

• 实验研究 •

助阳药对可的松大鼠模型下丘脑单胺类神经递质作用的研究

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内容提要 用高效液相色谱——电化学检测联用,以梓醇磺酸为离子对试剂,测定可的松阳虚大鼠及正常大鼠下丘脑单胺类神经递质,并观察用助阳药附子、肉桂、肉苁蓉、仙灵脾后的效果。发现:可的松阳虚大鼠下丘脑去甲肾上腺素(NA)较正常大鼠下降,肾上腺素(A)升高(P 均 <0.05),用助阳药后能恢复正常。助阳药使可的松阳虚大鼠及正常大鼠多巴胺(DA)均升高(P 分别 <0.05 及 $P<0.01$),3,4-二羟基苯乙酸(DOPAC)下降($P<0.001$),并使正常大鼠5-羟色胺(5-HT)升高($P<0.001$),用药后阳虚及正常大鼠都表现为DA/DOPAC及5-HT/5-羟吲哚醋酸(5-HIAA)比值升高($P<0.01\sim0.001$)的共同特点,提示助阳药似有抑制下丘脑单胺氧化酶活性的作用。

关键词 助阳药 可的松大鼠模型 单胺类神经递质 高效液相色谱——电化学检测

60年代邝安堃等用大剂量的可的松造成小鼠的虚损耗竭状态,并证实助阳药如附子、肉桂、肉苁蓉、仙灵脾对此虚损状态有显著的对抗作用^①,当时限于技术条件,未能进一步研究其机理。本实验从下丘脑单胺类神经递质的角度来观察可的松阳虚状态时该神经内分泌环节的生化变化及中药的可能作用机制。

材料和方法

一、动物及分组: Wistar 雄性大鼠200~300g,随机分成4组,每组5~6只。(1)正常对照组:每日肌肉注射生理盐水0.3ml/只,1周后灌生理盐水,每鼠每日0.5ml。(2)正常助阳药组:于注射生理盐水1周后灌饲助阳药,每鼠每日0.5ml。(3)可的松阳虚模型组:每日肌肉注射醋酸可的松(上海第九制药厂,批号850804,5ml含125mg)3mg/100g体重;1周后灌生理盐水,每鼠每日0.5ml。(4)模型加助阳药组:同第3组肌肉注射醋酸可的松,并于1周后灌饲助阳药,每鼠每日0.5ml。

二、助阳药: 附子、肉桂、肉苁蓉、仙灵脾各100g,水煎1小时,将药液滤出,药渣加水再煎1小时,两次药液浓缩至200ml,置4℃保存,每1ml含生药总量2.0g。

三、处死动物及取材方法: 经灌中药或生理盐水后28天(即注射可的松或生理盐水后35天)将所有大鼠快速断头,于1分钟内开颅取出鼠脑,在冰台上解

剖,按自然分部标记取出下丘脑,称湿重后加0.1mol/L过氯酸(含0.1%半胱氨酸)匀浆,高速离心,上清液当即用高效液相色谱(HPLC)——电化学检测器(EC)测单胺类神经递质及其代谢产物。

四、生化测定

1. 仪器: Waters 208 system, 510泵, 680梯度控制仪, BAS电化检测工作电压0.7V, 检测灵敏度为5nA, 微处理器为Data Model 730。

2. 色谱条件: 色谱柱为 μ -Bondapak C18, 30×0.5cmID, 颗粒度10 μ m, 另加保护柱10×0.8cmID, 填料RP-18, 10 μ m。

3. 流动相: A液为氢氧化钠——乙酸缓冲液, B液为甲醇。A液90%、B液10%等比梯度, 流速1.5ml/min, 进样量为200 μ l。

4. 用外标法计算。

5. 共测定8种单胺类神经递质及其代谢产物的浓度, 按洗脱顺序依次为: (1)去甲肾上腺素(NA), (2)肾上腺素(A), (3)3-甲氧-4-羟基苯乙二醇(MHPG), (4)3,4-二羟基苯乙酸(DOPAC), (5)多巴胺(DA), (6)5-羟吲哚醋酸(5-HIAA), (7)高香草酸(HVA), (8)5-羟色胺(5-HT)^②。并计算DA/DOPAC及5-HT/5-HIAA比值。

结 果

一、动物的一般状态: 注射可的松的动物, 开始1

周内表现为多动、活跃、警觉、多食、多饮,似临床阴虚状态。1周后渐趋安静。2周后虚损状态明显,拱背、少动、竖毛、毛少泽、不光滑、畏寒、颤抖、蜷缩一角、少食、少饮、消瘦,类似临床阳虚状态。注射生理盐水大鼠开始用助阳药第1周期间表现为活

跃,大便次数增多,以后与不用药组差别不显。

二、生化改变

1.正常大鼠及可的松阳虚大鼠下丘脑单胺类神经递质及其代谢产物的浓度,见表1。

表1 助阳药对可的松大鼠模型下丘脑单胺类神经递质及其代谢产物的影响 ($\bar{x} \pm S\bar{x}$, ng/g组织)

组别	NA	A	MHPG	DOPAC	DA	5-HIAA	HVA	5-HT
对照 (n=6)	3907.9 ± 178.3	96.5 ± 15.3	41.0 ± 3.4	733.6 ± 21.6	462.8 ± 31.5	862.5 ± 24.5	159.6 ± 53.6	189.8 ± 12.9
正常加助阳药 (n=5)	4247.4 ± 153.4	56.1 ± 10.6	34.0 ± 11.4	385.3** ± 69.1	732.2* ± 91.9	1102.3** ± 29.7	84.3 ± 13.6	443.1** ± 32.0
阳虚模型 (n=6)	3232.4 Δ ± 151.0	216.2 Δ ± 48.7	145.6 ± 49.8	738.0 ± 17.6	403.6 ± 46.1	879.6 ± 45.7	118.1 ± 5.9	138.3 ± 32.2
阳虚模型加助 阳药(n=6)	4504.9** ± 88.1	79.2* ± 25.8	36.3 ± 11.0	327.6** ± 11.6	789.2** ± 69.9	607.8 ± 38.8	63.9** ± 5.1	210.5 ± 19.5

注:与对照组相比 $\Delta P < 0.05$; 与相应不用药组相比 ** $P < 0.001$, * $P < 0.05$; n为动物数

阳虚大鼠NA下降, A升高(P 均 < 0.05), DA及5-HT有轻度下降但未至显著程度,余代谢产物也均未见有显著差别。

2.用助阳药后,神经递质及其代谢产物的变化:

(1)助阳药使阳虚模型大鼠下降的NA得以升高,使原先增高的A下降,用药4周后与对照大鼠无显著差别,见表1。(2)对照大鼠及阳虚大鼠用助阳药4周后均发现DA显著增高(正常助阳药组 $P < 0.05$,阳虚助阳药组 $P < 0.001$),此外使正常助阳药组5-HT及5-HIAA均升高,使阳虚助阳药组儿茶酚胺代谢产物HVA显著下降,见表1。(3)无论是对照组或阳虚组,用药后DA/DOPAC及5-HT/5-HIAA均显著升高($P < 0.01 \sim 0.001$),见表2。

表2 用助阳药后阳虚模型大鼠及对照大鼠DA/DOPAC及5-HT/5-HIAA的变化 ($\bar{x} \pm S\bar{x}$)

组别	DA/DOPAC	5-HT/5-HIAA
不用药对照	0.63 ± 0.004	0.22 ± 0.01
阳虚模型	0.55 ± 0.06	0.16 ± 0.04
用对照	1.96 ± 0.12 **	0.40 ± 0.03 **
药阳虚模型	2.43 ± 0.26 **	0.35 ± 0.02 *

注:与不用药组相比 ** $P < 0.001$, * $P < 0.01$

讨 论

关于虚证动物模型脑神经递质变化的报告较少,以前多用荧光比色法^[3],易受各种因素影响,尤其是儿茶酚胺类不够稳定。本研究用HPLC—EC方法,提

高了测定的灵敏度与准确性。从测定的结果可见可的松阳虚大鼠下丘脑单胺类神经递质的变化,既与(甲充)阴虚大鼠^[4,5]有所不同,也和其他原因所致阳虚大鼠有很大区别^[5,6]。主要表现为NA下降, A升高。NA是A合成的前体,这种浓度的改变可认为是NA甲基化作用的增强,其生理意义尚待研究。不同方法所造成的类似阳虚证的动物模型在中枢神经系统单胺类神经递质浓度表现上的异质性与临床表现上所具有的共同性是不一致的。尽管如此,助阳药的作用可使阳虚大鼠下丘脑NA及A的浓度接近正常,说明助阳药对这种阳虚动物在下丘脑这一特定的环节上有扶正复元之作用。

本实验另一重要发现是助阳药附子、肉桂、肉苁蓉、仙灵脾可升高正常大鼠及阳虚大鼠下丘脑中多巴胺浓度,而使其脱氨的代谢产物DOPAC浓度降低,同时使阳虚大鼠儿茶酚胺代谢产物HVA降低,使正常大鼠5-HT及代谢产物5-HIAA均升高。进一步分析这些变化,可归结为DA/DOPAC及5-HT/5-HIAA比值升高,这无论在正常大鼠或阳虚大鼠都是一致的,与相应不用助阳中药组相比,差异极其显著。DA脱氨生成DOPAC以及其他代谢产物或5-HT脱氨生成5-HIAA均需要单胺氧化酶(MAO)。曾有报告左归丸可使老年大鼠脑干MAO-B活力下降^[7],温里助阳药使下丘脑NA、纹状体DA、脑干5-HT升高^[8]。从本实验结果看,助阳药的作用似是抑制MAO的活性,而与机体本身状态关系不大。

这是一个有巨大潜在意义的研究领域,因为动物和人进入老年后脑内MAO活性都可能增强,多巴胺

含量下降,是脑功能衰老的表现之一。某些退行性病变尤其是神经系统退行性病变引起的疾病如Parkinson病的发病原理是由于产生多巴胺的黑质萎缩使纹状体中多巴胺含量下降,乙酰胆碱活动相对亢进而出现多动症状⁽⁸⁾。多巴胺又对多种内分泌激素释放起重要作用,如可抑制泌乳素的释放⁽⁹⁾。所以如何提高脑中某些部位多巴胺的含量是多科(神经科、内分泌科、如产科、老年病科等)医师所关心的问题,中药是一个有待进一步探索的宝库。

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穴位快速注射治疗急性腰踝扭伤 184 例

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笔者自1973~1988年采用穴位快速注射5%葡萄糖氯化钠治疗急性腰扭伤及踝扭伤患者共184例,均获满意疗效,现总结如下。

一般资料 184例急性扭伤患者中,男170例,女14例。年龄:17~55岁,平均36岁。其中腰扭伤135例(右侧扭伤者113例,左侧扭伤者22例)均有不同程度的疼痛和被迫体位,活动受限。急性踝扭伤49例(外踝扭伤者44例,内踝扭伤者5例)均有肿胀、疼痛,活动时疼痛加剧,行动受限。病程:1小时~7天。

治疗方法 (1)取穴部位:腰扭伤者取患侧气海俞穴,外踝扭伤者取丘墟穴,内踝扭伤取照海穴。(2)操作方法:先用75%酒精常规消毒皮肤,取10~20ml注射器连接7号针头,吸取5%葡萄糖氯化钠15ml,腰扭伤患者取俯卧位,选准穴位后快速进针,针尖应向内下,直达肌肉深层,回抽无回血即快速注射,患者有一种电麻感,并向周围和臀部放射;踝扭伤患者可取端坐位或仰卧位,吸取5%葡萄糖氯化钠6~8ml,在选定穴位上垂直进针,进针深度应达关节韧带,回抽无血即快速注射。以上均为每天注射1次,每次只选1

个穴位,3天为1个疗程,休息1天可进行第2个疗程。

结果 疗效标准:痊愈:治疗后功能活动完全恢复正常,无任何不适感;显效:治疗后功能活动明显好转,疼痛基本消失,能作弯腰动作或下地缓慢行走;无效:治疗后功能活动无改善。结果184例患者均治愈,经1次治愈者91例,2次治愈者68例,3次治愈者25例。49例急性踝扭伤患者经1次治愈者9例,2次治愈者33例,3次治愈者7例。本组腰、踝扭伤患者均在3天内治愈,治疗期间无任何不良反应。

体会 对急性腰、踝扭伤患者一般在临床上是以舒筋活血、散瘀止痛的药物或推拿、按摩、针灸、烤电等方法治疗,但疗效均不够理想。采用穴位快速注射5%葡萄糖氯化钠治疗急性腰、踝扭伤,其止痛消肿的效果非常显著,而且痛苦小,价廉,方法简便易行,安全可靠。受伤时间愈短,治疗效果愈好。在治疗期间无须辅以任何任何药物,值得在厂矿、农村等基层医疗单位推广应用。

Effect of Qigong(气功) on Sympathetico-Adrenomedullary Functions of Patients with Liver Yang(阳) Exuberance Type Hypertension

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By using the differential diagnosis of traditional Chinese medicine to determine the types of hypertension, using the diagnosis of western medicine(WM) to determine the phases of hypertension, 61 inpatients of Liver Yang exuberance type hypertension were randomly divided into Qigong group and WM group. The patients in the Qigong group were treated with both Qigong and antihypertensive drugs at low dosage, but those in the WM group were treated with the drugs alone. Several laboratory tests concerning sympathetico-adrenomedullary functions were conducted twice respectively at 1st and 9th week after hospitalization of the patients. The results indicated that the Qigong group after treatment of 9 weeks had more cases with normal sympathetico-adrenomedullary functions than it had before the treatment, and that their urinary CA, E, NE decreased, MHPG-SO₄ increased, plasma cAMP and cGMP got down, but cAMP/cGMP ratio got up. It suggested that Qigong could modulate the sympathetico-adrenomedullary functions of patients with Liver Yang exuberance type hypertension.

(Original article on page 283)

A Correlative Study for Urine Osmotic Pressure and Deficiency-Excess Syndrome of Renal Disease: Analysis of 428 Cases

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By means of TCM differentiation of symptom-complexes, the authors tested and analysed the urine osmotic pressure (UOP) and the urine and plasma osmotic ratio (UPOR) for 428 cases of renal disease, with the conclusion that the UOP and the UPOR were within the normal value range for not only the 36 cases lack of clinical symptoms so as to be unable to have TCM classification identified, but also for 24 cases of Wind edema excess syndrome mainly caused by pathogenic Wind's invasion to the Lung. But for 74 cases of damp-heat Kidney impairment and 294 cases with the main symptom being Kidney deficiency (including weakness of Qi(气) of Kidney, Yang(阳) deficiency of Spleen and Kidney, Yin(阴) deficiency of Liver and Kidney), the value of their UOP and the UPOR had the tendency of reduction ($P < 0.01$), among which the value of the patients of Kidney Yang deficiency reduced most obviously.

The further observation showed that, for the nocturia patients caused by renal disease, the value of UOP and the UPOR reduced more obviously than usual. Therefore the authors assert that the test on UOP and UPOR will offer an objective index to patients' nocturia and Kidney-Qi weakness.

60 cases with renal disease of Kidney deficiency syndrome and 27 cases of damp-heat Kidney impairment syndrome under the diagnosis and treatment based on an overall analysis of symptoms and signs leads to the following conclusion: With the elimination of pathogenic factors and recovery of kidney, the damp-heat Kidney impairment patients' UOP will be increased. The low UOP of patients caused simply by Kidney deficiency, however, will recover slower.

(Original article on page 286)

Observation on the Treatment of Rheumatoid Arthritis Patients with Low Dose of Multi-Glycosides of *Tripterygium wilfordii*

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Thirty two cases of rheumatoid arthritis were treated with multi-glycosides of *Tripterygium wilfordii* (T₂) in a dosage of 30 mg daily for 12 weeks. Significant improvements in clinical and laboratory variables were observed. In comparison with the results of routine dose of T₂ (60 mg per day) treatment, there was no significant difference to be seen between the two treatment plans. Meanwhile, symptom of rash and slightly alimentary canal lower incidences and milder manifestations of side effects of T₂ were found in the patients of low dose group than in that of routine dose