Journal of Steroid Biochemistry, 1977, Vol. 8, pp. 735–736. Pergamon Press. Printed in Great Britain

NEONATAL IMPRINTING OF SERUM TRANSCORTIN LEVELS IN THE RAT

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(*Received* 11 *November* 1976)

SUMMARY

The role of neonatal androgen in the sexual difference of rat serum transcortin levels was investigated. Male rats castrated at birth develop in adult life transcortin levels similar to those of female animals spayed at birth or to those of adult intact female animals. Animals of both sexes gonadectomized at birth and treated neonatally with a single injection of testosterone propionate show transcortin concentrations significantly lower than those of intact female animals.

INTRODUCTION

Gala and Westphal have extensively studied the hormonal regulation of rat corticosteroid-binding globulin (CBG) or transcortin [1]. From these studies it appears that the sexual difference in serum transcortin concentration can not be abolished by gonadectomy in adult life nor by testosterone treatment of adult rats. Since the sexual differentiation of many cellular steroid-metabolizing enzymes is predetermined by androgen in neonatal life [2–4] we investigated the effect of neonatal castration and neonatal treatment with testosterone on serum transcortin. These levels were measured in adult life by single radial immunodiffusion (Mancini technique).

MATERIAL AND METHODS

Rats of an inbred Wistar R strain were kept in standard conditions of temperature and humidity and received a constant pellet diet and tap water *ad libitum*. Neonatal gonadectomy was performed under hypothermia within 24 h after birth. Adult rats were castrated on day 58 under ether anesthesia. Testosterone propionate, usually 1 mg dissolved in 0.1 ml of olive oil, was administered subcutaneously. Control animals received the vehicle only. Blood was collected on repeated occasions by cutting a small piece of the tail.

Transcortin was determined by single radial immunodiffusion on 5 μl samples of rat serum. The method is similar to the one described for human transcortin [5]. The reference standard transcortin solution was pooled rat serum showing a cortisol-binding capacity of 32.3 μg/100 ml as measured by gel filtration at 4°C on charcoal treated serum [6]. Assuming one binding site and a molecular weight of 53,000 this capacity corresponds to a transcortin concentration of 47.3 mg/l. This value was used as basis for the calculation of the unknowns.

RESULTS

The evolution of serum transcortin levels as a function of age in normal rats and rats gonadectomized at the age of 58 days is shown in Fig. 1. A pronounced sexual difference is observed between normal male and female rats. After gonadectomy the transcortin levels in male rats markedly increase but never reach the levels of normal females. Gonadectomy of female rats results in a slight but significant increase of the serum transcortin levels. These results together corroborate those obtained by Gala and Westphal using multiple equilibrium dialysis as the method for

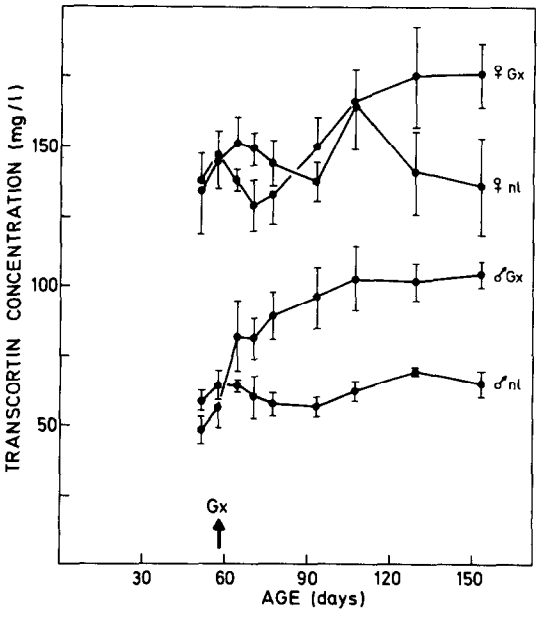

Fig. 1. Evolution of serum transcortin levels as a function of age in normal (nl) and gonadectomized (Gx) male and female rats. Each group consists of 4 animals and the amount of transcortin, expressed in mg/l (mean ± S.D.), was measured by single radial immunodiffusion on blood samples taken at the indicated age.

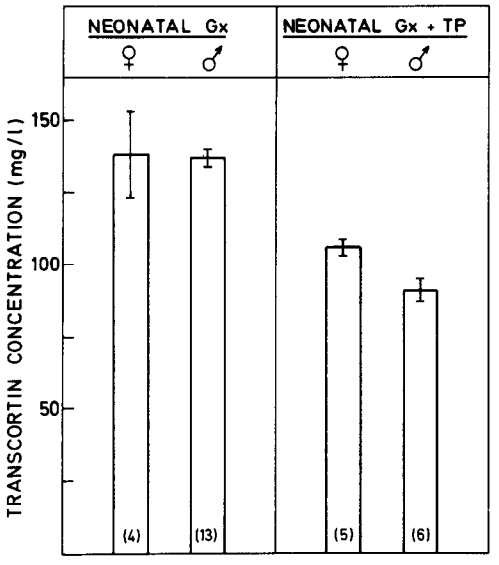

Fig. 2. Effect of neonatal gonadectomy (Gx) and neonatal administration of testosterone propionate (TP) on the serum transcortin level of adult rats. Blood was collected on day 62 and the amount of transcortin, measured by single radial immunodiffusion, was expressed in mg/l (mean ± S.E.M.). The number of animals in each group is given at the bottom of the bars.

measurement of transcortin activity [1]. Since the persistence of a sexual difference after gonadectomy in adulthood is often due to neonatal androgenisation the effect of gonadectomy at birth was investigated. As shown in Fig. 2 neonatal gonadectomy results in the abolition of the sexual difference as measured on day 62 of life. Indeeed both male and female rats now have identical serum transcortin levels which are not different from those of normal females of the same age (see Fig. 1). Further evidence for the intervention of neonatal androgens in the development of the sexual difference in adulthood was obtained after gonadectomy at birth followed by a single injection of testosterone propionate within 25 h after surgery. As shown in Fig. 2 such a treatment results in a marked decrease of the serum transcortin levels in both sexes as measured on day 62 of life. Neonatal gonadectomy followed by an injection of estradiol benzoate had no effect (not shown).

DISCUSSION

Our results clearly demonstrate that the sexual difference of the serum transcortin concentration in the rat is predetermined in neonatal life by the testis. Since a single injection of testosterone abolishes the effect of neonatal gonadectomy in the male, it is probable that this steroid is neonatally responsible for the sexual difference of the serum transcortin level in adulthood. In this respect transcortin bears strong resemblance with a score of cellular steroid-metabolizing enzymes [2–4]. By analogy with these enzymes it can be assumed that the perinatal differentiation of transcortin most probably takes place in the hypothalamo-hypophyseal system [7, 8].

This is the first example of a circulating plasma protein whose concentration is imprinted by androgen in neonatal life. Since transcortin can be studied serially on small amounts of blood and with a high degree of precision it is our opinion that this finding will facilitate and stimulate the further study of this form of sexual differentiation.

Acknowledgement—The authors appreciate the excellent technical assistance of Mrs. K. Vandecauter-De Ruddere.

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