性痛中的作用及其机制目前尚不清楚。本研究通过完全弗氏佐剂(complete Freund's adjuvant, CFA)诱发的慢性炎性痛模型来研究BAM22在炎性痛中的作用及可能的机制，以期为炎性痛的预防与治疗提供新思路。

1 材料与方法

1.1 实验动物 成年雄性Sprague-Dawley大鼠，由福建医科大学实验动物中心提供，体重230~280 g，符合国家清洁实验动物健康标准。动物饲养在昼夜周期各为12 h的恒温、恒湿的十万级洁净动物室内，供给充足食物和水。动物实验经福建师范大学实验动物伦理委员会认可，并符合国家《实验动物管理条例》。

1.2 试剂 BAM22 为瑞士Bachem公司产品；Vectastain ABC kit为美国Vector Laboratories公司产品，编号PK-4001；兔抗降钙素基因相关肽(calcitonin gene-related peptide, CGRP)和神经元型一氧化氮合酶(neuronal nitric oxide synthase, nNOS)多克隆抗体为Santa Cruz Biotechnology 公司产品；CFA为美国Sigma公司产品；浓缩型DAB 试剂盒和正常羊血清为北京中山生物技术有限公司产品；注射用头孢曲松钠为福建省福抗药业股份有限公司生产；其余试剂均为分析纯。

1.3 仪器 Plantar Test (7340型)为意大利UGO BASILE仪器设备有限公司制造；Microm HM550型冷冻切片机为德国制造；Olympus BX51型显微镜和Olympus DP70型数码照相机均为日本制造；PE-10插管为美国 Stoelting公司产品；微量进液器为Switzerland Hamilton Bonaduz AG 产品；Mettler Toledo (梅特勒-托利多) AL104型电子天平为中国上海产品；大鼠固定装置为成都泰盟仪器厂产品。

1.4 方法

1.4.1 椎管插管 椎管内插管参照文献[7,11]进行。所不同的是，将长15 cm的PE-10插管在7.0 ~ 8.0 cm处打一环结，并用缝合线固定，插管后将其固定于浅层肌肉上，以防脱落；术后皮下注射250 mg/mL头孢曲松钠1 mL以防感染。

实验全部结束后，称量体重，并用过量麻醉药进行麻醉处死，检查插管位置。选择体重下降不超过20%、插管位置正确的动物所得的数据进行统计。

1.4.2 CFA炎症痛模型建立 从后脚掌的前两个趾垫稍下方插入27号针头并沿皮下推进针头到脚掌中心位置，皮下注射150 μL CFA溶液，使药物到达足底的敏感区域，并尽量使其损伤程度降到最小。

1.4.3 鞘内注射 鞘内给药时，动物在清醒状态下，放置于大鼠固定装置内，并限制其活动，PE-10细管暴露在装置外。通过PE-10细管用微量进液器往椎管内蛛网膜下腔(以下简称鞘内)注射相关药品。药物容量为10 μL，药品注射完毕后都需再注入10 μL生理盐水缓慢冲洗，确保药品全部进入大鼠椎管内蛛网膜下腔。

实验设对照组(CFA致炎)、生理盐水组(CFA致炎+生理盐水)和不同剂量BAM22处理组(CFA致炎+BAM22)。在足底注射CFA前5 min，鞘内预先注射BAM-22或生理盐水；于24 h和48 h时再分别注射一次。

1.4.4 行为学观察

1.4.4.1 撤足潜伏期(paw withdrawal latency, PWL)

测试 实验前将大鼠置于Plantar Test仪器内，连续训练3 ~ 5 d，使其适应环境与操作。以PWL作为疼痛指标[7,12,13]。调节热刺激强度，使其基础潜伏期平均值位于6.5 ~ 8.5 s之间。CFA致炎前间隔3 min测量1 次，取2 次测定的平均值作为正常PWL；致炎后24 h和48 h测其潜伏期作为致炎后PWL基础阈值；然后鞘内注射相关药物，每隔20 min测量1次PWL，持续60 min，方法同上。

将CFA致炎后的PWL基础阈值与致炎前的正常PWL相比，作为评定药物对CFA炎性大鼠的热痛觉过敏程度的影响，以变化百分数(% Baseline of PWL, % BPWL)=100% × (致炎后PWL基础阈值/致炎前正常PWL)表示。

将给药后PWL与致炎后PWL基础阈值相比作为评定药物的最大抗伤害作用，结果以最大可能作用的百分数[(% maximal possible effect, % MPE)=100%×(给药后PWL－致炎后PWL基础阈值)/致炎后PWL基础阈值]表示。

1.4.4.2 炎症水肿测试 以大鼠脚掌厚度变化作为炎症水肿程度的指标。0 h用游标卡尺测量大鼠正常脚掌厚度，以2次平均值为基础值。于致炎后24、48 h (鞘内注射相关药物前)再次测定脚掌厚度。将CFA致炎后脚掌厚度与正常大鼠脚掌厚度基础值相比作为评定药物抑制脚肿的作用，以脚肿变化百分数[% Edema=100% × (致炎后脚掌厚度－正常脚掌厚度) / 正常脚掌厚度]表示。

1.4.5 免疫组织化学实验 行为学实验后(CFA致炎后48 h)，用5%戊巴比妥钠(50 mg/kg)麻醉，剪开胸腔，暴露心脏，经升主动脉，先用200 mL磷酸盐缓冲液(0.1 mol/L PBS, pH 7.4)快速冲洗血液，继以用4%多聚甲醛500 mL (4℃, pH 7.4)持续灌注30 ~ 40 min。剪开椎管，暴露脊髓，分别取出脊髓腰膨大及大鼠L3−L5脊髓节段对应的背根神经节(dorsal root ganglion, DRG)。继续在4℃、4%多聚甲醛中固定6 h后，转而浸入30%蔗糖溶液中，置4℃冰箱直至组织块下沉。标本经OCT包埋，用冰冻切片机冰冻冠状连续切片，脊髓片厚40 μm (每4片挑1片)，切好贴于载玻片上；DRG片厚10 μm，切好直接贴于涂有0.01%多聚赖氨酸的载玻片上。所有切片均置于–20℃冰箱中保存，留做免疫组织化学实验。

脊髓切片用PBS洗脱后行免疫组织化学实验。步骤参见文献[14]：(1) PBS冲洗3次，每次10 min；(2) 3%过氧化氢孵育15 ~ 25 min以消除内源性过氧化氢酶的活性；(3)蒸馏水冲洗，PBS浸泡10 min；(4) 滴加5%正常羊血清和5%牛血清，孵育4 h；(5) 滴加抗CGRP (1:10 000)或nNOS (1:2 500)一抗，4℃冰箱过夜；(6) PBS冲洗3次，每次10 min；(7)滴加生物素标记羊抗兔IgG (1:200 in PBS)，室温反应2 h；(8) PBS冲洗3次，每次10 min；(9)加ABC试剂(2滴A试剂加入10 mL PBS中，再加2滴B试剂，立即混匀，4℃孵育30 min)，室温孵育2 h；(10) PBS冲洗3次，每次10 min；(11)加DAB，呈色1.5 ~ 2 min后，自来水冲洗终止反应。呈色后的脊髓切片裱于涂有明胶的载玻片上，自然晾干，梯度酒精脱水，二甲苯透明，中性树胶封片。

DRG切片直接进行在片免疫组织化学实验，步骤同上。显色后直接进行梯度酒精脱水，二甲苯透明，中性树胶封片。

1.5 图像分析 用Olympus BX51型显微镜进行观察，并用Olympus DP70型数码照相机图像采集系统拍照。应用Image-pro plus 6.0图像分析软件进行图像统计分析。

1.5.1 脊髓腰膨大CGRP免疫反应阳性产物分析 每组动物随机选取8 ~ 10张切片，对所有切片中脊髓背角浅层(I−II层) CGRP免疫反应阳性产物进行图像分析处理，对免疫反应阳性产物的平均光密度值进行统计分析。

1.5.2 DRG中CGRP阳性细胞统计 每张DRG切片任选6 ~ 7个视野(20倍)，每个视野随机计数200 ~ 300个细胞核清晰的细胞，对CGRP阳性细胞进行分类筛选：面积大于1 200 μm^{2}的细胞为大型细胞，面积介于600 μm^{2}至1 200 μm^{2}的为中型细胞，面积小于600 μm^{2}的为小型细胞。统计大鼠DRG以上三类细胞中CGRP阳性细胞占总细胞的比例。

1.5.3 脊髓腰膨大nNOS阳性细胞统计 每组动物随机选取8~10张切片，分别统计脊髓炎症侧、非炎症侧和中央管阳性细胞数量。

1.6 数据统计分析 各组数据均以means ± SEM表示，多组数据间以SigmaStat软件采用单因素方差分析(one way ANOVA)并继之以Tukey's 检验进行统计分析，组间比较用*t*检验，同组内比较用配对*t*检验。以$P < 0.05$表示差异有显著性。

2 结果

2.1 BAM22对CFA炎性大鼠热痛觉过敏的影响

我们参照文献[15-18]复制CFA炎性痛模型。从图1

可以看出，大鼠足底注射CFA (对照组)后24 h和48 h，炎症侧PWL基础阈值分别为3.8 s和4.3 s，明显低于致炎前的正常PWL (7.3 s)，与文献报道的结果类似[16-18]，说明产生了痛觉过敏，模型复制成功；而非炎症侧PWL基础阈值在24 h和48 h时分别为7.1 s和7.2 s，与正常PWL没有差异(图中未显示)，显示没有“镜像痛”的产生，与文献报道相似[19]。方便起见，在研究BAM22对CFA炎性大鼠影响的行为学实验中以炎症侧作为研究对象。图1显示鞘内注射不同剂量的BAM22对CFA炎性大鼠PWL基础阈值的影响。CFA致炎前，各组大鼠PWL基础值均在7.2 ~ 7.4 s左右。致炎后24 h和48 h时，生理盐水组大鼠PWL基础阈值分别为3.6 s和4.4 s，仅为正常大鼠PWL基础值的50.3%和60%左右，与对照组的炎症侧相似，表明生理盐水对CFA导致的痛觉过敏没有影响。在1.1 nmol BAM22组，CFA炎性大鼠PWL基础阈值在24 h和48 h时分别为3.4 s和4.2 s，与生理盐水组比较没有显著性差异($P > 0.05$)；3.3 nmol BAM22作用后48 h时，大鼠PWL恢复至5.8 s，为正常基础潜伏期的80.0%，与生理盐水组相比有显著性差异($P < 0.001$)。而在高剂量的10 nmol BAM22组，24 h和48 h时大鼠的PWL分别为4.6 s和6.0 s，分别为正常大鼠基础潜伏期的63.9%和83.2%，PWL的恢复程度均明显高于生理盐水组($P < 0.05$，$P < 0.001$)。

2.2 BAM22对CFA炎性大鼠抗伤害作用的影响

图2显示在CFA炎性大鼠中，鞘内注射不同剂量BAM22对伤害性热刺激作用的效果与时程。图2*A*显示，在CFA致炎0 h、24 h和48 h时，不同剂量BAM22作用20 min后对伤害性热刺激的作用。在0 h时，注射药物组大鼠的PWL均维持在正常PWL的–60% ~ –66% MPE之间，与生理盐水组没有统计学差异($P > 0.05$)。CFA致炎后24 h时，BAM22的作用显现剂量依赖效应。3.3 nmol和10 nmol BAM22

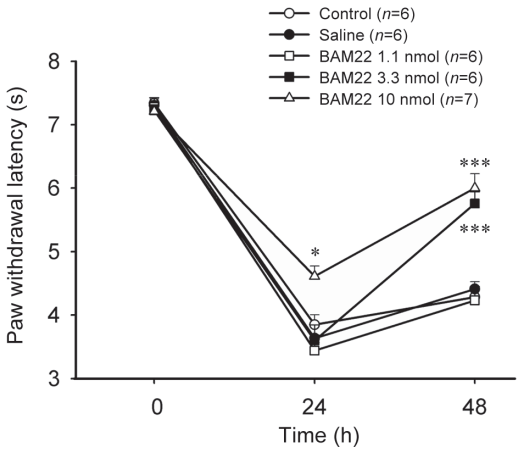

图 1. 鞘内注射BAM22对CFA炎性大鼠撤足潜伏期基础阈值的影响

Fig. 1. Effects of intrathecal (i.t.) administration of BAM22 on baseline paw withdrawal latency (PWL) in CFA model. PWL was measured to determine baseline before i.t. injection of saline or BAM22 at 0 h, 24 h and 48 h. Data were analyzed statistically by using one-way ANOVA followed by Tukey's test. Data are expressed as means ± SEM. $^{*}P < 0.05$, $^{***}P < 0.001$ compared with saline group.

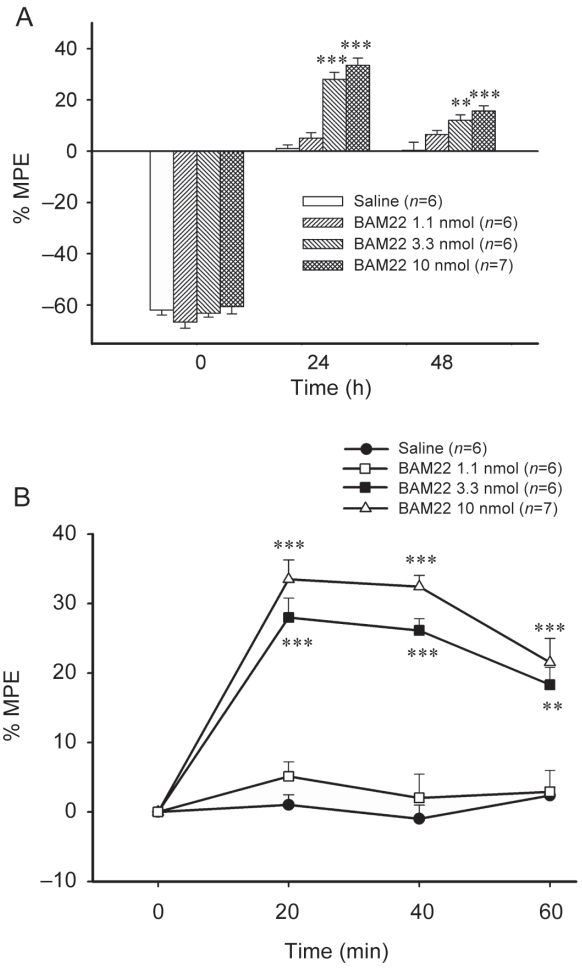

图 2. 鞘内注射BAM22对CFA炎性大鼠抗伤害作用的影响

Fig. 2. Antinociceptive effects of intrathecal (i.t.) administration of BAM22 on CFA model. *A*: Data were normalized as % MPE that was recorded 20 min after i.t. administration of saline or BAM22 at 0 h, 24 h and 48 h. *B*: Time courses of BAM22-induced responses at 24 h. Data are expressed as means ± SEM. $^{**}P < 0.01$, $^{***}P < 0.001$ compared with saline group.

组在注射20 min后PWL明显延长，分别延长28.0%和33.5% MPE，与生理盐水组比较有显著性差异($P < 0.001$)；48 h也有类似现象。图2*B*图进一步显示24 h时BAM22作用的时程变化。3.3 nmol和10 nmol BAM22组大鼠在20 min时对热刺激的抗伤害作用达最大，以后逐渐减小，其PWL在测试的1 h内均明显高于生理盐水组($P < 0.001$或$P < 0.01$)。

2.3 BAM22对CFA炎性大鼠脚肿的影响

图3显示鞘内注射BAM22对CFA炎性大鼠脚肿的影响。从图中可以看出：在致炎后24 h时，不同剂量的BAM22 (1.1 nmol, 3.3 nmol, 10 nmol)均不能抑制CFA引起的足跖红肿，脚肿比正常鼠增加81.3%～86.7%，与生理盐水组比较没有显著性差异($P > 0.05$)；在48 h时，低剂量的BAM22 (1.1 nmol, 3.3 nmol)不能抑制CFA引起的足跖红肿，但高剂量的BAM22 (10 nmol)能有效降低足跖红肿程度，肿胀程度仅增加60%左右，与生理盐水组(75%)比较存在显著性差异($P < 0.05$)。

2.4 BAM22对CFA炎性大鼠脊髓背角CGRP样免疫活性物质表达的影响

由图4可见，CFA致炎48 h后，CGRP免疫组织

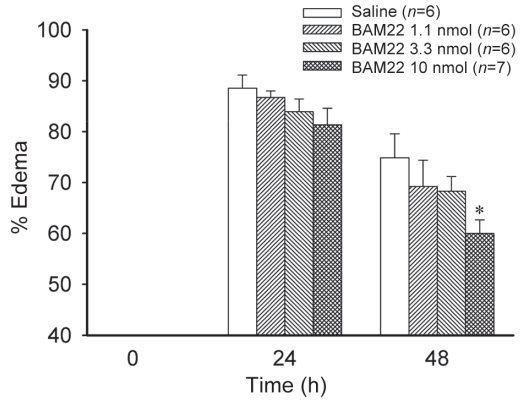

图 3. 鞘内注射BAM22对CFA炎性大鼠脚肿的影响

Fig. 3. Effects of intrathecal (i.t.) administration of BAM22 on CFA-induced paw edema. Data were normalized as % Edema that was recorded before i.t. administration of saline or BAM22 at 0 h, 24 h and 48 h. Data are expressed as means ± SEM. $^{*}P < 0.05$ compared with saline group.

化学染色显示大鼠腰段脊髓背角浅层都有CGRP样免疫活性物质(CGRP-IR)的分布，其中以I–II层的分布最密集，着色最深；III–V层阳性反应产物较稀疏，着色较淡，在其余灰质各层，CGRP-IR极弱或

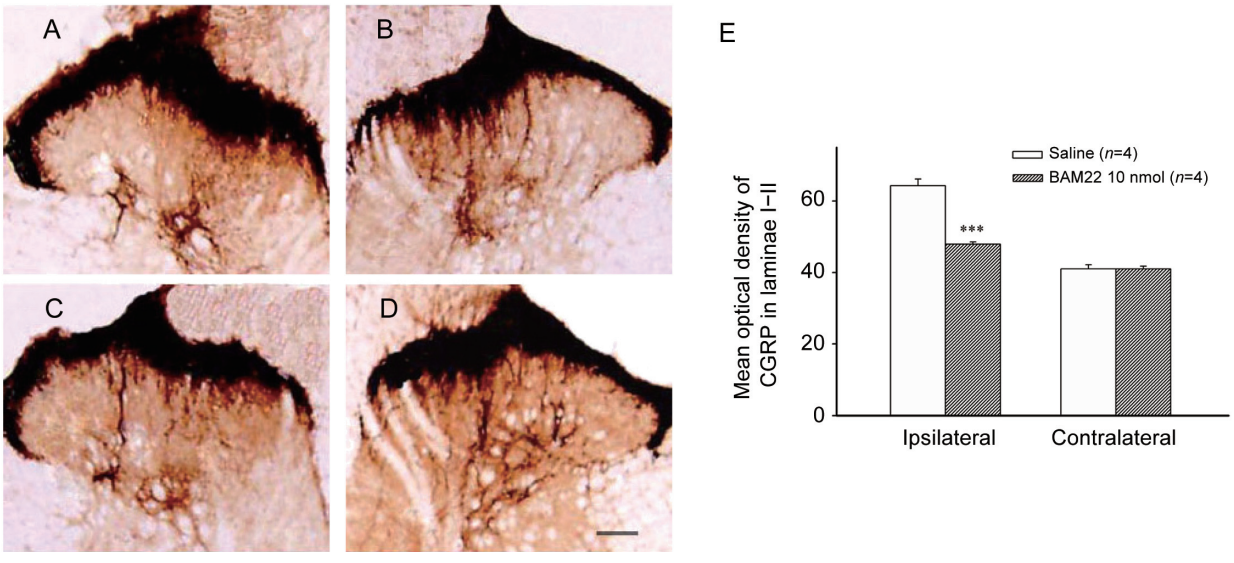

图 4. 鞘内注射BAM22对CFA炎性大鼠脊髓背角CGRP样免疫活性物质表达的影响

Fig. 4. Effects of intrathecal (i.t.) administration of BAM22 on the CFA-evoked expression of CGRP-immunoreactivity positive nerve fibers in the spinal dorsal horn. CFA was injected intraplantarly (i.pl.) at 0 h, and 10 nmol BAM22 / saline were administered i.t. at −5 min, 24 h and 48 h. The spinal cord at L3−L5 was harvested at 49 h. Photomicrographs of transverse sections of the spinal cord show CGRP expression following treatment with saline (*A*, *B*) or BAM22 (*C*, *D*). *A* and *C* were in the side ipsilateral to CFA injection, while *B* and *D* were the contralateral side of *A* and *C*. Histograms (*E*) show mean optial density (± SEM) of CGRP-like immunoreactivity in laminae I–II of the spinal cord. $^{***}P < 0.001$ compared with saline group. n=4 in each group, Scale bar, 100 μm.

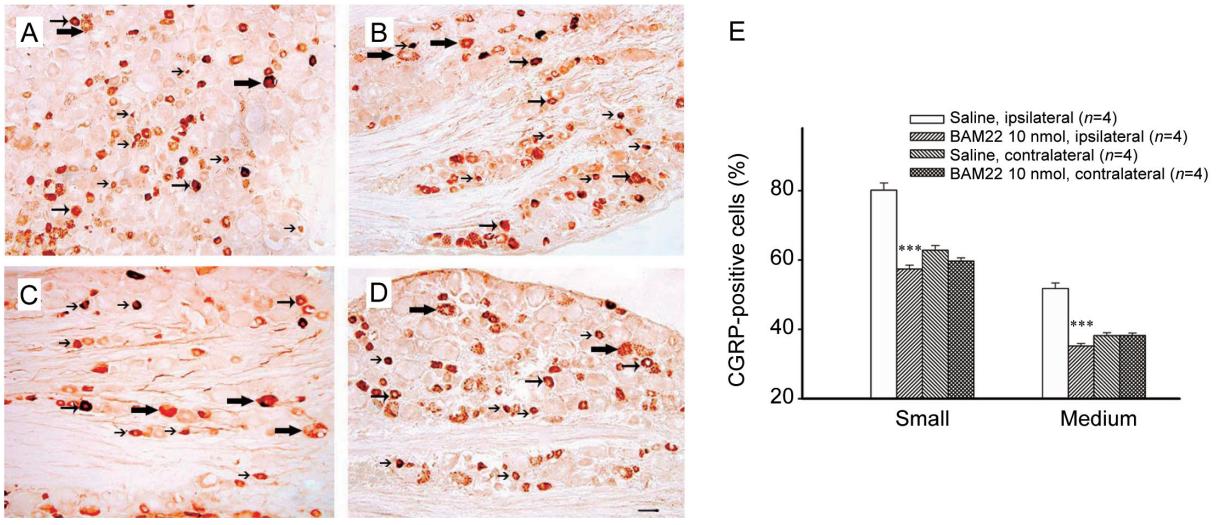

图 5. 鞘内注射BAM22对CFA炎性大鼠DRG中CGRP免疫阳性细胞表达的影响

Fig. 5. Effects of intrathecal (i.t.) administration of BAM22 on the CFA-evoked expression of CGRP-positive cells in DRG. CFA was injected intraplantarly (i.pl.) at 0 h, and 10 nmol BAM22 / saline were administered i.t. at −5 min, 24 h and 48 h. The DRG at L3−L5 was harvested at 49 h. Each photomicrograph is a representative example of CGRP-positive cells in DRG following treatment with saline (*A*, *B*) or BAM22 (*C*, *D*). *A* and *C* were in the side ipsilateral to CFA injection, while *B* and *D* were the contralateral side of *A* and *C*. CGRP-immunoreactivity (IR) is expressed in small (small arrows), medium (medium arrows) and large (large arrows) subpopulations of DRG cells. Histograms (*E*) show the percentage of CGRP-IR-positive cells in small- and medium-diameter subpopulations. $^{***}P < 0.001$ compared with saline group. n =4 in each group, Scale bar, 50 μm.

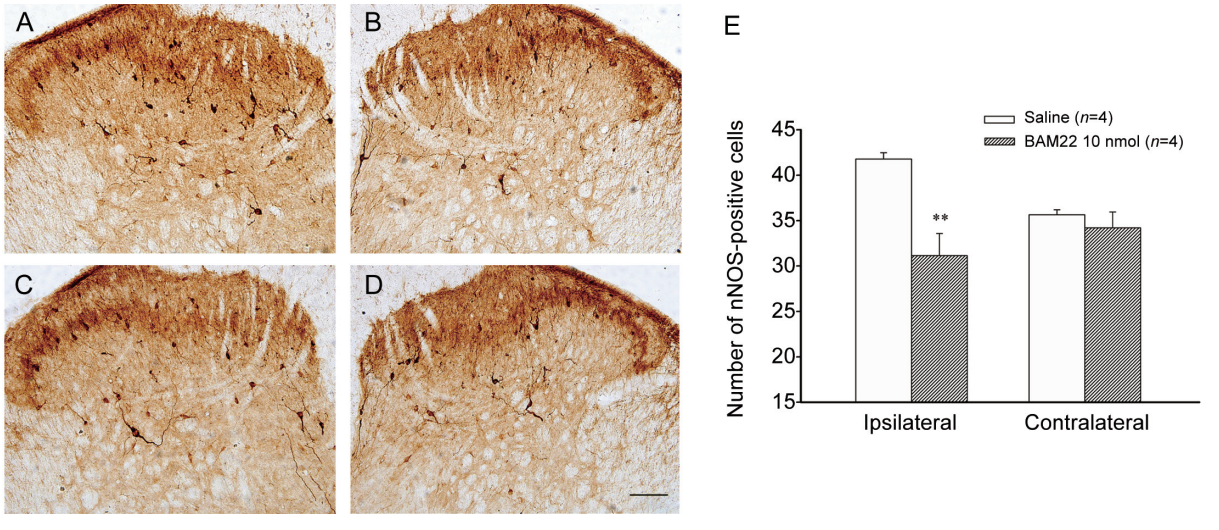

图 6. 鞘内注射BAM22对CFA炎性大鼠脊髓背角nNOS阳性细胞表达的影响

Fig. 6. Effects of intrathecal (i.t.) administration of BAM22 on the CFA-evoked expression of nNOS-positive cells in the spinal dorsal horn. CFA was injected intraplantarly (i.pl.) at 0 h, and 10 nmol BAM22 / saline were administered i.t. at −5 min, 24 h and 48 h. The spinal cord at L3−L5 was harvested at 49 h. Each photomicrograph is a representative example of nNOS-positive cells in the spinal dorsal horn following treatment with saline (*A*, *B*), or BAM22 (*C*, *D*). *A* and *C* were in the side ipsilateral to CFA injection, while *B* and *D* were the contralateral side of *A* and *C*. Histograms (*E*) represent the means ± SEM number of nNOS-positive cells in spinal dorsal horn. $^{**}P < 0.01$ compared with saline group. n=4 in each group, Scale bar, 100 μm.

阴性。因此，仅计算脊髓背角I−II层CGRP-IR的平均光密度值。

生理盐水组大鼠脊髓背角炎症侧CGRP-IR平均光密度值为64.3，明显高于非炎症侧的41.0 ($P < 0.001$，图4*A*、*B*、*E*)；10 nmol BAM22组脊髓背角炎症侧CGRP-IR 平均光密度值为47.9，与生理盐水组相比降低了25.2%，存在显著差异($P < 0.001$，图4*C*、*E*)；非炎症侧为41.0，与生理盐水组相比没有显著性差异($P > 0.05$，图4*D*、*E*)。

2.5 BAM22对CFA炎性大鼠DRG中CGRP阳性细胞表达的影响

免疫组织化学染色显示(图5)，DRG中的CGRP样免疫活性物质分布在中、小型神经元的细胞质中。在生理盐水组，CFA炎症侧CGRP小、中型阳性细胞表达分别为80.1%和51.8%，非炎症侧分别为62.8%和38.2%，炎症侧的阳性率明显高于非炎症侧($P < 0.01$)。10 nmol BAM22组炎症侧CGRP阳性细胞在小、中型细胞中表达分别为57.4%和35.2%，与生理盐水组相比存在显著差异($P < 0.001$)；非炎症侧表达分别为59.7%和38.3%，与生理盐水组相比没有显著性差异($P > 0.05$)。BAM22组与生理盐水组中CGRP大细胞的阳性率没有显著性差异(统计图中未显示)。

2.6 BAM22对CFA炎性大鼠脊髓背角nNOS阳性细胞表达的影响

图6显示鞘内注射BAM22对CFA引起的脊髓背角nNOS阳性细胞表达的影响。在CFA致炎 48 h后，生理盐水组大鼠脊髓背角炎症侧nNOS阳性细胞数量平均为41.8，非炎症侧为35.6 ，炎症侧明显高于非炎症侧($P<0.01$)。10 nmol BAM22组脊髓背角炎症侧nNOS阳性细胞数量平均为31.1, 与生理盐水组相比减少了25.6%，存在显著差异($P < 0.01$)；非炎症侧nNOS阳性细胞数量为34.2，与生理盐水组没有显著性差异($P > 0.05$)。此外，各组中央管周围nNOS阳性细胞数量均没有差异(图未显示)。

3 讨论

痛觉过敏是炎症痛的共同特征。足底注射CFA引起的伤害性信息传入类似于临床的炎症痛情形，是目前疼痛研究中一种理想的慢性痛模型[15]。CFA产生的热痛觉过敏一般在4 h内发展，2 d内痛觉过敏维持在最高水平[16-18]，之后慢慢恢复，痛觉过敏和水肿可持续3 周左右[20]。本研究从行为学角度以伤害性热刺激诱发的PWL和脚掌厚度作为实验指标，研究CFA致炎早期大鼠产生热痛觉过敏及水肿情况，结果与前人所建立的CFA模型吻合[21]。行为学研究显示，大鼠非炎症侧的PWL和脚掌厚度与正常鼠没有差异，说明不存在“镜像痛”现象，这与Omote等[19]在关节内注射CFA观察到对侧没有反应的结果是一致的。

由于DRG所处的解剖位置特点，目前在体实验中还没有适当的方法把药物直接作用于DRG，而外周给药(如皮下注射)时药物扩散到DRG的浓度极低，无法研究DRG的药理学机制。鞘内注射用于研究脊髓背角的神经药理学机制已得到广泛应用[7-11]，通过鞘内给药研究DRG神经元功能的变化是目前在体DRG药理学机制研究的常用方法[14,22]。我们参照以上文献进行鞘内给药来研究DRG神经元的功能变化。在脊髓背角浅层存在大量初级感觉神经元的传入末梢，鞘内注射BAM22很可能通过作用于这些传入末梢(即突触前膜)上的感觉神经元特异性受体(sensory neuron-specific receptor, SNSR)，进而逆向影响DRG中神经元的功能，也有可能通过局部作用于DRG，但具体作用途径有待进一步探讨。

前期对BAM22镇痛作用的研究主要集中在急性痛[7,9,10]，那么BAM22在慢性炎性痛中的作用又是如何？因为慢性炎性痛的治疗更具有临床意义。本研究结果显示，与生理盐水组相比，鞘内注射BAM22能剂量依赖地延长CFA炎性痛引起的PWL基础阈值，足趾红肿也得到一定抑制，同时在第二天和第三天，还具有明显的抗伤害作用。以上结果说明，BAM22参与抑制早期慢性炎性痛的产生和发展过程。

BAM22与μ-[23]、δ-[24]和κ-[25]三类阿片受体亚型和SNSR均有很高的亲合力[24]。阿片肽通过阿片受体起到镇痛作用是众所周知的。而SNSR是新近发现的一类受体，其cDNA独特地分布在DRG中管理痛觉的中、小型神经元中，推测与痛觉信息的传递有关[24]。Hong等[8]证明了纳络酮能部分阻断BAM22的作用，证实了SNSR参与了痛觉调制，Chen等[26]运用SNSR特异性激动剂BAM8-22和MSH发现能降低福尔马林引起的疼痛，而且其作用不受纳络酮影响，进一步证明BAM22可通过SNSR的非阿片途径参与痛觉信息的调制过程。BAM22是目前发现的体内唯一一种SNSR的天然配体，研究其抗痛觉过敏及作用机制有重要临床意义。

我们进一步探讨BAM22抑制CFA引起痛觉过敏

的机制。CGRP是一种由37个氨基酸组成的内源性生物活性神经多肽，是疼痛信息处理中的重要分子，在脊髓痛觉信息传导以及痛觉的形成与维持中发挥重要作用。脊髓背角灰质浅层(I-II层)和DRG中的中、小型神经元胞体均含有丰富的CGRP[27,28]。CGRP主要由DRG中的神经元产生[29]。正常大鼠足底注射生理盐水时，DRG中小型和中型CGRP阳性细胞分别占细胞总数的(54.6 ± 2.5)%和(30.5 ± 1.0)%[22]。当外周炎症时，CGRP合成增高[30]，一方面从外周感觉神经末梢释放[31]，去激活炎性细胞[32]，增强外周感受器的敏感性[33]；另一方面从初级传入末梢的中枢端释放，去敏化脊髓背角疼痛神经元[34]，同时也促进脊髓背角的其它疼痛递质的释放，如P物质(substance P, SP)[35]和谷氨酸[36]。阻断脊髓CGRP受体则可以抑制与疼痛相关的脊髓背角神经元的活性[37]和痛觉过敏[38]。以上结果已经表明CGRP在炎性痛中起到关键作用。本研究在足底皮下注射CFA导致注射侧DRG中的中、小型神经元中的CGRP阳性细胞明显增加，初级神经元传入纤维所在的脊髓背角浅层的CGRP样免疫活性物质的表达也明显增加，而鞘内注射BAM22则会降低DRG中CGRP阳性细胞的比例和脊髓背角浅层CGRP样免疫活性物质的含量。进一步研究显示，应用SNSR的特异性激动剂BAM8-22和(Tyr^6)-γ2-MSH-6-12也得到相同的结果(本文未显示)。以上结果提示，BAM22通过SNSR抑制CGRP的合成和释放来削弱CFA引起的痛觉过敏。

Lembo等[24]的原位杂交实验显示，仅有7%表达SNSR mRNA的细胞表达CGRP和SP，约76%的SNSR阳性细胞结合植物凝集素B4 (plant lectin isolectin B4, IB4)；Hager等[39]的免疫荧光染色显示，SNSR主要表达于IB4阳性中、小型DRG神经元中，仅有11%与CGRP共表达。可见SNSR主要表达于非肽能神经元中，在肽能神经元中极少表达；而CGRP主要表达于肽能神经元。由于SNSR与CGRP有少量(7%~11%)共存，激活SNSR可能通过自身细胞内信号诱导CGRP的表达增加，但更有可能是通过细胞间某种机制间接调节。

NO是小分子生物活性气体分子，在体内半衰期只有3~5 s，故很难直接准确测定其含量，而NO的合成主要受一氧化氮合酶 (nitric oxide synthase, NOS)的影响，测定NOS的量可以间接反映NO的生成速度和量。NO作为一种新型的神经递质，广泛参与外周及中枢神经系统痛觉信息传递和调制过程[40,41]，它能诱导DRG神经元的持续性放电[42]，还能通过刺激邻近细胞增加神经肽[43]和兴奋性氨基酸[44]的释放来促进感觉神经元的敏感化，还能参与介导吗啡戒断大鼠脊髓神经元敏感化[45]。NOS有三种亚型，分别是诱导型一氧化氮合酶(inducible nitric oxide synthase, iNOS)、nNOS和内皮型一氧化氮合酶(endothelial nitric oxide synthase, eNOS)，研究表明nNOS是NO在神经系统中生成的关键酶，参与慢性炎症痛的形成与发展[46]。Chen等[47]研究表明，在CFA炎性痛中，DRG中的nNOS mRNA表达明显增加，进一步确认nNOS参与了慢性炎性痛的调制过程。免疫荧光显示，在DRG中NOS阳性细胞中有74.6%与CGRP共存[48]，这为NOS调节CGRP合成提供细胞学证据。Boettger等[49]研究表明，在CFA炎性鼠的DRG中，nNOS缺乏会导致其下游的CGRP免疫阳性神经元和CGRP基因表达明显减少，证实了nNOS对CGRP合成具有直接的调制作用。Garry等[50]进一步研究表明，辣椒素引起脊髓CGRP释放是由NO调制的，而不是由cGMP调制的。本研究结果显示，BAM22能显著降低CFA注射侧DRG中CGRP阳性细胞以及脊髓背角CGRP样免疫活性物质和nNOS阳性细胞的表达。据此，我们推测，SNSR可能通过DRG和脊髓水平抑制CFA引起的痛觉过敏等过程：(1)通过DRG水平调节。当伤害性信息传到DRG中非肽能中、小型神经元时，首先激活SNSR，一方面可能直接通过细胞内机制直接调制CGRP的合成与释放(如上所述)；另一方面，SNSR更可能通过细胞间机制抑制nNOS合成NO，进而抑制其下游的CGRP的合成和释放[49]。(2)通过脊髓水平调节。DRG中非肽能神经元接受到伤害性信息时，产生BAM22，通过传入神经纤维运输到脊髓背角[14]，作用于脊髓背角神经元，通过抑制NO的合成，一方面可降低兴奋性氨基酸的释放，进而降低神经元的敏感性，抑制炎症痛信息的传入[42-44]；另一方面可直接抑制脊髓CGRP的释放[50]。

以上结果有助于我们了解早期炎性痛产生的可能机制。BAM22在炎性痛觉过敏中起着重要作用，不仅表现在激活SNSR以下调DRG中CGRP阳性神经细胞比例以及脊髓背角CGRP样免疫活性物质和nNOS阳性细胞数量，还表现在降低痛觉过敏、减少炎性部位红肿和增强抗伤害作用。由此可以得出结论，BAM22可以通过SNSR导致nNOS和CGRP下调进而抑制早期炎性痛觉过敏的发生与发展。

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