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川芎嗪对神经病理痛大鼠的镇痛作用及对
背根节神经元电压门控钙通道的影响

Analgesic effect of tetramethylpyrazine
on neuropathic pain rats and its effects on voltage gated
calcium channels in dorsal root ganglion neurons

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摘要

目的：应用脊神经结扎（Spinal nerve ligation, SNL）制作神经病理痛（Neuropathic pain, NP）大鼠模型，给予不同剂量川芎嗪（Tetramethylpyzine, TMP）治疗，检测神经病理痛大鼠的机械缩足反应阈值（Mechanical withdrawl threshold, MWT）和热缩足潜伏期（Thermal withdrawl latency, TWL）及伴随的抑郁行为，探讨川芎嗪对神经病理痛的镇痛作用，神经病理痛模型大鼠是否表现出抑郁行为，及川芎嗪是否对伴随神经病理痛的抑郁症状具有抗抑郁效果。采用全细胞膜片钳技术检测川芎嗪对背根神经节（Dorsal root ganglion, DRG）电压门控钙通道电流的影响，从电压门控钙通道角度探讨川芎嗪镇痛的作用机制。

方法：取健康成年清洁级雄性 SD 大鼠 50 只，体重 180-220g，采用随机数字表法，将大鼠分为 5 组：假手术组（Sham 组）、模型组（DMSO 组）和川芎嗪低剂量组、川芎嗪中剂量组、川芎嗪高剂量组，每组 10 只。Sham 组大鼠仅分离坐骨神经但不结扎，DMSO 组、川芎嗪低剂量组、川芎嗪中剂量组和川芎嗪高剂量组采用脊神经结扎（SNL）手术法建立神经病理痛模型。川芎嗪各组于 SNL 手术后开始腹腔注射川芎嗪，每天一次，低剂量组给药剂量 10mg/kg，中剂量组给药剂量 20mg/kg，高剂量组给药剂量 40mg/kg，直至处死的当天；Sham 组以等容量生理盐水替代，DMSO 组以等量 3% DMSO 代替。各组大鼠分别于手术前第 1 天、手术后第 3 天、7 天、14 天、30 天，检测其机械痛阈值和热痛阈值，并于术后第 15 天、19 天、21 天、23 天分别测定糖水偏好、旷场实验、强迫游泳、食物消耗等抑郁行为学指标。另外取正常大鼠，急性分散背根神经节（DRG），采用全细胞膜片钳技术记录 DRG 电压门控钙通道电流的 I-V 曲线、激活曲线、失活曲线及非 L 型电压门控钙通道电流，分析川芎嗪对 DRG 电压门控钙通道的影响。

结果：与 DMSO 组比较，川芎嗪低剂量组、川芎嗪中剂量组、川芎嗪高剂量组均能使神经病理痛模型大鼠的热痛阈值升高，只有川芎嗪高剂量组可以使神经病理痛模型大鼠机械痛阈值升高；各组神经病理痛模型大鼠在 30 天内糖水偏好率、旷场实验中大鼠活动的总路程及食物消耗量变化均没有统计学差异，没有表现出抑郁行为。川芎嗪还可以使 DGR 电压门控钙通道电流 I-V 曲线上移和激

活曲线右移，呈浓度依赖性抑制 DRG 电压门控钙通道电流，对非 L 型电压门控钙通道电流也有抑制作用，但对失活曲线没有影响。

结论:川芎嗪能够改善大鼠神经病理痛模型大鼠的疼痛行为，其机制可能与抑制 DRG 电压门控钙通道有关。

关键词: 川芎嗪 背根神经节 神经病理痛 电压门控钙通道 脊神经结扎

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Abstract

Objective: The rat model of neuropathic pain was made by sciatic nerve ligation (SNL). In order to study the analgesic effect of tetramethylpyrazine on neuropathic pain, whether the neuropathic pain model will show depressive behaviors and whether tetramethylpyrazine has antidepressant effects on depressive symptoms associated with neuropathic pain, different doses of tetramethylpyrazine was given to detect the thermal withdrawal latency and mechanical withdrawal threshold and concomitant depressive behaviors in rats with neuropathic pain. The effects of tetramethylpyrazine on calcium current in dorsal root ganglion were examined by whole cell patch clamp technique. The mechanism of analgesia of tetramethylpyrazine was explored from the perspective of calcium channels.

Methods: 50 healthy adult male Sprague-Dawley rats, weight 180-220g, were randomly divided into 5 groups: Sham group (control), DMSO group (model) and low dose tetramethylpyrazine group, medium dose tetramethylpyrazine group and high dose tetramethylpyrazine group, 10 in each group. In Sham group rats sciatic nerve were exposed only, but not ligated, in DMSO, low dose tetramethylpyrazine group, medium dose tetramethylpyrazine group and high dose tetramethylpyrazine group rats sciatic nerve ligation (SNL) method was adopted to establish the neuropathic pain model. tetramethylpyrazine group rats after SNL began to be given tetramethylpyrazine through intraperitoneal injection, 1 time/day, low dose group 10mg/kg, medium dose group 20mg/kg, high dose group 40mg/kg, until to the day of death; Sham group rats were injected with normal saline instead, DMSO group with equal volume of 3% DMSO instead. Mechanical withdrawal threshold and thermal withdrawal latency of each group of rats were measured on the first day before operation, 3rd day after operation, 7th day after operation, 14th day after operation, 30th day after operation, The effects on depressive behaviors were measured on the 15th day after operation, 19th day after operation, 21st day after operation and 23rd day after operation, by sugar water preference, open field experiment, forced swimming test, food

consumption test. Also taking normal rats, scattered dorsal root ganglion (DRG) acutely, recorded the I-V curve of DRG calcium current, the activation curve, the inactivation curve, the concentration curve, and the non L-calcium current with whole cell patch clamp technique to analyze the effect of tetramethylpyrazine on DRG calcium currents.

Results: Compared with DMSO group, low dose, middle dose, and high dose of tetramethylpyrazine can increase thermal withdrawl latency, but only high dose of tetramethylpyrazine can increase mechanical withdrawl threshold. The SNL neuropathic pain model show no depressive behaviors. Tetramethylpyrazine has no effect on depressive behaviors of neuropathic pain rats. Tetramethylpyrazine can also dose-dependently inhibit I-V curve, activation curve of calcium current in DRG, inhibit non L-type calcium current, but has no effect on the inactivation curve.

Conclusion: Tetramethylpyrazine can play an analgesic effect on neuropathic pain rats, which mechanisms may be related to inhibiting effect on DRG calcium current.

Key Words: Tetramethylpyrazine; DRG; Neuropathic pain; voltage gated calcium channels; spinal nerve ligation

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英文缩略词表

英文缩写	英文全称	中文全称
IASP	The International Association for the Study of Pain	国际疼痛研究学会
NP	neuropathic pain	神经病理痛
CCI	Chronic constriction injury	慢性坐骨神经压迫
TNF- α	Tumor necrosis factor- α	肿瘤坏死因子 α
IL-1 β	Interleukin-1 β	白细胞介素-1 β
IL-6	Interleukin-6	白细胞介素-6
VGCC	Voltage-gate calcium channel	电压门控钙通道
HVA	High voltage activated	高电压激活
LVA	Low voltage activated	低电压激活
SNL	Spinal nerve ligation	脊神经结扎
COX-2	Cyclooxygenase-2	环氧化酶-2
DRG	Dorsal root ganglion	背根神经节
LTP	Long-term potentiation	长时程增强
PSNL	Partial sciatic nervous ligation	部分坐骨神经结扎
SNI	Spared nerve injury	保留神经损伤
TMP	Tetramethylpyzine	川芎嗪
OFT	Open-field test	旷场实验
MWT	Mechanical withdrawl threshold	机械缩足反应阈值
TWL	Thermal withdrawl latency	热缩足潜伏期

第一章 前言

疼痛是一种令人不愉快的感觉体验,同时还可能发生生理上和心理上的变化,往往由机体组织损伤或者病变引起。有时在没有组织损伤的情况下也可能出现疼痛,这种疼痛往往与心理因素有关。伤害性刺激传递到外周神经痛觉感受器后,形成动作电位,传递到大脑相关感受区,产生痛觉。根据疼痛原因,疼痛可分为生理性疼痛和病理性疼痛。生理性疼痛是一种防御反应,当机体组织受到损伤或即将受到损伤时,通过一系列神经调节产生疼痛感觉来向机体发出警戒信号,以避免进一步的伤害,当机体组织发生一定的病变,形成长期甚至超过3个月的疼痛,这对患者来说是一种难以忍受的精神折磨,严重影响了患者的工作和生活质量等,这时的疼痛便失去了防御反应的意义,成为严重的经济和社会问题^[1]。病理性疼痛是由于机体得了某种疾病而引起的疼痛。

除上述分类,根据不同分类标准,疼痛类型多种多样。根据时间长短,疼痛分为急性疼痛和慢性疼痛。急性疼痛是指持续时间较短的疼痛,比如皮肤划伤痛、癌症疼痛、医学手术疼痛等。慢性疼痛是长时间的疼痛,例如创伤后神经病变痛、神经病理痛、带状疱疹后神经病理痛、三叉神经病理痛、糖尿病周围神经病理痛、术后神经病变痛、中枢性卒中后神经病理痛等。根据疼痛的性质,可以将疼痛分为钝痛、闷痛、胀痛、刺痛、切割痛、酸痛、锐痛、绞痛、灼痛等。根据疼痛程度可将疼痛分为微痛、轻痛、甚痛、剧痛等。根据躯体部位可将疼痛分为头痛、颌面痛、颈项痛、肩背痛、胸痛、上肢痛、腹痛、腰骶痛、盆痛、髌髁痛、下肢痛等^[2]。

1 神经病理痛概述

国际疼痛研究学会(The International Association for the Study of Pain, IASP)在2014年发表的报告中将神经病理痛(neuropathic pain, NP)定义为一类感觉神经损伤性疼痛或者感觉神经病变性疼痛,包括外周纤维(A β , A δ 和C纤维)和中枢神经元受损或病变性疼痛^[3]。发生在中枢的为中枢性神经病理痛,发生在外周的为外周性神经病理痛^[4]。流行病学研究显示,神经病理痛约占人群的7-10%^[5],女性比男性发病率较高(女性为8%,男性为5.7%),年老者发病率比年轻

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