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大鼠处理普奈洛尔后改变 α_1 受体亚型介导的心功能

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关键词 乳头状肌；心房；心肌收缩；心率；α_1 肾上腺素受体；普奈洛尔；苯肾上腺素；可乐定；乌拉地尔；卡巴胆碱

目的：研究普奈洛尔(Pro)作用后，大鼠心肌 α_{1A} 和 α_{1B}受体亚型介导正性肌力和正性频率变化. 方法：测定 Pro 大鼠和正常鼠左心室乳头状肌和右心房收缩力和心率. 结果：给予 Pro 后，苯肾上腺素(Phe)使乳头状肌收缩力由 53±17 mg 增加到 90±18 mg ($P<0.05$). Pro 和对照组收缩力分别增加 20±12 和 5±5 mg ($P<0.05$). 氯乙基可乐定使两组收缩力变化无区别. 5-甲基乌拉地尔存在时 Phe 使 Pro 组收缩力增加 13±5 mg，对照组无变化. 正常和心率抑制时，Phe 使两组动物 α_{1B}介导心率增加无差别. 结论：β 受体阻断，α_1 介导正性肌力增加主要由 α_{1B}作用增强引起.

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Inhibition of 11β-hydroxysteroid dehydrogenase obtained from guinea pig kidney by some bioflavonoids and triterpenoids[1]

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KEY WORDS hydroxysteroid dehydrogenases; kidney; microsomes; naringenin; emodin; fisetin; astramembranin I; oleanolic acid

AIM: To study the inhibitory effect of some bioflavonoids and triterpenoids on 11β-hydroxysteroid dehydrogenase (11β-OHSD) from guinea pig kidney. **METHOD**: The 11β-OHSD of kidney cortex microsomes in addition of cortisol was incubated in the presence of NADP, Triton DF-18, and the test compounds at 37 ℃ for 1 h. The enzyme activity was assayed by measuring the rate of conversion of cortisol to cortisone eluted with HPLC gradient analysis. **RESULTS**: The IC_{50} (95 % confidence limits)values of glycyrrhizic acid, naringenin, fisetin, emodin were 254 (202 - 320), 336 (270 - 418), 470 (392 - 564), and 527 (425 - 653) $\mu mol \cdot L^{-1}$, respectively. The inhibitory effect of oleanolic acid was 2-fold stronger than that of astramembranin I. The mode of action of naringenin was competitive inhibition. **CONCLUSION**: The test compounds inhibited the 11β-OHSD in kidney cortex with different potencies as glycyrrhizic acid did.

The syndrome of apparent mineralocorticoid excess, first described by Ulick *et al* in 1977, has led to much research on the enzyme 11β-hydroxysteroid dehydrogenase (11β-OHSD). Deficiency of 11β-OHSD in children leading to their

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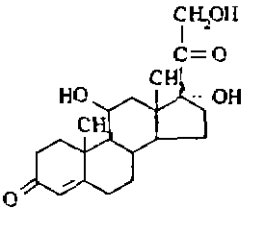
failure to oxidize cortisol to inactive cortisone enabled high cortisol level in the kidney to activate the renal mineraloocorticoid receptor and cause hypertension and hypokalemia, subsequently, the mechanism of licorice-induced hyperminaloocorticoidism was proposed to be the inhibition of 11β-OHSD by the active principle of licorice, glycyrrhizic acid. 11β-OHSD played an important role in regulating the interactions of cortisol with mineraloocorticoid and glucocorticoid receptor[1-2].

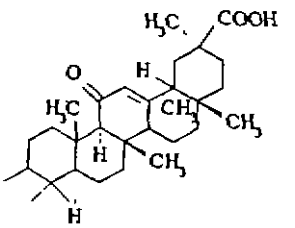
Various compounds with steriod and flavonoid structures showed inhibitory effects on 11β-OHSD *in vivo* and *in vitro*[3]. Gossypol inhibited 11β-OHSD from guinea pig and human renal cortex microsomes[4,5]. We found the furosemide also inhibited 11β-OHSD from guinea pig[6]. These studies may elucidate the reason for gossypol and furosemide to cause hypokalemia[6]. Therefore, we decided to test some compounds with steroidal or triterpenoid and flavonoid structure extracted from different plants and to study whether they had inhibitory effect on 11β-OHSD to evaluate the significance of these compounds in clinical use.

MATERIALS AND METHODS

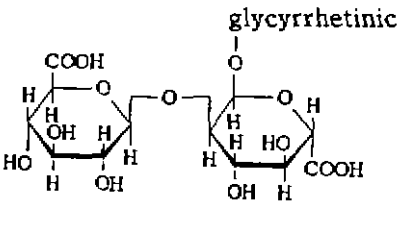
Chemical and Solution Cortisol, cortisone, corticosterone, NADP, glycyrrhizic acid (GA), naringin (NRG), and naringenin (NRGN) were purchased from Sigma Co. Astramembranin I (ASI), oleanolic acid (OA), were triterpenoids NRG, NRGN, emodin, fisetin, daphnin, daphnetin, adrographalide (AGP), which were bioflavonoids and adrographalide was a diterpenoids lactone. These compounds were identified by Department of Phytochemistry



Hydrocortisone



Glycyrrhetinic acid



glycyrrhetinic acid

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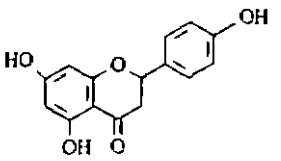
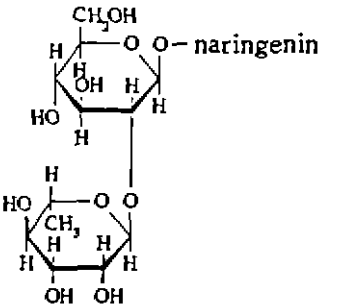
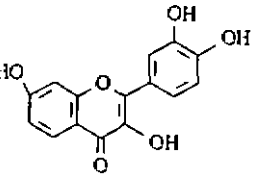


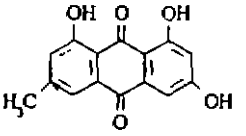
Naringenin



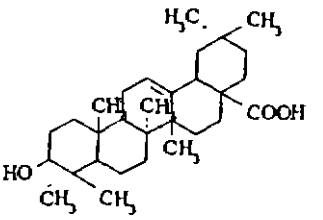
Naringin



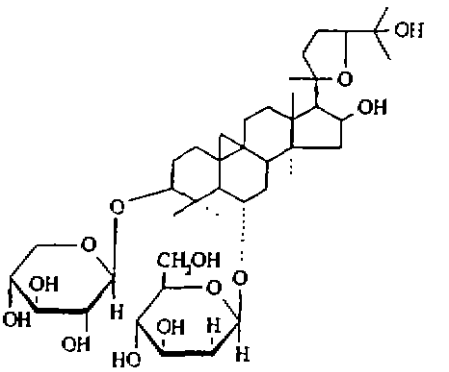
Fisetin



Emodin



Oleanolic acid



Astramembranin I

in China Pharmaceutical University and Department of Chemistry in Nanjing Medical University.

Emodin, ASI, OA, AGP, and daphnin were dissolved in methanol. GA was dissolved in distilled water. NRG, NRGN, and fisetin were dissolved in ethanol and diluted with methanol. Daphnetin was diluted in distilled water (pH 10). Corticosterone cortisol and cortisone were in methanol (144 $\mu mol \cdot L^{-1}$) and kept at −4 ℃.

Microsome preparation and assay for 11β-OHSD Kidney cortex from ♂ Hartley guinea pigs with hair was homogenized in Krebs-Henseleit buffer[4], but using a Tekmer Tissuemiger (Cincinnati OH). The microsomes were prepared as described previously[5] except that they were diluted to a concentration of 1.25 g protein · L^{-1} prior to storage at −70 ℃.

The activity of 11β-OHSD in guinea pig renal cortex microsomes was determined by measuring the rate of conversion of cortisol to cortisone. Five min before incubation, concentrated Triton DF-18 2 μL was added to each mL of the microsome suspension. The assay mixture contained Krebs-Henseleit buffer 500 μL (pH 7.2), 50 μL NADP 5 mmol · L^{-1}, 40 μL cortisol 144 $\mu mol \cdot L^{-1}$ in methanol, 20 − 50 μL (25 − 62.5 μg) of microsome suspension in phosphate-sucrose buffer 0.01 mol · L^{-1} containing various concentrations of test compound. The mixture was incubated in duplicate or triplicate. The total volume was 700 μL.

Methanol was kept at < 10 % in the incubation medium. Methanol at this concentration did not inhibit the reaction after 1 h of incubation at 37 ℃. The reaction was terminated with the addition of 3 mL methylene chloride and 20 μL corticosterone 144 $\mu mol \cdot L^{-1}$ as the internal standard for assay of cortisone and cortisol.

The enzyme inhibition constant for naringenin was determined by adding naringenin 25 − 200 $\mu mol \cdot L^{-1}$ and cortisol concentrations of 4, 8, and 16 $\mu mol \cdot L^{-1}$. The constants were obtained from a Dixon plot and a kinetic programme (Chou J Chou T-C: Michaelis-Menton analysis with microcomputers Disk # 1, Elsevier-Biosoft, 1993, Cambridge, UK).

Extraction of steroid on HPLC Cortisone, cortisol, and corticosterone were measured[5] in the microsomal incubation mixture. The steroids were extracted into methylene chloride with vortexing for 1 min, then centrifuged at 750 × g for 15 min. The aqueous layer was removed by aspiration. NaOH 300 μL 0.1 mol · L^{-1} was added to the organic phase followed by vortexing for 30 s. After centrifugation of the mixture, the organic phase was washed with 500 μL of Milli-Q water (Millipore Corp, Bedford MA). The 1.5 mL organic phase were transferred to clean glass tube and dried by evaporation at 45 − 50 ℃ water bath. The residue was dissolved with 200 μL of methanol and 5 μL of this solution was used for HPLC. A standard for cortisol and cortisone was assayed in parallel to each enzyme experiment after boiling to inactivate the enzyme. Standard curves were plotted as the ratio of peak height of cortisone (or cortisol) divided by the peak height of the internal standard *vs* steroid concentration. All unknown concentrations. IC_{50} were estimated from at least 3 concentrations of each compound tested by a dose-response programme (Chou J, Chou T-C: Dose-effect analysis with micro-computers. Disk # 2, Elsevier-Biosoft, 1994, Cambridge, England).

The HPLC apparatus consisted of a Waters Model 6000, a solvent delivery system, U6K injector, model 680 automated gradient controller, Water 486 tunable absorbance detector, and a BBC chart recorder (Model SE 120). The mobile phase contained methanol-water (30:70 vol/vol) at a flow rate of 1.0 mL · min^{-1}. The Waters stainless steel Novapak C_{18} column (3.9 mm × 150 mm, 4 μm) was kept at 20 ℃. The retention time for cortisone, cortisol, and corticosterone were 6.5, 7.0, and 9.0 min, respectively.

RESULTS

Naringenin was the most potent inhibitor tested, having potency similar with GA. The inhibitory potency in decreasing order was NRGN, fisetin, emodin, OA, ASI, and NRG. The oxidative activity of 11β-OHSD was inhibited in a dose-dependent manner. Daphnin, daphnetin, and AGP were failed to inhibit the activity of 11β-OHSD as high as 2 mmol · L^{-1} of maximum concentration tested (Tab 1).

The correlation coefficients (r) for the computed values agreed with the measured values of the inhibitors: 0.99 for GA, NRGN, and ASI, 0.98 for emodin, 0.95 for fisetin and NRG, and 0.93 for OA (Fig 1).

The effect of inhibition by naringenin at varying concentration of cortisol on the enzyme was shown as a double reciprocal plot (Fig 2). Most of the lines converged near the ordinate, which indicated that the inhibition by naringenin was competitive. The kinetic constants for enzyme were: $K_m = 3.30$ $\mu mol \cdot L^{-1}$ and $V_{max} = 26.21$ $\mu mol \cdot h^{-1}/g$ microsomal protein. The inhibitor constant, K_i, for naringenin was 16.13 $\mu mol \cdot L^{-1}$, nearly 4 times more than the K_m for cortisol.

DISCUSSION

We have tested a number of compounds for

Tab 1. Inhibition of 11β-hydroxysteroid dehydrogenase. $n=3-5$, $\bar{x}\pm s$.

Compound	Concn/ $\mu mol\cdot L^{-1}$	Inhibition/ %	IC_{50} $\mu mol\cdot L^{-1}$ (95 % confidence limits)
Glycyrrhizic Acid	132	23.2±4.4	
	246	52.4±5.3	254
	529	78.5±3.4	(201.5-320.5)
NRGN	12.5	16.3±2.0	
	25	25.1±1.8	
	50	44.0±7.9	
	100	62.5±1.7	336
	1 000	72.0±1.8	(270.4-417.5)
	2 000	86.4±1.4	
	5 000	92.9±2.8	
Fisetin	500	48.0±2.7	
	1 000	78.3±1.0	470
	2 000	84.2±3.2	(391.5-564.2)
Emodin	500	41.7±0.1	
	1 000	89.5±2.1	527
	2 000	97.2±0.7	(425.0-652.9)
OA	500	22.7±2.4	
	1 000	76.7±0.2	770
	2 000	84.3±0.1	(536.5-1 105.2)
ASI	1 000	19.8±3.0	
	1 500	51.9±4.1	1 547
	2 000	67.5±6.4	(1 272.4-1 881.0)
NRG	582	8.8±1.7	
	1 163	16.7±0.8	2 373
	1 744	42.1±4.5	(1 846.4-3 050.0)
	2 908	59.9±1.5	

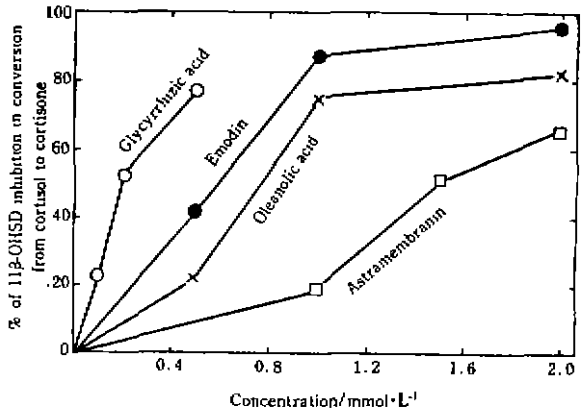


Fig 1. Inhibition of guinea pig renal 11β-OHSD.

their ability to inhibit the utilizing-NADP form of 11β-hydroxysteroid dehydrogenase from guinea pig renal cortex using cortisol as the substrate. The

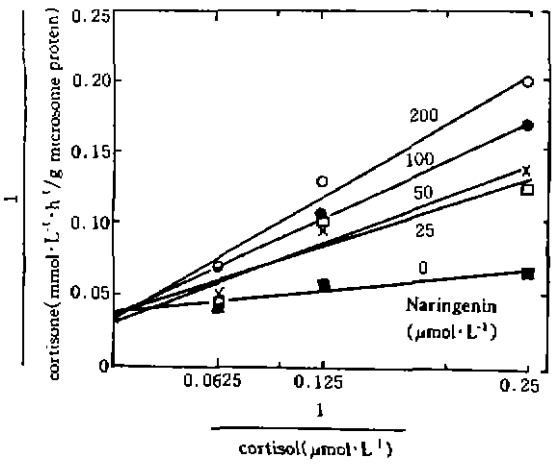


Fig 2. Effect of naringenin on 11β-OSHD, mean value from the lowest two batches assayed.

IC_{50} value of GA and K_m constant for cortisol were comparable to the data which were found in Buhler[3] and Monder, *et al*[7]. NRG and NRGN were active compounds in grape fruit juice. NRG was hydrolyzed to aglycon, NRGN, then absorbed completely. These two compounds inhibited a certain pathway of drug oxidation (cytochrome P-450 3A4)[8,9]. Emodin inhibited many enzymes such as Na^+-K^+ ATPase, cyclooxygenase, NAPH dehydrogenase and increased in potassium excretion in the urine[10-12]. The present observations demonstrated that the IC_{50} values of NRGN, fisetin, and emodin were very similar with GA. NRGN was a much more potency inhibitor than NRG and had a potent competitive inhibitory effect on the activity of 11β-OHSD in our study.

ASI and OA shared triterpenoid structure and had similar effect on prevention of exprimental liver injury induced by CCl_4[13,14]. OA was an inhibitor of 11β-OHSD which had a IC_{50} value two times to ASI, but much less potency than GA.

Recently, evidence has been presented that the extract of ginseng root also exerted an inhibitory effect on 11β-OHSD of rat renal microsomes[15]. It was known that the preparations of ginseng would induce hypertension and edema, if the person had taken them for a long period. The importance, if, any, of these compounds we studied as *in vivo* inhibitors of this enzyme remained to be determined.

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某些黄酮类和三萜皂苷类化合物对豚鼠肾的11β-羟化类固醇脱氢酶的抑制作用[1]

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关键词 羟化类固醇脱氢酶; 肾; 微粒体; 黄酮; 柚皮素; 大黄素; 非瑟素; 黄芪甲苷; 齐墩果酸; 三萜皂类

目的: 研究某些生物黄酮和三萜皂苷对豚鼠肾皮质11β羟化类固醇脱氢酶(11β-OHSD)的抑制作用. 方法: 肾皮质微粒体制备中加入氢化可的松, NADP, triton DF-18 和被研究的化合物分别经 37 ℃, 1 h 孵育,在 HPLC 梯度洗脱条件下, 测定氢化可的松成为可的松的转化率来表示 11β-OHSD 的活性. 结果: 甘草酸、柚皮素、非瑟素、大黄素对 11β-OHSD 抑制的 IC_{50}分别为 254, 336, 470, 527 μmol·L^{-1}. 齐墩果酸抑制此酶的 IC_{50}值为黄芪甲苷的两倍. 柚皮素对此酶的抑制是竞争性的. 结论: 柚皮素、非瑟素、大黄素等类似甘草酸, 它们对肾皮质的 11β-OHSD 有不同程度的抑制作用.