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CORRECTION OF DEVELOPMENTAL DEFECTS IN ADRENAL STEROID METABOLIZING ENZYMES OF THE GENETICALLY MALE RAT PSEUDOHERMAPHRODITE BY PROLACTIN

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SUMMARY

The effects of exogenous prolactin alone and with testosterone propionate on the activity of adrenal enzymes metabolizing androstenedione and testosterone were determined in genetically androgen-insensitive pseudohermaphrodites and their littermate males and females at 60 days of age and in adult pseudohermaphrodites. A significant correction of the elevation of both steroid 5α-reductase and 3β-hydroxysteroid oxidoreductase was produced in rat pseudohermaphrodites by prolactin at all ages. This correction was enhanced by the addition of testosterone propionate only in the pubertal pseudohermaphrodites. These treatments also lowered both enzymatic activities in males and females at 60 days of age. The results indicate that the adrenal enzyme defects occurring in this genetic model of prepubertal castration, the pseudohermaphrodite, is corrected by prolactin. Moreover, it appears that prolactin may be involved in the pubertal differentiation of the normal adrenal.

INTRODUCTION

The development of adrenal androgen metabolizing enzymes in the genetically male androgen-insensitive rat pseudohermaphrodite (Stanley and Gumbreck) differs from that of its littermate primarily by a persistent postpubertal elevation of activity of steroid 5α-reductase and a failure of a postpubertal rise in steroid 17-ketoreductase [1]. The developmental pattern of the activity of adrenal steroid 5α-reductase in the pseudohermaphrodite is quite similar to that observed in prepubertally gonadectomized rats [2]. The elevation of the level of adrenal steroid 5α-reductase can be corrected by androgen replacement of orchidectomized adult male rats or by estrogen replacement of ovariectomized adult female rats [3]. These corrective actions of the gonadal steroids require the presence of the pituitary and the only pituitary hormone which lowers the adrenal 5α-reductase in castrated animals is prolactin [4]. Since some correction of the elevated levels of 5α-reductase in the pseudohermaphrodite is produced by large doses of androgen [1], it was determined in the present report whether prolactin can alter the levels of adrenal steroid 5α-reductase in the pseudohermaphrodite during pubertal development and in adulthood.

EXPERIMENTAL

Animals. Pseudohermaphrodites and their normal King X Holtzman littermate males and females (Introgen, Oklahoma City, Okla.) were housed in air conditioned quarters under controlled environmental conditions of light and dark (14 and 10 h, respectively) and temperature (25°C). Male, female and pseudohermaphrodites, 8 each, were injected, daily, with 400 μg NIH-PS-10 ovine prolactin in 20 μl saline from 30 to 59 days of age. Eight more male and pseudohermaphrodite rats received the same prolactin treatment, and in addition were injected for the same period with 2 mg. testosterone propionate (TP) in 30 μl dimethylsulfoxide (DMSO)/d. Control animals were daily injected with 20 μl saline and 30 μl DMSO from 30 to 59 days of age. At 60 days of age the rats were quickly decapitated, the adrenals were removed, cleaned, weighed, and incubated.

Five month old pseudohermaphrodites, 4 each, were injected for 8 days with either 600 μg prolactin/d in 150 μl saline, prolactin plus 3 mg. TP/d in 60 μl DMSO or 150 μl saline and 60 μl DMSO, and decapitated on day 9. The adrenals were quickly removed, weighed and incubated. Four male and 4 female rats receiving only the diluents served as controls.

Substrates. Substrates were [1,2-^{3}H]-androstenedione (48 Ci/mmol) and [1,2-^{3}H]-testosterone (51 Ci/mmol). The commercially prepared labeled steroids were obtained from New England Nuclear Corp., Boston, MA. Unlabeled steroids were obtained from Sigma Chemical Co., St. Louis, MO or Steraloids, Pawling, NY. The labeled steroids were found to be better than 98% radiochemically and chemically pure and the unlabeled compounds were found to have no contaminants by thin-layer and gas–liquid chromatography prior to use.

Tissue preparation. Adrenal homogenates were prepared using glass tissue grinders at a tissue concentration of 8 mg/ml^{-1} in a buffered medium. The medium consisted of 66 M potassium phosphate buffer, pH 7.4.

Enzymatic activity. The conversion of labeled androstenedione or testosterone as substrate into the tritiated products: 1. (A) 3β, 17β-dihydroxy-5α-androstane; 2. (B) 3β, 11β-dihydroxy-5α-androstan-17-one; 3. (C) 3α-17β-dihydroxy-5α-androstane; 4. (D) 11α-hydroxy-4-androstene-3,17-dione; 5. (E) testosterone; 6. (F) 11α-hydroxy-5α-androstane-3,17-dione; 7. (G) 3β-hydroxy-5α-androstan-17-one; 8. (H) 17β-hydroxy-5α-androstan-3-one; 9. (I) 3α-hydroxy-5α-androstan-17-one; 10. (J) 4-androstene-3,17-dione; and 11. (K) 5α-androstane-3,17-dione, was quantified by a slight modification of our previously published micromethod for determination of these conversions [5]. 150 μl of homogenate containing 200 ng (100,000 c.p.m.) substrate in 20 μl of DMSO, 60 nmol NADP, 250 nmol glucose-6-phosphate and 5 U glucose-6-phosphate dehydrogenase was taken up in a total vol. of 250 μl with phosphate buffer, pH 7.4. Incubation was for 20 min at 37°C. The reaction was terminated with 500 μl ethanol–acetone (1:1 v/v). The labeled products were separated by a partition system on t.l.c. using propylene glycol as stationary phase and carbon tetrachloride–toluene–cyclohexane (45:45:10 by vol.) as mobile phase. Label peaks were also quantitated and identified by radio gas–liquid chromatography using 3% SP2401, and by recrystallization of representative samples to contrast S.A. as previously described [5]. A product was not considered identified unless it had fulfilled the criterion of no contamination with any other metabolite by the above methods.

The conditions of incubation were chosen as optimum after kinetic studies had established saturating amounts of substrate and that the production of products was proportional to the amount of homogenate, as well as to the time of incubation up to 1 h.

Calculation of enzymatic activity. Activity of 5α-reductase was determined as the sum of all 5α-reduced product formed from each substrate. The 5α-reduced steroids formed in 60 min by 10 mg of adrenal homogenate from androstenedione included: A + G + I + K. Using the substrate testosterone the 5α-reduced products included: A + C + H + I + K. Activity of 3β-hydroxysteroid oxidoreductase (3β-HSOR) using the substrate androstenedione was calculated as the sum of the 3β-hydroxysteroids which included: A + G. The only 3β-hydroxy metabolite formed from testosterone was A. Activity of 17β-ketoreductase was the sum of all 17β-hydroxysteroids formed from androstenedione: A + E. 17β-Hydroxysteroid oxidoreductase (17β-HSOR) activity was calculated as the sum of all 17-ketosteroids formed from testosterone: D + I + J + K. The total products from incubates using androstenedione included: A + D + E + G + I + K, while steroids A + C + H + I + J + K were formed from testosterone. Steroids B + F were not detected with either substrate. Significance was calculated by Student's t test.

RESULTS

The levels of adrenal 5α-reductase and 3β-HSOR were unaffected by the choice of substrate, regardless of the treatment or sex of the animal. This suggests that these adrenal enzymes are unable to distinguish, *in vitro*, between androstenedione and testosterone (Table 1 and 2).

Control rats. During the pubertal period (60-day-old rats) adrenal 5α-reductase and 3β-HSOR were significantly higher in the pseudohermaphrodites than in either males or females, while 17-ketoreductase was highest in the male adrenals and there was no difference in 17β-HSOR activities (Table 1 and 2).

In the adult males and females (150 days of age) adrenal 5α-reductase and 3β-HSOR declined significantly from pubertal levels, while 17β-HSOR and 17-ketoreductase levels remained unchanged. In the

Table 1. Effects of prolactin and prolactin plus testosterone propionate (TP) on the activities of adrenal enzymes metabolizing androstenedione

	No. of rats	Treatment	Age (days)	Enzymes* 5αReductase	3β-HSOR† (ng/h/10 mg adrenal)	17-Ketoreductase
Male	8	Diluent	60	2328 ± 75‡	1813 ± 100	445 ± 133
	8	Prolactin	60	1665 ± 290***	1133 ± 628*	340 ± 103
	8	Prolactin + TP	60	478 ± 60***	128 ± 29***	388 ± 75
Pseudo	8	Diluent	60	4410 ± 305	3855 ± 200	225 ± 60
	8	Prolactin	60	3885 ± 355**	2855 ± 570***	255 ± 75
	8	Prolactin + TP	60	2990 ± 835*	1760 ± 923*	565 ± 70***
Female	8	Diluent	60	1933 ± 538	1053 ± 415	165 ± 30
	8	Prolactin	60	1053 ± 385**	583 ± 145**	180 ± 75
Male	4	Diluent	150	643 ± 88	219 ± 51	371 ± 112
Female	4	Diluent	150	853 ± 181	270 ± 71	297 ± 87
Pseudo	4	Diluent	150	4315 ± 530	1590 ± 145	115 ± 20
	4	Prolactin	150	2600 ± 990*	950 ± 350*	205 ± 25**
	4	Prolactin + TP	150	2520 ± 83	755 ± 400	150 ± 50

* See *Experimental* for procedures determining enzyme activities. † 3β-Hydroxysteroid oxidoreductase. ‡ Mean ± 1 S.D. § Statistical analysis compares groups: Diluent to Prolactin, and Prolactin to Prolactin + TP for each sex. * $p < 0.02$, ** $p < 0.01$, *** $p < 0.001$.

Table 2. Effects of prolactin and prolactin plus testosterone propionate (TP) on the activities of adrenal enzymes metabolizing testosterone

	No. of rats	Treatment	Age (days)	Enzymes* 5α-Reductase	3β-HSOR† (ng/h/10 mg adrenal)	17β-HSOR‡
Male	8	Diluent	60	2010 ± 95§	1470 ± 160	470 ± 105
	8	Prolactin	60	1475 ± 293***	690 ± 471***	755 ± 305*
	8	Prolactin + TP	60	215 ± 105***	53 ± 28***	655 ± 302
Pseudo	8	Diluent	60	4525 ± 170	3115 ± 325	595 ± 60
	8	Prolactin	60	3570 ± 625***	1965 ± 1010**	1190 ± 505**
	8	Prolactin + TP	60	1755 ± 860***	475 ± 200***	1220 ± 545
Female	8	Diluent	60	1990 ± 275	910 ± 315	548 ± 165
	8	Prolactin	60	1448 ± 120***	923 ± 305	420 ± 160
Male	4	Diluent	150	722 ± 365	325 ± 158	575 ± 209
Female	4	Diluent	150	1187 ± 580	301 ± 162	581 ± 187
Pseudo	4	Diluent	150	4155 ± 540	645 ± 150	1200 ± 40
	4	Prolactin	150	1970 ± 840**	435 ± 355	1210 ± 175
	4	Prolactin + TP	150	2250 ± 1330	520 ± 230	1180 ± 95

* See *Experimental* for procedures determining enzyme activities. † 3β-Hydroxysteroid oxidoreductase. ‡ 17β-Hydroxysteroid oxidoreductase. § Mean ± 1 S.D. Statistical analysis compares groups: Diluent to Prolactin, and Prolactin to Prolactin + TP for each sex. * $p < 0.02$, ** $p < 0.01$, *** $p < 0.001$.

adult pseudohermaphrodites, adrenal 5α-reductase remained elevated and 17-ketoreductase remained subnormal. Although 3β-HSOR activity in the pseudohermaphrodite declined from puberty through adulthood, the levels remained significantly higher than that found in adrenals of adult males or females. Adrenal 17β-HSOR activity doubled from puberty to adulthood in the pseudohermaphrodite.

Prolactin treatment. Prolactin administration through puberty significantly depressed adrenal 5α-reductase and 3β-HSOR levels in 60-day-old male, female, and pseudohermaphrodites. However, in males and females prolactin was unable to depress completely the elevated enzyme levels found during puberty to the much lower levels of the adult.

In adult pseudohermaphrodites prolactin reduced adrenal levels of both 5α-reductase and 3β-HSOR to levels found in control pubertal males and females, but still significantly higher than adrenal enzyme levels in adult males and females. Furthermore, prolactin administration increased 17-ketoreductase levels in adult pseudohermaphrodites to near control values.

Prolactin plus TP treatment. The addition of TP injections with prolactin had an enhancing effect by further reducing the levels of adrenal 5α-reductase and 3β-HSOR in pubertal males and pseudohermaphrodites below values produced by prolactin alone. In fact, the combined treatment of prolactin plus TP in the males reduced pubertal levels of adrenal 5α-reductase and 3β-HSOR to adult values. In the pubertal pseudohermaphrodite the enhancing effect of TP was seen in the further depression of adrenal 5α-reductase and 3β-HSOR to levels found in untreated, pubertal males and females. Interestingly, in the adult pseudohermaphrodite the added treatment with TP had no additional effect on adrenal enzymes than had prolactin alone.

DISCUSSION

The present report has shown that prolactin partially corrects the elevation in adrenal 5α-reductase and 3β-HSOR in the pseudohermaphrodite throughout puberty and in adulthood and reduces the elevated levels of these enzymes found during puberty in male and female rats. Kitay and coworkers have shown that after neonatal or prepubertal gonadectomy adult animals have a postpubertal elevation of adrenal 5α-reductase with either corticosterone, androstenedione, or testosterone as substrates [2]. This defect can be corrected by administration of gonadal hormones in the adult human [3]. The corrective actions of the gonadal steroids require the presence of the pituitary and the only pituitary hormone which can lower the adrenal 5α-reductase in castrated animals is prolactin [4].

Serum concentrations of prolactin in males, females and pseudohermaphrodites are not different [6], and the present report shows that the magnitude of change of adrenal 5α-reductase and 3β-HSOR in response to exogenous prolactin is similar in all 3 sexes. It is conceivable that levels of these enzymes in the pseudohermaphrodite would be even further elevated in the hypophysectomized animal, and that endogenous prolactin normally regulates adrenal 5α-reduction, 3β-HSOR, 17-ketoreductase, and 17β-HSOR activities. Although prolactin has been shown to regulate adrenal 5α-reductase and corticoid production in castrate rats of both sexes [4], identical responses to prolactin in both sexes such as, the control of mammary growth and lactation, is relatively uncommon in mammals [7, 8]. However, orchiectomy, as well as ovariectomy, decreases the circulating levels of prolactin [9] and serum levels of prolactin increase in both sexes during puberty [10] at a time of adrenal steroidogenic maturation [1].

The postpubertal elevation in activity of adrenal 5α-reductase and 3β-HSOR in the pseudohermaphrodite rat is probably due to the animal's genetic castration by virtue of its inherited defect of androgen-insensitivity [1] reflected at the cellular level by a deficiency of androgen receptor proteins [11]. Although the animal's target organs are generally unresponsive to physiologic doses of testosterone,

they do respond somewhat to pharmacologic doses [11], and indeed, the postpubertal elevation of 5α-reductase, as well as, the failure of a postpubertal rise in adrenal 17-ketoreductase are corrected to a great degree by large doses of testosterone or 5α-dihydrotestosterone [1]. Interestingly, in the pubertal pseudohermaphrodite TP administration enhanced the effects of prolactin by further reducing the levels of 5α-reductase and 3β-HSOR (though not as effectively as in the pubertal male) and elevating 17-ketoreductase activity. However, in the adult pseudohermaphrodite the pharmacological doses of TP in combination with prolactin had no additional effects on adrenal enzyme levels than had prolactin, alone. This suggests that the young pseudohermaphrodite is more responsive to TP than the adult and that the androgen insensitivity of the male rat pseudohermaphrodite may increase with age.

We have previously shown that female rats have a higher castration-induced level of 5α-reductase in the adrenals than that of the male and that this difference is programmed by neonatal testicular androgens as are hypothalamic functions and liver enzyme levels [12]. However, it has been shown that unlike its role in the regulation of adrenal steroid-metabolizing enzymes, prolactin does not affect this hepatic metabolism [13].

The present report indicates that the genetically male rat pseudohermaphrodite has the same biological mechanism (i.e. prolactin) controlling the maturational levels of adrenal 5α-reductase, 3β-HSOR and possibly 17-ketoreductase as does the pubertal rat.

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