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PROSTAGLANDIN E_2-9-KETOREDUCTASE OF RAT TESTIS

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SUMMARY: Prostaglandin E_2-9-ketoreductase, an enzyme which catalyzes the conversion of PGE_2 to $PGF_{2\alpha}$, occurs in both the cytoplasmic and microsomal fractions of rat testis. The cytosolic enzyme has a very high specific activity, 150-300 ng $PGF_{2\alpha}$ formed/mg protein/minute, about 20-40-fold higher than that reported for any other tissue. The apparent Km of this ketoreductase is 153 μM. Although either NADH or NADPH can function as cofactors, NADPH is a more effective coenzyme than NADH. Prostaglandin E_2-9-ketoreductase activity is inhibited by NAD^+, $NADP^+$, FAD and Cu^{++}. NADPH protects the inhibition of the enzyme by NAD^+, but not inhibition by $NADP^+$. The activity of the reductase is not affected by the addition of Mg^{++}, Ca^{++}, EDTA, EGTA, cyclic AMP or cyclic GMP. The specific activities of the cytosolic enzyme of the interstitial and seminiferous tubule compartments of the testis are approximately equal. This is the first report on the presence of this enzyme in testis.

INTRODUCTION: Although prostaglandins were originally discovered in secretions and tissues of male accessory glands of reproduction, little is known about the synthesis, turnover and function of prostaglandins in male reproduction. Polyenoic fatty acids of the n-6 family are abundant in testicular tissue (1). The principal prostaglandins of rat testis, PGE_2 and $PGF_{2\alpha}$, are formed from arachidonic acid (2,3). The synthesis of $PGF_{2\alpha}$ increases during the differentiation of this tissue (4). As PGKR has the potential to regulate the relative amounts of PGE_2 and $PGF_{2\alpha}$, the presence of this enzyme in testis was investigated. The cytosolic fractions of rat heart, kidney, liver brain and tissues from several other mammals, the pigeon, and the chicken (5-13) have been shown to have PGKR. The presence of this reductase in testis has not been previously reported.

PGKR: Prostaglandin E_2-9-ketoreductase
PGE_2: Prostaglandin E_2
$PGF_{2\alpha}$: Prostaglandin $F_{2\alpha}$
$PGF_{1\alpha}$: Prostaglandin $F_{1\alpha}$
RIA: Radioimmunoassay
$DKF_{2\alpha}$: 13,14-dihydro, 15-keto-prostaglandin $F_{2\alpha}$

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MATERIALS AND METHODS: [^{3}H]-$PGF_{1\alpha}$ and [^{3}H]-PGE_2 were purchased from New England Nuclear. [^{3}H] 13,14 dihydro-15-keto $F_{2\alpha}$ was prepared enzymatically using [^{3}H]-$PGF_{2\alpha}$ and guinea pig kidney cytosol. PGE_2 and $PGF_{2\alpha}$ were kindly provided by Dr. John Pike, and $PGF_{2\alpha}$ and $DKF_{2\alpha}$ antisera by Dr. Ken Kirton of the Upjohn Co., Kalamazoo, Michigan. NADPH and other chemicals were purchased from Sigma Chemical Co., Missouri.

Preparation of testis cytosol: Adult Holtzman-Sprague Dawley rats, 400-500 g, fed a Purina chow diet were sacrificed; the testis excised and the tunica albugenia removed. The tissue was homogenized in 3 volumes of 0.092 M phosphate buffer, pH 7.3, containing 0.1 mM dithiothreitol. After centrifugation at 4°C for 20 min at 10,000 x g the pellet was discarded, and the supernate centrifuged at 105,000 x g for 60 min. The supernate (cytosol) was used to assay PGKR activity. The pellet was used to prepare twice-washed microsomes. Protein concentrations were determined by the method of Lowry, et al. (14) using bovine serum albumin as a standard. Separation of the seminiferous tubules and the interstitium of the testis was performed according to the method of Christensen and Mason (15).

Measurement of enzyme activity: PGKR activity was assayed in a reaction system containing 2.27 mM (200 µg) PGE_2, 5 mM NADPH, 0.1 M Tris-HCl buffer, pH 7.4 and testis cytosol (0.2-0.5 mg protein) in a total volume of 250 µl. After incubation at 37°C for 30 min in a Dubnoff Metabolic Shaker, the reactions were stopped by boiling for 3 min. Following centrifugation at 2000 RPM, for 10 min, the amount of $PGF_{2\alpha}$ in the supernate was estimated by RIA after appropriate dilution. The specific activity is expressed in ng of $PGF_{2\alpha}$/mg of cytosolic protein/min. Boiled cytosol was added to blank incubations containing all other reagents. In some experiments, a NADPH generating system consisting of 0.1 M glucose-6-phosphate and 1 unit of glucose-6-phosphate dehydrogenase, was used.

Measurement of $PGF_{2\alpha}$ by radioimmunoassay: Aliquots of the incubation mixtures were diluted and mixed with antiserum in assay buffer (0.5 M sodium phosphate, 0.15 M NaCl, and 0.1% gelatine, at pH 8.0). [^{3}H]-$PGF_{1\alpha}$ or [^{3}H]-$DKF_{2\alpha}$, about 2000 cpm were added and the mixture incubated for 1 hr at 4°. After the addition of dextran-treated charcoal, the mixture was centrifuged (1000 x g, 10 min), the supernate decanted into scintillation vials containing 10 ml of cocktail (4% Omnifluor, and 15% Bio-Solv in toluene), and counted using a Packard 2450 counter Counting efficiency was 30-50%. Standard curves, 20-200 pg $PGF_{2\alpha}$, 50-300 pg $DKF_{2\alpha}$, were run with each assay. Sample values were calculated from the standard curves which were plotted on log probit paper. The standard error was ±1.5%. Since PGE_2 was used as substrate, the PGF values are reported as $PGF_{2\alpha}$. PGE_2 in the incubation mixtures did not cross-react with $PGF_{2\alpha}$ antiserum. RIA values of solvent extracted, silicic acid column partitioned, $PGF_{2\alpha}$ of the incubations did not differ significantly from those of aliquots of diluted incubation mixtures.

RESULTS AND DISCUSSION: Testicular cytosol or washed microsomes incubated in the presence of 5 mM NADPH and 200 µg of PGE_2, produced $PGF_{2\alpha}$. No activity was observed in the absence of PGE_2, tissue, or when subcellular fractions were heated (100°C for 3 min). As the activity in the cytoplasm was unusually high, the properties of the PGKR of this fraction have been studied.

Studies have been performed to determine the properties of this activity in the cytosol of rat testis. The enzymatic activity reached a plateau at a sub-

TABLE 1

EFFECT OF PYRIDINE NUCLEOTIDES ON

PGE_2-9-KETOREDUCTASE OF TESTES CYTOSOL

NADPH (5 mM)	INHIBITORS (5 mM)	SPECIFIC ACTIVITY (ng $PGF_{2\alpha}$/mg/min)	$PGF_{2\alpha}$ (ng/assay)	$DKF_{2\alpha}$ (ng/assay)
-	-	28	231	10
-	NAD^+	12	96	8
-	$NADP^+$	10	84	7
-	NADH	316	2565	209
+	-	424	3439	439
+	-	386	3132	ND*
+	NAD^+	317	2570	ND*
+	$NADP^+$	110	893	ND*

*ND: Not Determined
Incubations were carried out as described in "Methods" section.

strate concentration of between 1.1 and 4.5 mM (Fig. 1a), the apparent Km for PGKR is 153 μM. The Km for PGKR in other tissue is 15 μM-320 μM (8-12,17,18). The routine substrate concentration used by other workers has varied from 1 nM (19) to 1.2 mM (18), usually much smaller than the apparent Km. The enzyme in bovine thyroid gland may be regulated by substrate concentration (PGE_2) (12). Testicular PGKR exhibits normal Michaelis-Menten kinetics. Concentrations of PGE_2 up to 4.54 mM did not inhibit PGKR in the standard assay system. In the presence of a saturating concentration of substrate and 5 mM NADPH, the production of $PGF_{2\alpha}$ was linear for 1 hr (Fig. 1b), and increased in proportion to the concentration of cytosolic protein (Fig. 1c). Testicular PGKR has a broad pH optimum, between pH 7.0 - pH 8.5. Other PGKR's share this property (7,8).

The activity of PGKR is low in the absence of reduced pyridine nucleotide (Table 1). Addition of either NADPH or NADH stimulates the activity more than 10-fold (Fig. 1d). NADPH appears to be 30% more effective than NADH. Both cofactors reach optimal concentrations at 2 mM; neither cofactor is inhibitory in amounts up to 10 mM. The activity of the reductase is low in the absence of exogenous pyridine nucleotides; both NAD^+ and $NADP^+$ are effective inhibitors and reduce the activity about 60% (Table 1). The degree of inhibition with 5 mM

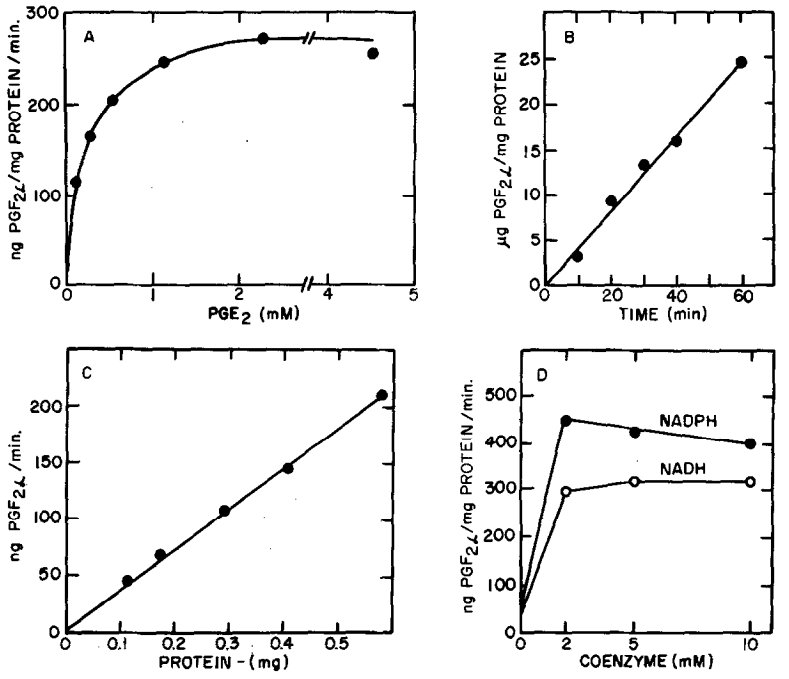

Fig. 1. Properties of testicular PGE_2 9-ketoreductase: Incubations were carried out as described in the "Methods" section.

$NADP^+$ in the presence of 5 mM NADPH, is 72%, while 5 mM NAD^+ inhibits only 18%. Since NADPH reverses the inhibitory effect of NAD^+ more effectively than that of $NADP^+$, the mechanism of inhibition appears to be different. NAD^+ may be functioning as a competitive inhibitor and $NADP^+$ as a non-competitive inhibitor.

Formation of $DKF_{2\alpha}$ by the testis (2) demonstrates that prostaglandin 13,14 reductase and prostaglandin 15-hydroxydehydrogenase activities occur in testis. In most tissues these enzymes are located in the cytosol, and either NAD^+ or $NADP^+$ serve as cofactors (19). Thus, $PGF_{2\alpha}$ newly formed from PGE_2 by PGKR could be metabolized to 13,14 dihydro $PGF_{2\alpha}$ and $DKF_{2\alpha}$. Addition of NAD^+ or $NADP^+$ might enhance the formation of these compounds causing an apparent decrease in $PGF_{2\alpha}$. Conversion of $PGF_{2\alpha}$ to $DKF_{2\alpha}$ was relatively constant, about 10% of the amount of $PGF_{2\alpha}$ formed (Table 1). The degradation of $PGF_{2\alpha}$ in the cytosol did not interfere with the estimation of $PGF_{2\alpha}$, and the inhibitory effects of NAD^+ and $NADP^+$

TABLE 2

EFFECT OF 17α HYDROXY PROGESTERONE ON PGE_2-9-KETOREDUCTASE

17α HYDROXY PROGESTERONE μg	SPECIFIC ACTIVITY ng $PGF_{2\alpha}$/mg/min
0	152
20	130
32	141
40	128
60	139

Incubation system: Testis cytosol (0.375 mg protein), 5 mM NADPH, 50 μg PGE_2, 0-60 μg 17α hydroxy progesterone, 0.1 M Tris-HCl buffer, pH 7.4, in a total volume of 250 μl. Incubations were carried out as described in "Methods" section.

appear not to be due to an enhanced degradation of $PGF_{2\alpha}$ to metabolites.

Apparently, there is no metal requirement for the reductase, for neither chelating agents, EGTA and EDTA, (250 μM), nor Ca^{++} (1 mM), and Mg^{++} (1 mM) additions had any effect on the activity. Cyclic AMP or cyclic GMP, 0.72 mM, had no effect on the enzyme. Pyrophosphate addition (10 mM), which enhances the activity in chicken heart muscle (7), stimulates the activity in rat testis. Inhibitors of prostaglandin cyclooxygenase, aspirin (50 μg) and indomethacin (10 μg), had essentially no effect on testicular PGKR. Testicular PGKR is completely inactivated by FAD (2 mM). The production of $PGF_{2\alpha}$ by sheep seminal vesicle is enhanced by Cu^{++} (21), but Cu^{++} (1 mM) inhibits the PGKR of rat testis.

The high Km for PGE_2 of the cytoplasmic testicular PGKR raised the question that the enzyme could be a non-specific steroid reductase. In testis the only steroid reductase that is not membrane-bound is 20α-hydroxy steroid dehydrogenase, an enzyme of the Leydig cells stimulated by EDTA (22,23). However, 17α-hydroxy progesterone, the substrate for the 20α-hydroxy steroid dehydrogenase did not inhibit PGKR (Table 2). Since testis PGKR is not affected by either EDTA or 17α-hydroxy progesterone, it appears to differ from the steroid reductase.

The activity of PGKR is located primarily in the cytoplasmic fraction; the specific activity in the particulate fraction is less than 2% of that in the

TABLE 3

DISTRIBUTION OF PGE_2-9-KETOREDUCTASE IN RAT TESTIS

ENZYME SOURCE	SPECIFIC ACTIVITY ng $PGF_{2\alpha}$/mg/min
WHOLE HOMOGENATE	118
10,000 X G SUPERNATE	330
CYTOSOL	410
MICROSOMES	6
WHOLE TISSUE CYTOSOL	339
TUBULE CYTOSOL	283
INTERSTITIUM CYTOSOL	230

Incubations were carried out as described in "Methods" section

cytosol (Table 3). Since the microsomal fraction was washed twice, contamination by cytoplasmic PGKR is unlikely. Activity of the reductase in testis microsomes responds to increasing amounts of substrate and to NADH, but is higher in the presence of NADPH. Microsomal NADH-dependent PGKR activity occurs in monkey liver microsomes, but not in a wide variety of other tissues (9). Microsomes of rat skin have PGKR activity which is also very low as compared to that of skin cytoplasm (20). Although microsomal PGKR has a low specific activity, the location in the same compartment as the prostaglandin-synthesizing complex suggests that it could play a role in the production of $PGF_{2\alpha}$ by testis.

Cytoplasmic fractions of both the interstitium and seminiferous tubules of testis have high specific activities (Table 3). Since interstitial protein constitutes only about 5% of the total testicular protein, the primary activity measured in total testicular cytosol represents the tubular component. The compartmentization of PGKR is of interest as the function of the interstitial compartment is primarily androgen synthesis, and that of the tubules gametogenesis. Both compartments synthesize prostaglandins (3). Prostaglandins have been shown to have a role in steroidogenesis as well as differentiation.

The presence of PGKR activity has not previously been reported in testicular tissue. The testicular enzyme has a much higher specific activity, 20-40-fold greater, than that reported for any other tissue (5-13). The activity of

PGKR may be related to the predominance of $PGF_{2\alpha}$ synthesis by rat testis (2). Further investigations to determine the potential role of PGKR in spermatogenesis and steroidogenesis are in progress.

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