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The Effect of Starvation, Diabetes or Hypophysectomy on the Testicular Function in Rat

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Serum levels of testosterone, *in vitro* production of testosterone and the activity of testicular Δ^5-3β-hydroxysteroid dehydrogenase (3β-HSD) were measured in normal, starved, streptozotocin diabetic and hypophysectomized rats seven days after initiation of the experiment. Similar impaired testicular functions were found in the starved and diabetic animals. The greatest alteration corresponded to the hypophysectomized group. The 3β-HSD activity correlates in all groups with hormone levels or hormone production *in vitro*. The impaired testicular function in the starved and diabetic groups is discussed.

It has been reported that the adrenal cortex, corpora lutea, placentae and interstitial cells of the testis contain an enzyme which would oxidize a 3β-hydroxy group in a steroid to a 3-ketone, while a wide range of other tissues did not show this activity (21). Early works demonstrated that 3β-HSD in the testis is primarily a constituent of the Leydig cells and not of the germinal epithelium, and that the activity of the enzyme decreases after hypophysectomy (22). Both LH and prolactin can influence the activity of 3β-HSD in hypophysectomized rats or in the genetically prolactin-deficient dwarf mice (2, 12, 25).

Impaired gonadal function in the male rat have been reported after starvation (7) or diabetes (13-15, 19). The present investigation was undertaken in order to

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elucidate whether food deprivation or diabetes may influence 3β-HSD activity in the testis of the rat and whether the activity of the enzyme can be related to serum levels of T in these animals when compared with control or hypophysectomized rats.

Materials and Methods

Sprague-Dawley rats, 3 months old, from our own colony, were housed individually in metabolic cages in an air conditioned, temperature controlled (24 ± 2° C) room. Light was provided daily from 700-1,900 h. Five days later, animals were randomly divided into 4 groups (n = 5 animals per group) and sham-operated (groups I-III) or hypophysectomized (group IV), and either injected once only with streptozotocin (group III) (40 mg/kg body weight i.p.) or vehicle (1 ml 0.05 M citrate buffer, pH = 4.5) (groups I, II and IV). All groups received tap water and a standard pelleted diet (Pan-Lab) *ad libitum*, except group II, which received no food for seven days. The criteria used to assess the severity of the diabetic state included the daily measurement of glucosuria (Reactive Strips, Boehringer-Mannheim), polydipsia and polyuria. The rate and direction of body weight and serum glucose over the experimental period were also followed.

Seven days following hypophysectomy, streptozotocin administration or food deprivation animals were killed by decapitation and trunk blood was collected for steroid assays. The pituitary fossa of each hypophysectomized rat was examined under magnification and found to be free of pituitary tissue.

The testes were rapidly removed, weighed and decapsulated and used either for *in vitro* testosterone production (8), or after homogenization, in tris-sucrose buffer, pH 7.4, assayed for 3β-HSD by a microassay procedure in which 0.1 ml homogenate is added to 1.9 ml Hank's media containing 1 μCi of [^{3}H] pregnenolone (The Radiochemical Center) (20). Serum testosterone and *in vitro* testosterone production were assayed in triplicate by RIA using a commercial kit (The Radiochemical Center). Quadriplicate determinations were carried in 3β-HSD assays (12, 20), and activity was calculated as μmol pregnenolone depleted in 2 h at 33° C/20 mg wet tissue or total testis. Total 3β-HSD was calculated as μmol/testes × 100 g body weight (12). Results were compared by analysis of variance (ANOVA) and the least significant difference (LSD) method.

Results

The present study indicates that pituitary ablation resulted in lower body weight than those of controls, starved and diabetic rats (table I). The reduced weight of the gonads and sex accessory glands in hypophysectomized animals are well-known effects after hypophysectomy, and the moderated polyuria and polydipsia in these animals are consequent with the absense of the neurohypophysis (23).

Food deprivation, as well as diabetes resulted in moderate body weight loss and a significantly reduced weight of sexual accessory glands when compared with controls ($p < 0.05$). Polydipsia, polyuria and serum glucose levels of streptozotocin injected animals (group III) were consistent with the diabetic state.

Serum levels of testosterone and total testosterone production *in vitro* was significantly lower in hypophysectomized animals if compared with controls, starved or diabetic rats (table II). After seven days starvation or diabetes (groups II and III), both serum and *in vitro* production of testosterone were significantly lower than in control animals ($p < 0.05$). The activity of 3β-HSD follows a similar pattern whether expressed per 20 mg/tissue,

Table I. *Body Weight, Organ Weights, Water Intake, Urine Output and Serum Glucose in Rats Starved for 7 Days, Diabetics 7 Days After Streptozotocin Administration or Hypophysectomized Animals 7 Days After Ablation of the Pituitary.*

Experimental groups	Body weight (% initial)	Testis weight (g)	G.S.I.[b]	Prostate (mg)	Seminal vesicles (mg)	Epydidymus (mg)	Water intake (ml/day)	Urine output (ml/day)	Serum[c] glucose (mM)
I. Controls[a]	102	1.86±0.16[d]	0.48±0.10	348± 9	516±60	564±35	35±12	2.5± 0.5	5.2±0.3
II. Starved	96	1.67±0.19	0.65±0.08	250±11 *	370±46 *	566±60	47± 8	2.0± 0.9	2.9±0.2 ††
III. Diabetics	97	1.52±0.22	0.60±0.11	185±30 *	307±27 *	486±25 *	321±12 **	176.0±15 **	24.6±0.9 **
IV. Hypophy-sectomized	82	0.35±0.11 †	0.14±0.02 †	105±11 †	77±12 †	154±54 †	87± 5 †	48.0± 8 †	5.9±0.6

a Control, starved and diabetic rats were sham operated at the beginning of the experiment.
b Testis weight (g)/body weight × 100.
c The day of sacrifice.
d $\bar{x}$ ± S.E.M. (n = 5 animals per group).
† $p < 0.05$ compared to groups I, II and III.
†† $p < 0.05$ compared to groups I, III and IV.
* $p < 0.05$ compared to groups I and IV.
** $p < 0.05$ compared to groups I, II and IV.

Table II. *Serum Testosterone, Testosterone Production* in vitro *and Testicular 3β-HSD Activity In Rats Starved for 7 Days, Diabetics 7 Days After Streptozotocin Administration of Hypophysectomized Animals 7 Days After Ablation of the Pituitary.*

Experimental groups	Serum T (ng/ml)	T production in vitro		3β-HSD activity		Total activity [b]
		(ng/testis/h)	(ng/g testis/h)	(μmol preg/ 20 mg tissue)	(μmol preg/ testis)	
I. Controls [a]	6.34±0.45 [c]	12.67±0.70	6.90±0.40	8.74±1.01	807±149	206±13
II. Starved	2.01±0.30 *	8.01±0.66 *	4.40±0.50 *	4.02±0.35 *	300±35 *	140±12 *
III. Diabetics	2.14±0.28 *	7.77±0.76 *	4.81±0.38 *	3.22±0.63 *	259±30 *	150±18 *
IV. Hypophysectomized	0.78±0.07 †	2.84±0.52 †	5.10±0.60 *	1.76±0.24 †	23± 4 †	14± 2 †

[a] Control, starved and diabetic rats were sham operated at the beginning of the experiment.
[b] Total activity: μmol PREG/testes/body weight × 100.
[c] $\bar{x}$ ± S.E.M. (n = 5 animals per group).
† $p < 0.05$ compared to groups I, II and III.
* $p < 0.05$ compared to groups I and IV.

total testis or total activity (100 g body weight).

Discussion

Reduced food intake has been reported to result in decreased secretion of anterior pituitary hormones (7, 15, 18), accompanied by a reduction in weight and function of target organs. The term «pseudohypophysectomy» sometimes has been applied to this state (17). Diabetes, as well, is known to produce marked impairment of reproductive function in the male (10, 11) frequently accompanied by testicular lesions in the diabetic patients and experimental animals (24). It has been assumed that these changes are due in part to impaired pituitary gonadotropin secretion (13, 19). Our results for serum levels of T in starved and diabetic animals agree well with the activity of the regulatory enzyme 3β-HSD (table II) and with the fact that LH appears to act on numerous sites in the testis (5), and is considered responsible also for the maintenance of many of its steroidogenic enzymes. The enzyme complex concerned with the cleavage of the cholesterol-side-chain (16, 26, 29), as well as 3β-HSD can be restored to physiological levels by LH in hypophysectomized animals (16, 21, 25). Impaired serum levels of T and *in vitro* T production by the testis of streptozotocin diabetic rats have been reported (19) and correlated with low levels of reduced NADPH generating enzymes in Leydig cells of diabetic rats (6), since androgen biosynthesis requires reductive energy in the form of NADPH (3). The activity of these enzymes is diminished in a similar way in the diabetic state and under starvation (4, 27) and insulin or refeeding restores them to even supranormal levels in the liver (1,28). The diminished supply of NADPH in interstitial tissue is not the unique factor in the control of steroidogenesis in the gonads (6, 26, 29) and comparative results presented in this paper agree with the fact that in the absence of pituitary hormones (group IV) lower activity of 3β-HSD and serum T or T production can be found if compared with diabetic and starved animals with low NADPH supply (6) but appreciable, if lower than control, levels of gonadotropins (13). Furthermore, results from this laboratory have demonstrated that refeeding restores serum T levels as well as 3β-HSD activity (9) in the same way

as has been reported for anterior pituitary hormones (18), but testosterone production is not restored in diabetic animals treated with insulin alone (6,19).

Our results show that impaired gonadal function during starvation or diabetes may be due to low activity of testicular 3β-HSD, and that serum and *in vitro* production of testosterone correlates with 3β-HSD activity in the testis of normal, starved, diabetic or hypophysectomized rats.

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Resumen

Se miden los niveles séricos de testosterona (T), producción de testosterona *in vitro*, y la actividad testicular del enzima 3-β-hidroxiesteroide deshidrogenasa, en ratas normales, sometidas a ayuno, diabéticas por estreptozotocin o hipofisectomizadas, siete días antes de iniciado el experimento.

Se encuentran alteraciones semejantes en los animales diabéticos y en los sometidos a ayuno, correspondiendo como era de esperar las mayores alteraciones de la función testicular al grupo de los hipofisectomizados.

En todos los grupos experimentales, los niveles de testosterona y la producción de testosterona *in vitro* son correlacionables con la actividad en testículo del enzima 3-β-HSD. Se discute la alteración de la función testicular, en los grupos de animales diabéticos y sometidos a ayuno.

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