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GOSSYPOL, OUABAIN AND VANADATE ON THE ISOLATED PERFUSED RAT KIDNEY AND Na,K-ATPase

Hridayesh K Pathak, Clinton N Corder, Nirmala Suman
(*Dept of Pharmacology, Oral Roberts University, Tulsa OK 74171, USA*)

ABSTRACT Gossypol, ouabain, and vanadate were tested on the isolated perfused rat kidney preparation (IPRK). Gossypol decreased Na reabsorption, urinary K concentration, and glomerular filtration rate(GFR). Ouabain decreased Na reabsorption, K concentration, and GFR, but increased urine flow, renal vascular resistance and osmolar clearance. Ouabain was also tested in combination with vanadate in the IPRK. The effect of gossypol was tested on rat kidney microsomes: half-maximal inhibition of Na,K-ATPase occurred at 2.4 μ*M* gossypol, and Mg-ATPase was half-maximally inhibited at 36 μ*M* gossypol. On the same microsomes ouabain inhibited Na,K-ATPase, but not Mg-ATPase. When gossypol was administered to intact rats, it caused a diuresis and a decrease in plasma K levels. It is concluded that gossypol exerts effects that are different

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Reprint requests to Dr C N Corder, 3921 S Florence Place, Tulsa OK 74105, USA

from those of ouabain and vanadate.

KEY WORDS gossypol; ouabain; vanadate; kidney function tests; kidney microsomes; Na,K-ATPase; Mg-ATPase

Gossypol, isolated from the cotton seed plant, is of primary interest to the food industry[1], and shows promise as an effective male antifertility agent[2]. Hypokalemia induced by gossypol may be observed clinically and attributed to inhibition of Na,K-ATPase in the kidney[3,4]. Its pharmacological effects on the isolated kidney have not been reported.

Ouabain is a cardiac glycoside that inhibits Na,K-ATPase. In the isolated perfused rat kidney (IPRK), ouabain caused effects on electrolyte reabsorption that were attributed to inhibition of Na,K-ATPase[5]. Somewhat similar conclusions were drawn from the studies of vanadate in the IPRK[6].

The present report describes the effects of gossypol in the IPRK and on Na,K-ATPase, compares those effects to that elicited by ouabain and vanadate, and suggests dissimilarities in the mechanism of action of gossypol and ouabain.

METHODS

Conditions of the isolated perfused rat kidney (IPRK) were similar to previous studies in this laboratory, except the bovine serum albumin concentration was reduced to 55 mg/ml[6]. During the stabilization period, the perfusate was cycled through a Gilman 0.5 μm pore size in-line filter; thereafter the filter was glass-wool. The control perfusion flow rate averaged 28.4 ml/min with the pressure always set at 17.3 kPa (130 mm Hg) during the control period. Each control and drug observation was taken for a 10 min period. Ouabain octahydrate (lot 59C-0102, Sigma Chem Co) was dissolved in distilled water. Gossypol (Sigma Chem Co) was dissolved in aqueous 50 m*M* sodium carbonate. Sodium orthovanadate was purchased from Fisher Scientific Co. Appropriate aliquots of the test drugs were added to the IPRK for the desired drug concentrations. [*Carboxy*-^{14}C] inulin (New England Nuclear, lot 1141-229, sp. act. 1.91 μCi/mg of inulin supplied at 13.1 mg/ml) was added at 1 ml/l of perfusate to measure the glomerular filtration rate (GFR) as inulin clearance.

Where indicated, clearance and tissue levels of ouabain were measured with 0.1 μ*M* [^{3}H]ouabain (New England Nuclear, lot 1162-075, 19.5 Ci/mmol added as a tracer to unlabeled ouabain). At the end of the 15 min clearance study, the kidneys were submerged in liquid nitrogen without prior interruption of pressure or perfusate flow. The kidneys were powdered in a liquid nitrogen cooled mortar and pestle. Samples were weighed and homogenized in 100 m*M* tris at pH 7.5 at 4℃.

Radioactivity was measured by liquid scintillation[6].

Na,K-ATPase activity was assayed at pH 7.2 and 37℃ in a medium containing 0.1-0.5 mg protein/ml, 100 m*M* imidazole, 6 m*M* ATP, 6 m*M* $MgCl_2$, 100 m*M* NaCl and 20 m*M* KCl; the difference between total ATPase and that in the presence of 1 m*M* ouabain was taken as Na,K-ATPase[7]. Na,K-ATPase from rat kidney microsomes was prepared by methods previously described[7] and had a specifica ctivity of 32 mol ATP hydrolyzed/kg protein/h.

Effects of gossypol were also studied in intact ♂ Sprague-Dawley rats housed individually in metabolic cages (Maryland Plastic, Inc). The rats were accustomed to the cages over a 24 h period, then a 24 h control period was recorded and followed by a sc injection of gossypol at 25 mg/kg. Consecutive 24 h urine collections were made. Blood (0.5 ml) was drawn from the tail vein into syringes containing 150 U lithium heparin (Sigma Chem Co) at the beginning of each day, centrifuged and the plasma was stored at −20℃ until assayed for K. Urine was only assayed for volume because of excessive contamination by food and feces.

RESULTS

Gossypol in IPRK Gossypol caused dose-dependent decreases in fractional reabsorption of Na, urinary K concentration and GFR (Table 1). Na reabsorption decreased to 88% of control at 0.6 μ*M* gossypol. Fractional K absorption did not change despite a significant decrease in the concentration of K in the urine. Diuresis was noted in 3 perfusions and decreased urine flow occurred in three. However, the GFR was consistently decreased at 0.3 and 0.6 μ*M* gossypol. Vascular resistance was unaffected.

Ouabain in IPRK Ouabain caused increases in urine flow, Na excretion, osmole clearance, and resistance (Table 2). The increase in resistance occurred within 30–60 s of addition of ouabain to the IPRK. Conversely, fractional Na reabsorption and urinary K concentration decreased. GFR remained unchanged. The responses became less consistent at the 10 and 100 μ*M* drug concentrations. The concentration of ouabain for half-maximal effect (0.01 – 0.8 μ*M*) was approximately as follows: Na excretion, 0.15 μ*M*; Na reabsorption, 0.2 μ*M*; diuresis, 0.11 μ*M*; K concentration, 0.09 μ*M*; and osmole clearance, 0.11 μ*M*. Therefore, approximately 0.13 μ*M* ouabain gave the half-maximal responses in the IPRK.

Clearance of [^{3}H]ouabain was measured in three IPRK studies with 0.1 μ*M* drug to approximate the half-maximal responses to ouabain. The clearance was 71 ± 58 μl/min, approximately 18 % of the 416 μl/min observed for [*carboxy*-^{14}C]inulin (Table 2).

The concentration of [^{3}H]ouabain in the kidneys taken from the clearance studies was 0.254 μmol/kg wet weight.

Gossypol-Ouabain in IPRK The combination of 0.6 μ*M* gossypol and 0.3 μ*M* ouabain was examined (Table 1). Diuresis was absent

Tab 1. Gossypol and gossypol-ouabain on isolated perfused rat kidney. Following a 30 min equilibration period, a 10 min control was followed by successive 10 min periods of 0.1, 0.3, 0.6 μ*M* gossypol then 0.3 μ*M* ouabain. Responses to the drugs are shown as % of control. $\bar{x} \pm SD$. ** $p < 0.05$ compared to control. n = 6

Observation	Control values (actual units)	μ*M* Gossypol 0.1	0.3	0.6	0.6 μ*M* Gossypol 0.3 μ*M* Ouabain
Urine Flow	58.0 μl/min (19.0)	135 (62)	234 (267)	240 (286)	116 (170)
GFR (Inulin Clear.)	425.1 μl/min (66.4)	95 (10)	89** (22)	77** (28)	49** (22)
Sodium in Urine	84.9 mEq/l (11.1)	102 (7)	101 (7)	103 (7)	103 (9)
Sodium Excreted	5.0 μEq/min (1.8)	134 (52)	229 (251)	245 (293)	167 (170)
Sodium Reabsorp.	90.9 percent (2.3)	97** (2)	91** (9)	88** (12)	84** (18)
Potassium in Urine	36.2 mEq/l (8.6)	74** (14)	56** (25)	52** (23)	50** (27)
Potassium Excreted	1.5 μEq/min (0.2)	94 (20)	86 (57)	78 (55)	54** (36)
Potassium Reabsorp.	42.4 percent (5.4)	110 (21)	103 (30)	105 (43)	107 (65)
Osmols in Urine	257.3 mOsmol/l (9.9)	99 (6)	98 (9)	96 (12)	97 (13)
Osmol Clear.	46.3 μl/min (14.4)	133 (63)	221 (247)	223 (269)	159 (167)
Free Water Clear.	11.7 μl/min (4.8)	129 (73)	299 (424)	310 (430)	177 (216)
Renal Resistance	609.4 kPa/l/min (25)	101 (17)	105 (9)	105 (13)	109 (19)

in the combination, even though ouabain alone induced diuresis (Table 2). The depression of GFR by gossypol was potentiated by ouabain (Table 1), whereas ouabain alone had no effect (Table 2). K excretion was 54.1 % of control, not seen by either drug alone, but urine K concentration was depressed at all drug levels tested. The increase in Na excretion at 0.3 μ*M* ouabain (Table 2) was not observed in the drug combination (Table 1), nor was osmolar clearance increased in the combination. However, reabsorptions of Na was decreased.

Ouabain-Vanadate in IPRK The effect of 0.05 μ*M* ouabain in combination with 0.3 μ*M* vanadate was examined in six IPRK studies. Vanadate caused a further reduction in urine K concentration from 68±12 to 58±12% of control, and Na reabsorption decreased slightly from 98±2 to 96±3%. No other responses to this level of ouabain were affected by 0.3 μ*M* vanadate.

Gossypol in Intact Rats Gossypol injected sc to intact rats caused a decrease in plasma K that was noted over the 24 to 72 h post-injection period (Table 3). Diuresis was evident at the 72 h period.

ATPase Activity Ouabain and gossypol were tested on rat kidney microsomal ATPase. Na,K-ATPase was inhibited at a half-maximal concentration of 2.4 μ*M* gossypol (Fig 1). Mg-ATPase was also inhibited half-maximally at 36 μ*M* gossypol. Ouabain elicited half maximal inhibition of Na,K-ATPase at 100 μ*M* (Fig 1), but 1000 μ*M* ouabain had no effect on Mg-ATPase activity.

Gossypol was also tested on a preparation of human kidney Na,K-ATPase in which Mg-ATPase had been removed by purification: inhibition was observed half-maximally at 1 μ*M* gossypol.

Na,K-ATPase activity was measured in kidneys from the three [^{3}H]ouabain IPRK and

Tab 2. Ouabain on isolated perfused rat kidney. Following a 30 min equilibration period, a 10 min control period was followed by 2–4 ten-minute periods of incremental increasing concentrations of ouabain: control n = 13; 0.05 μ*M* n = 6; all others n = 4. Responses are % of control. $\bar{x}$ ±SD. ** $p<0.05$

Observation	Control Values	μ*M* Ouabain 0.01	0.05	0.1	0.3	0.8	10	100
Urine Flow	33.1 μl/min (16.2)	114 (14)	185 (166)	256** (126)	361** (193)	432** (249)	273 (174)	464 (368)
GFR (Inulin Clear.)	415.6 μl/min (153.2)	95 (19)	97 (33)	109 (30)	128 (46)	106 (58)	115 (42)	105 (64)
Sodium in Urine	79.1 mEq/l (28.8)	99 (4)	94** (6)	141 (139)	173 (108)	199 (135)	103 (4)	109** (3)
Sodium Excreted	2.7 μEq/min (1.8)	112 (12)	167 (129)	338** (186)	550** (350)	714** (470)	283 (182)	520** (388)
Sodium Reabsorp.	94.4 percent (5.4)	99** (1)	98** (2)	95** (3)	90** (4)	84** (6)	92** (4)	81** (3)
Potassium in Urine	46.0 mEq/l (20.9)	83** (8)	68** (13)	59** (15)	39** (15)	30** (16)	59** (17)	29** (14)
Potassium Excreted	1.4 μEq/min (0.7)	95 (19)	115 (75)	136 (31)	130 (57)	123 (78)	139 (60)	108 (58)
Potassium Reabsorp.	38.4 percent (29.9)	99 (12)	87 (34)	47 (56)	69 (23)	83 (78)	101 (25)	265 (313)
Osmols in Urine	272.2 mOsmol/l (41.8)	98 (6)	92** (5)	94 (10)	95 (10)	96 (15)	94 (12)	94 (10)
Osmol Clear.	28.7 μl/min (14.1)	108 (9)	156 (111)	231** (86)	329** (146)	392** (191)	247 (142)	419 (305)
Free Water Clear.	4.9 μl/min (5.1)	138 (40)	324 (423)	641 (816)	846 (948)	1027 (1168)	335 (277)	512 (365)
Renal Resistance	619.9 kPa/l/min (82.6)	101 (4)	105 (7)	109** (7)	109 (8)	108 (7)	120** (10)	158** (40)

in 3 control IPRK. The residual ouabain level in the assay was about 1 n*M*. Control Na,K-ATPase was 5.7±2.2, whereas the ouabain perfused kidneys were not significantly different at 5.1±4.0 mol/kg h. The addition of 254 n*M* ouabain, the concentration in kidney tissue found in these [^{3}H]ouabain IPRK, caused inhibition to 78% of that in the absence of ouabain.

DISCUSSION

Gossypol suppressed GFR, urine K and fractional Na reabsorption in the IPRK. Ouabain, the potent inhibitor of Na,K-ATPase, caused diuresis, but no effect on GFR. Like gossypol, ouabain decreased urine K concentration and fractional Na reabsorption. Therefore, gossypol acting on the IPRK elicited responses that were different from that elicited by ouabain. Gossypol in combination with ouabain decreased Na reabsorption and the rate of K excretion to a greater degree than observed with either drug alone.

Ouabain was previously studied in the IPRK using 0.05 – 24 m*M* ouabain, much higher than the lower levels of the present study[5,8,9] At 860 μ*M* ouabain, Na transport was decreased 40%and glucose reabsorption was suppressed by 30%[8]. The half-maximal effect on Na and K excretion occurred at about 200 μ*M* ouabain[9]; much higher than the 0.13 μ*M* level in the present study. Another report of 12 to 24 m*M* ouabain in IPRK noted diuresis of 50% above control, a 38% reduction in GFR and a 47% decrease in reabsorbed filtrate[5].

Na,K-ATPase in homogenates of whole kidney taken from IPRK studies was inhibited to 78% by 254 n*M* ouabain. Since 0.13 μ*M* ouabain generally gave half-maximal effects in the present IPRK which was considerably below the 100 μ*M* ouabain level for half-maximal inhibition of rat kidney Na,K-ATPase, it is concluded that inhibition of Na,K-ATPase is only part of the mechanism of impaired Na reabsorption in IPRK elicited by ouabain. The half-maximal concentration of 100 μ*M* ouabain for inhibition of rat kidney Na,K-ATPase has also been noted previously[10].

Tab 3. Gossypol in Intact Rats. $\bar{x}\pm$SD. $^{**}p<0.05$ by paired *t* test compared to baseline –24 h.

Time after sc (h)	–24	24	72	96
Control: Plasma K (m*M*)	4.7 (0.4)	4.9 (0.2)	4.7 (0.4)	4.5 (0.2)
Control: Urine flow (ml/24 h)	12.1 (1.6)	10.4 (2.5)	10.9 (0.9)	11.3 (0.8)
Gossypol: Plasma K (m*M*)	4.5 (0.3)	4.2** (0.2)	4.0** (0.4)	4.1 (0.3)
Gossypol: Urine flow (ml/24 h)	11.2 (1.1)	12.2 (1.8)	17.5** (2.0)	13.0 (0.8)

Gossypol inhibited Na,K-ATPase uncompetitively and half-maximally at approximately 30 μ*M* gossypol[3,4]. The present study showed half-maximal inhibition at 2.4 μ*M* gossypol for Na,K-ATPase and 36 μ*M* gossypol for Mg-ATPase. Although gossypol was only studied at a maximum concentration of 0.6 μ*M* in the IPRK, the marked effect on urine K concentration, 52% of control suggests that part of the gossypol mechanism may be through Na,K-ATPase, but it is unlikely that all of the effect can be explained this way.

Vasoconstrictor properties of ouabain have been described in dogs and in humans[11], as well as for vanadate in the IPRK[6]. In the present study, ouabain increased resistance whereas gossypol did not. Either the pre- or postglomerular constriction may increase resistance to flow. Postglomerular vasoconstriction has been associated with an increase in GFR, as concluded from experiments with angiotensin II and noradrenaline in the IPRK[12,13], The postglomerular site may be a more likely

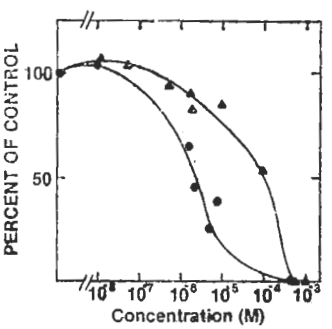

Fig 1. Effects of gossypol (●) and ouabain(▲) on Na,K-ATPase. Control Na,K-ATPase = 32 mol/kg h^{-1}.

mechanism of vanadate, because the GFR was unchanged with ouabain. The reduction in GFR induced by gossypol is not explained by preglomerular vasoconstriction, because resistance was not increased.

Gossypol may induce hypokalemia in humans[3]. In the present study on intact rats, the effect on plasma K may have involved renal mechanisms, but in the IPRK there was no diuresis and K concentration in urine was decreased. The major route of elimination of gossypol is the gut and it is concentrated in the liver[1]. Its effect on liver catechol-*O*-methyl transferase is affected by plasma proteins and perhaps derangements in catecholamine metabolism may contribute to *in vivo* as well as *in vitro* effects of the drug[14]. Chronic administration of gossypol to rats caused weight loss and decreased intracellular muscle K and Mg.[15] Therefore, the relative contribution of renal versus non-renal mechanisms in the electrolyte disturbances induced by gossypol in the intact rat needs further clarification.

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REFERENCES

1 Abou-Donia MB. *Residue Rev* 1976; 61:125
2 Saksena SK, Salmonsen RA. *Fertil Steril* 1982; 37:686
3 Qian SZ, Jing GW, Wu XY, Xu Y, Li YQ, Zhou ZH. *Chin Med J* 1980; 93:477
4 Bi XF, Zheng YJ, Yang HF, Zhang ZR. *Sci Sin* 1981; 24:573
5 Ross R, Leaf A, Silva P, Epstein FH. *Am J Physiol* 1974; 226:624
6 Kumar A, Corder CN. *J Pharmacol Exp Ther* 1980; 213:85
7 Braughler JM, Corder CN. *Biochim Biophys Acta* 1978; 524:249
8 Ruedas G, Weiss C. *Pfluegers Arch* 1976; 298: 12
9 Bowman RH, Dolgin J, Coulson R. *Am J Physiol* 1973; 224:1200
10 Allen JC, Schwartz A. *J Pharmacol Exp Ther* 1969; 168:42
11 Braunwald E, Bloodwell RD, Goldberg LI, Morrow AG. *J Clin Invest* 1961; 40:52
12 Davalos M, Frega NS, Saker B, Leaf A. *Am J Physiol* 1978; 35:F605
13 Corder CN, Pathak H, Kumar A, Wells A. *Indian J Physiol Pharmacol* 1984; 28:in press
14 Tang F, Tsang AYF, Lee CP, Wong PD. *Contraception* 1982; 26:515
15 Qian SZ, Xu Y, Chen ZC, *et al*. *Acta Pharm Sin* 1979; 14:513