

“阳虚”动物模型肝胞液糖皮质激素受体改变及助阳药的作用

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内容提要 本文以 [^3H] 地塞米松为配体, 用交换法测定糖皮质激素受体 (GCR) 的结合容量 (Ro) 及解离常数 (Kd)。以氢化考的松造成大鼠“阳虚”模型后, 在末次注射后 5 或 24 小时处死者, 肝胞液中 GCR 的 Ro 均明显减少, Kd 明显增大。但末次注射后 48 小时处死者已近于恢复。助阳药对此种 GCR 改变无影响。

临床所见阳虚病人大多有下丘脑—垂体—肾上腺皮质轴功能低下的表现⁽¹⁾, 而血中皮质醇浓度有时却并不减少, 原因不明。近年阐明皮质醇要发挥生物效应, 必须通过和靶细胞胞液中的糖皮质激素受体 (简称 GCR) 结合, 才能起作用。即使血中皮质醇浓度是正常的, 或甚至是高的, 但胞液中 GCR 减少, 仍可能表现为肾上腺皮质功能低下。因此很有必要在阳虚的发病机理中检查这一重要环节。我们利用近年开展的 GCR 测定技术, 测定“阳虚”动物模型肝细胞胞液中 GCR 的改变, 并初步探索了助阳药对 GCR 的作用。

方 法

一、动物: 纯种 Wistar 大鼠, 雌性, 实验开始时体重 90~110g, 日龄 40 天左右。

二、“阳虚”动物模型: 每日肌肉注射醋酸氢化考的松 2mg/100g 体重, 共 20 天。

三、助阳药: 附子、肉桂、淫羊藿、肉苁蓉, 附子先煎 1 小时 (h), 再入其他药, 共煎 6 h, 浓缩至每毫升含生药 2g。动物隔日一次胃管灌喂 1 ml, 共 10 次。

四、分组: 除一般对照外, 考虑到肌肉注射也是应激, 设盐水肌肉注射对照。又因氢化考的松对 GCR 可能有直接影响, 又增加对照动物在处死前 5 及 24h 分别注射一次氢化考的松的对照组。而“阳虚”模型组平时为每日晨 8 时注射, 又按最后一次注射时间在处死前 5、24、48h 不同而分为三组。其余详见附表。

五、肝胞液的制备和 GCR 测定: 大鼠一律在早晨断头处死, 由肝静脉灌注 0°C 生理盐水后作肝匀浆, 超速离心分出肝胞液, 以 [^3H] 地塞米松 (Dex) 为配体, 用交换法测定 GCR 的结合容量 (Ro) 和解离常

数 (Kd), 详细步骤见另文⁽²⁾。

结 果

一、“阳虚”动物模型: 动物肌注氢化考的松后一周左右体重明显下降 (至 3 周后处死时约下降 10g), 精神萎靡, 背弓, 不活泼, 皮毛无光泽, 以后有部分动物死亡。加用中药组的外观基本同上。各种对照及单服中药组均无上述改变, 体重在处死时约增加 30g 左右。“阳虚”动物模型在处死后检查多有肺炎, 肺出血和轻度的胸膜粘连。

二、肝胞液 Dex 特异结合结果: (1) 4 种对照组及单用助阳药组之间的 Ro 与 Kd 无明显差别, 与以前报告⁽²⁾ 结果 (Ro 为 $293.8 \pm 25.6 \text{ fmol/mg 蛋白}$, Kd 为 $0.54 \pm 0.15 \times 10^{-8} \text{ M}$) 相近。(2) 5h 和 24h “阳虚”组的 Ro 均明显减少, Kd 明显增大, 与相应对照组比较, 统计学上均有显著意义。(3) 48h “阳虚”组的 Ro 已经正常, Kd 也近于恢复。(4) “阳虚”加助阳药组仍和 5 及 24h “阳虚”组的数值相近, 但和对照组有显著差异见附表。

附表 各组肝胞液 [^3H] Dex 特异结合结果

	动物数	[^3H] Dex 特异结合	
		Ro fmol/mg 蛋白	Kd $\times 10^{-8} \text{ M}$
对 照 组	5	284.1 ± 36.0	0.40 ± 0.12
盐水对照组	5	258.1 ± 68.4	0.53 ± 0.26
5h 氢考对照组	7	258.5 ± 44.2	0.39 ± 0.09
24h 氢考对照组	6	276.2 ± 60.1	0.43 ± 0.10
5h 阳虚组	9	$155.5 \pm 36.9^{**}$	$1.43 \pm 0.57^{**}$
24h 阳虚组	10	$174.0 \pm 40.6^*$	$1.66 \pm 0.96^{**}$
48h 阳虚组	3	308.7 ± 89.0	0.83 ± 0.32
阳虚加助阳药组	9	$151.2 \pm 45.7^{**}$	$1.87 \pm 0.73^{**}$
单用助阳药组	4	277.4 ± 30.5	0.33 ± 0.05

分别与 5h、24h 氢考对照组比较, *P<0.01, **P<0.001

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讨 论

一、“阳虚”动物模型的 GCR 改变：临床见一部分阳虚病人的症状及肾上腺皮质功能低下表现很明显，但血中皮质醇浓度及节律正常。对这部分病人有理由推测其 GCR 可能有改变。从以上结果来看，以慢性应用氢化考的松形成的“阳虚”模型，其 GCR 减少。虽在最后一次注射后 48 小时的 GCR 已近正常，但本模型是每天注射一次，故可以认为 GCR 基本上处于低水平。Kd 的改变提示 GCR 在分子结构上也可能有改变。至于其他方法产生的模型及阳虚病人的 GCR 是否有改变，尚待进一步研究。

二、此种模型 GCR 减少的原因：由于此种模型是由糖皮质激素(GC)形成的，故首先要考虑 GC 对 GCR 的影响。Koch 等⁽³⁾及 Mayer 等⁽⁴⁾的动物实验见 GC 可使垂体前叶和骨骼肌的胞液 GCR 减少。但 Murakami 等⁽⁵⁾用山羊白细胞作实验，给动物注射 ACTH 使血皮质醇增加 11 倍，但白细胞上 GCR 数却无变化。如把白细胞和 100nM 皮质醇孵育 3 小时，虽对受体数无明显影响，但可使 Kd 增高。

至于人的 GC 与 GCR 关系，Doe 等⁽⁶⁾认为内源性血皮质醇浓度和单核白细胞胞浆 GCR 数之间有显著相关，即浓度高者 GCR 少。Голиков 等⁽⁷⁾以人淋巴细胞作培养，加入皮质醇后可使 GCR 稍有减少，但 Kd 不变。Kontula 等⁽⁸⁾检查了肾上腺皮质功能亢进或减退病人的淋巴细胞 GCR，见两组的绝对值及 Kd 均与健康对照组无显著差异。但一例临床表现较轻的肾上腺腺瘤其 GCR 只有正常的 30%。他们认为临床表现较轻的原因可能为 GCR 减少。另有 2 例神经性厌食患者 GCR 亦偏低。

本组 GCR 减少并非因为每天肌肉注射的应激，也不是因最后一次注射氢化考的松后使胞液的 GCR 转移到核内，因为几组对照组都属正常。而且氢化考的松在血浆中的半衰期只有 80~100 分钟，24h 后对血中浓度影响很小。Varma 等⁽⁹⁾报告低蛋白饮食可使动物肝胞液中 GCR 显著减少，本组看来不是这个原因，因为同样体重减轻的模型组最后一次注射后 48h 测 GCR 数却并不减少。而且低蛋白饮食所致者 Kd 并无改变，而本组则明显增高。我们⁽¹⁰⁾过去曾见此动物其 DNA 合成率减少。Голиков 等⁽⁷⁾也见氢化考的松抑制 DNA 的合成。而受体本身是一种蛋白质，因此通过减慢 DNA 合成的影响，也可能使 GCR 减少。总之，GC 与 GCR 的关系比较复杂，但“反向调节(down regulation)”，很可能起主要作用。可能此种模

型的 GCR 合成较正常为慢，且受到 GC 的反向调节，故处于低水平。

三、助阳药对 GCR 影响：从本组结果看，助阳药本身对 GCR 并无作用，也不能纠正此种模型的 GCR 减少。助阳药虽能促使合成减少的 DNA 恢复⁽¹⁰⁾，但对 GCR 作用却不明显。可能 GCR 的改变是几个因素综合所致。助阳药本身并非甾体激素类物质，其纠正阳虚的作用很可能是通过下丘脑—垂体轴的影响，因其对另外两种靶腺也有作用。因而助阳药对 GC 所致的 GCR 改变影响不大，也是可以理解的。我们所用助阳药仅 4 味，药物剂量和用药时间等条件有待进一步探索，故不能认为其他助阳药及其他用药方法也一定无作用。

(本工作承内科龚念慈教授及病理生理学教研室吴中立教授、徐仁宝副教授大力支持及指导，许士科同志参加了部分动物模型工作，特此致谢)

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Changes of mast cells: The mast cells were filled with bright orange or brick-red granules. The total number of mast cells of hydrocorticoid-treated mice decreased markedly. Its small-type cells decreased while the large ones increased. The total number of mast cells of the mice treated with Chinese medicines increased markedly. Its small-type cells increased and the large ones decreased.

The B-lymphocyte originates in the pluripotential stem cells of bone marrow. The mast cell probably originates in the cell of thymus and bone marrow. The Chinese medicines mainly nourish the "kidney" and warm the "kidney" Yang in this experiment. These Chinese medicines probably improve the function of the bone marrow which produces immune activated cells and mast cells. These cells probably may regulate and decrease the syndrome of deficiency to some degree.

(Original article on page 104)

Changes in Glucocorticoid Receptor of Liver Cytosol in "Yang Deficiency" Animal Models and Effects of "Yang-Tonifiers"

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The binding capacity (Ro) and the apparent dissociation constant (Kd) of glucocorticoid receptor (GCR) of liver cytosol in "Yang deficiency" animal models were estimated by way of exchange assay, using ^3H dexamethasone as the ligand. The levels of Ro from control groups were about 270 fmol/mg protein, and Kd were about 0.40. But the levels of Ro of "Yang deficiency" animals, which were produced by chronic injections of hydrocortisone for 20 days, were significantly decreased. The value of rats decapitated in 5 hours after the last injection of hydrocortisone was 155.5, and that in 24 hours was 174.0. Kd was markedly elevated, the figures being 1.43 and 1.66 respectively. There were statistically significant differences between the "Yang deficiency" groups and the control. The Ro and Kd of "Yang deficiency" rats decapitated in 48 hours after the last injection, however, almost returned to normal values.

The addition of 4 "Yang-tonifiers" (prepared aconite root, cinnamon bark, epimedium and cistanche) had no effect on the Ro and Kd of GCR of "Yang deficiency" animals, suggesting that the 4 "Yang-tonifiers" were of little avail with respect to the decrease in GCR.

In general, the GCR level of the animal model is low, and there is an increase in Kd. This suggests that there may be some variation in the molecular structure of GCR. As for the cause of the decrease in GCR, the authors suggest that this may be due to the lowered synthesis rate of GCR as well as the influence of "down regulation" of glucocorticoids.

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Experimental Study of Antitumor Effect of an Extract Derived from *Patrinia Scabra* Bge

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Development of transplanting sarcoma 180 (S₁₈₀) in mice was significantly inhibited by local intralesional injection of *Patrinia Scabra* Bge (PSB) extract. The inhibition rate of tumor weight was 62.5%. These mice with the regression of tumor were implanted again with sarcoma cells after one and five months: none of these mice showed signs of tumor growth. Local intralesional injection was more effective than intraperitoneal injection. Significant antitumor effect was observed when the extract was combined with an immunostimulant of *C. parvum*.

In vitro experiment showed that by peritoneal injection with the PSB extract, peritoneal macrophages were increased in the ability of phagocytosis of erythrocyte and in the effect of cytotoxic activity of S₁₈₀ cell (with ^{125}I -UdR release assay).

The acute i.p LD₅₀ of PSB in mice was $0.6 \pm 0.028 \text{ ml/10g}$, subacute toxicity in dog i.p 3-6 ml/kg of PSB once every other day for 7 times produced obvious toxic actions, including leukocytosis, temporal thrombopenia and the cloudy swelling of liver cells in the two groups.

Transmission electron microscopy demonstrated that in vivo PSB-treated S₁₈₀ exhibited severe alterations. The nuclear chromatin was markedly reduced and vacuoles appeared in the mitochondria in the cytoplasm. Microvilli decreased in number and size.

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A Physiological Interpretation of Pulse Feeling

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This paper, from the point of modern physiological theory, studied mainly the relationship between pulse feeling in traditional Chinese medicine and cardiovascular hemodynamics. Following views are held by the authors: wiry pulse (弦脉) results from the increase of arteriolar resistance which leads to the increase of content and pressure in arterial store and tensility of arterial wall; thready pulse (细脉) results from the decrease of blood content in arterial store which leads to the decrease of the inflation amplitude of the arterial blood vessel; full pulse (洪脉), contrary to thready pulse, results from the increase of blood content in arterial store; smooth pulse (滑脉) is characterized by the rapid movement of blood stream; forceful pulse (实脉) and feeble pulse (虚脉) are the reflection of the volume of cardiac output.

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