THE SEQUENCE OF CHANGES IN PREPUBERTAL MALE RAT SERUM TESTOSTERONE FOLLOWING INTRAVENOUS INJECTION OF RAT LH

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Summary. Mean serum testosterone concentration in the 27-day-old prepubertal Holtzman rat was 0.25 ng/ml, much lower than that observed in the adult male, 5.5 ng/ml. Within 15 min after the intravenous injection of rat LH (in a dose of 400 ng-equiv. NIH-LH-S1/100 g body weight) serum testosterone concentration in the prepubertal rat increased approximately fivefold. Maximal elevation of serum testosterone to levels as high as those observed in the adult male occurred 1 hr after intravenous injection of rat LH. Serum testosterone returned to 'control' levels within 2 hr of the intravenous injection of a small dose of rat LH. A dose-response relationship existed between serum testosterone and the dose of rat LH injected intravenously, over the range 25 to 1600 ng-equiv. NIH-LH-S1/100 g body weight.

Until recently, assessment of the responsiveness of the prepubertal rat testis to the effect of gonadotrophins with respect to stimulation of steroid secretion usually required measurement of weight gain of the prostate gland or seminal vesicles at a long period of time after chronic administration of the gonadotrophins. With the advent of sensitive radioimmunoassay methods for measuring serum levels of testosterone, such as that developed in our laboratory (Coyotupa, Parlow & Abraham, 1972), it has become feasible to evaluate the acute effect of LH on prepubertal male rat serum testosterone within minutes of the intravenous injection of the hormone.

Prepubertal male rats of the Holtzman strain arrived in the laboratory at 22 days of age. Five days later, at a body weight range of 68 to 90 g, the rats were utilized for the following experimental studies: (1) the effect of graded doses of a rat LH preparation on serum testosterone, measured 45 min after a single intravenous injection of the rat LH; (2) the sequence of changes in serum testosterone at 5, 15, 30, 60, 120 and 240 min following a single intravenous injection of rat LH.

The rat LH used was a partially purified preparation having a biological potency, according to the OAAD assay (Parlow, 1961), of $0.2 \times \text{NIH-LH-S1}$. The rat LH preparation was dissolved in 0.9% NaCl solution just before use. Doses of rat LH were administered per 100 g body weight. Intravenous injections

TEXT-FIG. 1. The effect of graded doses of a rat LH preparation ($0.2 \times \text{NIH-LH-SI}$, OAAD assay) on serum testosterone concentration (mean $\pm$ S.D.) in the 27-day-old Holtzman rat, 45 min after intravenous injection. Doses were administered per 100 g body weight. The level of serum testosterone in 'control' rats injected with saline is indicated by the horizontal 'interrupted' line.

TEXT-FIG. 2. The sequence of changes in serum testosterone concentration in the 27-day-old Holtzman rat during a period of 4 hr following a single intravenous injection of either a small dose ($2 \mu\text{g}/100 \text{ g}$) or a large dose ($47 \mu\text{g}/100 \text{ g}$) of a rat LH preparation ($0.2 \times \text{NIH-LH-SI}$, OAAD assay.) Each dot represents the serum testosterone concentration of one animal. Mean serum testosterone concentration is indicated, at each time interval measured, by the 'interrupted' line.

were performed and blood samples were obtained from the vena cava, under light ether anaesthesia. There were three rats in each experimental group.

Blood samples were obtained without anticoagulant. After overnight clotting of the blood sample in the refrigerator, the serum was separated by centrifugation, and stored frozen at -20°C . Measurement of serum testosterone was accom-

plished by a specific radioimmunoassay method, as previously described (Coyotupa *et al.*, 1972.)

Forty-five minutes after the intravenous injection of 1 ml 0.9% NaCl solution, the mean serum testosterone concentration in the 27-day-old male rat was 0.25 ng/ml. Injection of rat LH, in a dose of 0.125 μ g/100 g body weight, increased the serum testosterone level considerably. Greater increases in serum testosterone concentration, in excess of 4 ng/ml, were attained with larger doses of rat LH. A dose-response relationship existed between the serum testosterone concentration and the dose of rat LH administered over the range 0.125 to 8 μ g/100 g body weight (Text-fig. 1).

No significant increase in serum testosterone concentration was discernible within 5 min of an intravenous injection of either the small or large dose of rat LH, but a considerable elevation in serum testosterone, just short of 4 ng/ml, was attained 15 min after an intravenous injection of the small dose of rat LH. A maximal response was apparently reached 60 min after the injection (Text-fig. 2). Thereafter, the serum testosterone fell rapidly, nearly reaching pre-injection values within 120 min of an intravenous injection of the small dose of rat LH. On the other hand, the serum testosterone appeared to be maximally elevated 120 min after the intravenous injection of the large dose of rat LH, and was still well above the baseline at 240 min after injection of the large dose (Text-fig. 2).

The mean serum testosterone concentration (0.25 ng/ml) recorded in the 27-day-old prepubertal male Holtzman rat was quite low compared to the mean value (5.5 ± 2.7 ng/ml) observed in the adult animal (J. Coyotupa, A. F. Parlow & N. Kovacic, unpublished observations). However, within 45 min of an intravenous injection of 0.125 μ g of rat LH (25 ng-equiv. NLH-LH-S1)/100 g body weight, the prepubertal male rat serum testosterone was greatly increased and within 1 hr of the intravenous administration of 2 μ g rat LH (400 ng-equiv. NIH-LH-S1)/100 g body weight, prepubertal male rat serum testosterone was elevated to levels of approximately 4 ng/ml. This serum testosterone level was as high as that observed in the adult male rat but within 2 hr the concentration had declined essentially to its preinjection level.

These data indicate that the 27-day-old prepubertal Holtzman rat testis is poised to respond to rat LH stimulation with a rapid elevation of serum testosterone to levels as high as those observed in the adult animal. The maintenance of such elevated levels of serum testosterone is apparently dependent on continuous stimulation with LH.

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