·实验研究·

阴虚内热证初探

——知母对钠泵作用的大鼠体内实验

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中医的"阴虚内热"，"阳虚外寒"和能量代谢有否内在联系？是许多研究工作者所关注的问题。

七十年代初，Edelman 等[1]提出细胞中消耗能量最多的是钠泵(Na^+、K^+—ATP酶)。钠泵所消耗的能量估计占整个细胞总能量的 40～60%[2]。因此，在机体热生成中占重要地位。这个理论为许多实验所证实[3]。那么，阴虚内热是否与钠泵相关呢？这是一个很值得探讨的问题。如果它们之间存在着内在联系，这不仅可以解释阴虚内热的"热"，也可以理解阴虚内热的另一些症状，如大便干结，小便短赤。因为钠泵在肠粘膜上皮细胞中以及肾脏的肾曲小管中是促进水份重吸收的。中医有以方验证的理论，比如说阴虚内热则可用滋阴泻火药来治疗，所以我们从研究滋阴泻火药的作用来了解阴虚内热。从这一设想出发我们制定了以知母这一典型滋阴泻火药作为研究对象，研究它对动物钠泵的作用。

知母中的一个成份—知母皂甙及其水解产物知母菝葜皂甙元的体外实验结果[4]，证明它们不论对组织切片的耗氧率或是对提纯的兔肾 Na^+、K^+—ATP 酶都有极为明显的抑制作用。本文用知母菝葜皂甙元喂大鼠，观察它在整体动物身上对各种脏器中钠泵的作用。

材料与方法

一、知母菝葜皂甙元的制备，Na^+、K^+-ATP 酶测定及耗氧率测定均同前文[4]。

二、实验动物：纯种大白鼠 18 只，体重在 200～250g，分成三组。正常对照组，通过胃管每只大白鼠每天灌入 2ml 生理盐水。甲状腺素组，每只大白鼠每天灌喂含有甲状腺素 2.5mg 的溶液 2ml。甲状腺素加知母菝葜皂甙元组，每只大白鼠每天灌喂甲状腺素 2.5mg 加知母菝葜皂甙元 25mg 的混合液 2ml。共喂药三周，然后在乙醚麻醉下，处死动物，迅速取出脏器，制备实验用材料。

三、测定 Na^+、K^+-ATP 酶前各脏器的预处理，预处理方法因各脏器不同而异，是按文献中已有的报道进行，（略有修改）。预处理的简要步骤列于表 1。最后获得的 Na^+、K^+-ATP酶制剂置冰箱中保存待用。

表 1　各脏器的预处理

脏　器	肝　脏	小肠粘膜	肾　脏	脑组织
方法学根据	David M 等方法[5]	Yehuda Gutman 等方法[6]	JC Skou 等方法[7]	John Jarnefelt 等方法[8]
匀浆介质	0.001M $NaHCO_3$ pH7.5	0.25M蔗糖（含 2mM EDTA）pH7.4	0.25M蔗糖（含 30mM 组氨酸，0.1% Doc）pH7.4	0.25M蔗糖 pH7.4
低温离心 0～4℃	4,000r.p.m. 15′	3,500r.p.m. 10′	11,000 r.p.m. 15′	2,500r.p.m. 10′
上清液①	弃　去	保　存	保　存	保　存
沉　淀	各加相应介质，重复匀浆一次			
低温离心 0～4℃	4,000 r.p.m 15′	3,500r.p.m. 10′	11,000 r.p.m. 15′	2,500r.p.m. 10′
上清液②	弃　去	保　存	保　存	保　存
沉　淀	加 0.9% NaCl溶液 4℃保温 1h	弃　去	弃　去	弃　去
合并上清液①②低温离心，0～4℃		18,000 r.p.m. 60′	18,000 r.p.m. 60′	18,000 r.p.m. 60′
低温离心 0～4℃	4,000r.p.m. 10′			
沉　淀	各加 0.25M蔗糖（约 1 ml）			

注：上列各脏器酶制剂置冰箱中保存待用，弃去部分经检查不含 Na^+、K^+-ATP 酶；Doc 为脱氧胆酸。

实验结果

知母菝葜皂甙元对整体大鼠肝、小肠粘膜、肾脏及脑组织中 Na^+、K^+-ATP 酶活性的影响，结果见表 2。

大鼠在喂以甲状腺素后肝、肾、小肠粘膜中 Na^+、

表 2　大白鼠灌喂甲状腺素、甲状腺素加知母菝葜皂甙元后各脏器中 Na^+、K^+-ATP 酶活性比较

	肝细胞	小肠粘膜	肾脏	脑组织
正常对照组	0.73±0.06 (n=7)	2.94±0.22 (n=7)	15.32±1.74 (n=6)	3.96±0.41 (n=6)
甲状腺素组	1.59±0.25 (n=6)	5.37±0.39 (n=6)	27.46±1.66 (n=6)	3.49±0.49 (n=6)
甲状腺素加知母菝葜皂甙元组	0.65±0.13 (n=6)	1.44±0.63 (n=6)	15.40±1.55 (n=6)	3.04±0.30 (n=6)
P 值*	<0.05	<0.01	<0.01	>0.05

注：反应介质A：0.175M NaCl，0.0175M KCl，0.007M $MgCl_2$，0.002M ATP，0.14M Tris，pH7.2
反应介质 B：反应介质 A 中再加 1 mM 乌本甙
反应体系：介质 A(或 B)0.9ml，酶液 0.1ml，37℃保温 25 分钟
n：动物只数，M±SE
酶活性单位：μmoles Pi/mg 蛋白/小时
* P 值为喂甲状腺素的两组对比

K^+-ATP 酶活性显著地升高，但脑组织无明显变化。此结果表明甲状腺素对脏器的作用有一定的选择性。喂食甲状腺素加知母菝葜皂甙元后，Na^+、K^+-ATP 酶活性除小肠粘膜低于正常对照外，其余三种脏器均和正常对照组数值相近。可见喂食知母菝葜皂甙元的整体动物实验和体外加入知母菝葜皂甙元的实验结果是相同的[4]，都有抑制 Na^+、K^+-ATP 酶的作用。

肾脏切片的耗氧率(切片包括肾皮质与髓质)，结果见表 3。

表 3　大鼠灌喂甲状腺素、甲状腺素加知母菝葜皂甙元后对肾脏切片耗氧率的影响

	正常对照组	甲状腺素组	甲状腺素加知母菝葜皂甙元组
耗氧率	3.80±0.26 (n=7)	7.10±0.10 (n=6)	3.33±0.25 (n=7)
P 值*	<0.001		

注：反应体系：Krebs' Ringer 磷酸缓冲液 (pH7.4) 2 ml，肾脏切片约 100mg，总体积为 2.3ml。
Qo_2：μlO_2/mg 肾脏切片干重/小时
* 甲状腺素组与其他两组比较
n：动物只数，M±SE

喂食甲状腺素后的大鼠肾切片耗氧率显著地升高。喂食甲状腺素加知母菝葜皂甙元后，其耗氧率相当于正常对照组，这一结果说明由于知母菝葜皂甙元对 Na^+、K^+-ATP 酶活性的抑制作用所致。

讨　论

本文报道的整体动物实验结果，同样证明知母菝葜皂甙元对 Na^+、K^+-ATP 酶有明显的抑制效应。

肝脏是体内最大的实质性脏器，肝脏细胞内 Na^+、K^+-ATP 酶的变化会影响整体的热与寒。肾与肠粘膜细胞中此酶的变化可解释阴虚内热时大便干结，小便短赤。

最近我们采用 ^{86}Rb 放射性同位素法测定了红细胞钠泵活性，分析了 21 例肾阴虚病人，发现其红细胞钠泵全都低于正常人水平，阴虚病例现正收集中。

当然，中医的"内热外寒"其含义甚广，但从实验结果可以认为钠泵活性与"寒、热"有相当密切关系。

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X-Ray Research of Gastro-Entero-Functional Examination for the Deficiency Syndrome of Chronic Bronchitis

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This paper reports results from GI examination of the barium meal in 123 cases of chronic bronchitis and 20 cases of healthy subjects as control. The results obtained have shown that abnormality in the GI tract for the patients with Qi deficiency in the lung is insignificant. However, patients with Yang deficiency in the spleen and kidney have quite a few varied abnormal phenomena: retention of moderate quantity of gastric juice in the empty stomach; folds of the gastric membrane being thickened; gastric hypotension; decrease in speed, number and frequency of the gastric peristalsis wave; and prolongation of empty period in the stomach and the intestine. However, colon's motor function is increased. This may explain pathologically why patients with Yang deficiency in the spleen and kidney appear to possess symptoms typical of the digestive system. (Original article on page 225)

Observations on Cerebral Functions in the Aged

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This paper tries to provide simple methods and parameters for the prevention of aging. We have tested nearsighted visions, auditory function, transient memory of various shapes of drawings and function of extrapyramidal system (tremor of hands) in 53 healthy persons aged over 60. 121 healthy persons below 60 were grouped according to their age scale as controls. Our study has indicated that the functions mentioned above decreased markedly over 50 years of age. In those over 60, they had a negative correlation with the increase of age. These results suggest that the prevention of the regression of cerebral functions should be started at the age of 50. Because of the waning of the kidney in most of the aged, which is the main cause of regression of the cerebral functions, it is reasonable to replenish vital energy or essence of the kidney for the prevention of regression of the cerebral functions. (Original article on page 227)

Analysis of Blood Flow Dynamics of Taut Pulse and Slippery Pulse

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Examination of the radial pulse has been one of the important items of clinical diagnosis in TCM. The physiological and pathological implications of the radial pulse have not been fully studied until recent years. We have reported the establishment of a mathematical model according to non-linear elastic chamber hypothesis and derivation of the relative equations for the stroke volume of the heart (SV), the total peripheral resistance (TPR) and arterial compliance (Co) from the pulse wave form by using strain gauge method and impedance rheogram.

In this clinical study, 28 normal pulse, 32 slippery pulse of pregnancy, and 47 taut pulse of hypertension were investigated. A comparison between slippery pulse was classified according to hardness into three grades, namely 1, 2 and 3 respectively. It was found that the predicrotic pulse wave of the taut pulse ascended with increasing hardness, whereas in the case of slippery pulse, the predicrotic pulse descended. The higher the taut pulse, the greater the increase of TPR, arterial elastic modulus and the decrease of the stroke volume. This turned out opposite in the case of slippery pulse.

Animal experiment in dogs has demonstrated that infusion of nor-epinephrine, a vasoconstricting agent, induced a pulse pattern similar to taut pulse, while *viscum coloratum*, a vasodilating agent, induced a pulse pattern similar to slippery pulse. (Original article on page 232)

A Preliminary Study of Internal Heat Due to Deficiency of Yin — The Sodium Inhibitory Action of Anemarrhena Rhizome in Vivo

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It was mentioned in our previous report that a kind of saponin from Anemarrhena Rhizome had been isolated. The saponin and its hydrolytic product, sapogenin, are potential inhibitors of sodium pump in vitro. In this paper, the sodium pump inhibitory action of the sapogenin in vivo is reported. Eighteen rats were divided into three groups—control, thyroxine group and thyroxine plus sapogenin group. The duration of drug administration was three weeks. Then the animals were killed and the sodium pump activity of four organs (liver, kidney, the mucous membrane of the small intestine and brain) were measured. The results revealed that the activity of the three organs (liver, kidney and the mucous membrane of the small intestine) were markedly induced by the thyroxine and the induced enzyme can be inhibited totally by the sapogenin in vivo. Significances of the result are discussed.

(Original article on page 235)