The Renal Effects of Prenatal Testosterone in Rats

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Abbreviations and Acronyms

BUN = blood urea nitrogen

Ctrl = control

Flu = flutamide

Tst = testosterone

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Purpose: Previous studies have shown that prenatal testosterone affects the development of not only reproductive organs but also the brain and even glucose metabolism. Whether prenatal testosterone influences the kidney development is largely unknown. We analyzed whether testosterone modulation during prenatal development would affect renal function and the number of nephrons in adult offspring.

Materials and Methods: Pregnant rats were treated with olive oil, testosterone (2 mg/kg), the androgen receptor blocker flutamide (5 mg/kg) or testosterone plus flutamide via daily intramuscular injections from gestation day 14 until delivery. Renal histology and functional parameters were assessed in male and female adult offspring. Macerated kidneys were used for nephron counting.

Results: Prenatal testosterone administration increased proteinuria in male rats by 256%. A similar 134% effect in female rats was not statistically significant. This effect was prevented when flutamide was co-administered. In male rats prenatal testosterone increased blood urea nitrogen. In female rats flutamide increased creatinine clearance. In male rats prenatal testosterone and flutamide led to higher and lower, respectively, interstitial collagen deposition in adulthood.

Conclusions: Prenatal testosterone induces proteinuria in adulthood. This effect is mediated via androgen receptor. Additional effects seem to be sex specific. Further studies should focus on the timing and dosing of testosterone as well as the applicability to human development.

Key Words: kidney, androgens, embryonic and fetal development, female, male

PRENATAL Tst affects various physiological and pathophysiological developmental processes. It is not surprising that Tst affects prenatal development of the reproductive system with long-term effects in adulthood.[1] The number of Sertoli cells, and sperm count and motility are affected by Tst during a sensitive period such as the last week of gestation in rats. Most research aimed at the effects of prenatal Tst is focused on the brain. For example, fetal brain exposure to increased concentrations of Tst during gestation is associated with an increased risk of autism.[2] Several experiments have been done on the metabolic effects of prenatal Tst, of which most focused on postnatal glucose and lipid metabolism.[3] To our

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knowledge similar studies of the renal effects of prenatal Tst are lacking.

It was hypothesized that prenatal insults result in fewer nephrons as well as cardiovascular and renal complications in adulthood.[4] This part of the hypothesis was confirmed, especially the fact that a lower number of nephrons is associated with a high risk of renal failure and essential hypertension.[5] However, the clinically relevant factors that disrupt nephrogenesis and cause a decreased number of nephrons are unknown. The Barker hypothesis suggests that poor nutrition in pregnant women might be responsible for later cardiovascular and metabolic diseases in the offspring.[6] These pathological conditions might also indirectly affect kidney structure and functions but it is also possible that the causation is the opposite and renal dysfunction leads to some of the mentioned systemic diseases.[7]

Interspecies differences exist in kidney development. In experimental rodents kidneys are not fully developed at birth. Neonatal pups can be seen as prematurely born humans, at least in regard to nephrogenesis.[8] The last week of gestation in rats represents approximately the first and second trimesters of human gestation. In a systematic study of prenatal dexamethasone administration the most vulnerable period was gestation days 15 to 18.[9] This treatment led to a 20% decrease in the number of nephrons and to higher systolic blood pressure in adulthood.

In most prenatal androgen exposure experiments the molecular mechanism was not studied. It was only suggested that effects on fat tissue are mediated by estrogen receptors since Tst can be converted to estradiol by aromatase.[3] In contrast, modulation of postnatal insulin signaling seems to be mediated by androgen receptor.[10] As a Tst metabolite, dihydrotestosterone is a strong activator of androgen receptor. An alternative pathway beyond classic androgen or estrogen receptors is activation of so-called nongenomic effects.[11,12]

We determined whether administering exogenous Tst or inhibiting endogenous androgen signaling via pharmacological androgen receptor blockade with Flu during prenatal development would affect renal function and the number of nephrons in adult rat offspring. In addition, we analyzed the molecular mechanism of the potential effects by co-administration of Tst and Flu.

MATERIALS AND METHODS

Animals

Ten male and 20 female Lewis rats at age 3 months were housed in a room with a controlled environment (humidity and air temperature) with a 12/12-hour light-dark cycle and free access to standard rodent chow and tap water. The experiment was approved by the ethical committee of the Institute of Molecular Biomedicine, Comenius University.

Experimental Design

Two virgin females were mated with 1 male per cage. Vaginal smears were performed daily at 7:00 a.m. The presence of sperm in the smears was considered day 0 of gestation. Pregnant rats were randomized into 4 groups of 5 per group, including Ctrl, Tst, Flu and Tst plus Flu groups. From gestation day 14 to delivery the pregnant rats were treated daily with hind limb intramuscular injection of Tst propionate (2 mg/kg body weight), Flu (5 mg/kg body weight) or a combination of Tst plus Flu (Sigma-Aldrich®) dissolved in olive oil. Pregnant Ctrl rats received olive oil in the same manner. The litter was normalized to decrease bias due to litter size variability. Blood from pregnant dams was collected from the tail vein daily from day 18 of gestation to delivery. Blood was always collected before applying the next injection.

At age 4 months adult offspring were placed in metabolic cages (Tecniplast®) for 24 hours to collect urine. Systolic blood pressure was measured using the tail cuff method. Rats were weighed and sacrificed under general anesthesia using ketamine and xylazine. Blood was collected from the abdominal aorta in heparinized tubes. After centrifugation plasma was stored at −20C until analysis. The 2 kidneys were removed, weighed, snap frozen in liquid nitrogen and stored at −80C until analysis.

Nephron Quantification

The total number of nephrons was determined using the previously described maceration technique[13] with slight modification. A preliminary experiment was done to investigate whether freezing in liquid nitrogen and subsequent storage at −80C would influence the quantified number of nephrons. No significant difference was found (data not shown). For maceration the kidneys were refrozen and incubated in 5 N HCl at 37C for 90 minutes. After washing with distilled water they were incubated in 50 ml distilled water at 4C overnight. The kidneys were gently macerated with a pestle in 9 ml distilled water until a homogenous suspension was achieved. The suspension (30 μl) was loaded on slides (7 fields per kidney and glomeruli were counted at 100× magnification. The total number of nephrons was calculated for the whole volume of the kidney macerate.

Analysis

Biochemical. Plasma creatinine and BUN were measured spectrophotometrically using commercially available kits (Arbor Assays®). Urinary creatinine was measured using the Jaffe method.[14] Proteinuria was measured using the pyrogallol red-molybdate method.[15] Tst was measured using a commercially available enzyme-linked immunoassay method (DRG, Marburg, Germany).

Histomorphological. Kidneys fixed in methyl Carnoy solution were embedded in paraffin and sectioned at 4 μm. After deparaffinization in xylol and rehydration in ethanol the sections were stained with hematoxylin

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