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ENHANCEMENT OF RENAL MEDULLA PROSTAGLANDIN SYNTHETASE ACTIVITY BY DEXAMETHASONE TREATMENT IN THE RAT

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We studied in rats the effect of dexamethasone (2.5 mg/kg per week) on the conversion of radiolabeled arachidonic acid to prostaglandins by renal medulla slices, microsomes, and homogenates. The steroid did not affect the rate of conversion of arachidonic acid to prostaglandins by renal medulla slices, but significantly increased the rate of conversion by both the microsomes and the 10,000 x g supernatatant of renal medulla homogenates. We conclude (a) that dexamethasone treatment increases the activity of renal medulla prostaglandin synthetase measured in broken cells preparations, and (b) that such a change in enzyme activity is not manifested by augmentation of prostaglandin synthesis in renal medulla slices incubated with exogenous arachidonic acid.

Recent studies suggest influence of glucocorticoids on both the degradation and the synthesis of renal prostaglandins. For example, there are reports that dexamethasone reduces renal 15-hydroxyprostaglandin dehydrogenase (1,2), induces phospholipase A_2-inhibitory proteins leading to decrease of the arachidonic acid available for prostaglandin synthesis in cultured renomedullary cells (3), and lowers the activity of microsomal cyclooxygenase in the rat kidney (4). However, the reported action of dexamethasone to depress renal cyclooxygenase activity (4) is difficult to reconcile with our recent finding that chronic treatment with this glucocorticoid does not reduce the release of prostaglandins from rat renal medulla slices incubated with exogenous arachidonic acid in Krebs solution (2). One possibility to consider is that the reduction of renal medulla cyclooxygenase in dexamethasone-treated rats lacks expression during experimental conditions that maintain the integrity of the renal cells. Therefore, the present study was designed to contrast the effects of dexamethasone treatment on the rate of conversion by renal medulla slices and microsomes of exogenous arachidonic acid to prostaglandins.

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MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats weighing 270-330 g were injected subcutaneously with dexamethasone acetate (Decadron-LA, 2.5 mg/kg; Merck, Sharp & Dohme) or with vehicle only (0.15 M NaCl, 1.0 ml/kg) every seven days. On the 14th day after commencing treatment, the rats were anesthetized with ether, the kidneys were removed, and each inner medulla pair was excised and placed in ice-cold Krebs solution until further use. Three protocols were implemented.

Conversion of Arachidonic Acid to Prostaglandins by Renal Medulla Slices. Each inner medulla pair was segmented into slices and the pooled slices were placed into flasks containing 2 ml of Krebs solution with both [^{3}H] arachidonic acid (2 µCi; New England Nuclear; Specific activity, 86.6 Ci/mmol and unlabelled arachidonic (14 µM; Nu-Chek). After incubation for 30 min at 37°C with 100 cycle/min agitation under an atmosphere of 95% O_2-5% CO_2, the tissue slices were dried to constant weight, and the medium was acidified to pH 3-3.5 with 2 M citric acid and extracted with chloroform-methanol (2:1, vol/vol). The organic phase was evaporated, and the lipid residue was dissolved in chloroform-methanol (2:1, vol/vol), applied to silica gel G plates (Synbron-Brinkmann), and chromatographed concurrently with authentic arachidonic acid and prostaglandin standards (Upjohn Co.) using as a solvent system the organic phase of ethyl acetate-isooctane-acetic acid-water (11:5:2:10, vol/vol). The radioactivity in zones of the plate corresponding to the position of arachidonic acid and the various prostaglandin standards was determined by liquid scintillation counting.

Conversion of Arachidonic Acid to Prostaglandins by Renal Medulla Microsomes. Inner medulla pairs from each of five rats were pooled and homogenized (7.5 ml/g of tissue) in 0.1 M Tris-HCl buffer (pH 8.0; 4°C) using a Polytron homogenizer. The homogenate was centrifuged at 10,000 x g for 15 min and the resulting supernatant was centrifuged at 105,000 x g for 60 min; the pellet and the supernatant so obtained were designated as the microsomal and the cytosolic fractions, respectively. The microsomal fraction was suspended in 0.1 M Tris-HCl buffer (pH 8.0) to give a protein concentration of 1.2 mg/ml. Aliquots (0.25-0.50 ml) of this suspension were incubated for 5 min at 37°C with both [1-^{14}C] arachidonic acid (0.15 µCi; New England Nuclear; specific activity, 55 mCi/mmol) and unlabelled arachidonic acid (14 µM) in the presence of L-epinephrine (1 mM) and reduced glutathione (1 mM); the final volume of the mixture was adjusted to 1 ml with 0.1 M Tris-HCl buffer (pH 8.0). In some experiments, renal medulla microsomes were incubated with arachidonic acid as described above both in the absence and the presence of the cytosolic fraction (0.25-0.50 ml) from either control or dexamethasone-treated rats. The reaction was stopped by additon of twenty volumes of chloroform-methanol (2:1, vol/vol), the mixture was acidified (pH 3.0-3.5) with 2 M citric acid and extracted, the organic phase was evaporated, and the radioactive metabolites of arachidonic acid in the lipid residue were separated and quantitated as described above.

Conversion of Arachidonic Acid to Prostaglandins by the 10,000 x g Supernatant of Renal Medulla Homogenates. Each inner medulla pair was homogenized as described above and the homogenate was centrifuged at 10,000 x g for 15 min. An aliquot of the resulting supernatant containing 1.3 mg of protein was incubated for 5 min at 37°C with both [1-^{14}C]arachidonic acid (0.15 µCi) and unlabelled arachidonic acid (14 µM) in the presence of L-epinephrine (1 mM) and reduced glutathione (1 mM); the volume of the mixture was adjusted to 1 ml with 0.1 M Tris-HCl (pH 8.0). The reaction was terminated, and the radioactive metabolites of arachidonic acid were extracted, separated, and quantitated according to the procedures described above.

Protein Determination. Protein was determined by the method of Lowry et al. (5) with bovine serum albumin as standard.

Statistical Analyses. Results are expressed as mean ± standard error. The data were analyzed by unpaired Student's t-test.

RESULTS AND DISCUSSION

Incubation of arachidonic acid with renal medulla slices or microsomes resulted in metabolism of the fatty acid to products having the chromatographic mobility of PGE_2, $PGF_{2\alpha}$, and PGD_2 (Table 1 and 2). The rate of conversion of [^{3}H]arachidonic acid to prostaglandins by renal medulla slices from rats treated with dexamethasone for 14 days did not differ significantly from the rate of conversion by renal medulla slices from untreated control animals (Table 1). However, the rate of arachidonic acid metabolism to prostaglandins by renal medulla microsomes from dexamethasone-treated rats exceeded by 47% ($P<0.01$) the rate of arachidonic acid metabolism by renal medulla microsomes from control rats (Table 2), suggesting glucocorticoid-induced elevation of microsomal prostaglandin synthetase activity. The increased prostaglandin synthetase activity of renal medulla microsomes from animals receiving dexamethasone was manifested by augmented synthesis of $PGF_{2\alpha}$, PGE_2, and PGD_2, and was not accompanied by significant alterations in the renal medulla concentration of microsmal protein (Table 2).

That treatment with dexamethasone increased the metabolism of arachidonic acid to prostaglandins by renal medulla microsomes but not by renal medulla slices calls attention to the possibility that soluble cell constituents prevent the expression in intact cell systems of the steroid-induced augmentation of prostaglandin synthetase. In this regard there are reports that the soluble fraction of the renal medulla contains factors capable of inhibiting

Table 1

Conversion of [^{3}H]arachidonic acid to prostaglandins by renal medulla slices from control and from dexamethasone-treated rats

Treatment	Radioactivity (cpm/mg dry weight)		
	$PGF_{2\alpha}$	PGE_2	PGD_2
Control (N=6)	3978±486	5739±694	1845±206
Dexamethasone (N=6)	4480±527	6608±359	1830±126

Values are the mean ± SE; N = number of experiments. See text for details.

Table 2

Conversion of [1-^{14}C]arachidonic acid to prostaglandins by renal medulla microsomes from control and from dexamethasone-treated rats

Treatment	Prostaglandin (nmole/mg microsomal protein)				Microsomal Protein (mg/g wet medulla)
	$PGF_{2\alpha}$	PGE_2	PGD_2	Total	
Control (N=5)	2.14±0.17	0.98±0.06	0.35±0.01	3.47±0.22	4.78±0.43
Dexamethasone (N=5)	2.79±0.16	1.79±0.27	0.57±0.08	5.10±0.57	4.13±0.44
P	<0.05	<0.02	<0.05	<0.01	<0.30

Values are the mean ± SE; N = number of experiments; P indicates the level of significance. See text for details.

the activity of microsomal prostaglandin synthetase (6). Figure 1 depicts the effect of renal medulla cytosol from control and from dexamethasone-treated rats on the metabolism of arachidonic acid to prostaglandins by renal medulla microsomes. The conversion of arachidonic acid to prostaglandins by renal medulla microsomes from untreated or from dexamethasone-treated animals was

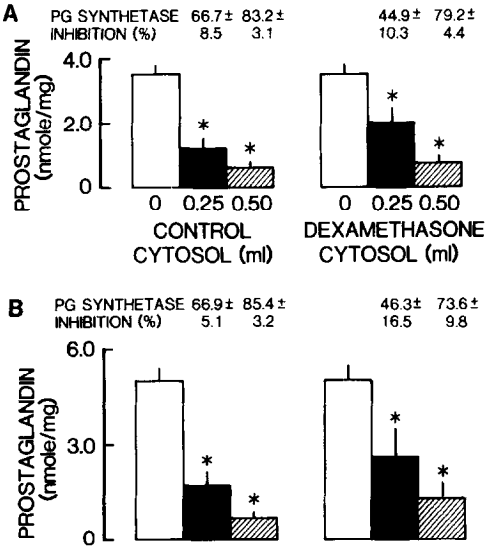

Figure 1. Effect of renal medulla cytosol fraction on microsomal prostaglandin synthetase activity. Renal medulla microsomes from control rats (A) or dexamethasone-treated rats (B) were incubated with [1-^{14}C] arachidonic acid and cofactors in the absence (open bars) and the presence of 0.25 ml (solid bars) or 0.50 ml (dashed bars) of renal medulla cytosolic fraction from control and from dexamethasone-treated rats. Results are expressed as means ± SE; the asterisk indicate P<0.05 relative to value without cytosol. See text for details.

Table 3
Conversion of [1-^{14}C]arachidonic acid to prostaglandins by renal medulla 10,000 x g supernatant from control and from dexamethasone-treated rats

Treatment	Prostaglandin (nmole/mg 10,000 x g supernatant protein)			
	$PGF_{2\alpha}$	PGE_2	PGD_2	Total
Control (N=5)	0.79±0.08	0.63±0.11	0.32±0.08	1.75±0.22
Dexamethasone (N=5)	1.75±0.30	0.95±0.07	0.53±0.05	2.92±0.21
P	<0.05	<0.05	<0.05	<0.01

Values are the mean ± SE; N = number of experiments; P indicates the level of significance. See text for details.

inhibited by adding to the reaction mixture the 100,000 x g supernatant of renal medulla homogenates. However, the degree of prostaglandin synthetase inhibition effected by the cytosolic fraction obtained from dexamethasone-treated rats did not differ significantly from that achieved by the cytosolic fraction from control rats (Figure 1). Moreover, a shown in table 3, the metabolism of arachidonic acid to prostaglandins by the 10,000 x g supernatant of renal medulla homogenates from dexamethasone-treated rats exceeded ($p<0.01$) the rate of metabolism by the supernatant of renal medulla homogenates from control animals. These data argue against the possibility that soluble constituents of renal medulla cells prevent the expression of increased microsomal prostaglandin synthetase activity on the conversion of radiolabeled arachidonic acid to prostaglandins by renal medulla slices from dexamethasone treated rats. However, it is possible that in the experiments with renal medulla slices the dexamethasone-induced augmentation of prostaglandin synthetase activity is offset by other attributes of the cellular enviroment that are not duplicated by the experiments with microsomes or homogenates.

The present observation that treatment with dexamethasone for 14 days increases the activity of prostaglandin synthetase measured in broken cell preparations of rat renal medulla contrasts with the report by Moore and Hoult (4) that treatment with glucocorticoids including dexamethasone reduces the prostaglandin synthetase activity of microsomes prepared from whole kidney

homogenates. These conflicting results are not strictly comparable as the respective experiments differed from each other with regard to the source of the microsomes and the assay procedures. In general agreement with our findings, however, Chandrabose et al. (7) reported that the prostaglandin synthetase activity of cultured fibroblasts is induced by dexamethasone.

In conclusion, this study demonstrates that chronic treatment with dexamethasone causes augmentation of prostaglandin synthetase activity in the renal medulla of rats. This action of the glucocorticoid may have a bearing on our previous finding of increased urinary excretion of PGE_2 and $PGF_{2\alpha}$ in rats treated with dexamethasone (2).

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REFERENCES

1. Blackwell, G.J., and Flower, R.J. (1975) Br. J. Pharmacol. 56, 343-344.
2. Nasjletti, A., Erman, A., Cagen, L.M., and Baer, P.G. (1984) Endocrinolgoy 114, 1033-1040.
3. Rothhut, B., Russo-Marie, F., Wood, J., DiRosa, M., and Flower, R.J. (1983) Biochem. Biophys. Res. Comm. 117, 878-884.
4. Moore, P.K., and Hoult, J.R.S. (1980) Nature 288, 269-270.
5. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.
6. Tai, H.H., Tai, C.L., and Hollander, C.S. (1976) Biochem. J. 154, 257-264.
7. Chandrabose, K.A., Lapetina, E.G., Schmitges, C.J., Siegel, M.I., and Cuatrecasas, P. (1978) Proc. Natl. Acad. Sci. USA 75, 214-217.