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一种环境激素联合冷应激大鼠模型阴茎组织中 Sprouty 2、ERK1/2 表达的研究 *

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摘要 目的:研究 Sprouty 2(Spry 2)和细胞外调节蛋白激酶(ERK1/2)在环境激素联合冷应激大鼠模型阴茎组织中的表达,并观察伊木萨克片对二者表达的影响。**方法:**选用 30 只正常的雄性 SD 大鼠,其中 10 只为正常对照组(N 组),余 20 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),其中 10 只大鼠采用伊木萨克片干预 2 w(Y 组),剩余 10 只为模型组(M 组)。采用免疫组化方法检测各组大鼠阴茎组织中 Spry 2、ERK1/2 的表达。**结果:**M 组大鼠阴茎组织中 Spry 2 表达低于 N 组($P<0.05$),Y 组大鼠阴茎组织中 Spry 2 表达明显高于 M 组($P<0.05$)。三组大鼠阴茎组织中总 ERK1/2(t-ERK1/2)的表达比较差异均无统计学意义($P>0.05$)。M 组大鼠阴茎组织中磷酸化 ERK1/2(p-ERK1/2)表达高于 N 组($P<0.05$),Y 组大鼠阴茎组织中 p-ERK1/2 表达明显低于 M 组($P<0.05$)。**结论:**Spry 2 表达下调和 ERK1/2 活化可能促进应激反应的发生发展,伊木萨克片可能通过上调 Spry 2 的表达和减少 ERK1/2 的活化发挥抗应激作用。

关键词:环境激素;冷应激;Sprouty 2;细胞外调节蛋白激酶;伊木萨克片

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The Sprouty 2 and ERK1/2 Expression in the Penile Tissue of Rats Model Induced by Environmental Hormones Combined with Cold Stress

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ABSTRACT Objective: To study the Sprouty 2 (Spry 2) and extracellular regulated protein kinases1/2 (ERK1/2) expression in the penile tissue of rats model induced by environmental hormones and cold stress, and observe the effect of Yimusake. **Methods:** A total of 30 mature male Sprague-Dawley (SD) rats were randomly divided into 2 groups: normal control group (N group, n=10) and model group (n=20), the model rats were treated with environmental estrogen-like diet and cold stress for 20 weeks until establishment of stress rats model. Then the rats were randomly divided into 2 groups: stress model group (M group, n=10) and Yimusake intervention group (Y group, n=10), intervened for 2 weeks, the Spry 2 and ERK1/2 expressions were detected by immunohistochemistry. **Results:** (1) The Spry 2 expression in penile tissue of rats: M group was decreased compared with group N ($P<0.05$), Y group was increased compared with M group ($P<0.05$). (2) The total ERK1/2 (t-ERK1/2) expression in penile tissue had no statistical difference among three groups ($P>0.05$). (3) Phosphorylated ERK1/2 (p-ERK1/2) expression in penile tissue: B1 group was obviously higher compared with N group ($P<0.05$); B3 group was lower compared with M group ($P<0.05$). **Conclusion:** Downregulation of Spry 2 expression and activity of ERK1/2 can enhance the development of stress response, Yimusake play a anti-stress role by upregulating the Spry 2 expression and inhibiting the activation of ERK1/2.

Key words: Environmental hormones; Cold stress; Sprouty 2; Extracellular regulated protein kinases1/2; Yimusake

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前言

急性或慢性的应激暴露(定义为对体内平衡的持续或预期

的威胁)会引发一系列的生理和行为反应,显著改变人类和实验动物的新陈代谢和行为状态^[1]。导致应激的刺激可以是环境的、食物的、社会文化等的诸因素^[2]。应激时,内脏器官,如脑、血

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管、阴茎等会发生一系列变化,进而导致抑郁、勃起功能障碍等疾病^[3,4]。本课题组前期研究^[3]成功建立了复合性应激大鼠模型,随着造模时间的延长,甚至出现勃起功能障碍、早泄等。维药伊木萨克片(国药准字 Z65020144)虽已广泛应用于勃起功能障碍,但其现代医学作用机制尚未清晰,因此在很大程度上限制了其应用范围和接受程度。本研究拟检测环境雌激素样饮食联合冷应激大鼠模型阴茎组织中 Sprouty 2(Spry 2)和细胞外调节蛋白激酶(extracellular regulated protein kinases1/2, ERK1/2)表达的变化,并观察维药伊木萨克片对二者的影响,探讨其作用机制。

1 材料与方法

1.1 实验动物

正常雄性 SD 大鼠 30 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

BIC-400 型人工气候箱(上海博迅实业有限公司),伊木萨克片(新疆和田维吾尔药业有限责任公司,国药准字 Z65020144,规格 $0.5 \text{ g} \times 6 \text{ s}$),兔单克隆抗体 Spry 2(美国 Abcam 公司,ab180527)、兔多克隆抗体 ERK1/2(总-ERK1/2, t-ERK1/2,美国 Abcam 公司,ab17942),小鼠单克隆抗体 Erk1(pT202/pY204)+ Erk2(pT185/pY187)(磷酸化 ERK1/2, p-ERK1/2,美国 Abcam 公司,ab50011),DAB 显色试剂盒(北京中杉金桥公司),免疫组化检测试剂盒(北京中杉金桥公司)。

1.3 方法

1.3.1 大鼠分组和模型制备 复合性应激大鼠模型的建立和

伊木萨克给药方法见前期研究报道^[5],将 30 只大鼠分为 3 组,包括正常对照组(N 组)10 只、应激模型组(M 组)10 只、Yimusake 给药组(Y 组)10 只。

1.3.2 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^[6]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 $\times$ 着色强度 $\times 25$,免疫反应值在 0~300。

1.4 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 $\pm$ 标准差 $\bar{x} \pm s$ 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 伊木萨克片对复合性应激大鼠模型阴茎组织中 Spry 2 表达的影响

成功制备复合性应激大鼠模型后随机分为 M 组和 Y 组,药物干预 2 w 后,免疫组化方法检测各组大鼠阴茎组织中 Spry 2 的表达。与 N 组比较,M 组大鼠阴茎组织中 Spry 2 表达量明显下调,差异有统计学意义 ($P < 0.05$)。Y 组大鼠阴茎组织中 Spry 2 表达较 M 组明显增高($P < 0.05$),差异有统计学意义。见图 1、表 1。

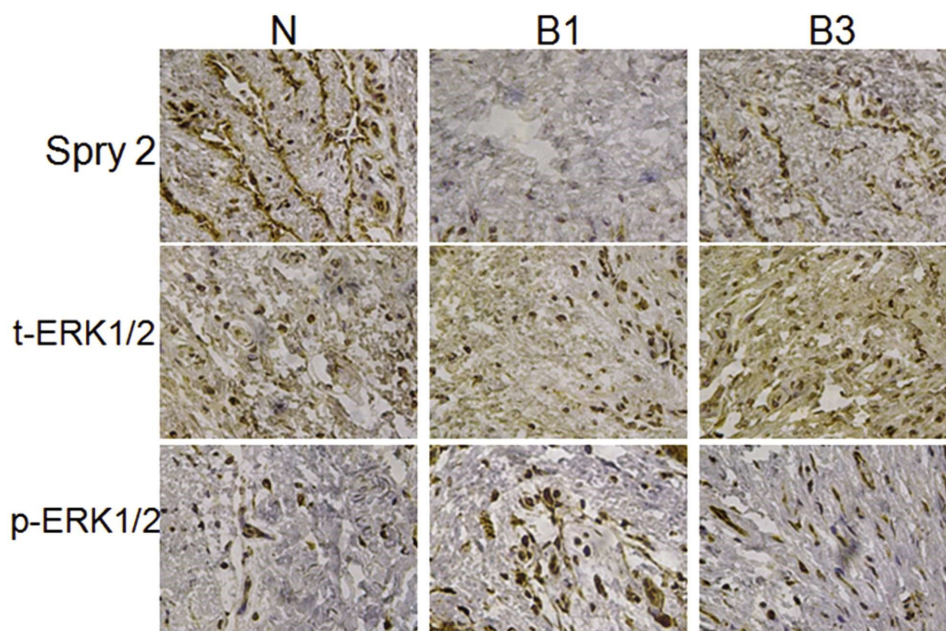


图 1 免疫组化检测大鼠阴茎组织中 Spry 2、t-ERK1/2 和 p-ERK1/2 表达($\times 400$)

Fig.1 The Spry 2, t-ERK1/2 and p-ERK1/2 expressions in the penile tissue of rats detected by immunohistochemistry($\times 400$)

2.2 伊木萨克片对复合性应激大鼠模型阴茎组织中 ERK1/2 表达和磷酸化的影响

如图 1、表 1 所示,三组大鼠阴茎组织中 t-ERK1/2 表达比较差异均无统计学意义 ($P > 0.05$),M 组大鼠阴茎组织中

p-ERK1/2 表达较 N 组显著增高 ($P < 0.05$),Y 组大鼠阴茎组织中 p-ERK1/2 表达较 M 组显著降低($P < 0.05$),差异有统计学意义,见图 1、表 1。

表 1 免疫组化检测大鼠阴茎组织 Spry 2、t-ERK1/2 和 p-ERK1/2 表达

Table 1 The Spry 2, t-ERK1/2 and p-ERK1/2 expressions in the penile tissue of rats detected by immunohistochemistry

Groups	n	Spry 2	t-ERK1/2	p-ERK1/2
Group N	10	202.3± 21.5	222.7± 44.0	122.7± 34.3
Group B1	10	145.6± 26.1 ^a	190.6± 46.3	190.6± 43.0 ^a
Group B3	10	189.0± 35.7 ^a	195.0± 42.2	155.0± 32.1 ^a
P value		0.000	0.251	0.001

Note: compared with group N, ^aP<0.05; compared with group M, ^aP<0.05.

3 讨论

应激源包括损伤、温度、湿度、饮食等。冷刺激作为一种常见应激因素,在心血管疾病和生殖系统疾病的基础研究中应用较多。研究表明对大鼠进行冷刺激能导致大鼠阴茎勃起功能受损^[67]。而环境雌激素摄入过多导致的应激反应也是影响阴茎勃起功能的潜在因素,无论是长期用雌激素治疗的病人,还是经常暴露于高雌激素环境下的普通人群均可使雌激素受体功能异常而致勃起功能障碍^[89]。本课题组前期研究成功建立复合性应激大鼠模型^[5],对其性功能与交配行为等生殖生物学和阴茎形态学方面的变化及伊木萨克片的干预效果做了研究,结果显示该药可显著增强大鼠模型的勃起功能和性行为能力,其作用机制涉及神经内分泌网络、信号通路等。此外,大鼠模型的关键靶器官(阴茎)有水肿、纤维化等炎症反应,伊木萨克片对其有显著的修复作用。

Spry 是一类在全身组织广泛表达的软脂酰化磷蛋白,包括 4 种亚型(Spry 1-4)。Spry 表达减少能激活细胞 ERK 通路,进而传递受体酪氨酸激酶(Receptor tyrosine kinase, RTK)信号,使多种靶蛋白发生磷酸化,引起组织生长发育失调、细胞异常增生及转化^[10]。Spry 2 敲除后,失去对 ERK1/2 通路的负反馈调控,活化的 p-ERK1/2 增多,导致细胞增殖、迁移增多和上皮细胞间充质转化,影响乳腺结构的形成^[11]。而且上皮细胞间充质转化在慢性炎症、组织重建和纤维化疾病中都发挥了关键作用^[12,13]。丝裂原活化蛋白激酶通路要包括 ERK1/2, c-Jun 氨基末端激酶和人 p38 蛋白激酶,可以被细胞外信号或刺激所激活,如炎症细胞因子、细菌复合物等,参与细胞各种生理及病理过程^[14-16]。在饮食诱导肥胖的小鼠肝脏内敲除 ERK,可提高胰岛素和葡萄糖耐受性,增加肝脏 ERK 活性可能有助于增加肝脏糖原含量,减少肥胖的能量消耗,导致血管内皮功能障碍,引发各种心血管疾病,如动脉粥样硬化等^[17,18],且 ERK1/2 信号通路在血管氧化应激和内皮功能障碍的保护中发挥着关键的作用^[19-22]。

本研究在前期研究工作基础上检测了伊木萨克片对环境激素联合冷应激大鼠模型阴茎组织中 Spry 2 和 ERK1/2 (t-ERK1/2 和 p-ERK1/2)表达的影响,结果显示模型组大鼠阴茎组织中 Spry 2 表达低于对照组,伊木萨克片处理的大鼠阴茎组织中 Spry 2 表达明显高于模型组,提示 Spry 2 可能在雌激素样饮食和冷环境诱导的应激反应中发挥重要的作用,伊木萨克片可能通过提高 Spry 2 表达发挥作用。此外,各组大鼠阴茎组织中总 ERK1/2 的表达差异均无统计学意义,模型组大鼠阴茎组织中 p-ERK1/2 表达高于正常组,伊木萨克片给药组大鼠阴

茎组织中 p-ERK1/2 表达明显低于模型组,提示雌激素样饮食和冷环境诱导的应激反应使阴茎组织中 Spry 2 表达减少,可能导致 ERK1/2 通路活化,促进勃起功能障碍的发生发展,而经过维药伊木萨克片干预后,能够显著改善 Spry 2-ERK1/2 通路的这些变化。结合本团队前期研究,我们推测伊木萨克片可能通过调节 Spry 2-ERK1/2 通路发挥抗应激、抗炎和保护血管内皮等作用,促进大鼠勃起功能和交配能力,这可能也是该药物发挥作用的关键作用机制之一。

综上所述, Spry 2 表达下调和 ERK1/2 活化可以促进雌激素样饮食和冷环境诱导的应激反应,维药伊木萨克片可能通过上调 Spry 2 的表达,抑制 ERK1/2 的活化发挥抗应激、抗炎和保护血管内皮作用,促进大鼠勃起功能和交配能力,但其更具体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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