Vol. 95, No. 1, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 16, 1980 Pages 236-242

PROSTAGLANDIN METABOLISM IN THE RAT ADRENAL CORTEX: CHARACTERIZATION OF PROSTAGLANDIN-9-KETOREDUCTASE AND 15-HYDROXYPROSTAGLANDIN DEHYDROGENASE

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Received May 23,1980

SUMMARY: The cytosol fraction of rat adrenocortical tissue contains comparatively high levels of two prostaglandin metabolizing enzymes. The first, prostaglandin-9-ketoreductase, utilizes NADPH more effectively than NADH as cofactor, is inhibited by NADP, and exhibits an apparent K_m of 304 μM for PGE_1. 15-hydroxyprostaglandin dehydrogenase, tentatively identified as the type II NADP-dependent isozyme, is inhibited by NADPH but not NADH, and exhibits an apparent K_m of 157 μM when PGE_1 is used as substrate. Changes in specific activities of the two enzymes following ACTH, hypophysectomy, or dexamethasone treatment are inconclusive in defining a chronic regulatory role for adrenocorticotropin.

INTRODUCTION: Prostaglandins (PG's), particularly of the E type have been shown to stimulate adrenocortical steroidogenesis in several species in *in vitro* systems (1-5). Additionally, prostaglandins have been shown to augment both adrenocortical cAMP accumulation (1,4,6-8) and protein kinase activity ratios (7,8). Although the role of cAMP in the mediation of adrenocortical steroidogenesis has remained somewhat tentative, a recent series of elegant experiments by Dufau's group (8) is strongly supportive of an intermediate role for the nucleotide.

Quantitative studies of prostaglandin synthesis in the adrenal cortex have been reported on both rat and cat adrenocortical cell preparations (9,10). The PGE and PGF content of human adrenocortical tissue, as measured by bioassay, has also been reported (11). Whereas Ramwell and Shaw have reported that ACTH lowered PGE and PGF synthesis in superfused rat adrenals (12), Vahouny *et al* (10) have subsequently reported that acute ACTH challenge preferentially augments PGE_2 release from rat adrenocortical cells. The

PGE, prostaglandin E; PGF, prostaglandin F; cAMP, adenosine 3':5'-monophosphate; ACTH, adrenocorticotropin; PG9KR, prostaglandin-9-ketoreductase; 15HPDH, 15-hydroxyprostaglandin dehydrogenase.

0006-291X/80/130236-07$01.00/0

latter report is consistent with Laychock's findings with cat adrenocortical cell preparations as measured by both a radiolabeled arachidonate conversion technique and radioimmunoassay (9,13).

Given the capacity of PGE to stimulate adrenocortical steroidogenesis and the putative positive modulation of PG synthesis by ACTH in the rat, we found it to be of interest to undertake a study of PGE metabolism in the rat adrenal cortex. In this paper we report on the metabolism of PGE_1 to $PGF_{1\alpha}$ via prostaglandin-9-ketoreductase and PGE_1 to 15-keto-PGE_1 via 15-hydroxyprostaglandin dehydrogenase.

MATERIALS AND METHODS: Animals: Male Holtzman rats weighing 150-200 g were maintained on standard laboratory chow and tap water. The diet of hypophysectomized rats (Hormone Assay Laboratories, Chicago, IL) was supplemented with orange slices. Following sacrifice, the sella turcica was examined to ascertain the completeness of hypophysectomy. Rats treated with dexamethasone (20 mg/l drinking water) ingested approximately 500 µg of dexamethasone per day. Rats treated with ACTH (Acthar^R; Armour, Phoenix, AZ) received 10 U ip twice daily at 10:00 AM and 4:00 PM for 3 days (2 x 10 U x 3 days). Animals were sacrificed 18 hours following the last injection.

Tissue Preparation: Adrenocortical tissue was homogenized 1:15 (W/V) in 10 mM Tris-HCl/150 mM NaCl pH 7.3 using a Polytron^R (Brinkman) at speed 3 for 10 s. The homogenate was centrifuged at 10,000 x g for 20 min and the supernatant subsequently centrifuged at 100,000 x g for 60 min. The 100,000 x g supernatant fraction was used for all assays other than subcellular distribution studies. Protein was assayed by the method of Lowry *et al* (14) using recrystallized BSA (Sigma, St. Louis, MO) as a standard.

Enzyme Assays: Prostaglandin-9-ketoreductase was assayed in a reaction mixture containing 0.2 µCi[5,6-^{3}H(N)]-PGE_1 (74.8 Ci/mmole; New England Nuclear, Boston, MA), 25 µg PGE_1 (Dr. John Pike, The Upjohn Co., Kalamazoo, MI) 0.25 µmoles NADPH, 2 µg glucose-6-phosphate dehydrogenase, 50 µmoles glucose-6-phosphate, 14 µmoles Tris-HCl pH 7.3, 7.5 µmoles NaCl and 100-175 µg of cytosol protein in a final volume of 0.5 ml. For 15-hydroxyprostaglandin dehydrogenase, the reaction mixture contained 0.2 µCi [5,6-^{3}H(N)]-PGE_1, 25 µg PGE_1, 0.25 µmoles NADP, 14 µmoles Tris-HCl pH 7.3, 7.5 µmoles NaCl and 100-175 µg of cytosol protein in a final volume of 0.5 ml. Following incubation at 37 °C for 10-20 minutes, the reaction was terminated by adjusting the pH of the mixture to 3.0 with 1 N HCl. The samples were extracted, chromatographed, and product formation determined as described elsewhere (15).

Radioimmunoassay: Radioimmunoassay of PGE_2 equivalents was performed using antisera prepared in our laboratory (16).

Statistical Analyses: Regression lines were determined using the method of least squares and data were analyzed for statistical significance using Student's *t* test.

Table 1.

Subcellular Distribution of Prostaglandin-9-Ketoreductase and 15-Hydroxyprostaglandin Dehydrogenase Activities in the Rat Adrenal Cortex

Fraction	PG9KR (ng $PGF_{1\alpha}$/mg/min)	15HPDH (ng 15-keto-PGE_1/mg/min)
Whole Homogenate	252 ± 3	170 ± 35
10,000 x g Pellet	19 ± 9	3 ± 4
100,000 x g Pellet	47 ± 52	0
100,000 x g Supernatant	608 ± 16	585 ± 14

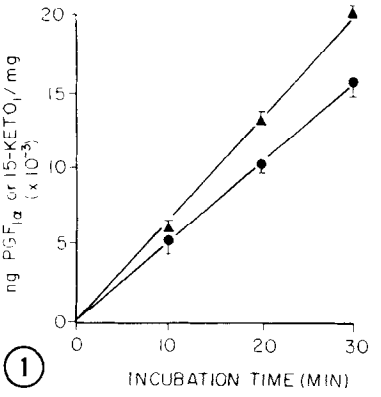
Enzyme assays were performed as described in "Methods"

RESULTS AND DISCUSSION: As shown in Table 1, rat adrenocortical PG9KR and 15HPDH are localized almost exclusively in the soluble fraction. Both enzymes have previously been reported to be localized in the cytosol fraction of other tissues (15, 17-20), although an NADH-dependent PG9KR has been reported in monkey liver microsomes (19). Both enzymes are linear with respect to time to 30 minutes (Figure 1) and with respect to protein over the range used (data not shown).

The endogenous concentration of PGE_2 equivalents in the soluble fraction of rat adrenocortical tissue was determined by radioimmunoassay to be 250 ± 22 pg/mg protein (x ± SE; n = 5). Since this value is negligible relative to the amount of PGE_1 added to the incubation mixture, endogenous PGE_2 was disregarded in our calculations.

PG9KR utilizes NADPH more effectively than NADH as cofactor (Figure 2), is optimum at 0.1 mM, and is inhibited by NADP (Figure 3). In comparison to our findings, the previously reported specific activity of rat adrenal PG9KR by Levine (22) was comparatively low, vis-à-vis other rat tissues because NADH was used as cofactor.

Rat adrenocortical cytosol assayed in the absence of exogenous cofactor displays predominantly 15HPDH activity and is not stimulated significantly by exogenous NAD or NADP (data not shown). 15HPDH is inhibited by NADPH but not NADH when assayed in the presence of 0.5 mM NADP (Figure 4). The specificity



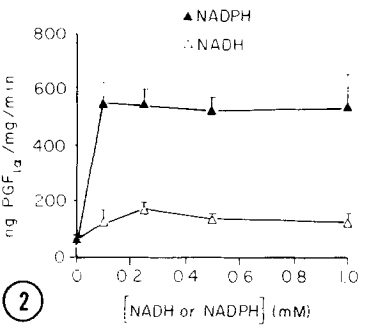
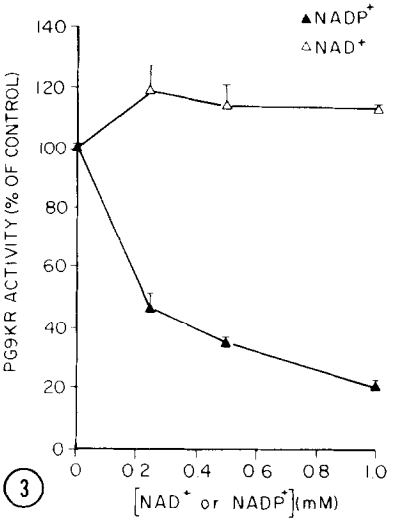


Figure 1. Prostaglandin E_1-9-ketoreductase (▲—▲) and 15-hydroxyprostaglandin E_1 dehydrogenase (●—●) time courses. Enzymes were assayed as described in "Methods". Bars indicate 1 SEM.

Figure 2. Reduced pyridine nucleotide dependence of rat adrenocortical prostaglandin E_1-9-ketoreductase. PG9KR was assayed as described in "Methods" using NADH (△—△) or NADPH (▲—▲).



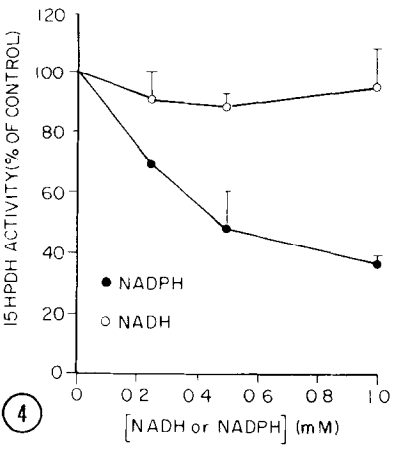


Figure 3. Inhibition of prostaglandin E_1-9-ketoreductase by oxidized pyridine nucleotides in the rat adrenal cortex. PG9KR was assayed in the absence of exogenous glucose-6-phosphate and glucose-6-phosphate dehydrogenase. The reaction mixture contained 0.5 mM NADPH and varying concentrations of NAD^+ (△—△) and $NADP^+$ (▲—▲).

Figure 4. The effect of reduced pyridine nucleotides on rat adrenocortical 15-hydroxyprostaglandin E_1 dehydrogenase. 15HPDH was assayed as described in "Methods" in the presence of varying concentrations of NADH (○—○) or NADPH (●—●).

of NADPH inhibition is qualitatively identical when 15HPDH is assayed in the absence of exogenous oxidized cofactor (data not shown). We tentatively conclude, therefore, that rat adrenocortical 15HPDH is predominantly the type II NADP-dependent isozyme. Type II 15HPDH has been reported to have a greater affinity for PGF than PGE in other tissues (23), however, PGF was not tested as substrate in this study.

Both rat adrenocortical PG9KR and 15HPDH exhibit Michaelis-Menten kinetics. Double reciprocal plots yield K_m values of 304 μM and 157 μM for PG9KR and 15HPDH, respectively (Figure 5). These values compare with 200 μM for monkey brain 15HPDH with PGE used as substrate (23) and 153 μM for rat testicular PGE-9-ketoreductase (18).

Several PG metabolizing enzymes including 15HPDH have been shown to exhibit ontogenic changes in several rat tissues (24). Additionally, 15HPDH has been shown to exhibit a relatively short half-life in both rat lung and kidney (25). We, therefore, decided to investigate the possibility that rat adrenocortical PG9KR and 15HPDH are subject to chronic regulatory control by ACTH. Rats were either hypophysectomized, dexamethasone treated, or treated with ACTH. The data presented in Table 2 are inconclusive. The changes in specific activities following hypophysectomy and ACTH treatment favor a chronic positive regulatory role for adrenocorticotropin; however, the failure of

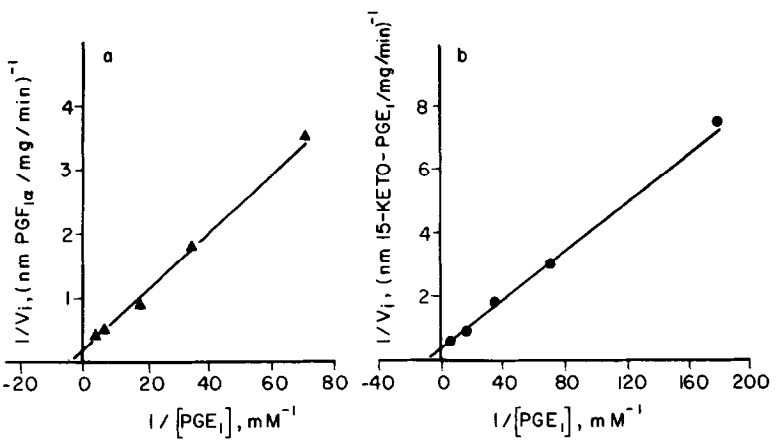


Figure 5. Lineweaver-Burk plots of: (a) rat adrenocortical prostaglandin E_1-9-ketoreductase and (b) 15-hydroxyprostaglandin E_1 dehydrogenase.

Table 2.

The Effect of Hypophysectomy, Dexamethasone, and ACTH Treatment on Rat Adrenocortical Prostaglandin-9-Ketoreductase and 15-Hydroxyprostaglandin Dehydrogenase Activities

Treatment	PG9KR (ng $PGF_{1\alpha}$/mg/min)	p	15HPDH (ng 15-keto-PGE_1/mg/min)	p
Control (12)	624 ± 11		564 ± 16	
Dexamethasone (5)	619 ± 14	n.s.	558 ± 94	n.s.
Hypophysectomy (6)	562 ± 28	<0.01	455 ± 33	<0.005
ACTH (5)	827 ± 69	<0.005	613 ± 47	n.s.

Animals were treated and enzyme assays performed as described in "Methods". Numbers in parentheses indicate the number of duplicate determinations performed on adrenocortical tissue from groups of 4-6 rats.

dexamethasone treatment to decrease the specific activity of either enzyme is puzzling. Dexamethasone, a corticosteroid analogue, has been shown to depress corticosterone levels (26) by inhibiting adrenocorticotropin release (27). Further studies needed to resolve the question of the chronic regulatory role of ACTH in PG metabolism are currently in progress.

The data reported herein suggest that the major mechanism available to regulate PGE metabolism in the rat adrenal cortex is the NADP/NADPH ratio. Previous reports that the prostaglandin precursor, arachidonate, is stored primarily as cholesterol arachidonate esters (6,10) make it tempting to suggest that PG synthesis and metabolism is coupled to NADPH-dependent corticosterone biosynthesis. This conclusion, however, will necessitate defining more precisely the role of PG's in corticosteroid biosynthesis.

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