Plasma concentrations of LH and testosterone in castrated rams treated with testosterone or testosterone propionate

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Summary. After injection of castrated rams with 600 mg testosterone or testosterone propionate, plasma LH levels were biphasic, with significant decreases at 6–10 and 96 h after treatment. These results support the suggestion that testosterone acts at the hypothalamo-pituitary level by two different mechanisms.

The injection of testosterone propionate induces a biphasic decrease in the plasma LH levels of castrated rams at 12 and 72 h after treatment (Pelletier & Ortavant, 1972). These two phases have been attributed to two different actions of testosterone, one of which induces a decrease in the pituitary response to LH-RH (Pelletier, 1974) and the other a block to the synthesis of LH-RH (Pelletier, 1970). To demonstrate clearly that testosterone acts on LH release by two different mechanisms, it is necessary to know the variation in plasma testosterone levels after treatment with testosterone propionate. For example, it is possible that the two phases of LH decrease may be related to a biphasic resorption of the injected steroid. In the present study, therefore, plasma testosterone and LH concentrations were measured concurrently after injection of testosterone propionate or testosterone.

Methods

Fourteen Préalpes du Sud rams, 15 months old and castrated 3 months before treatment, were divided into two equal groups. Seven rams received 600 mg testosterone propionate (Group 1) dissolved in 8 ml olive oil and injected i.m. into two sites in the neck, i.e. approximately 7.5 mg/kg bodyweight. Similarly, the rams in Group 2 received 600 mg testosterone. Jugular blood samples were collected by venepuncture three times at 1-h intervals before treatment, every 2 h for 30 h, and at 36, 48, 60, 72, 90 and 120 h after injection. Plasma samples were kept frozen until assayed. LH was measured by the radioimmunoassay described by Pelletier, Kann, Dolais & Rosselin (1968). The mean of the three pretreatment samples was taken as the initial LH level.

Testosterone was directly assayed in 50 µl of a 1 in 50 dilution of plasma by means of an antiserum raised against testosterone 3-carboxy-methoxime-bovine serum albumin. The bound and free fractions were separated by immunoprecipitation (Terqui, Dray & Cotta, 1973; Dray, 1975). The sensitivity for undiluted plasma was 0.2 ng/ml. The only cross-reaction of the antiserum was for dihydrotestosterone (28%) but the level in ovine peripheral plasma is very low (<1 ng/ml). Other steroids had a negligible cross-reaction; that for testosterone propionate was <0.01%. Two standard plasma samples (H and L) were assessed by radioimmunoassay and by gas-phase chromatography (Attal, 1970); the levels found were 6.8 and 7 ng/ml, respectively, for the H sample and 1.3 and 1.6 ng/ml for the L sample. The interassay variability was 13% for plasma containing 1.5 ng testosterone/ml and 7% for plasma containing 13 ng/ml. Results are expressed as the mean $\pm$ S.E.M.

Results and discussion

LH. The mean plasma LH level in rams of Group 1 (Text-fig. 1a) declined after treatment with a maximum decrease by 10 h (4.0 ± 0.04 versus 8.1 ± 0.7 ng/ml at 0 h; $P < 0.001$). Levels then increased with oscillations to the initial level 20–22 h after treatment. A second decrease occurred and the

minimum level was reached at 96 h (2.0 ± 0.7 ng/ml). Although increasing by 120 h, levels had not returned to initial values. The decrease of plasma LH concentration was steeper in Group 2 rams (Text-fig. 1b) with a significant decrease 4 h after treatment (3.9 ± 0.3 versus 6.7 ± 0.6 ng/ml at 0 h; $P < 0.01$). The maximum LH decrease was observed 6 h after testosterone injection. However, the increase in plasma LH which then took place was slower than in Group 1 and returned to the initial level only by 30 h after treatment.

Testosterone. The plasma levels in Group 1 (Text-fig. 1a), once undetectable, increased sharply and reached maximum levels (approx. 390 ng/ml) 12–22 h after treatment. They then decreased steadily and by 120 h the values were close to the initial levels (9 ± 4 ng/ml). In Group 2, plasma testosterone levels (Text-fig. 1b) showed a sharp increase and the maximum level (400 ± 61 ng/ml) was reached 6 h after treatment. During the next 4 h concentrations decreased to 150 ng/ml but then, 10–24 h after treatment, showed a second but more moderate increase up to 220 ng/ml. Thereafter, plasma values decreased again slowly and were relatively high by 120 h (42 ± 9.3 ng/ml).

Text-fig. 1. Mean plasma LH (Δ , $\blacktriangle$) and testosterone ($\circ$, $\bullet$) concentrations in castrated rams after an injection (time 0) of (a) 600 mg testosterone propionate or (b) 600 mg testosterone.

Three phases were apparent from the results in Text-fig. 1. (1) 8–10 h after steroid injection testosterone increased as LH decreased and the LH decrease was faster in Group-2 than in Group-1 animals because of the earlier increase in testosterone levels. It has been previously shown (Pelletier, 1974) that the LH response of castrated rams to LH-RH was decreased 6 h after injection of testosterone propionate. The present results indicate that at this time the plasma testosterone level is already high and thus may be responsible for the decrease in the pituitary response to LH-RH. (2) Between 10 and 36 h after steroid injection testosterone and LH concentrations fluctuated widely, and no correlation between these two hormones could be demonstrated, as Sanford, Palmer &

Howland (1974) found in the entire ram. This could be due to the sampling interval which was greater than the half-lives of the two hormones. Nevertheless, the mean plasma LH level increased (the present study) and the pituitary response to LH-RH is restored (Pelletier, 1974) at a time when the plasma testosterone level is still high, suggesting that there is a change in the ability of plasma testosterone to inhibit the release of LH at this time. (3) LH levels decrease as the plasma testosterone declines 36–120 h after steroid treatment. No decrease in the pituitary response to LH-RH was observed during this period (Pelletier, 1974), but a decrease in the hypothalamic content of LH-RH assessed by an in-vitro method, has been shown (Pelletier, 1970).

Two unexpected findings were noted. Firstly, the magnitude of the testosterone fluctuations indicates that resorption rate and/or metabolism are highly variable. Such fluctuations have been also observed by Knobil (1974) in oestrogen-implanted monkeys. The methodology may be excluded as a major source of error since similar results have been obtained by a very specific gas chromatography method ($r = 0.87$ between methods) (Attal, 1970). Secondly, although testosterone levels increased more rapidly after testosterone than after testosterone propionate, testosterone appears to inhibit the release of LH for a longer period (Text-fig. 1b). It is possible, since the solubility of testosterone is lower than that of testosterone propionate, that some of the injected steroid might have crystallized at the site of injection and total resorption therefore occurred more slowly. Consequently, the high testosterone levels at 120 h could have been responsible for the low LH level at this particular time. However, in spite of that and the multiphasic pattern of plasma testosterone in the testosterone-injected animals it is clear that the plasma LH patterns are similar whatever the androgenic treatment. Thus, a multiphasic resorption of testosterone cannot account for the biphasic LH pattern since it also occurred in the testosterone propionate-treated animals in which plasma testosterone was clearly monophasic.

The hypothesis that there are two actions of testosterone, with different latencies, on pituitary and/or hypothalamic cells remains the most likely to account for these results.

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