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SEX DIFFERENCES IN PROSTAGLANDIN METABOLISM

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SUMMARY:

Prostaglandin E2 (PGE2), PGF2alfa and PGD2 were synthetized from [1-^{14}C]-arachidonic acid by rat kidney medulla microsomal fraction. The formation of each prostaglandin was significantly less in female animals than in males. The rate of inactivation of [^{3}H]-PGF2alfa by kidney cortex cytosol was almost linear with the time of incubation during the first 30 min. The production of PGF2alfa metabolite (13,14-dihydro-15-keto PGF2alfa) was higher in male rats than in females.

INTRODUCTION:

Prostaglandin synthesis by rat testicular tissue was observed to be gonadotropin and adrenal dependent (1). Prostaglandin administration resulted in a diminished androgen synthesis and a reduced testicular weight (2,3). Testosterone release induced by PGF_{2alfa} is mediated through LH-RH and LH (4,5).

Prostaglandin E_2 was demonstrated to be more potent in stimulating LH and FSH release than prostaglandin analogs or PG endoperoxides (6). Prostaglandins may be involved in the feedback regulation of gonadotropin secretion (7). The primary effect of prostaglandins on gonadotropin secretion was proposed to be an indirect stimulatory one exerted at the hypothalamic supraoptic level (8).

Prostaglandins of the E type may provide an essential link in the coupling of the membrane-bound receptors for tropic hormones LH, FSH, ACTH to adenylate cyclase (9).

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These data prompted us to investigate the role of sexual steroids on the metabolism of prostaglandins in kidney.

MATERIALS AND METHODS:

Materials: arachidonic acid (grade I), [1-^{14}C] -arachidonic acid (55 mCi/mM), beta-NAD were purchased from Sigma Chemical Co., St. Louis, Mi. Glutathione reduced form (research grade) was product of Serva Feinbiochemica, Heidelberg. Norepinephrine was obtained from Rhone-Poulenc, France. [^{3}H]-PGF2alfa (120 Ci/mM) was donated by the Isotope Institute of the Hungarian Academy of Sciences. PGE2 and PGF2alfa were generously provided by Dr J.E. Pike of the Upjohn Co., Kalamazoo, Mich. All chemicals were analytical grade and obtained commercially unless otherwise stated.

Assay for prostaglandin synthetase: kidneys from Sprague-Dawley CFY (180-220 g) rats were removed rapidly to ice after the animals had been killed. The kidney cortex and medulla was separated. The medulla was homogenized with an Ultra-Thurrax in 2 vol. of 0.05 M Tris/HCl buffer (pH 8.05, containing 0.25 M sucrose) for 30 sec. The homogenate was centrifuged at 800 g for 10 min then the supernatant was centrifuged at 10 000 g for 30 min. The supernatant was further centrifuged at 105 000 g for 60 min in an MSE Superspeed 50. The microsomal pellet was homogenized with a Potter-Elvehjem homogenizer in 0.05 M Tris/HCl buffer (pH 8.05). This microsomal fraction was used for the study of prostaglandin synthetase. Prostaglandin synthetase was assayed with [^{14}C] -arachidonic acid (0.1 µM) as substrate in the presence of 2 mM glutathione and 1 mM norepinephrine as cofactors. Microsomal fraction (2-3 mg protein) was used for enzyme source in the assay system. Prostaglandin formation and extraction was done by the method of Tai et al. (10).

Assay for prostaglandin dehydrogenase: kidney cortex was homogenized with an Ultra-Thurrax in 2 vol. of phosphate buffer (K_2HPO_4 40 mM, KH_2PO_4 10 mM, 4 µM $MgCl_2$, pH 7.5). Supernatant fraction was prepared as above (105 000 g for 60 min) and used for prostaglandin dehydrogenase determination. The assay mixture contained phosphate buffer pH 7.5, [^{3}H]-PGF2alfa (0.5 µM) and NAD (0.5 µM)(11). The extracted prostaglandin and metabolite was spotted on silica-gel G t.l.c. plates. Chromatograms were developed in solvent system benzene:dioxan: acetic acid (20:10:1).

The radioactivity of each 5 mm band of chromatogram was determined in an LKB 81 000 liquid scintillation counter using 5 ml scintillant dioxan containing 10 % w/v naphthalane, 0.4 % PPO, 0.005% POPOP, 10 % v/v ethanol .

Authentic prostaglandin compounds were detected with anisaldehyde reagent (12).

Protein concentrations were determined by microbiuret method (13).

Statistical analysis was made by Student's *t* test.

RESULTS:

Rat kidney medulla microsomal fraction incubated with [^{14}C]-arachidonic acid in the presence of the cofactors norepinephrine

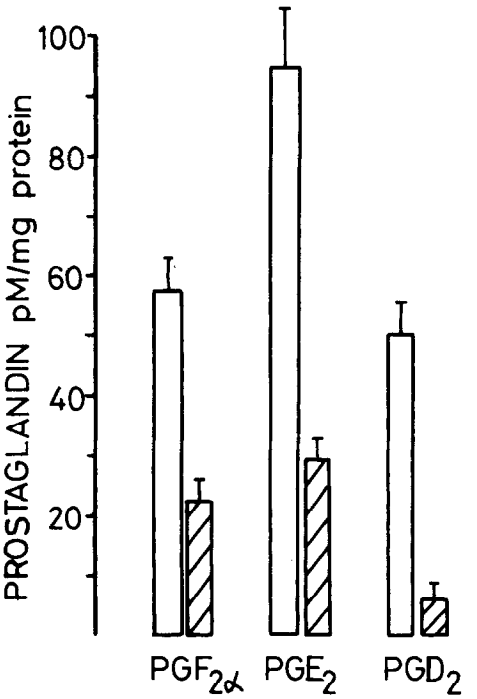

Fig. 1. Prostaglandin synthetase activity in the microsomal fraction of kidney medulla of male and female rats. The assay system contained [^{14}C]-arachidonic acid (0.1 μM), 2 mM glutathione, 1 mM norepinephrine and 2-3 mg microsomal protein. Incubations were performed at 37 °C for 20 min. □ male rats, ▨ female rats. Mean value ± S.D. of 8 animals in each group is presented.

and glutathione resulted in the production of PGE_2, PGF_{2alfa} and PGD_2. A minor fraction located next to arachidonic acid was probable hydroxy fatty acid, but it was not identified. The rate of formation of each of the prostaglandins was not precisely linear with time at any given period, but was nearly linear during the first 30 min of incubation. The incubation was therefore 20 min in each experiment. There was a great difference in the kidney of male and female animals. Each synthetized prostaglandin fraction was much higher in male rats. The PGD_2 was the smallest fraction both in male and female rats (Fig. 1).

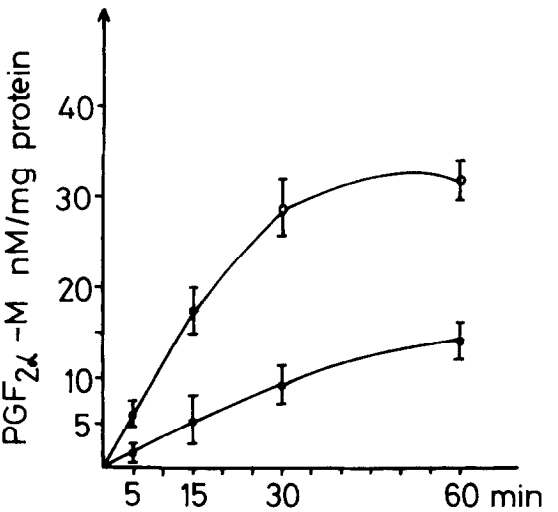

Fig. 2.. The activity of prostaglandin dehydrogenase in the kidney cortex 105 000 g supernatant of male and female rats. The incubation mixture contained [^{3}H]-PGF2alfa (30 000 cpm), NAD (0.5 µM), phosphate buffer (containing 4 µM $MgCl_2$, pH 7.5) and 105 000 g supernatant (1.5-2.5 mg protein). Incubations were on 37°C. Mean value ± S.D. of 5 rats is represented in each point. o——o = male rats; ●——● = female rats

The prostaglandin F_{2alfa} inactivation was determined in rat kidney cortex both in female and male rats. The rate of formation of main PGF_{2alfa} metabolite (14) was linear with the time during the first 30 min of incubation. The 13,14-dihydro-15-keto PGF_{2alfa} production was significantly higher in male animals than in females (Fig. 2).

DISCUSSION:

There is a sexual difference in the metabolism of prostaglandins. Both the synthesis and inactivation of prostaglandins is elevated in male rats compared to females. It is not clear yet what is the significance of the increased prostaglandin metabolism in male rats. The results show, that not only prostaglandins can affect testicular function (2, 3, 15), or LH and FSH release (4, 5, 6), but the sexual steroids might have a modifying

action on the metabolism of prostaglandins too. Further experiments are needed to elucidate the physiological significance of the observed phenomenon.

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