DIURNAL FLUCTUATIONS OF TESTOSTERONE AND LH IN THE RAM: EFFECT OF HCG AND GONADOTROPHIN-RELEASING HORMONE

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Summary. To examine a possible relationship between the release of LH and increasing testosterone levels, blood samples were collected at 2-hr intervals for 24 hr from six mature rams with indwelling jugular cannulae. Although high levels of LH often preceded increases of testosterone, a consistent time relationship between these two hormones was not observed, nor was a diurnal rhythm established for either hormone. The following day, two rams were each injected intravenously with saline, 500 i.u. HCG, or 100 µg synthetic gonadotrophin-releasing hormone (Gn-RH) respectively. Immediately after the injection, blood was collected every 15 min for approximately 4 hr. After Gn-RH, the plasma LH rose 25- to 50-fold within 12 min and was elevated for more than 3 hr. A twofold rise of plasma testosterone followed this increase of LH and testosterone remained high until the end of the experiment. After HCG treatment, the level of testosterone rose threefold within 30 min and did not decline during the experiment, but there was no effect on the LH level. Saline treatment did not influence levels of LH or testosterone.

It was concluded that daily fluctuations of testosterone and LH may occur independently, and no pattern is common to all rams. Treatment with Gn-RH results in a rise in both the testosterone and LH levels, but only testosterone is affected by HCG.

INTRODUCTION

The concentrations of testosterone and LH are known to fluctuate in the peripheral plasma of the male, but the existence of diurnal rhythms and the

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temporal relationship between these two hormones have been examined in only a few species. In the bull, peaks of testosterone follow peaks of LH (Katongole, Naftolin & Short, 1971) and sexual stimulation, and occasionally ejaculation, caused an increase of LH in the peripheral plasma, but these LH peaks were not always followed by a testosterone peak. In the male miniature pig, a clear response of plasma testosterone to major changes in plasma LH induced by releasing factor was seen but there was a low correlation between normal LH release and changes in plasma testosterone (Pomerantz, Ellendorf, Elsaesser, Konig & Smidt, 1974). Copulation, or the mere presence of the doe, resulted in a significant rise of testosterone in the peripheral plasma of the male rabbit (Saginor & Hortin, 1968; Haltmeyer & Eik-Nes, 1969). Studies in men have shown rapid fluctuations of both LH and testosterone in the peripheral plasma, and there are conflicting data showing both the presence and an absence of a diurnal rhythm of these two hormones (Resko & Eik-Nes, 1966; Nankin & Troen, 1971; Alford & co-authors, 1973).

Recent data on the ram have shown that LH and testosterone are released episodically but there is no evidence of a diurnal rhythm for either hormone (Bolt, 1971; Katongole *et al.*, 1974; Purvis, Illius & Haynes, 1974; Sanford, Winter, Palmer & Howland, 1974). In the ram, the secretory pattern of these hormones is also affected by the season. Peaks of LH appeared more frequently but the amplitude was either similar or depressed during the breeding season (Katongole *et al.*, 1974; Sanford *et al.*, 1974). Testosterone peaks during the breeding season displayed both higher amplitude and frequency (Katongole *et al.*, 1974; Purvis *et al.*, 1974; Sanford *et al.*, 1974). Furthermore, in studies where both LH and testosterone were measured, testosterone peaks were always shown to be preceded by an LH peak (Katongole *et al.*, 1974; Sanford *et al.*, 1974).

The present study was designed to examine changes of LH and testosterone in the peripheral plasma of adult rams during a 24-hr period. In addition, HCG and synthetic gonadotrophin-releasing hormone (Gn-RH) were administered to observe the effects of these hormones on LH and testosterone in the peripheral plasma. These experiments were designed to determine the temporal relationship, if any, between LH release, either naturally occurring or artificially induced, and testosterone secretion.

MATERIALS AND METHODS

During the breeding season in September, six adult Hampshire rams each weighing 110 kg were placed in outdoor pens, isolated from ewes, 1 week before the experiment. Blood samples were collected from indwelling jugular cannulae which had been inserted 1 day previously. Samples were taken every 2 hr for 24 hr. After the 24-hr collection, two rams each were injected intravenously with saline, 500 i.u. HCG (APL:Ayerst Laboratories, New York) or 100 µg Gn-RH respectively. Blood was collected every 15 min for 1 hr before and for approximately 4 hr after an injection.

Plasma LH was assayed using the double antibody radioimmunoassay (RIA) as described previously by Niswender, Reichert, Midgley & Nalbandov (1969), with some modifications. Ovine LH (LER-1056-C2) was iodinated using ^{125}I ,

according to the method of Greenwood, Hunter & Glover (1963). The first antibody used in these assays was No. 15 of Niswender which has been shown to be specific for ovine LH (Niswender, Midgley & Reichert, 1968). Samples of plasma were assayed in duplicate at 100 or 200 μ l and NIH-LH-S13 was used as the ovine standard. Using these volumes of plasma, the acceptable range of the assays was 0.17 to 30.0 ng/ml. For statistical purposes, values below the

TEXT-FIG. 1. Daily (24-hr) fluctuations of testosterone (●) and LH (■) in the peripheral blood of six rams. N.D. = not detectable.

sensitivity of the assays were assigned a value of 0.17 ng/ml and those above the upper range of the assays, a value of 30.0 ng/ml. The inter-assay coefficient of variation was 11%.

The RIA used for plasma testosterone was similar to that of Bartke, Steele, Musto & Caldwell (1973), the modifications used in our laboratory having been described previously (Falvo, Buhl & Nalbandov, 1974). The antiserum used was specific to testosterone and dihydrotestosterone. All samples were run in duplicate using either 10 or 20 μ l plasma without chromatography. This procedure and validation for the testosterone RIA in rams has been previously published by our laboratory (Falvo & Nalbandov, 1974).

TEXT-FIG. 2. The response of plasma testosterone (●) and LH (■) to an intravenous injection of saline. The average and range for testosterone and LH was measured the day previously in the same ram and served as the control (vertical bars, right side of figure). N.D. = not detectable.

When [3 H]testosterone was added to 10- μ l aliquots of wether plasma, the mean recovery of 20,000 ct/min was 97.0%. Standard curves from six different assays showed a coefficient of variation of 2.4% at the lowest concentration on the curve (3.7 pg) to 11.9% at the highest point (500.0 pg). The recovery (Mean $\pm$ S.E.) of 31.2, 62.5 and 125.0 pg testosterone from 10 μ l wether plasma was 20.7 ± 6.8 ($n = 4$), 48.7 ± 2.1 ($n = 8$), and 105.8 ± 4.3 ($n = 4$) pg/ml, respectively. The lowest amount of testosterone which was measurable repeatedly and distinguishable from zero was 13.4 ± 1.7 pg. The specificity of the method was examined by analysis of twenty samples of wether plasma. All samples contained non-detectable quantities of testosterone. The variations between duplicates was less than 10% in all cases and therefore the plotted values will not show the standard errors.

RESULTS

Text-figure 1 shows the changes for both LH and testosterone in samples collected every 2 hr for 24 hr. Concentrations of testosterone ranged from 1.6 to 26.0 ng/ml, and the levels of LH ranged from non-detectable to 1.8 ng/ml. In Rams 2 and 3, an LH-testosterone relationship was seen which was not observed in other rams. The time interval from a 'peak' of LH to a peak in testosterone varied from 2 hr to 6 hr. However, because of the absence of this relationship in most of the rams, the lack of any time consistency between LH and testosterone, and the infrequent sampling time, the physiological importance of these observations is not clear. The mean LH and testosterone

TEXT-FIG. 3. The response of plasma testosterone (●) and LH (■) to an intravenous injection of Gn-RH. The average and range for testosterone and LH was measured the day previously in the same ram and served as the control (vertical bars, right side of figure). N.D. = not detectable.

concentrations over the 24-hr period were used as control values for comparison with the levels observed after administration of saline, HCG, or Gn-RH. These control values (average and range) appear at the right side of Text-figs 2, 3 and 4.

During the 24-hr collection period, a diurnal rhythm could not be established for either hormone in individual rams. When the mean levels of plasma testosterone and LH of all six rams were taken together, a diurnal rhythm was still

not apparent. Text-figure 2 shows the levels of testosterone and LH following saline injection. In Ram 3, no difference was observed in plasma testosterone and LH following saline administration compared to the control values of these hormones measured the previous day (right side of figure). With frequent sampling, however, a time relationship between plasma LH and testosterone was apparent. There was a rise in plasma testosterone within 48 to 50 min following an LH peak. The two LH peaks occurred 4 hr apart. In Ram 6, no time relationship between LH and testosterone could be established. A similar relationship to that found in Ram 3 may have been present but, owing to the low circulating levels of LH, definitive peaks could not be measured.

TEXT-FIG. 4. Response of plasma testosterone (●) and LH (■) to intravenous injections of HCG. The average and range for testosterone and LH was measured the day previously in the same ram and served as the control (vertical bars, right side of figure). N.D. = not detectable.

Following the injection of 100 μ g Gn-RH (Rams 2 and 4, Text-fig. 3), there was a rise in plasma LH within 12 min to a level which was 30-fold above the control values shown at the right side of the figure. The LH concentrations in Ram 4 were elevated for over 4 hr after Gn-RH injection. Testosterone concentrations following Gn-RH rose to significantly higher levels in both rams within a mean $\pm$ S.E. time of 55 ± 5 min or within 43 ± 5 min after the induced LH peaks.

After administration of HCG to Ram 1 and Ram 5 (Text-fig. 4), no rise in LH was observed, but the testosterone concentrations in both rams rose significantly above the control values, three- to fivefold within 30 min and four- to tenfold 66 min after HCG. The second HCG injection did not elicit any further increase in the testosterone levels in either ram.

DISCUSSION

No consistent time relationship between peripheral testosterone and LH was noted over a 24-hr period in rams under the conditions of this study. The LH release observed appears to represent only small excursions from basal levels, confirming earlier work which showed that LH was released in brief surges rather than at a continuous rate with no diurnal variation during the collection period (Bolt, 1971). The physiological significance of this release is not understood. Furthermore, in order realistically to determine a possible relationship between LH and testosterone, more frequent sampling may be necessary. The fluctuations of the peripheral testosterone concentrations appeared to be independent of the LH peaks in all except two animals, and showed no variation with respect to the time of day. In more recent studies in which blood was collected at 20 min intervals, a consistent pattern between these two hormones was found (Sanford *et al.*, 1974). These workers noted that although, among rams, LH releases were occurring at random, in individual rams they occurred at regular intervals and were always followed by increases in plasma testosterone.

The response of plasma LH following injection of Gn-RH was both rapid and prolonged. These responses to synthetic Gn-RH appear to be greater than those reported in the intact ram by Reeves, Arimura & Schally (1970) in response to a purified porcine hypothalamic LH-releasing hormone extract.

This pattern of response to Gn-RH injection (LH increase followed by testosterone increase) observed in the present studies with the ram is similar to that reported by other workers for the male miniature pig (Pomerantz *et al.*, 1974), and the responses of plasma testosterone to injection of HCG are similar to those found in other animals. Marked increases in plasma testosterone in response to HCG treatment have been reported for the human male (Lipsett & co-authors, 1966), the bull (Katongole *et al.*, 1971), the rabbit (Saginor & Hortin, 1968) and the rat (Bardin & Peterson, 1967).

It is concluded that when blood samples were taken every 2 hr for 24 hr in the ram and measured for LH and testosterone, no consistent time relationship between these two hormones was found, and a diurnal rhythm could not be established for either hormone during this 24-hr period. With more frequent sampling, however, a time relationship between LH and testosterone was seen in one of the rams. With Gn-RH and HCG a consistent time interval of 30 to 40 min was demonstrated between administration of these two hormones and testosterone secretion. This appeared to be similar to the interval observed between endogenous LH and testosterone peaks in one animal. This LH-to-testosterone time interval is in agreement with that found previously in the ram after frequent blood sampling (Sanford *et al.*, 1974).

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