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降钙素基因相关肽、血管活性肠肽在复合应激大鼠模型阴茎组织中表达及伊木萨克干预的研究*

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摘要 目的:检测复合应激大鼠模型阴茎组织中降钙素基因相关肽(CGRP)、血管活性肠肽(VIP)的表达,并观察伊木萨克片对二者表达的影响。**方法:**选用 56 只正常雄性 SD 大鼠,其中 10 只为正常对照组(N),余 46 只为造模组,采用富含环境雌激素饲料+寒冷环境的干预条件建立复合性应激大鼠模型(20 w),并随机将其分为模型组(B1)、自然恢复组(B2)和伊木萨克干预组(B3),药物干预 2 w 后,免疫组化及 Western blot 方法检测大鼠阴茎组织中 CGRP、VIP 的表达。**结果:**① 大鼠阴茎组织中 CGRP 表达:B1、B2 组较 N 组明显减少($P<0.05$);B3 组较 B1、B2 组明显增多($P<0.05$)。② 大鼠阴茎组织中 VIP 表达:B1、B2 组较 N 组显著降低($P<0.05$);B3 组较 B1、B2 组显著升高($P<0.05$)。**结论:**复合应激大鼠模型阴茎组织中 CGRP、VIP 明显减少,伊木萨克片干预可抑制此变化。

关键词:勃起功能障碍;降钙素基因相关肽;血管活性肠肽;伊木萨克

中图分类号:R-33; Q593; R697.1 **文献标识码:**A **文章编号:**1673-6273(2019)08-1446-04

Calcitonin Gene Related Peptide and Vasoactive Intestinal Peptide Expression in Penis Tissue of Complex Stress Rats Model and the Yimusake Intervention Effect*

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ABSTRACT Objective: To detect the expression of CGRP、VIP in the penis tissue of complex stress rats model, and explore the role of Yimusake. **Methods:** A total of 56 mature male Sprague-Dawley rats were randomly divided into 2 groups: normal control group (N, n = 10) that had normal environment and diet, and model group (n = 46) were treated with spinach and coriander diet in a cold-wet environment for 24 w. Then, the model rats were randomly divided into 3 groups (B1, B2, B3), after treated by Yimusake (250 mg/kg) for 2 weeks, CGRP, VIP were detected by immunohistochemistry (IHC) and Western-blot analysis. **Results:** ① CGRP expression in rat penis tissue: B1, B2 groups were decreased compared with N group($P<0.05$); B3 group was increased compared with B1, B2 group ($P<0.05$). ② VIP expression in rat penis tissue: B1, B2 groups were decreased compared with N group ($P<0.05$); B3 group was increased compared with B1, B2 group ($P<0.05$). **Conclusion:** CGRP and VIP expression were decreased in the complex stress rats model, Yimusake can promote the CGRP and VIP expression.

Key words: Erectile dysfunction; Calcitonin gene related peptide; Vasoactive intestinal peptide; Yimusake

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前言

应激为持续或预期的对体内平衡或健康的威胁,急性或慢性暴露于应激会引起一系列生理和行为反应,显著改变人类和实验动物的代谢和行为状态^[1]。应激诱导的神经内分泌下丘脑-垂体-肾上腺轴的激活增加糖皮质激素的合成和葡萄糖的利用度,以满足其他生理和行为应激反应的代谢需求^[2]。大量来自

临床和动物学研究证实,慢性应激可引起机体过度的炎症反应和氧化应激损伤^[3,4],与心血管疾病、肥胖、勃起功能障碍等多种疾病发生发展密切相关,主要通过影响机体神经-内分泌-免疫系统的紊乱对机体中枢神经系统和外周组织产生广泛的影响^[5-9]。降钙素基因相关肽(calcitonin gene-related peptide, CGRP)是一种具有强大血管舒张功能的神经肽,对应激损伤具有保护作用^[10,11]。血管活性肠肽(vasoactive intestinal peptide,

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VIP)具有刺激血管扩张、降低动脉血压、抗炎和抗应激损伤等多种生理作用^[12-14]。本课题组前期研究发现复合应激大鼠模型出现勃起功能障碍,伊木萨克片(国药准字 Z65020144)干预后可促进勃起功能恢复。因此,本研究拟检测复合应激大鼠模型阴茎组织中 CGRP 和 VIP 表达,并观察伊木萨克对二者表达的影响,探讨该药促进勃起功能的可能机制。

1 材料与方法

1.1 实验动物

正常雄性 SD 大鼠 56 只,鼠龄 70 d,体质量 200 ± 20 g,购自新疆医科大学实验动物中心。

1.2 主要仪器与试剂

阿朴吗啡(Apomorphine,APO,美国 Sigma 公司),黄体酮(浙江仙琚制药股份有限公司),雌二醇(宁波市三生药业有限公司),伊木萨克片(新疆和田维吾尔药业有限责任公司),Anti-CGRP 抗体(ab47027)、Anti-VIP 抗体(ab8556)和 Anti-beta Actin 抗体(ab8227,美国 Abcam 公司),BCA 蛋白定量试剂盒(百泰克公司),WesternBreeze 免疫检测试剂盒(Invitrogen 公司),二步法免疫组化检测试剂盒(北京中杉金桥公司),免疫组化染色试剂盒(北京中杉金桥公司)。

1.3 动物分组和复合应激大鼠模型的制备

56 只正常雄性 SD 大鼠,随机抽取 10 只为正常对照组(N),余 46 只为造模组,采用环境雌激素样饮食联合冷刺激的干预条件建立 ED 大鼠模型,并随机将其分为复合应激大鼠模型组(B1,16 只)、自然恢复组(B2,15 只)、伊木萨克给药组(B3,15 只)。

1.3.1 环境雌激素样饲料的制备 按每 1 kg 大鼠常规饲料中加入茺萎实和菠菜实各 150 g,购自新疆医科大学实验动物中心。

1.3.2 复合应激大鼠模型的造模方法 N 组于常规环境中饲养(温度 $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$,湿度 55%-65%),普通饲料饲养。造模组置于人工气候箱中饲养(设定温度 6°C ,湿度 85-95%),环境雌激素样饲料饲养。

1.3.3 伊木萨克干预方法 ① N 组:大鼠予以等剂量蒸馏水灌胃,每天 1 次,连续 2-3 w;② B1 组:维持造模条件不变,大鼠予以等剂量蒸馏水灌胃,每天 1 次,连续 2-3 w;③ B2 组:去除环境雌激素样饮食联合冷刺激的干预条件,大鼠予以等剂量蒸馏水灌胃,每天 1 次,连续 2-3 w;④ B3 组:去除环境雌激素

样饮食联合冷刺激的干预条件,大鼠予以伊木萨克片 250 mg/kg 灌胃,每天 1 次,连续 2-3 w。

1.4 免疫组化检测

严格按照试剂盒步骤进行,封片后显微镜观察并采集图像,结果按文献^[4]方法量化,每个阴茎组织样本随机选择 10 个视野,阳性细胞所占视野中的百分比为阳性范围,包括 5 级,即 0(<5%)、1(6%-25%)、2(26%-50%)、3(51%-75%)、4(>75%);着色强度是指阳性细胞染色的深浅为,包括 4 级,即 0(几乎不着色)、1(着色浅)、2(着色深)、3(着色非常深),综合结果 = 阳性范围 × 着色强度 × 25,免疫反应值在 0~300。

1.5 Western-blot

阴茎组织块称重并研碎,加入 RIPA 裂解液,离心收集上清,并测定蛋白质浓度。取 50 μg 蛋白质于 10% 十二烷基磺酸钠聚丙烯酰胺凝胶中,25 mA/胶恒定电流电泳至分离胶底端;恒定电压、常温转至 PVDF 膜上,封闭 1 h。加入 Anti-CGRP 抗体(ab47027)、Anti-VIP 抗体(ab8556)和 Anti-beta Actin 抗体(ab8227)4 $^{\circ}\text{C}$ 过夜,PBS 缓冲液清洗。加入通用型二抗(Invitrogen 公司),室温孵育 2 h,PBS 缓冲液清洗。后加入 DAB 显色液,直至目的蛋白质条带出现,Quantity one 软件进行灰度扫描。

1.6 统计学处理

采用 SPSS 17.0 统计软件包分析数据,计量资料用均数 ± 标准差表示,多组数据做正态检验和方差齐性检验,满足条件采用单因素方差分析(ANOVA),如不满足条件求出秩次进行方差分析,以 $P < 0.05$ 为差异具有统计学意义。

2 结果

2.1 大鼠阴茎组织中 CGRP 表达

成功制备复合应激大鼠模型后随机分为 B1、B2 和 B3 组,伊木萨克干预 2 w 后,免疫组化方法检测各组大鼠阴茎组织中 CGRP 的表达。CGRP 主要表达在大鼠阴茎海绵体平滑肌和血管。与 N 组比较,B1、B2 组大鼠阴茎组织中 CGRP 表达明显减少,差异有统计学意义($P < 0.05$)。与 B1 组比较,B3 组大鼠阴茎组织中 CGRP 表达明显增多($P < 0.05$),差异有统计学意义,见图 1、表 1。Western blot 结果与免疫组化结果趋势一致,见图 2、表 2。

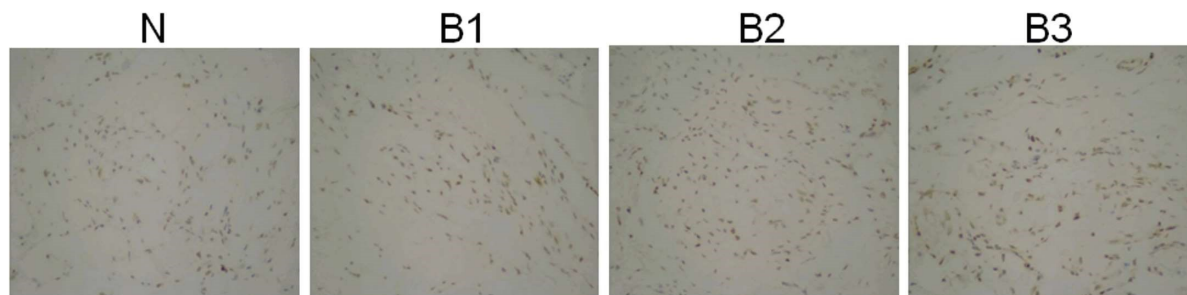


图 1 免疫组化检测大鼠阴茎组织中 CGRP 表达($\times 400$)

Fig. 1 CGRP expression in penis tissue detected by immunohistochemistry($\times 400$)

注:N:正常对照组,B1:模型组,B2:自然恢复组,B3:伊木萨克干预组

Note: N: normal control group, B1: model group, B2: spontaneous recovery group, B3: Yimusake intervention group

2.2 大鼠阴茎组织中 VIP 表达

VIP 主要表达在大鼠阴茎海绵体平滑肌和血管。与 N 组比较,B1、B2 组大鼠阴茎组织中 VIP 表达显著降低,差异有统计

学意义($P<0.05$)。与 B1、B2 组比较,B3 组大鼠阴茎组织中 VIP 表达显著升高 ($P<0.05$), 差异有统计学意义, 见图 3、表 1。Western blot 结果与免疫组化结果趋势一致,见图 4、表 2。

表 1 免疫组化检测大鼠阴茎组织中 CGRP、VIP 表达
Table 1 CGRP and VIP expression in penis tissue detected by immunohistochemistry

Group	n	CGRP	VIP
N	10	227.3± 42.5	175.0± 45.6
B1	16	143.2± 35.5 ^a	50.0± 19.4 ^a
B2	15	164.3± 45.3 ^a	77.8± 36.3 ^a
B3	15	272.2± 42.3 ^{ab}	162.5± 42.3 ^{ab}

注: 与 N 组相比, $n: P<0.05$; 与 B1 组相比, $a: P<0.05$; 与 B2 组相比, $b: P<0.05$

$n: P<0.05$ versus N group; $a: P<0.05$ versus B1 group; $b: P<0.05$ versus B2 group。

N: 正常对照组, B1: 模型组, B2: 自然恢复组, B3: 伊木萨克干预组

Note: N: normal control group, B1: model group, B2: spontaneous recovery group, B3: Yimusake intervention group.

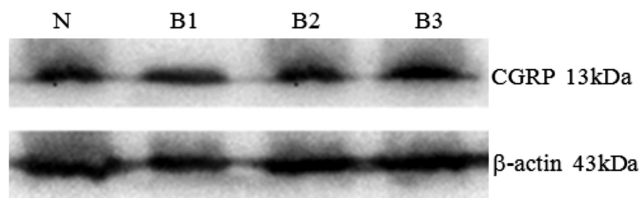


图 2 Western-blot 方法检测大鼠阴茎组织中 CGRP 表达

Fig.2 CGRP expression in penis tissue detected by Western-blot analysis

注: N: 正常对照组, B1: 模型组, B2: 自然恢复组, B3: 伊木萨克干预组。

Note: N: normal control group, B1: model group,

B2: spontaneous recovery group, B3: Yimusake intervention group.

3 讨论

应激是机体受到一定强度的体内外刺激时对环境的一种反应。应激源包括环境的、躯体的、心理的和社会文化的诸因素。不同环境刺激信号经大脑加工处理, 出现以神经和内分泌反应为主的非特异性全身性反应, 包括警觉期、抵抗期、衰竭期, 使人处于虚弱甚至 " 崩溃 " 状态, 造成心理的或躯体的各种疾病的发生, 多表现为紧张、忧虑、烦躁、易怒、不安和食欲不振等^[15,16]。人类每天都对许多未预见到的外在或内在的压力刺激做出应激反应, 这些应激不断挑战着我们复杂的内部平衡,

如果不及及时给予干预或治疗, 平衡将逐渐失调, 甚至引起组织、器官发生病理性改变, 导致各种疾病^[17]。

CGRP 是一种 37- 氨基酸肽, 具有强效血管舒张和保护作用^[18], 能够保护应激损伤诱导的抑郁^[19], 可通过调控 PI3K/Akt 和 MAPK 通路, 抑制应激损伤对心肌细胞起到保护作用^[20]。还有文献报道, 通过慢病毒转染过表达 CGRP, 可以减轻高糖诱导的氧化应激导致的细胞毒性, 对细胞起到保护作用。我们前期研究发现, 环境激素样饮食联合冷应激, 可以诱导大鼠发生应激和炎症反应, 而且勃起和性功能显著降低。关于 CGRP 在环境雌激素样饮食联合冷应激干预诱导的复合应激大鼠模型中的研究还未见报道。有资料显示, 外源性褪黑素可以通过激活与谷胱甘肽代谢相关的抗氧化酶和直接的自由基清除活动, 保护卵巢切除诱导的雌激素缺乏大鼠肝脏不受氧化应激的影响^[21]。另一项不同的研究认为, 雌激素治疗会干扰细胞内铁的代谢, 导致 MCF-7 细胞氧化应激^[22]。而本研究在前期基础上发现, 在环境激素样饮食联合冷应激诱导的复合应激大鼠模型阴茎组织中, CGRP 表达水平显著减少, 提示应激损伤可能通过抑制 CGRP 表达降低大鼠勃起功能和交配能力, 伊木萨克可保护此应激损伤, 促进大鼠阴茎组织中 CGRP 表达, 提升大鼠勃起功能和交配能力。

VIP 又名舒血管肠肽, 是含 28 肽的一种神经递质, 可通过

表 2 Western-blot 方法检测大鼠阴茎组织中 CGRP、VIP 表达
Table 2 CGRP and VIP expression in penis tissue detected, by Western-blot analysis

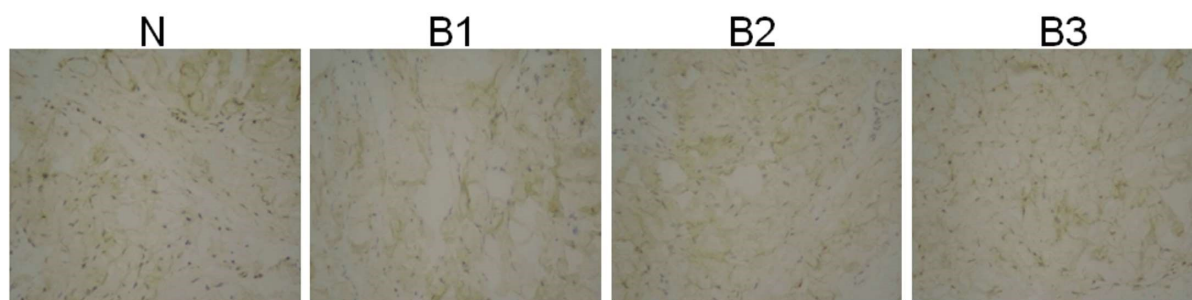
Group	n	CGRP/-actin	VIP/-actin
N	10	1.02± 0.24	1.24± 0.23
B1	16	0.88± 0.26	0.82± 0.18 ^a
B2	15	0.92± 0.28	0.86± 0.30 ^a
B3	15	1.11± 0.27 ^a	1.23± 0.33 ^{ab}

注: 与 N 组相比, $n: P<0.05$; 与 B1 组相比, $a: P<0.05$; 与 B2 组相比, $b: P<0.05$ 。

N: 正常对照组, B1: 模型组, B2: 自然恢复组, B3: 伊木萨克干预组

Note: $n: P<0.05$ versus N group; $a: P<0.05$ versus B1 group; $b: P<0.05$ versus B2 group.

N: normal control group, B1: model group, B2: spontaneous recovery group, B3: Yimusake intervention group.

图3 免疫组化检测大鼠阴茎组织中VIP表达($\times 400$)Fig. 3 VIP expression in penis tissue detected by immunohistochemistry($\times 400$)

注:N:正常对照组,B1:模型组,B2:自然恢复组,B3:伊木萨克干预组

Note: N: normal control group, B1: model group, B2: spontaneous recovery group, B3: Yimusake intervention group

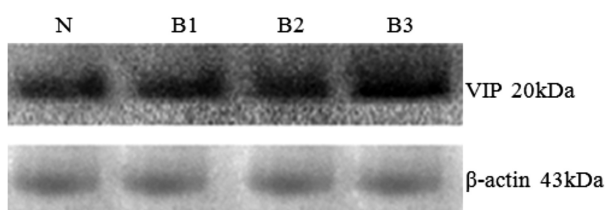


图4 Western-blot 方法检测大鼠阴茎组织中VIP表达

Fig.4 VIP expression in penis tissue detected by Western-blot analysis

注:N:正常对照组,B1:模型组,B2:自然恢复组,B3:伊木萨克干预组

Note: N: normal control group, B1: model group,

B2: spontaneous recovery group,

B3: Yimusake intervention group

蛋白激酶A和蛋白激酶C通路下调白介素-17a的表达,抑制巨噬细胞介导的炎症,对应激损伤起到保护作用^[23],也可通过抑制还原型烟酰胺腺嘌呤二核苷酸磷酸氧化酶,降低胰腺腺泡细胞的氧化应激^[24]。但VIP及其受体在冷应激下的脂肪组织中表达却无显著改变^[25]。在较低的环境温度下全身降温会加剧尿急、心情紧张、动脉粥样硬化等^[26,27]症状。关于VIP在环境雌激素样饮食联合冷应激干预诱导的复合应激大鼠模型中的研究还未见报道。本研究发现,在环境雌激素样饮食联合冷应激诱导的复合应激大鼠模型阴茎组织中,VIP表达水平显著减少,提示环境雌激素样饮食联合冷应激的干预条件抑制VIP在大鼠阴茎组织中表达,诱导复合性应激模型建立,并能降低大鼠模型的勃起功能和交配能力。伊木萨克片干预后,可能通过下调大鼠阴茎组织中VIP的表达,促进大鼠模型的勃起功能和交配能力。

综上所述,环境雌激素样饮食联合冷应激诱导的复合应激大鼠模型中CGRP和VIP表达显著降低,伊木萨克片干预可促进二者表达,可能通过抗炎、抗应激和保护血管作用,促进大鼠模型勃起功能和交配能力,但其更详细的作用机制还需要体内体外实验进一步深入研究。

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