Testicular Testosterone Response to Human Chorionic Gonadotropin in the Rat (33674)

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Testosterone is the androgen predominantly secreted from the testicles in many species of mammals, including the rat (1–4). Resko *et al.* (5) reported that testosterone is the major androgen secreted in the postnatal and adult stages of development in the rat. Human chorionic gonadotropin (HCG) has been shown to be effective in stimulating the rat testicle to secrete testosterone (1, 6). To determine the amount of HCG necessary to stimulate the testicles to produce testosterone, graded doses of HCG were given to rats and their testicular testosterone measured.

Materials and Methods. Reagents. The HCG was obtained from a commercial source. Testosterone-4-^{14}C (New England Nuclear Corp.), with a specific activity of 76 μCi/mg, was purified by thin-layer chromatography in systems of ether:chloroform (1:9) and benzene:ethyl acetate (4:1). Liquid scintillation fluid was prepared by adding 4 g of PPO (2,5-diphenyloxazole) and 40 mg POPOP (*p*-bis[2-(5-phenyloxazolyl)]benzene) to 1000 ml of toluene. Silica gel H was purchased from Research Specialties Co. All solvents were of analytical grade and were redistilled before use. Analytical grades of acetic anhydride and pyridine were distilled and stored in a dessicator.

Chromatography. Whatman filter paper no. 2, washed with methanol and cut in 50 × 2.5-cm strips, was used for paper chromatography in a modified Bush-type system. Thin-layer glass plates, 20 × 5 cm, were coated with silica gel H to a thickness of 0.75 mm. The plates were dried at room temperature and then activated at 110° for 1 hr. The first two thin layers were done in paper-lined tanks; however, the third system was run in an unlined tank, which gave better separations. Gas-liquid chromatography was performed on F & M Scientific Corp. instruments, model nos. 400 and 402, equipped with 6-ft glass U-tube columns (3 mm i.d.) and hydrogen flame ionization detectors. Individual columns were packed with Anakrom ABS (110–120 mesh), coated with 1% neopentyl glycol succinate (NGS) or Gas-chrom-Q (110–120 mesh), coated with 3% fluorosilicone (QF-1). The columns were kept at 215°, the flash heater at 250–260°, and the detector at 300°. Helium was used as the carrier gas, with a flow rate of 80 ml/min. The attenuations found most satisfactory were 1/16 or 1/32.

Animals. Male rats of the Sprague-Dawley strain were used (Berkeley Pacific Labs., Berkeley, Calif.) weighing 200–250 g at the time of sacrifice. The animals were fed Purina rat pellets and tap water *ad libitum*. The rats were sacrificed within 1 week after they were received.

Assay. The rats were lightly anesthetized with ether anesthesia. Hypophysectomy was performed by inserting an 18-guage needle on a syringe into the sella turcica through the auditory canal and applying suction, as described by Tanaka (7). Three hr after hypophysectomy they were again lightly anesthetized with ether anesthesia, a venous cutdown was done in the femoral area, and HCG was injected in a volume of 1.0 ml. Dilutions of HCG with saline were made prior to use for each group of rats, containing 0.1, 0.25, 0.5, 1.0, 2.5, 5.0, and 10.0 IU/ml. The rats were handled in groups of 4–6 animals. The first group were given 5 or 10 IU of HCG and then sacrificed by decapitation at 15, 30, 45, 60, and 90 min after the injection. The second group of animals were given varying amounts of HCG, as previously outlined, and were sacrificed by decapitation 30 min after injection. A control group of rats were hypophysectomized, and 3 hr later they were given 1.0 ml of saline. They were sac-

rificed 30 min after the injections. Immediately after decapitation, the testicles were removed, frozen, and stored at −20° until the determinations were performed. The testicles from each rat were weighed as a pair and then homogenized with 40 ml of absolute ethanol in a Virtis homogenizer. To calculate recoveries, a tracer amount of testosterone-4-^{14}C (11,000 dpm), 0.5 ml was added and the mixture was centrifuged at 2000 rpm in conical tubes. The supernatant fluid was decanted and the sediment was resuspended two more times in 40 ml of absolute ethanol, centrifuged, and decanted. The pooled supernate was dried on a rotary evaporator under vacuum at 55°. Distilled water (20 ml) and sodium hydroxide (6 *M*, 1.0 ml) solution was added to the dry samples and the aqueous phase was extracted three times with chloroform:ether (3:1, 40 ml). The pooled organic phase was dried under vacuum and the samples were spotted on silica gel H thin-layer chromatography plates. Each plate was run in benzene:methanol (99:1), dried at room temperature, and rerun in benzene:ethyl acetate (6:4). Each plate was scanned for radioactivity, using a Nuclear Chicago scanner, model 1002, with a thin-layer conveyer system, model 1006. The radioactive zone was eluted with a mixture of chloroform:methanol (2:1, 10 ml). After drying, this solution was applied to paper strips for the paper chromatography. The mobile phase for the paper chromatography was made by taking the upper layer of a mixture of dioxane (100), cyclohexane (100), methanol (10), and distilled water (1). The lower layer was used as the stationary phase. The paper strips were equilibrated in the tank for 2 hr before adding the mobile phase. The paper strips were run for 16–18 hr, using the descending technique, then removed and dried at room temperature. The strips were scanned for the radioactive peak on a Nuclear Chicago Actigraph III paper radiochromatogram, model 1002, and the band corresponding to testosterone cut out and eluted with absolute ethanol (10 ml).

The samples were dried in centrifuge tubes and acetylated overnight at 37° with acetic anhydride (0.2 ml) and pyridine (0.2 ml). Distilled water (1.0 ml) and dichloromethane (10 ml) were added to the reaction mixture, and after separation the organic layer was washed with water (1.0 ml). After drying, the sample was applied to a silica gel H thin-layer plate and run in cyclohexane:ethyl acetate (65:40). The plates were scanned to locate the radioactive testosterone peak, which was removed and eluted with a solution of chloroform:methanol (2:1, 10 ml). After drying, 16-α-methylprogesterone (1.0 μg) in ethanol (1.0 ml) was added. One-tenth vol (0.1 ml) was removed and placed in a scintillator counting vial and 10 ml of counting fluid was added. The samples were counted with a standard and blank for each assay group in a Nuclear Chicago Unilux liquid scintillation spectrophotometer. Calculated recoveries for the samples, carried through the entire procedure, ranged from 40 to 80%, with a mean of 60%. The remaining solution was dried and 18 μl of tetrahydrofuran was added before injection into the gas chromatograph. Using a Hamilton (model 701N) 10-μl syringe, 2.0 μl were injected into a QF-1 or 1% NGS column. Areas corresponding to the testosterone acetate and the internal standard (16-α-methylprogesterone) peaks were measured by planimetry and by comparing the areas with the standards of known concentrations. The testosterone is expressed as testosterone acetate per 100 g of testicular tissue.

Results. No detectable peaks of testosterone were seen in the samples from the saline-injected hypophysectomized rats (controls). Five untreated, decapitated rats had a mean testosterone level of 19.5 ± 2.3 μg/100 g (SEM) testicular tissue.

The results of the testosterone response to 5 and 10 IU of HCG at various intervals are shown in Table I. There is a significant difference in testosterone concentrations when the responses to 5 and 10 IU of HCG administration are compared at various timed intervals. Peak concentrations occurred at 45 min for both doses, and at 60 and 90 min had decreased. An interval of 30 min was used to

TABLE I. Response of Testicular Testosterone to HCG Administration in Rats.

Treatment (IU)	No. of animals	Testosterone (μg/100 g of tissue)	SEM	Time sacrificed (min after HCG administration)
Untreated control	5	19.5	2.3	
Saline control	6	Undetectable		30
HCG, 5	4	12.2	3.4	15
	6	49.9	5.8	30
	6	72.1	14.7	45
	7	60.4	10.3	60
	5	16.4	1.7	90
HCG, 10	6	61.2	14.1	15
	5	72.1	14.2	30
	6	82.8	15.1	45
	9	64.4	13.7	60
	6	53.0	8.4	90

evaluate the effect of graded doses of HCG. The response of testosterone to various doses of HCG is shown in Table II. There is no significant difference in the testosterone response to 0.1, 0.25, and 0.5 IU of HCG; however, when compared with the saline-treated controls this difference is significant. With graded increasing doses of HCG, there was an associated rise in the concentration of testicular testosterone.

TABLE II. Response of Testicular Testosterone to Varying Concentrations of HCG in Rats.

Treatment (IU)	No. of animals	Testosterone (μg/100 g of tissue)	SEM
Saline control	6	Undetectable	
HCG, 0.1	4	6.1	0.6
0.25	3	6.9	0.9
0.5	4	6.8	0.7
1.0	4	10.8	1.2
2.5	5	13.9	3.9
5.0	6	49.9	5.8
10.0	5	72.1	14.1

Discussion. Testosterone has been identified as the major steroid of the rat testicle (4, 5). Measurement of these steroids was done in samples from single rats (1).

The concentration of testosterone found in testicular tissue in normal, mature, untreated, decapitated rats is consistent with previously reported studies (1, 4), whereas the levels found in unstimulated hypophysectomized animals was not recoverable. The peak concentration of testicular testosterone was found at 45 min with the administration of both 5 and 10 IU of HCG. A significant difference in testicular testosterone response to graded doses of HCG and to 5 and 10 IU at timed intervals was shown by these studies.

Summary. The testicles from hypophysectomized rats respond to small doses of HCG, with the maximal concentration found at 45 min after injection of HCG. A method is reported for measuring testicular testosterone in the rat, using gas-liquid chromatography with a hydrogen flame ionization detector.

1. Hashimoto, I. and Suzuki, Y., Endocrinol. Japon. **13**, 326 (1966).
2. Linder, H. R., J. Endocrinol. **23**, 171 (1961).
3. Eik-Nes, K. B., Endocrinology **71**, 101 (1962).
4. Bardin, C. W. and Peterson, R. E., Endocrinology **80**, 38 (1967).
5. Resko, J. A., Feder, H. H., and Goy, R. W., J. Endocrinol. **40**, 485 (1968).
6. Suzuki, Y. and Eto, T., Endocrinol. Japon. **9**, 277 (1962).
7. Tanaka, A., Ann. Rept. Shionogi Res. Lab. **5**, 678 (1955).

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