Metabolism of Progesterone in the Kidneys of Male Rats

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Abstract

The metabolism of ^{14}C progesterone in the renal cortex and medulla of male wistar rats was investigated in vitro. The metabolites formed were 20a/βOHP, 3a/B 5aTHP, 5aDHP, 3a5 βTHP, 20a 3a 5a THP/20a 3β 5βTHP, and 20a 5a THP. NADPH was found to be the preferred cofactor in the incubations.

Keywords: progesterone, metabolites, metabolism, cortex, medulla

Introduction

Progesterone is secreted into the circulation in the greatest quantity among all of the steroid hormones produced in normal men and women. However, high progesterone secretion occurs only in women and only during the luteal phase of the ovarian cycle and pregnancy. Progesterone is known to be important for optimal endometrial development that precedes implantation of the blastocyst and for the maintenance of pregnancy.

Oelkers (1996) reported that the natriuretic effectiveness of progesterone has been known for 40 years although the very high mineralocorticoid receptor (MCR) affinity of progesterone as antagonist has only been demonstrated recently. The question as to why the very high extracellular progesterone concentrations in the luteal phase and during pregnancy do not completely block the MCR has not as yet been studied. It is assumed that progesterone is partially metabolized in the kidneys or near the MCR - containing cells (distal tubule/collecting tubule).

Various studies have been conducted on the metabolism of progesterone (P) in rat and human mammary tumors (Abul-Hajj, 1979), human parotid and submandibular glands (Blom et al. 1993), liver (Rao and Taylor, 1965) and placenta (Milewich, 1978). Very few studies have been done on P metabolism in the kidneys although Swart et al. (1993) investigated the metabolism of P by monkey kidney tumor cells and found that transfected cos 1 cells converted P to 17a OHP as well as 16a OHP. Casey et al. (1982) found that the deoxycorticosterone (DOC) which increases in the luteal phase and especially during pregnancy is also produced

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from progesterone in the kidney and not the liver. Lately, Oelkers (1996) studied P metabolism in human kidney sections and had detected considerable tritrated P metabolism in three metabolites. In this study, we examined the capability of the kidneys of male rats to metabolize progesterone in vitro. We wanted to find out if P can be reduced to probably inactive products and what are these metabolites formed.

Materials and Methods

Tissue Preparation

Progesterone metabolism was examined in the kidney cortex and medulla of male wistar rats. Fresh kidney tissues were pooled from four male rats. The kidneys were placed immediately in physiological saline solution after removal and cleaned of adhering fat and other tissues. One and a half grams of medullary tissue in 6.0 ml buffer and one and a half grams of cortical tissue in 6.0 ml. buffer containing 0.25 M sucrose were homogenized. All subsequent steps were performed at 0-4 degrees centigrade.

Incubations

Incubations were carried out for 120 minutes in a 20-well incubation plate placed in a steel chamber that was preheated to 37 degrees centigrade in a water bath under continuous shaking. The total incubation volume was 730 microliters for each well. The control consisted of 600ul buffer, 100ul NADH or NADPH, 10ul ^{14}C progesterone, 10ul glucose -6-phosphate, and 10ul G-6-P-dehydrogenase . Triplicates were prepared for medulla without cosubstrate, cortex without cosubstrate, medulla with NADH, cortex with NADH, medulla with NADPH, and cortex with NADPH. The triplicates contained 600ul homogenized tissue in buffer, NADH or NADPH, ^{14}C progesterone, glucose-6-phosphate and glucose-6-phosphate-dehydrogenase as regenerating system.

Incubations were terminated by rapid transfer of the incubation plate onto ice and transferring each well into vials containing 650ul of methylacetate.

Extraction of Steroid Metabolites

The methylacetate phase (upper phase) was removed after centrifugation for 10 minutes at 3,000 RPM at 4 degrees centigrade. The extraction with methyl acetate was done four times. The methyl acetate was then evaporated by putting the vials in the evaporating chamber. The lower phase buffer was checked for radioactivity using the liquid scintillation counter.

After evaporation, the upper phase was redissolved in 30ul of methanol and used for the two-dimensional thin layer chromatography (TLC).

Identification of Steroid Metabolites

A single and two-dimensional TLC was done for the nonradioactive standard steroids. The results were later used as reference for the identification of the radioactive metabolites.

A two-dimensional TLC was done for a representative sample in the triplicate of incubates. Nonradioactive steroid standards were put on the TLC plate and the radioactive sample was added to it. The TLC plates were then run two-dimensionally. The solvent systems were 1,2-Dichloroethane and methylacetate (65:35) for the first run and hexan:1-hexanol (75:25) for the second run.

The TLC plates were scanned for radiooactivity using the TLC analyzer. They were then sprayed with Liebermann-Burchards Reagents containing 2 ml. Essigsaure anhydrid, 8 ml. concentrated sulfuric acid, and 24 ml. ethanol. The spots were observed and marked under UV light. The TLC plates were photocopied using transparencies and the radioactive metabolites were identified by comparing the radioactive spots on the transparency with steroid standards on the plate as well as by measuring the actual distance of the radioactive region compared to the standard.

Determination of Radioactivity

The radioactivity of each metabolite was quantified by integrating the regions of radioactivity as scanned by the TLC analyzer. The radioactivity of the metabolites was summed up and converted to percentage.

The counting efficiency of the TLC analyzer was compared to the Liquid Scintillation Counter (LSC) and it was found that the TLC analyzer was counting 4.9% of the LSC count.

Results and Discussion

The results indicated active metabolism of progesterone in the kidneys of male rats. Table 1 shows the six metabolites formed: 20a/ß OHP, 3a/ß-5a THP, 5a DHP, 3a 5ßTHP, 20a 3ß 5ßTH / 20a 3a 5aTHP, and 20a 5aTHP. The metabolites 20a/ßOHP, 3a/ß5aTHP, and 5∝DHP were found in both cortex and medulla. The major metabolite in cortex and medulla without cosubsrate was 20a/ßOHP. Cortex and medulla with NADH and NADPH as cofactors had 3 a/ß5a THP as the major metabolite. More metabolites were formed when NADPH was used as cofactor in both the cortex and medulla.

The reduction of progesterone to 20a/ßOHP agrees with the findings of Mao et al. (1994) in rat ovaries where 20a hydroxysteroid dehydrogenase reduced P to 20a OHD Blom et al. (1993) reported the same finding in human parotid and submandibular glands. On the other hand, the preference for NADPH as cosubstrate confirms the findings of Laine and Harri (1990) in rat submandibular glands and Mao et al. (1994) in rat ovaries.

Table 1. Metabolites of ^{14}C Progesterone After Incubation with Renal Cortex and Medulla of Male Rats

METABOLITES	CORTEX			MEDULLA		
	Cosubstrate	NADH	NADPH	Cosubstrate	NADH	NADPH
Progesterone	1721.19	1185	615.91	2005.57	1143	188.54
20a/ß-OHP	119.91	68.41	133.04	203.7	145.18	346.05
3a/ß-5aTHP	76.31	247.4	925.36	99.16	415.74	552.5
5aDHP		230.9	349.94		282.35	194.55
a5ßTHP			121.25			
20∝3ß5ßTHP, 20∝3ß5ßTHP			157.29			189.99
20∝3∝5∝THP			90.43			151.5
Σ	1917.41	1732	2393.2	2308.43	1986.3	1623.1
added cpm	82000	82000	82000	82000	82000	82000
portion	1	1	1	1	1	1
recovery ROIs	2.34	2.11	2.92	2.82	2.42	1.98
relat. to TLC	47.72	43.11	59.56	57.45		40.40
Rc. (=4.9%)						

The metabolite 17∝0HP which was observed in human kidney sections (Oelkers, 1996) was not observed in this present study. This may indicate that the enzyme for the reduction of P to 17aOHP is not present in rat kidneys.

McDonald et al. (1991) reported that 40% of extrahepatic metabolism occurs by 5a, 3aß and 20a reduction. This appeared to be the pathway of metabolites formed in this present study. McDonald (1991) further reported that the metabolic transformations involved 5a reduction and reduction at C-20. The transfer constant of conversion of progesterone at 20a- DHP has been found to be 0.09. Plasma progesterone metabolism by 5a reduction in extrahepatic sites represents 80% of extrahepatic metabolism.

Table 2 shows that 89.8% of P in the kidney cortex and 86.9% of P in the kidney medulla both without cosubstrate remain unmetabolized. The result indicates that the enzymes necessary for the conversion of P to its metabolites require a cosubstrate. About 68.4% of

P in the cortex with NADH and 57.5% of P in the medulla remain unmetabolized. The highest degree of P metabolism occurred in the cortex and medulla with NAPH which used NADPH as cosubstrate. Only 25.7% and 11.6% of P in the cortex and medulla, respectively remained unmetabolized. NHDPH was observed to be the preferred cosubstrate in both the cortex and medulla. In vitro studies of Milewich et al. (1978) using human placenta and Nancarrow et al. (1984) also showed the preference for NADPH as cosubstrate.

The metabolites 20a/ßOHP and 3a/ß5aTHP were found in all incubations although 3a/ß 5aTHP was observed to be the metabolite with highest radioactivity. The findings of Milewich et. al. (1978), in human placenta Mao et. al. (1997) in rat ovaries, and Mao et. al. (1994) in rat corpus luteum support the present findings that progesterone can be metabolized to 20a -OHP in the kidneys. Blom et al. (1993) found that 20aOHP represented 50-60% of the P metabolites detected in parotid glands and about 70% in the submandibular glands.

The metabolite 5aDHP was observed to have lower percentage recovery of radioactivity compared to the 3a/ß 5∝THP and 20a/ß-OHP. This could be due to the further transformation of 5a - metabolite of P leading to low recovery of this metabolite. In the extrahepatic pathway of P metablism, 5a reduction of P is followed by 3aß reduction and this could explain the very high percentage radioactivity observed in 3a/ß 5∝THP.

Table 2. Percentage Radioactivity of Progesterone and Its Metabolites

METABOLITES	CORTEX			MEDULLA		
	Cosubstr. -	NADH	NADPH	Cosubstr.-	NADH	NADPH
Progesterone	89.8	68.4	25.7	86.9	57.5	11.6
20a/ß-OHP	6.3	3.9	5.6	8.8	7.3	21.3
3a/ß-5aTHP	4.0	14.3	38.7	4.3	20.9	34.0
5aDHP	0.0	13.3	14.6	0.0	14.2	12.0
3a5ßTHP	0.0	0.0	5.1	0.0	0.0	0.0
20a3ß5ßTHP,						
20a3a5∝THP	0.0	0.0	6.6	0.0	0.0	11.7
20a5aTHP	0.0	0.0	3.8	0.0	0.0	9.3
	100.0	100.0	100.0	100.0	100.0	100.0

The present results provide evidence that progesterone can be actively metabolized in the kidney cortex and medulla of rats in the presence of appropriate cosubstrate.

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