Pituitary FSH Increase in Male Protein-Deficient Rats Induced by Testosterone Propionate[1] (36721)

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Feeding of protein-free diets, continued for several weeks, causes severe gonadal atrophy and virtually eliminates follicle-stimulating hormone (FSH) from anterior pituitary glands of young male rats (2, 3). Short term injections of testosterone propionate (TP) elevate FSH levels in glands of normal female rats whose pretreatment stores also are very low, whereas they do not affect the already high pituitary FSH concentrations of normal males (4). In this study, the progressive reduction in pituitary FSH potency associated with protein deprivation has been followed over a period of time, and attempts have been made to reverse this depletion through administration of small amounts of TP.

Materials and Methods. Groups of male Long-Evans rats, average age 40 days at onset of experiment, were fed *ad libitum* either a synthetic protein-free diet [3] or an isocaloric high-protein control diet for 10, 21, or 31 days after which times they were killed with chloroform vapor. An onset control group consisted of well-nourished rats sacrificed at 40 days of age. Additional groups of protein-deficient (PD) and diet control (DC) animals received 10 daily subcutaneous injections of 60 or 400 μg of TP[4] beginning on or about day 21 of experiment: such animals were sacrificed 24 hr after the last injection. Testes and ventral prostate of all animals were weighed at the conclusion of experiment. Anterior pituitary glands were removed and either fixed in sublimate–formol and prepared for light microscopic examination (3) or weighed and kept at —20° until bioassayed for FSH by the ovarian weight-augmentation method (6). Pooled anterior lobes of donor rats were tested at two or more dose levels, and parallel assays were done with a standard FSH preparation.[5] Total amounts of test material or saline included 20 IU of HCG and were administered in six equal parts over a 3-day period. Test animals were killed on day 4 and both ovaries were removed and weighed to the nearest 0.1 mg. Each group of recipients consisted of three or more rats, and replicate assays were performed at all critical dosages. All bioassay data were subjected to the multiphasic statistical program of Sakiz and Guillemin (7) and are included only if the parallelism and indices of precision were within acceptable limits. FSH potencies of the different donor pi-

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[3] The protein-free diet was identical in all respects to the control diet (see below), except that casein was replaced isocalorically by sucrose, crystalline vitamins and choline chloride were doubled, and 0.05 mg of Vitamin B_2 was added/kg of diet.

The control diet consisted of alcohol-extracted casein 24%, sucrose 64%, hydrogenated cottonseed oil (Primex) 8%, modified salts No. 4 (5) 4%. Crystalline vitamins per kg of diet were *d*-biotin, 0.3 mg; 2-methyl-1,4-napthoquinone, 5 mg; thiamine HCl, 5 mg; pyridoxine, HCl 5 mg; pteroylglutamic acid, 5.5 mg; riboflavin, 10 mg; *p*-aminobenzoic acid, 10 mg; niacin, 20 mg; *d*-calcium pantothenate 50 mg; inositol, 400 mg; choline chloride, 1000 mg.

All rats received weekly a fat-soluable vitamin supplement containing 800 USP units of Vitamin A, 115 chick units of Vitamin D, 6 mg synthetic alpha-tocopherol, and 650 mg of corn oil.

[4] Testosterone proprionate, obtained from Matheson, Coleman and Bell.

[5] NIH-FSH-S8, a gift from the Endocrinology Study Section, NIH.

tuitary glands (μg of NIH-FSH-S8 equiv/mg of anterior pituitary tissue) were derived from the test results: they are presented in Table I as percentage of onset control values (100%).

Results. Body weight of animals placed on the protein-free diet declined rapidly, and after three weeks of deficiency one-third of the initial weight was lost. Testis weight became reduced only in the last 10 days of the experiment, but regression of the ventral prostate followed a rectilinear, logarithmic course. Pituitary FSH concentrations were normal for the first 10 days of the experiment (73 μg/mg of wet gland); they diminished thereafter to barely detectable levels.

Daily doses of 60 μg of TP stimulated ventral prostate growth and produced pituitary FSH concentrations which were greater ($p < .05$) than those of PD rats injected with vehicle only. The higher dose further increased ventral prostate weight and caused significant elevation of FSH concentration vis-à-vis residual hormone in glands of oil-injected controls ($p < .01$). When expressed in terms of preinjection values, FSH potency exceeded that of rats maintained on the protein-free regimen for 3 wk; however, the difference was not significant statistically for the number of assay rats in this group was small. The same high dose of TP had no effect on pituitary FSH concentrations of DC rats; although the prostate was considerably heavier ($p < .05$) in steroid-treated than in oil-injected animals (N.B., the same mean difference in organ weight, 128 g, was found in PD rats), and testis weight was lowered ($p < .01$), presumably reflecting inhibition of LH release (8). The administration of TP, which has no appreciable effect on the microscopic appearance of anterior pituitary glands from well-nourished rats (9) did not visibly improve the disarrayed pituitary morphology of PD animals (3 and unpublished observations).

Discussion. Daily doses of 60 μg of TP prevented the decline in pituitary FSH concentration which was observed in sesame oil-injected, PD rats; and daily injections of 400 μg of TP caused a measurable increase of FSH concentrations in PD animals, so that, at the conclusion of the experiment, their pituitaries contained more FSH than was present before injections were begun. Thus, a rise in pituitary FSH potency of intact male rats was clearly revealed after only 10 days of fairly mild TP treatment, presumably because preinjection levels of FSH had been drastically lowered by feeding of a protein-free diet. In gonadectomized rats, but not in normal males, short term injections of androgen also bring about FSH accrual in the pituitary (8–11); and, as FSH release from the gland continues apparently unchanged for several days (9, 11), the gain may well be due to net increase in FSH synthesis. If in the PD rats of this study FSH release, but not synthesis, was similarly unaffected by TP treatment then one may speculate that such androgen-induced FSH synthesis is dose-dependent: any number of different interpretations is possible, of course, if TP had an effect on FSH release rates. Starvation, which causes many endocrine anomalies resembling those of protein deprivation, significantly reduced hypothalamic FSH-releasing factor (FSH-RF) content as well as pituitary FSH potency of male rats (12). It is not unlikely, therefore, that the ability of TP to partially ameliorate the FSH depletion caused by protein deprivation reflects some similar alteration at the neural level of gonadotropin control. However, this question must remain unresolved until a more sensitive and reliable assay for FSH-RF becomes available. At the very least, however, it is plain that protein deprivation does not eliminate the feedback action of gonadal steroids—or the capacity of the pituitary to synthesize FSH—just as the dietary deficiency does not limit accessory organ sensitivity to low doses of androgen (13).

The results of this study also provide some insight into the time course of FSH disappearance from rat anterior pituitary glands during 1 month of protein deprivation. For the first 10 days of experiment hypophyseal FSH concentration probably did not vary from control levels. Accordingly, FSH stores did not begin to diminish appreciably until some time after this initial period; though when the animals had been on diet for 3 wk the

TABLE I. Effects of Testosterone Propionate (TP) on Organ Weights and Pituitary FSH Concentrations of Male Protein-Deficient Rats.[a]

Group	Treatment (day × 10 days)	Rats (no.)	Age at autopsy (days)	Wt change (g)	Testis (mg)	Ventral prostate (mg)	Ant. pit. (mg)	FSH concn. (% of onset)
Onset control	—	25	40	—	708 ± 36[b]	60 ± 4	4 ± 1	100
Protein deficient	—	23	50	−34	868 ± 47	48 ± 3	3 ± 1	103 ± 20
	—	25	61	−46	808 ± 42	31 ± 2	3 ± 1	31 ± 9
	oil	22	71	−56	533 ± 39	22 ± 1	4 ± 1	19 ± 4
	60 μg TP	25	71	−53	494 ± 43	89 ± 9	4 ± 1	39 ± 8[c]
	400 μg TP	22	71	−54	580 ± 38	151 ± 13	4 ± 1	50 ± 5[d]
Diet control	—	9	50	+57	1140 ± 41	157 ± 10	5 ± 1	89 ± 15
	—	9	61	+137	1430 ± 57	222 ± 27	7 ± 1	97 ± 14
	oil	8	71	+210	1629 ± 61	287 ± 23	10 ± 1	143 ± 9
	400 μg TP	8	71	+198	1354 ± 45	415 ± 47	9 ± 1	148 ± 14

[a] Long-Evans rats, 39–40 days of age and 137–153 g at onset.

[b] Mean ± standard error.

[c] Vs own oil-treated control, $p < .05$; [d] $p < .01$.

concentration of hormone was reduced to about one-third of its original value, and differed insignificantly from that at the end of experiment. Ventral prostate weight, however, declined steadily from the start, indirect evidence that circulating androgen titers became reduced very soon after the protein-free regimen was instituted. Experimental verification of this concept must await the results of plasma testosterone determinations which are in progress. In view of the prevailing belief [*e.g.* (14)] that pituitary ICSH, rather than FSH, is primarily responsible for sustaining androgenic function of the testis, the temporal relationships of ventral prostate atrophy in PD rats are of interest. The prompt regression of this structure may have been caused by a fall in circulating ICSH activity at a time when FSH secretion was seemingly still unaffected by the absence of dietary protein. It is entirely possible, however, that changes in FSH synthesis and/or release rates also occurred very early in this study and that pituitary potency estimates failed to register the shift in secretory dynamics.

Summary. Male rats were placed on a purified protein-free diet for 10, 21, or 31 days. Ventral prostate weights declined almost immediately, but pituitary FSH concentrations remained at preexisting levels for at least 10 days. After 21 days of deficiency, when FSH stores had become greatly reduced, daily injections of 60 or 400 μg of TP for 10 consecutive days produced partial restoration of ventral prostate weight and pituitary FSH concentrations. The same treatment increased ventral prostate weight, but not pituitary FSH potency, of control rats fed optimal amounts of protein.

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