

中 药 研 究

淫羊藿对大鼠下丘脑—垂体—肾上腺—胸腺轴
抑制模型的影响

张玲娟* 沈自尹* 蔡定芳* 陈晓红* 沈时谋*

摘要 本研究用皮质酮造成大鼠下丘脑—垂体—肾上腺—胸腺(HPAT)轴抑制模型,观察补肾助阳要药淫羊藿对受抑大鼠的调节作用。结果表明,模型大鼠垂体、肾上腺、胸腺重量明显减轻;下丘脑室旁核小细胞区促肾上腺皮质激素释放因子(CRF)阳性细胞及正中隆起CRF阳性神经纤维及垂体前叶促肾上腺皮质激素(ACTH)阳性细胞明显减少,胞体变小,染色变淡,肾上腺及胸腺小体明显减少;血浆ACTH、皮质酮以及淋巴细胞增殖反应、自然杀伤细胞活性(NKCC)、 γ -干扰素(γ -IFN)诱生能力较正常组明显降低。淫羊藿灌胃能有效改善皮质酮对HPAT轴形态与功能的抑制。提示淫羊藿对神经内分泌免疫的调节起了重要作用。

主题词 淫羊藿/药理学 下丘脑—垂体系系统 疾病模型,动物

淫羊藿为传统的补肾助阳中药,据现代药理研究,该药生物效应广泛,具有调节免疫,提高肾上腺皮质功能及抗衰老等作用^[1]。为进一步探讨肾虚与补肾的神经内分泌免疫网络的变化,本文用皮质酮(corticosterone, CORT)造成大鼠下丘脑—垂体—肾上腺—胸腺(HPAT)轴肾阳虚抑制模型,观察补肾助阳要药淫羊藿对其的调节作用。

1 材料与方法

1.1 药物制备:淫羊藿购自上海市药材公司,经水煎醇提,用蒸馏水调药物浓度至200%,10磅20分钟高压灭菌,4℃冰箱保存备用。

1.2 主要试剂:皮质酮(CORT)购自sigma公司,使用时溶于灭菌豆油中,浓度为20mg/ml;兔抗鼠促肾上腺皮质激素抗血清和兔抗羊促肾上腺皮质激素释放激素抗血清均购自美国UBC公司; ^3H -TdR购自中科院上海原子核研究所,放射性比强度22ci/mmol,放

射性浓度为1mci/ml; ^{125}I -ACTH放射免疫检测药盒购自美国DPC公司; ^3H -CORT放射免疫检测药盒购自上海市内分泌研究所。

1.3 动物分组:雄性SD大鼠30只,体重 $200\pm 15\text{g}$,随机分为淫羊藿、模型、正常对照三组,每组各10只。淫羊藿组10mg/kg体重皮下注射CORT,同时10g/kg体重淫羊藿灌胃,每天各1次,连续14天。正常对照组以等体积灭菌豆油代替CORT,余同模型组。实验第15天,所有动物断头处死,1分钟内取出下丘脑、垂体、胸腺,再依次取出脾脏、左侧肾上腺,所有组织标本均固定于无冰醋酸Bouin液中,分离包膜,4℃过夜。

1.4 下丘脑—垂体—肾上腺—胸腺轴的形态学观察:方法参见文献^[2]。

1.5 血浆CORT及ACTH放射免疫法检测:大鼠快速断头取血,CORT用肝素抗凝,ACTH用EDTA抗凝,其他按药盒说明操作。

1.6 淋巴细胞增殖试验:无菌取脾,Ficoll分离脾细胞,调细胞至 $1\times 10^7/\text{ml}$,加入96孔平底细胞培养板,每孔0.1ml,同时实验孔加入Con-A $5\mu\text{g}/0.1\text{ml}$,对照孔以等体积培养液补足,每鼠均设实验与对照各3孔,在5%CO₂孵育箱中培养20小时后每孔加入 ^3H -TdR 1 μci ,继续培养18小时,多头细胞收集仪收集细胞,测cpm值。

1.7 自然杀伤细胞活性测定:方法参照文献^[3]。

1.8 γ -IFN的诱生与检测:将 $5\times 10^6/\text{ml}$ 的脾细胞加入24孔培养板,每孔2ml,再加入Con-A $10\mu\text{g}$,CO₂孵育箱培养96小时,收集上清液检测 γ -IFN,方法参见文献^[4]。

2 结果

2.1 淫羊藿对抑制模型大鼠垂体—肾上腺—胸腺重量的影响:表1示,大鼠注射皮质酮后垂体、肾上腺、胸腺重量比正常组明显减轻($P<0.01$ 或 $P<0.001$);淫羊藿组重量显著上升($P<0.05$ 或 $P<0.001$)。

2.2 淫羊藿对抑制模型HPAT轴的形态学影响:外

* 上海医科大学中西医结合研究所,上海市乌鲁木齐中路12号(200040)

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源性CORT明显抑制大鼠HPAT轴,模型组大鼠下丘脑室旁核小细胞区CRF阳性细胞及正中隆起外层CRF阳性纤维明显减少,染色变淡。垂体缩小,垂体前叶ACTH阳性细胞数明显减少,胞体变小,染色变淡。肾上腺明显萎缩,特别是束状带明显变薄。胸腺萎缩,以皮质萎缩严重,皮质淋巴细胞数量显著减少,整个胸腺的苏木素染色变浅,髓质内胸腺小体数量减少。淫羊藿组的上述病理变化程度远较模型组轻。

2.3 淫羊藿对抑制模型大鼠血浆CORT、ACTH的影响:表2示,模型组大鼠血浆CORT、ACTH明显降低($P<0.001$);淫羊藿能有效提高模型大鼠的血浆CORT及ACTH含量($P<0.001$)。

2.4 淫羊藿对模型大鼠淋巴细胞增殖反应、自然杀伤细胞活性(NKCC)和 γ -IFN活性的影响:表3示,模型大鼠淋巴细胞对有丝分裂原Con-A的刺激反应明显减弱,NKCC及 γ -IFN活性明显抑制,与正常组比较有显著及极显著差异($P<0.05\sim 0.001$)。

表3 淫羊藿对模型大鼠淋巴细胞增殖反应、NKCC及 γ -IFN的影响

组别	鼠数	淋巴细胞增殖		NKCC	γ -IFN
		CPM	SI	(%)	(U/ml)
正常组	10	119716 $\pm$ 24856	8.21 $\pm$ 3.31	61.8 $\pm$ 8.2	85.20 $\pm$ 6.10
模型组	10	65187 $\pm$ 22802 Δ	4.29 $\pm$ 1.50 Δ	15.6 $\pm$ 7.8 Δ	40.50 $\pm$ 5.20 Δ
淫羊藿组	10	93966 $\pm$ 31675*	6.20 $\pm$ 1.96*	46.6 $\pm$ 8.3 Δ	59.93 $\pm$ 11.78 Δ

与正常组比较 $\Delta P<0.01$, $\Delta\Delta P<0.001$;与模型组比较* $P<0.05$, $\Delta P<0.001$

3 讨论

随着中医肾本质研究的不断深入,已表明中医肾虚证不仅表现在下丘脑—垂体—肾上腺轴、甲状腺轴、性腺轴功能的紊乱,同时存在免疫功能的低下,补肾中药能改善和提高神经内分泌免疫功能,具有多环节多途径的调节作用。

笔者曾自拟补肾益寿汤和温肾益气汤(寿而康),从甲状腺、性腺轴及免疫系统进行了临床与实验研究,结果表明补肾能改善和提高神经内分泌功能,而该类复方中均有淫羊藿;并又用外源性糖皮质激素造成肾上腺皮质功能抑制的细胞模型,在肾上腺细胞培养实验中设立了温肾复方与单味淫羊藿组,结果两组作用相似,可以在一定程度上保护肾上腺皮质细胞免受外源性激素的抑制。但是,有关淫羊藿对HPAT轴抑制模型的研究尚未见报道。

HPAT轴受抑模型又称“肾阳虚”模型。我们认为肾阳虚是HPAT轴功能低下或紊乱的重要原因。因此,温补肾阳不仅直接提高HPAT轴功能,而且还能间接纠正因HPAT轴失调导致的全身各系统功能的紊乱。淫羊藿为补肾助阳要药,《本草述》及《本草正义》都认为此

表1 淫羊藿对模型大鼠
垂体—肾上腺—胸腺重量的影响(mg)

组别	鼠数	垂体	肾上腺	胸腺
正常组	10	8.55 $\pm$ 1.10	24.02 $\pm$ 3.84	393.61 $\pm$ 65.01
模型组	10	6.90 $\pm$ 0.98 Δ	11.06 $\pm$ 2.04 Δ	204.52 $\pm$ 38.49 Δ
淫羊藿组	10	8.01 $\pm$ 1.10*	18.94 $\pm$ 3.35 Δ	256.39 $\pm$ 55.03*

注:(1)表中数据为均值 $\pm$ 标准差,下同

(2)与正常组比较 $\Delta P<0.01$, $\Delta\Delta P<0.001$;

与模型组比较* $P<0.05$, $\Delta P<0.001$

表2 淫羊藿对模型大鼠血浆CORT、ACTH水平的影响

组别	鼠数	CORT(μ g/dl)	ACTH(pg/ml)
正常组	10	10.70 $\pm$ 4.41	59.70 $\pm$ 6.94
模型组	10	1.18 $\pm$ 0.72 Δ	39.64 $\pm$ 4.85 Δ
淫羊藿组	10	4.05 $\pm$ 0.90*	47.52 $\pm$ 3.10*

Δ 与正常组比较 $P<0.001$; *与模型组比较 $P<0.001$

药入命门,专壮肾阳。现代研究提示此药能广泛作用于内分泌、免疫系统。本研究观察单味淫羊藿对抑制模型大鼠神经内分泌免疫功能的影响。结果表明,淫羊藿能改善外源性皮质酮对HPAT轴形态与功能的抑制,与模型组比较,下丘脑室旁核和正中隆起的CRF神经元及神经纤维明显增多,肾上腺皮质束状带增宽,结构完整;胸腺皮质萎缩减轻,胸腺细胞数量增多。血浆ACTH、CORT含量明显上升,淋巴细胞增殖反应、NKCC活性及 γ -IFN诱导能力显著提高,提示仙灵脾对实验性HPAT轴抑制大鼠有显著的改善作用。有关淫羊藿的有效组分的神经内分泌免疫学调节作用的研究目前正在进行中。

4 参考文献

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ABSTRACTS OF ORIGINAL ARTICLES

Clinical Observation on Angina Pectoris in CHD Treated with Guanxin Paste.

Miao Yang, Qian Hongyu, Xu Mingyu, et al

The Paste is refinely made of Radix Salviae Miltiorrhizae, Rhizoma Ligustici Chuanxiong and Borneolum. 120 cases were treated with another group of 50 cases treated by sticking membrane of nitroglycerine as control. The total ameliorated rate, changed EKG— ischemia rate of treatment group was 90% and 50% respectively, while for the controlled group, it was 39.2%, and the rapid amelioration rate for angina pectoris was 70%, being similar to the controlled group ($P > 0.05$). This paste also mitigated other symptoms, especially stuffy chest, short breath and palpitation, being better than the controlled group. It also lowered the whole blood viscosity, plasma specific viscosity and platelet aggregation.

Authors' Address: Xiyuan hospital, China Academy of TCM, Xiyuan, Beijing 100091

Key Words: diseases of coronary vessels/TCM therapy angina pectoris/TCM therapy

(Original article on page 604)

Observation on 30 Cases of Uro—lithiasis Treated with Compound Si—jin Decoction.

Cui Xiumei, Zhang xiaobo, Shen Guonan, et al

Thirty cases of urolithiasis were treated with a self—formulated Sijin Decoction. B—ultrasonic, X—ray and BUN, Cr, GPT and blood, urique routine exams were made before and after the treatment. The total effective rate after 3 months was 73.3% (22 cases), the rate of relapsing for one—year follow—up exma being 31.4%.

Authors' Address: Xiyuan Hospital, China Academy of TCM, Beijing 100091

Key Words: urolithiasis/TCM therapy @ Compound Sijin Decoction

(Original article on page 608)

Analysis on the Relation Between Blood Sugar and Diabetic Syndromes in 2080 Cases.

Zhang Yanqun, Han Qing, He Guizhang, et al

Epidemic investigation was made in 2080 diabetic cases in Henan Province. Results showed that TCM Xiaoke syndrome is not only related to the pathological

changes of the Lung in Upper—jiao, Spleen—stomach in middle—jiao and kidney in lower—jiao, but also closely related to pathological changes of the liver. During the early stage when mild hyperglycemia is present, the manifestations of deficient liver (of liver blood and liver Yin) are especially conspicuous with a frequency of occurrence only less than Qi—deficiency syndrome. This important new discovery reminds us that "liver" is not to be overlooked clinically. It also proves that application of nourishing, softening, converging, and soothing the liver are reasonable and scientific methods for diabetes treatment.

Authors' Address: First Affiliated Hospital, Henan College of TCM Zhengzhou, Henan 450000

Key Words: Diabetes mellitus/blood glucose/analysis

(Original article on page 617)

Inhibition of Herba Epimedii on Rat's Model of Hypothalamus—pituitary—adrenal—thymus axis.

Zhang Lingjuan, Shen Ziyin, Cai Dingfang, et al

Rat models of HPAT inhibition were made by corticosterone for observation on the adjusting action of a Yang—stimulant, Herba Epimedii. Results included marked reduction in weights of rats' pituitary, adrenal and thymus, the CRF positive cells from microcellular area of hypothalamic paraventricles, positive nerve fibres of CRF, positive cells of ATCH were also markedly reduced, with decreased cellular size, and light staining, marked decrease in the sizes of adrenals and thymus glands. There were also marked decrease of plasmal ACTH, cortisone, lymphocyte proliferative reaction, NKCC, γ —IFN induced growth capacity as compared with normal control group. Gastric infusion of Herba Epimedii effectively ameliorated the inhibition of HPAT morphorolgy and functions by cortisone, suggesting that this herb yields important action on neural and hormonal immunity.

Authors' Address: Institute of Integrated Medicine, Shanghai Medical University, Shanghai, 200040

Key Words: Herba Epimedii/pharmacology Hypothalamous—pituitary system diseased model, animal

(Original article on page 620)