J. Steroid Biochem. Molec. Biol. Vol. 39, No. 2, pp. 185–188, 1991
Printed in Great Britain.

0960-0760/91 $3.00 + 0.00

THE EFFECT OF GONADECTOMY AND AROMATASE INHIBITION ON THE EXCRETION OF 19-NORDEOXYCORTICOSTERONE IN RATS

ELISE P. GOMEZ-SANCHEZ* and CELSO E. GOMEZ-SANCHEZ
Research Service, James A. Haley V. A. Medical Center and the Department of Internal Medicine, University of South Florida, College of Medicine, Tampa, FL, 33612, U.S.A.

(Received 11 December 1990)

Summary—19-Nordeoxycorticosterone (19-norDOC) is a powerful mineralocorticoid, which has been postulated to be involved in the pathogenesis of some forms of hypertension. The urinary excretion of 19-norDOC by female rats is up to 20 times that of males. To demonstrate the influence of the gonads on the excretion of 19-norDOC, we measured the excretion of 19-norDOC in intact and gonadectomized male and female rats with and without replacement with testosterone (40 mg testosterone enanthate s.c.) or estrogen (4 mg estradiol valerate s.c.) and in intact animals receiving the aromatase inhibitor, 10-propargyl androstenedione (10-pA) (10 mg s.c.). Orchiectomy produced a significant increase in the urinary excretion of 19-norDOC in males. Testosterone treatment decreased 19-norDOC excretion by castrated males to below intact values, while estrogen administration increased its excretion. Oophorectomy had no consistent effect on 19-norDOC excretion. In oophorectomized females, testosterone administration significantly suppressed 19-norDOC excretion and estrogen replacement increased excretion slightly. 10-pA had little effect on the excretion of 19-norDOC in intact rats of either sex. In conclusion, it appears that 19-norDOC production is inhibited by testosterone, but is affected only slightly by estrogens.

INTRODUCTION

The steroid 19-nordeoxycorticosterone (19-norDOC) is a powerful mineralocorticoid which was originally identified in the urine of rats undergoing adrenal regeneration [1]. This steroid has been implicated in the pathogenesis of some forms of human and experimental hypertension [2–5], however this postulate is not universally accepted [6–9]. The mitochondrial cytochrome *P*-450-dependent 11β,18 hydroxylase in the adrenal gland is also responsible for the hydroxylation of deoxycorticosterone (DOC) at the 19 position to produce 19-hydroxydeoxycorticosterone (19-OH DOC) which is successively hydroxylated to 19-oxo, then 19-oicDOC [10, 11]. 19-NorDOC is thought to be formed by the decarboxylation of 19-oicDOC in the kidney [12] and is probably excreted soon after, as it does not circulate in appreciable amounts [5]. Estrogen synthesis also involves a related series of hydroxylations by a microsomal cytochrome *P*-450-dependent aromatase [13].

We studied the role of the gonads in the regulation of the excretion of 19-norDOC by measuring urinary 19-norDOC in intact and gonadectomized rats with and without replacement with testosterone or estrogen and in animals receiving an aromatase inhibitor, 10-propargyl androstenedione (10-pA) [13].

EXPERIMENTAL

Breeding stock for our colony of R/JR rats were kindly made available by Dr John Rapp from the Medical College of Ohio in Toledo, Ohio. Offspring are weaned at 4 weeks and maintained on an *ad libitum* diet containing 0.3% sodium chloride (Purina Formulab, No. 5008) and tap water. Gonadectomy was performed under halothane anesthesia using aseptic technique in male and female 14–17-week-old R/JR rats. 10–12 Days after surgery 40 mg testosterone enanthate or 4 mg estradiol valerate was injected s.c. 7 and 1 day before 24 h urine collections. The aromatase inhibitor, 10-pA, was injected s.c. at 10 mg/kg in a corn oil solution of 1 mg/0.1 ml (0.1 ml/100 gm body wt) 1 day before and every

*To whom correspondence should be addressed.

day of the urine collection. The other rats received equivalent volumes of oil s.c. every day that the 10-pA rats were injected if they were not scheduled to receive any other replacement. There were 6 or 7 rats in each group.

The rats were placed in the metabolic cages overnight to acclimatize them before collecting urine for 4 or 8 consecutive 24 h periods. Urine was collected in beakers containing 1 ml of a solution of 10 mg of sodium azide as preservative and 100 mg of ammonium bicarbonate to keep the pH around 7.1 to minimize bacterial or low pH-induced alterations of the excreted steroids. Cage floors were washed every morning after urine volume was measured and an aliquot frozen.

The radioimmunoassay for 19-norDOC has been described previously [14]. The results are expressed as the average ng/24 h/rat ± SD. The data was compared by ANOVA using the Dunnett, Fishers PLSD and Scheffé F-test for comparisons between means using a Statview 512 software program (Brainpower Inc, Calabazas, CA) for the Macintosh computer (Apple Computer Inc, Cupertino, CA).

RESULTS

Female rats excreted significantly greater quantities of 19-norDOC than male rats, as reported previously [7]. Orchiectomy consistently produced a significant increase in the excretion of 19-norDOC (Figs 1, 2 and 3). Administration of testosterone to castrated males resulted in a decrease of 19-norDOC excretion to below intact values (Fig. 1), while estrogen administration to castrated males increased 19-norDOC excretion slightly (Fig. 2).

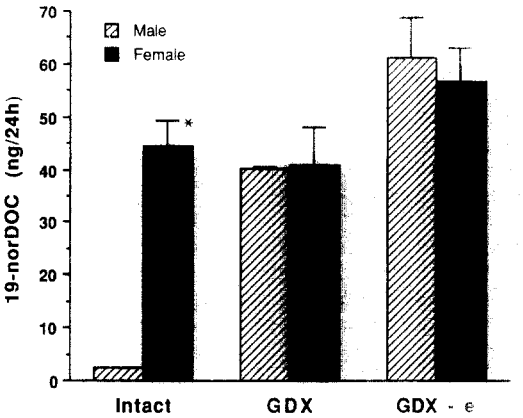

Fig. 2. The effect on urinary 19-norDOC excretion of gonadectomy and estrogen replacement at a rate of 4 mg estradiol valerate s.c. 7 and 1 days before urine collections. $n = 6$ or 7, $^*P < 0.01$.

Oophorectomy had no consistent effect on 19-norDOC excretion (Figs 1, 2 and 3). In 4 of 6 related experiments castration of the females had no effect on 19-norDOC excretion, in one it increased it, in the other there was an insignificant decrease. Testosterone treatment decreased the excretion of 19-norDOC in oophorectomized females to levels similar to those excreted by intact male rats (Fig. 1), while estrogen administration to oophorectomized females increased 19-norDOC excretion slightly compared to intact females (Fig. 2). 10-pA had no significant effect on the excretion of 19-norDOC in intact rats of either sex (Fig. 3).

DISCUSSION

Testosterone had a profound inhibitory effect on 19-norDOC excretion. Urinary 19-norDOC excretion increased to feminine levels with orchiectomy and decreased, in both castrated

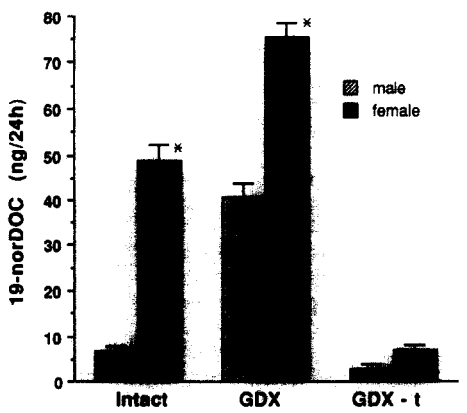

Fig. 1. The effect of urinary 19-norDOC excretion of gonadectomy and testosterone replacement at a rate of 40 mg testosterone enanthate s.c. 7 and 1 days before urine collections. $n = 6$ or 7, $^*P < 0.01$.

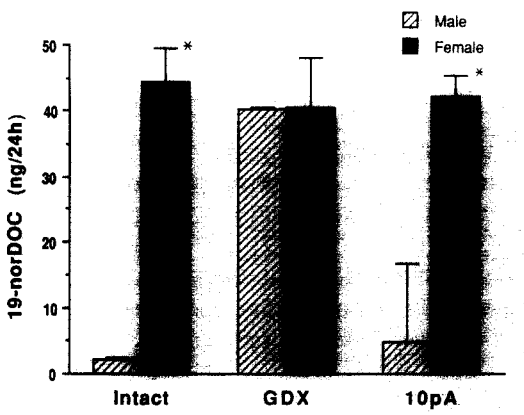

Fig. 3. The effect on urinary 19-norDOC excretion of gonadectomy or 10-pA at a rate of 10 mg s.c. 1 day before and each day of urine collection. $n = 8$, $^*P < 0.01$.

females and males, with the administration of testosterone. 19-NorDOC excretion was not affected by either oophorectomy or by 10-pA. 10-pA is an aromatase inhibitor which blocks estrogen synthesis by a mechanism based on the inhibition of the microsomal cytochrome *P*-450-dependent hydroxylation (aromatization) at the 19 position [13]. We have measured the daily excretion of 19-norDOC, aldosterone and corticosterone in intact male and female rats for 20 consecutive days. The excretion of 19-norDOC, which, as has been reported previously, was significantly higher in female than male rats, varied over 600% from day-to-day in some individual rats [14]. The variability in the excretion of 19-norDOC did not correlate with the excretion of aldosterone or corticosterone and did not appear to coincide with an estrous cycle. The difference in 19-norDOC excretion by gonadectomized females in Fig. 1 compared to Figs 2 and 3 is consistent with this huge day-to-day variation and would also explain disparate results from different labs. The lack of cyclical variations are also consistent with our findings that oophorectomy did not substantially alter 19-norDOC production. Our results are in contrast to those of Melby *et al.* [15, 16] who have reported that both 10-pA and a similar aromatase inhibitor suppressed 19-norDOC excretion, as well as the increase in blood pressure, in spontaneously hypertensive rats. The reasons for this discrepancy are as yet unclear.

Estrogens and androgens have multiple, interacting effects on the production, release and metabolism of corticosterone in rats [17]. In summary, the production of corticosterone and aldosterone has been found by others to correlate positively with levels of estrogen, and not to be affected much by testosterone. In contrast, we found that 19-norDOC levels correlate negatively with testosterone and are relatively independent of estrogen levels. Circulating corticosterone decreases in both sexes with gonadectomy, at least in part due to an increase in liver and adrenal 5α-reductase activity [17]. It may be speculated that the larger amount of metabolic machinery in the female zonas fasciculata and reticularis results in more 19-norDOC precursors and that by releasing the adrenal cortex from the inhibitory effect of testosterone, orchiectomy produces an increase excretion of 19-norDOC by the male. The finding that oophorectomy decreases and orchiectomy has no effect on corticosterone production, while orchiectomy increases and oophorectomy has no effect on 19-norDOC excretion demonstrates the complexity of the interactions between the pituitary–adrenal–gonadal axis and conversions from precursors in peripheral tissues such as the liver and kidney.

The excretion of 19-norDOC has been shown to be markedly greater in S/JR females in comparison to R/JR females consuming a high sodium diet and thus has been implicated in the development of hypertension in the S/JR rat [2]. While the course of the disease is greatly accelerated and exacerbated by a high sodium diet, the S/JR rat is spontaneously hypertensive on a normal diet, given adequate time. We measured the urinary excretion of 19-norDOC, 18-OH-DOC, and corticosterone in male and female S/JR and R/JR rats consuming a normal sodium diet. The excretion of corticosterone and 18-OH-DOC were significantly higher by S/JR of both sexes than by the R/JR, with the excretion by female rats being higher than by male rats within the same strain. However, the rates of excretion of 19-norDOC were: S/JR females > R/JR females > S/JR males > R/JR male rats. These studies indicate that, while S/JR rats of both sexes develop higher blood pressures than the R/JR even on a standard salt intake, the excretion of 19-norDOC does not correlate with their blood pressure elevation since the normotensive female R/JR rat excretes significantly higher quantities of 19-norDOC than the hypertensive male S/JR rat [8]. Thus, it is unclear if 19-norDOC is playing a significant role in the pathogenesis of the hypertension in the S/JR rat.

Acknowledgements—We are grateful to J. O. Johnston, Merrel Dow Research Institute, for the 10-pA and for the expert technical help of Mythili Venkataraman and Dwaine Thwaites. These studies were supported by Medical Research Funds from the Department of Veterans Affairs and by NIH Grants HL-27737 and HL-27255.

REFERENCES

1. Gomez-Sanchez C. E., Holland O. B., Murry B. A., Lloyd H. and Milewich L.: 19-Nor-deoxycorticosterone: a potent mineralocorticoid isolated from the urine of rats with regenerating adrenals. *Endocrinology* **105** (1979) 708–711.
2. Dale S. L., Holbrook M. M. and Melby J. C.: 19-Nordeoxycorticosterone excretion in rats bred for susceptibility and resistance to the hypertensive effects of salt. *Endocrinology* **117** (1985) 2424–2427.
3. Dale S. L., Holbrook M. M., Komanicky P. and Melby J. C.: Urinary 19-nordeoxycorticosterone in the spontaneously hypertensive rat. *Endocrinology* **110** (1982) 1989–1993.

4. Griffing G. T., Dale S. L., Holbrook M. M. and Melby J. C.: 19-Nordeoxycorticosterone excretion in healthy and hypertensive subjects. *Trans. Ass. Am. Physns* **94** (1981) 301–309.
5. Gomez-Sanchez C. E., Gomez-Sanchez E. P., Upcavage R. J. and Hall E. B.: Urinary free and serum 19-nor-deoxycorticosterone in adrenal regeneration hypertension. *Hypertension* **5** (1983) I-32–I-34.
6. Gomez-Sanchez C. E., Gomez-Sanchez E. P. and Holland O. B.: The role of 19-nor-pregnanes in human and experimental hypertension. In *The Adrenal Gland and Hypertension* (Edited by F. Mantero, E. G. Biglieri, J. W. Funder and B. A. Scoggins). *Serono Symp. Publ.* Raven Press, New York (1984).
7. Gomez-Sanchez E. P. and Gomez-Sanchez C. E.: 19-Nordeoxycorticosterone excretion in male and female Dahl's S/JR and R/JR rats. *Endocrinolgoy* **122** (1988) 1110–1113.
8. Gomez-Sanchez C. E., Bataillard A., Vincent M. and Sassard J.: Urinary mineralocorticoids in genetically hypertensive rats of the Lyon strain. *J. Hypert.* **5** (Suppl. 5) (1987) S227–S229.
9. Gomez-Sanchez C. E., Holland O. B. and Upcavage R.: Urinary free 19-nor-deoxycorticosterone and deoxycorticosterone in human hypertension. *J. Clin. Endocr. Metab.* **60** (1985) 234–238.
10. Ohta M., Fujii S., Wada A., Ohnishi T., Yamano T. and Okamoto M.: Production of 19-hydroxy-11-deoxycorticosterone and 19-oxo-deoxycorticosterone from 11-deoxycorticosterone by cytochrome *P*-450–11β. *J. Steroid Biochem.* **26** (1987) 73–81.
11. Watanuki M., Tilley B. E. and Hall P. F.: Cytochrome *P*-450 for 11β and 18-hydroxylase activities of bovine adrenocortical mitochondria: one enzyme or two. *Biochemistry* **17** (1978) 127–130.
12. Gomez-Sanchez C. E., Gomez-Sanchez E. P., Shackleton C. H. L. and Milewich L.: Identification of 19-hydroxy-deoxycorticosterone, 19-oxo-deoxycorticosterone and 19-oic-deoxycorticosterone as products of deoxycorticosterone metabolism by rat adrenals. *Endocrinology* **110** (1982) 384–389.
13. Johnston J. O., Wright C. L. and Metcalf B. W.: Biochemical and endocrine properties of a mechanism-based inhibitor of aromatase. *Endocrinology* **115** (1984) 776–785.
14. Gomez-Sanchez E. P. and Gomez-Sanchez C. E.: 19-Nordeoxycorticosterone, aldosterone and corticosterone excretion in sequential urine samples of male and female rats. *Steroids* **56** (1991). In press.
15. Melby J. C., Holbrook M., Griffing G. T. and Johnston J. O.: Antihypertensive effects of an aromatase inhibitor in the spontaneously hypertensive rat. *Hypertension* **10** (1987) 484–487.
16. Griffing G. T., Holbrook M., Melby J. C. and Brodie A. H.: Selective 19-hydroxylase inhibition by an aromatase inhibitor 4-hydroxyandrostenedione. *Clin. Physiol. Biochem.* **6** (1988) 171–178.
17. Kitay J. I.: Effects of estrogen and androgen on the adrenal cortex of the rat. In *Functions of the Adrenal Cortex Volume 2* (Edited by K. W. McKerns). Appleton-Century-Crofts, New York (1968) pp. 775–811.