

· 实验研究 ·

白芍对大鼠离体胰腺腺泡淀粉酶活力及分泌的影响

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内容提要 高浓度白芍水提液对大鼠胰淀粉酶活力有明显抑制作用；浓度 $\leq 16\text{mg/ml}$ 时不影响酶活力，但可使八肽胆囊收缩素(CCK-OP)诱导胰腺腺泡分泌淀粉酶的效价降低10倍，却不影响促胰液素刺激的酶分泌。这一选择性的拮抗作用说明白芍水溶性成分中可能有某种活性物质，可拮抗胰腺腺泡细胞膜上的CCK受体。这也许是白芍在清胰汤中对胰腺炎产生治疗作用的原因之一，但对其有效成分尚需进一步研究。

白芍是清胰汤Ⅰ号、Ⅱ号、Ⅲ号等中西医结合治疗急性胰腺炎有效方剂中的重要组成成分^(1~3)，我们利用大鼠离体胰腺腺泡制备的方法，研究了白芍水提液对胰淀粉酶活力以及对胆囊收缩素(Cholecystokinin, 简称CCK)和促胰液素(Secretin)诱导的大鼠胰腺腺泡淀粉酶分泌的影响。

材料和方法

一、试剂与溶液的配制

白芍(*Paeonia lactiflora* Pall)由本院药材料购于安徽。CLSPA型纯化胶原酶为美国Worthington公司产品，牛血清白蛋白(BSA)产自美国Miles实验室，BME多种氨基酸产自GIBCO实验室；Phadebas淀粉酶试剂片为瑞典Pharmacia Diagnostics公司产品；促胰液素为Squibb公司产品；八肽胆囊收缩素(简称CCK-OP, Peninsula公司产品)和胰蛋白酶抑制剂(Sigma产品)由美国国家卫生研究院JD Gardner博士赠送。

孵育液: 98mM NaCl, 6 mM KCl, 2.5mM NaH_2PO_4 , 1.2mM MgCl_2 , 0.5mM CaCl_2 , 24.5mM N-2-羟乙哌嗪-N'-2-乙基磺酸, 5 mM 丙酮酸钠, 5 mM 反丁烯二酸钠, 5 mM 谷氨酸钠, 2 mM 谷氨酰胺, 11.5mM 葡萄糖, 1% BSA, 0.01% 胰蛋白酶抑制剂, 1% 多种氨基酸液。pH7.4。

腺泡制备液: 含2 mM CaCl_2 , 0.2%及4% BSA, 余同孵育液。

细胞溶解液: 10mM NaH_2PO_4 , 1mM CaCl_2 , 0.1%十二烷基磺酸钠, 0.1% BSA, pH7.8。

淀粉酶测试液: 50mM NaCl, 20mM NaH_2PO_4 , 0.02%叠氮钠。用此液8 ml溶解1片Phadebas淀粉酶试剂片。

二、方法

1. 白芍水提液的制备: 将白芍生药研细过筛。称取20g, 加6~8倍蒸馏水, 浸泡15分钟后, 煮沸30分钟, 经4层纱布抽滤, 收集滤液。药渣加蒸馏水再次加热提取(同上)。合并两次滤液, 于80°C水浴浓缩至20ml, 则每ml相当于含生药1g。此液经 $2,000 \times g$ 离心20分钟, 取上清液于-20°C冻存。实验时用蒸馏水稀释。

2. 大鼠离体胰腺腺泡的制备^(4,5): 雄性Sprague-Dawley大鼠(由中国医学科学院医学实验动物中心提供), 体重150~200g, 实验前任意进食饮水。用颈椎脱臼法将动物处死后立即取出胰腺, 除净脂肪与系膜, 向胰腺组织内注入5 ml胶原酶液(溶于含0.2% BSA的腺泡制备液中, 浓度为25u/ml)。置37°C水浴中快速振荡(160次/分)消化30分钟, 其间每10分钟更换新鲜胶原酶液5 ml, 并充纯氧以维持细胞存活。再经剧烈振荡使腺泡分散, 迅即加入10ml含4% BSA的腺泡制备液中以中止胶原酶的水解作用, 于4°C, $800 \times g$ 离心30秒, 吸除上清液。用含0.2%及4% BSA的腺泡制备液依次反复清洗二次并吸除不均匀的小组织块。最后, 将由一只大鼠胰腺制得之分散的腺泡加入160ml孵育液中, 即成每毫升大约含1万个腺泡的均匀混悬液。

3. 淀粉酶活力测定: 仿Ceska等的方法^(6,7), 各管取0.1ml待测液, 加入2 ml淀粉酶测试液, 置37°C水浴中振荡20分钟, 迅速加入0.5N NaOH 0.5ml以中止反应并用8 ml蒸馏水稀释。于4°C, $800 \times g$ 离心15分钟, 用Hitachi 200A型分光光度计在620nm测定上清液的光吸收值。

三、实验步骤

1. 淀粉酶活力抑制实验: 取1 ml腺泡悬液加到

10ml 细胞溶解液中, 剧烈振荡, 使细胞及酶原颗粒膜破坏, 将淀粉酶全部释出。取此含酶液 1 ml (蛋白浓度约 30 μ g), 加各浓度白芍液使每 ml 含白芍的浓度分别为 1, 4, 16, 64mg (测定含有各浓度白芍的酶溶液的渗透压为等渗, pH7.4), 置 37°C 水浴中 30 分钟, 待药物与酶充分作用后测定酶活力。各实验管的淀粉酶活力以不加白芍对照管淀粉酶活力的百分率表示之。

2. 腺泡淀粉酶分泌实验: 取 1 ml 均匀腺泡悬液若干份, 加入 25 μ l 各浓度的白芍液、CCK-OP 或促胰液素 (浓度见图), 使药物和/或促分泌物与腺泡在 pH7.4、37°C 下孵育 30 分钟; 然后于 10,000 \times g 离心 30 秒, 取 0.1ml 上清液加入 0.5ml 细胞溶解液, 准备测定淀粉酶活力。孵育前另取 1 ml 腺泡悬液, 用前述方法 (见三、1) 使细胞内的淀粉酶全部释放, 测定酶的总活力。腺泡分泌的淀粉酶按下式计算:

$$\text{酶分泌}(\%) = \frac{\text{腺泡释出到孵育液中酶的活力}}{\text{孵育前细胞内淀粉酶总活力}} \times 100$$

每次实验均取自同一大鼠的胰腺腺泡, 实验结果为三份数值的平均。

结 果

一、白芍对大鼠胰腺淀粉酶活力的影响

附表 白芍对大鼠胰淀粉酶活力的影响

白芍(mg/ml)	淀粉酶活力(%)
1	101.4 \pm 4.1
4	97.1 \pm 3.4
16	97.2 \pm 2.2
64	73.0 \pm 2.7**
n=5	M \pm SE
	**P<0.01

我们首先测定了不同浓度白芍液对淀粉酶活力的影响。如附表所示, 高浓度白芍液对淀粉酶活力有明显抑制作用, 而低浓度白芍液 (≤ 16 mg/ml) 不影响酶活力。

二、白芍对大鼠离体胰腺腺泡淀粉酶分泌的影响

1. 为了研究白芍对胰腺腺泡淀粉酶分泌的影响, 我们首先采用对淀粉酶活力无抑制作用的各浓度白芍液, 检测它们对腺泡的基础分泌以及对 CCK-OP 或促胰液素诱导的大鼠胰腺腺泡淀粉酶分泌的影响。结果见图 1, 说明白芍浓度 ≤ 16 mg/ml 时, 对腺泡的基础分泌以及 1 μ M 促胰液素刺激的淀粉酶分泌无影

响, 但白芍浓度达 16mg/ml 时, 可明显抑制 0.1nM CCK-OP 引起的酶分泌。

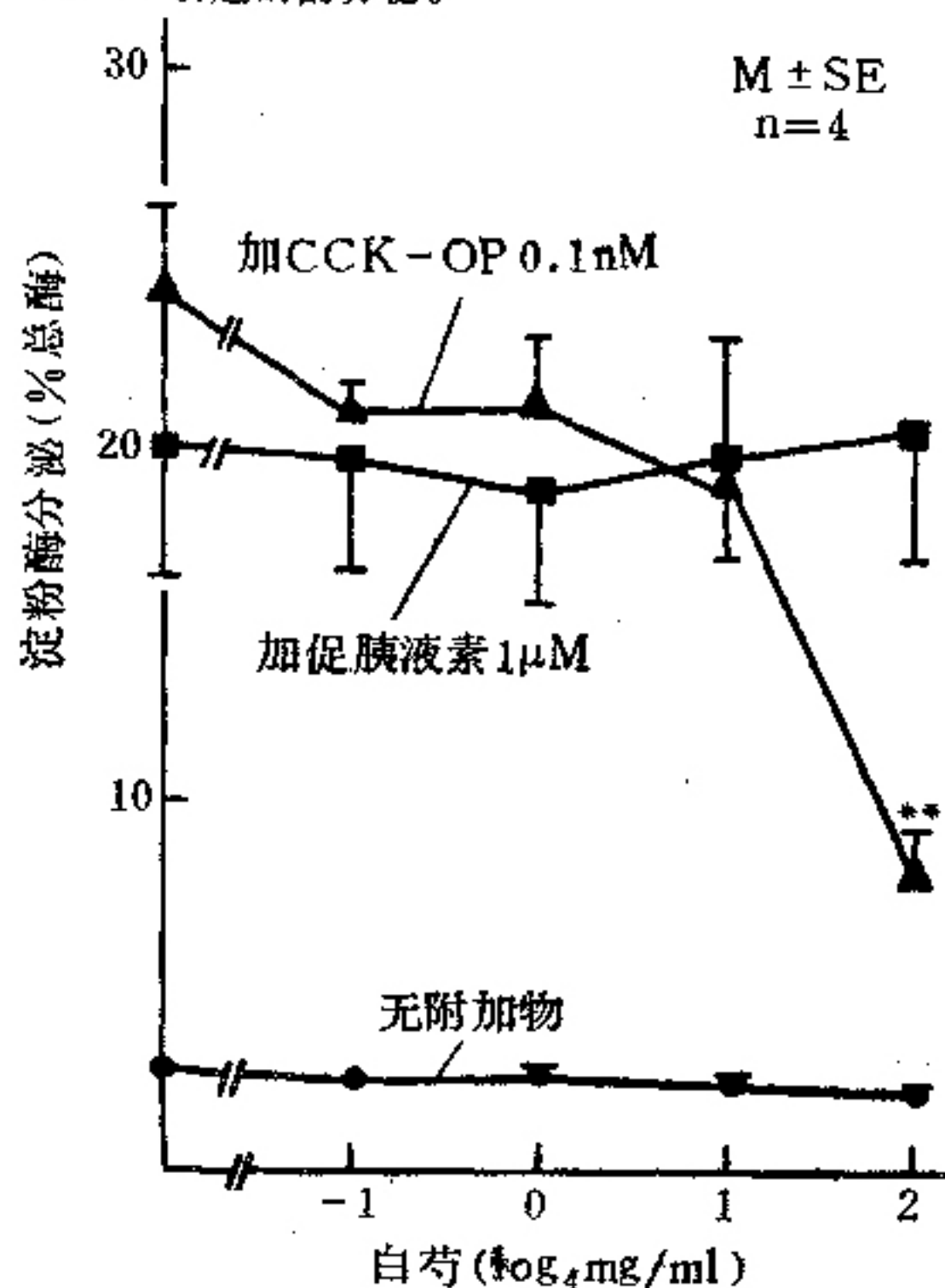


图 1 白芍对基础和刺激后大鼠胰腺腺泡淀粉酶分泌的影响
与对照相比 **P<0.01

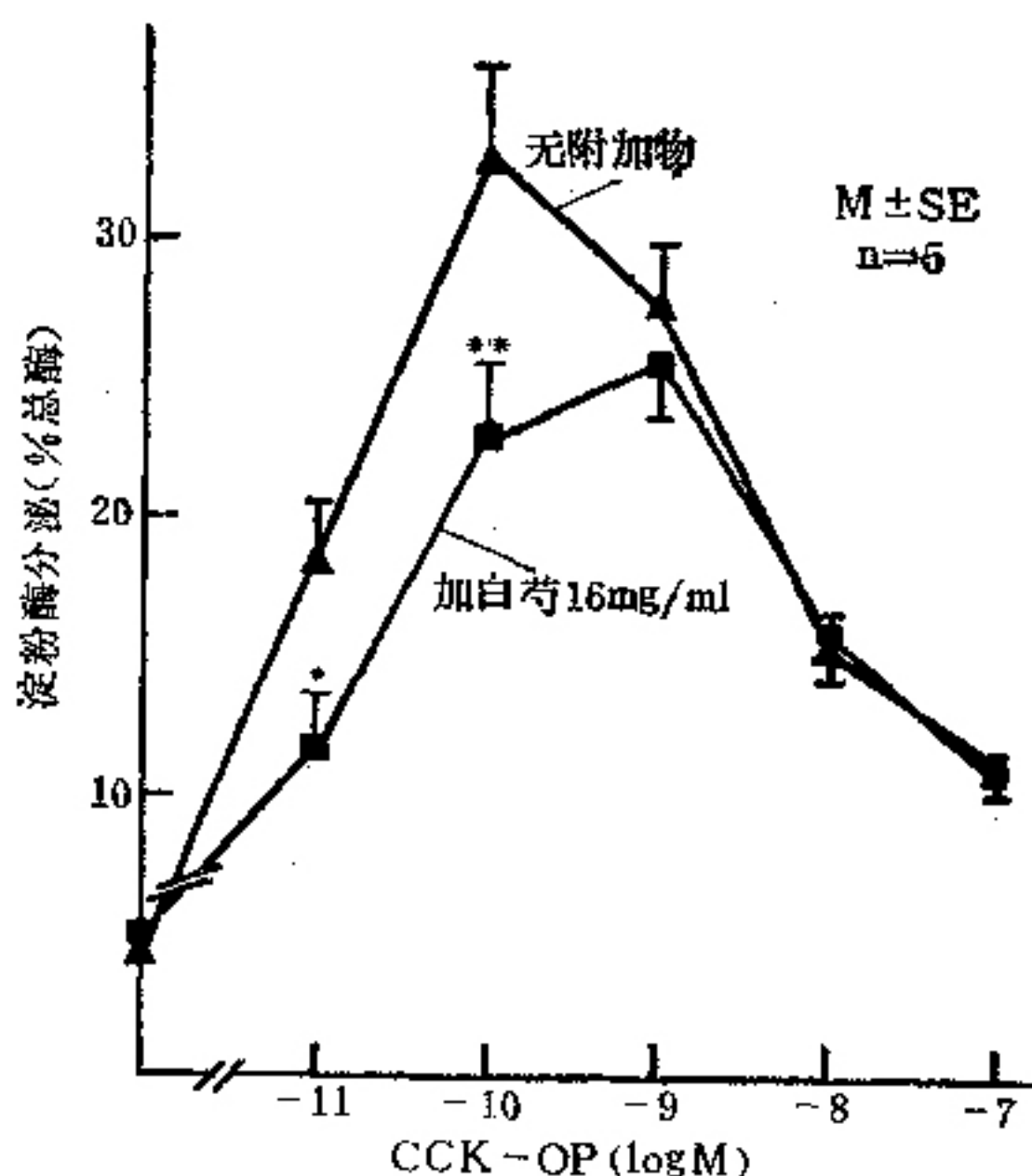


图 2 白芍对 CCK-OP 刺激淀粉酶分泌剂量反应曲线的影响
与不加白芍相比 *P<0.05, **P<0.01

2. 白芍对CCK-OP诱导的酶分泌剂量反应曲线的影响: 胰腺泡与不同浓度的CCK-OP在37°C条件下孵育30分钟, 或附加浓度为16mg/ml的白芍液共同孵育(pH7.4, 这一药物浓度已被证明不影响淀粉酶的活力)。结果显示白芍可以改变CCK释放淀粉酶的作用, 使其诱导酶分泌的剂量反应曲线的高峰右移, 分泌降低。图2示在腺泡孵育液中加入白芍不仅使CCK的效价降低10倍, 也影响激素的促分泌效应, 因为即使增加CCK的浓度也不能刺激淀粉酶的分泌达到最高的峰值。

3. 白芍对促胰液素刺激的酶分泌剂量反应曲线的影响: 图3示1 μ M促胰液素可使腺泡淀粉酶分泌明显增高, 浓度为16mg/ml的白芍液不能抑制此分泌, 对促胰液素诱导的淀粉酶分泌的剂量反应曲线亦无明显影响。

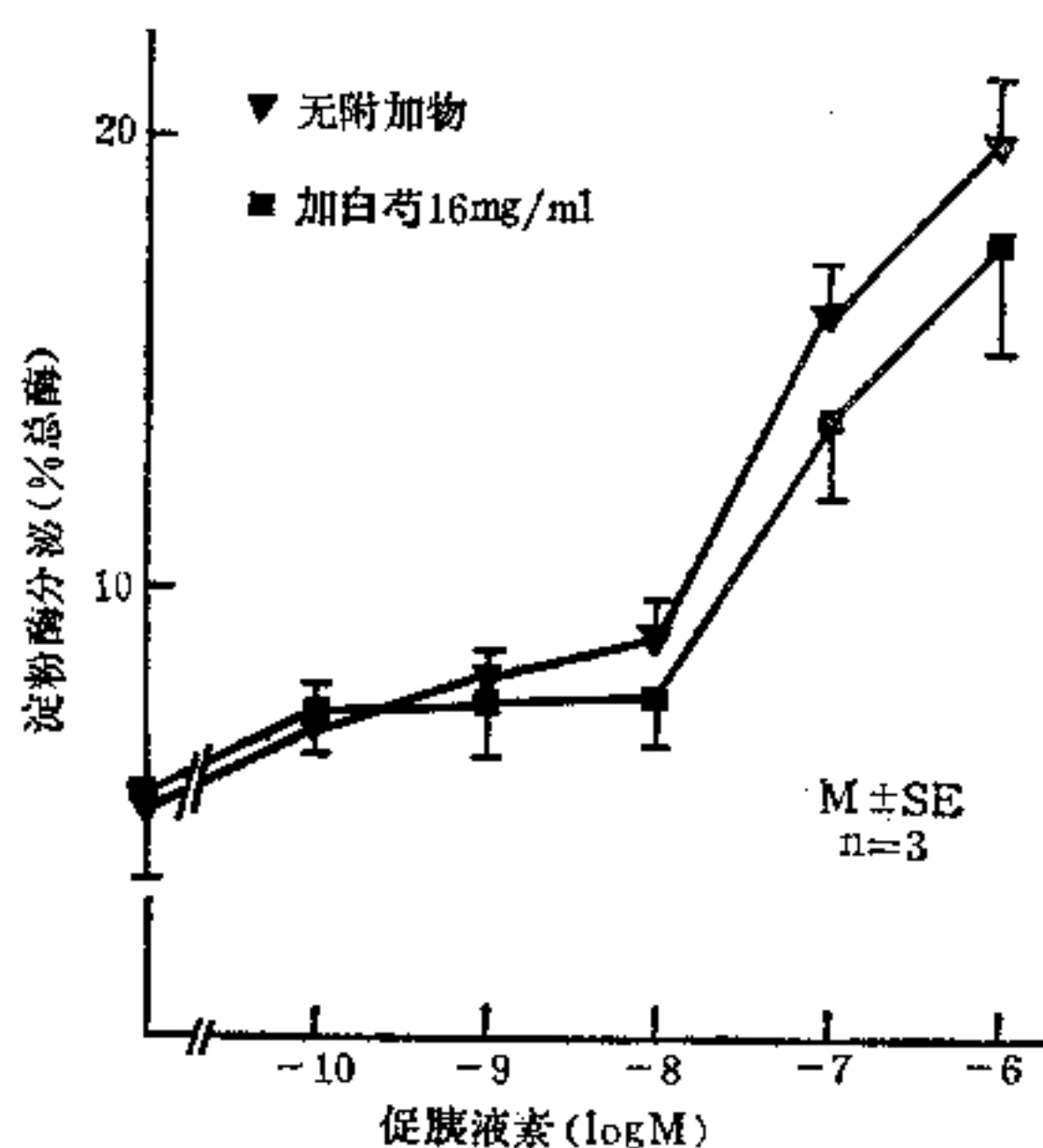


图3 白芍对促胰液素刺激淀粉酶分泌剂量反应曲线的影响

讨 论

白芍水溶性成分为芍药甙、苯甲酸、鞣质、 β -谷甾醇等。中医认为白芍在治疗急性胰腺炎方剂中起调气疏肝、缓急止痛的作用^[8]。临床观察发现, 用清胰汤治疗急性胰腺炎时, 血清淀粉酶能较快地降至正常。本工作显示清胰汤成分中的白芍水提液对胰酶有

两种作用: (1)高浓度白芍液能抑制大鼠胰淀粉酶的活力; (2)较低浓度时对CCK诱导的大鼠离体胰腺腺泡的淀粉酶分泌有抑制作用; 这或许是产生上述治疗作用的原因之一。但其中有效成分值得进一步探讨, 以上作用也需要在整体动物实验中继续研究证实。

目前认为CCK对胰腺的作用是刺激腺泡分泌消化酶, 而促胰液素主要作用于胰导管的上皮细胞, 引起水和碳酸氢盐的分泌, 对腺泡分泌消化酶也有轻度刺激作用。本实验结果发现白芍水提液能抑制CCK-OP刺激大鼠离体胰腺腺泡分泌的淀粉酶, 却不影响促胰液素的作用, 说明白芍对激素的这种拮抗作用有一定的特异性。为了检验白芍的上述作用是否使CCK分子失活, 我们将白芍液(16mg/ml)与CCK-OP(0.1nM)先行孵育15分钟, 然后再和腺泡共同孵育, 发现这样做并不使CCK-OP的活性降低。因此, 我们认为白芍对CCK-OP诱导的胰酶分泌的抑制是作用于细胞水平; 推测其水溶性成分中可能含有能拮抗胰腺腺泡细胞膜上CCK受体的活性物质, 对其有效成分还需进一步研究。

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Effect of *Paeonia Lactiflora* on Amylase Activity and Amylase Release from Pancreatic Acini in Rats

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The root of *Paeonia lactiflora* (PL) is an important component in the decoction of TCM for the treatment of acute pancreatitis. In the present study, we examined the effect of the water soluble extract of PL on amylase activity and amylase release induced by cholecystokinin octapeptide (CCK-OP) or secretin using in vitro preparation of dispersed pancreatic acini prepared from rat pancreas.

Results from the study indicated that: (1) At a high concentration, the drug prominently inhibited pancreatic amylase activity, but it had no effect on this activity at low concentrations ($\leq 16\text{mg/ml}$). (2) PL at a concentration of 16 mg/ml could modify the action of CCK-OP and produced a 10-fold decrease in its potency in stimulating amylase release from pancreatic acini, but it did not affect amylase secretion induced by secretin. (3) Because of this selective effect of PL in inhibiting the action of CCK on amylase secretion, we assume that certain components of the drug might possess the ability of antagonizing the action of CCK at the cell receptor level.

The result also indicates that the above mentioned action of PL may be related to its therapeutic effect in the treatment of acute pancreatitis. (Original article on page 100)

Hemorheologic Investigation on the Properties of Medicinal Herbs of Promoting Blood Circulation and Removing Stasis

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Traditional herbs of promoting blood circulation and removing stasis were divided into three groups according to their properties: circulation regulator, circulation promoter and stasis remover. Ten typical herbs were selected to feed Wistar rats with a dosage of 15g/kg for 4 days; the same volume of water was given to the control group. HAAKE Rotovisco RV100 and other related instruments were employed to determine nine hemorheologic parameters, including blood viscosity, plasma viscosity, hematocrit, index of RBC aggregation, platelet aggregation, recalcification time (tr), blood viscosity changing rate ($d\eta/dt$) during low shear coagulation, etc. The results showed that herb property had its significant influence ($P < 0.05$) on the process of coagulation characterized by tr and $d\eta/dt$. The anticoagulation property of stasis remover was most impressive. There was also a tendency of decreasing in blood viscosity (shear rate $21-210\text{s}^{-1}$) and the index of RBC aggregation when property of medicinal herbs was strengthened from regulating blood circulation to removing stasis. (Original article on page 103)

Temperature-Reducing Effect of Rhubarb and Its Relation to PGE in the Central Nervous System

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Rabbit model of fever was produced by means of subcutaneous inoculation of pneumococci. Decoction of Rhubarb (*Rheum officinale*) was given orally to the experiment group ($n=17$) and tap water to the control ($n=17$). Anal temperature was markedly reduced in the Rhubarb group and significant difference vs the control group was observed ($P < 0.001$). For comparison, anal temperature was measured in 10 normal rabbits before and after Rhubarb administration, but no marked change was recorded ($P > 0.2$). Third ventricular intubation and irrigation was carried out in 5 rabbits. Irrigation fluid of the third ventricle was collected during the stage of normal temperature, at the peak of fever and when the temperature was lowered by Rhubarb. Prostaglandin E was detected with RIA in each of the three portions of the irrigated CSF. The results showed that PGE level was raised during fever and decreased after Rhubarb administration (4.92 ± 2.2 to $1.5 \pm 1.2\text{ng/ml}$, $P < 0.001$). In 17 normal rabbits, the same happened after treatment with Rhubarb (2.3 ± 1.8 to $1.9 \pm 1.3\text{ng/ml}$, $P < 0.01$). Irrigation of CSF without medication had no significant influence to the PGE level either in febrile rabbits or in normal one ($P > 0.05$).

The above data reveal that Rhubarb can reduce the infective fever in rabbits and its antipyretic effect is closely related to the inhibition of PGE level in CSF, which is the mediator of the temperature center in the central nervous system. (Original article on page 106)