The stimulation of testosterone and LH secretion by synthetic GnRH in the male Cape porcupine

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The effects of GnRH stimulation on plasma testosterone and luteinizing hormone (LH) levels in Cape porcupine males were examined by analysing plasma collected before and after an intravenous injection of GnRH. In six mature males and one subadult, which were given an intravenous injection of 0,5 ml saline, levels of plasma testosterone and LH did not increase. Four weeks later an intravenous GnRH challenge (40 μg) caused plasma testosterone to rise three-fold and LH to rise 10–15-fold within 180 min in five of the mature males. Peaks of plasma testosterone and LH occurred 90 and 120 min, respectively, after stimulation, and baseline and peak levels of both hormones were significantly related.

Die invloed van GnRH stimulasie op plasma-testosteroon en luteïniseringshormoonvlakke (LH), in Kaapse ystervark mannetjies, is deur die analisering van plasma, wat versamel is voor en na die intraveneuse toediening van GnRH, ondersoek. In ses geslagsrype en een onvolwassene wat 0,5 ml van 'n soutoplossing ontvang het, het plasma-testosteroon en LH vlakke nie toegeneem nie. Vier weke later het 'n intraveneuse toediening van GnRH (40 μg) aanleiding gegee tot 'n drievoudige toename in testosteroon en 'n 10–15-voudige toename in LH vlakke binne die bestek van 180 min in vyf van die volwasse mannetjies. Pieke het respektiewelik op 90 en 120 min na stimulasie voorgekom en die verwantskap tussen grond- en piekvlakke van beide hormone was betekenisvol.

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The Cape porcupine *Hystrix africaeaustralis* is a seasonal breeder (Van Aarde 1987). Van Aarde & Skinner (1986) recorded seasonal changes in plasma testosterone levels in adult males but the large variance within each month reported by them is probably due to the known effects of stress, and the pulsatile nature of testosterone secretion (see Kreuz, Rose & Jennings 1972). Fluctuations in plasma testosterone concentrations in males over short periods result from the pulsatile secretion of LH (Katongole, Naftolin & Short 1971). Therefore, in order to define the testosterone milieu of an individual or to compensate for the pulsatile secretion of testosterone, it is necessary to take a large number of serial blood samples at short intervals over an extended period of time. However, without sedation frequent blood sampling over extended periods is not feasible for Cape porcupines and most other non-domesticated mammals. In this study it is attempted to establish a procedure whereby, through the collection of a single blood sample following sedation and GnRH stimulation, the testosterone and luteinizing hormone milieu of individual male Cape porcupines can be determined correctly. The response of the pituitary and the gonads to a single injection of GnRH, as reflected by luteinizing hormone and testosterone levels in serial plasma samples, is reported on in this paper.

Material and Methods

Experimental animals were housed in family groups in semi-outdoor enclosures on the experimental farm of the University of Pretoria (25°45'S / 28°12'E) as described by Van Aarde (1985). Between March and June 1987 the breeding male in each of six family groups and one subadult male (six months old) were anaesthetized with a mixture of xylazine hydrochloride (0,75 mg/kg — Rompun, Bayer Pharmaceuticals (Pty) Ltd, Johannesburg, South Africa) and fentanyl citrate (0,38 mg/kg — Ethor (Pty) Ltd, New Road, Halfway House, Transvaal, South Africa) ~15 min before treatment and the collection of blood samples. Animals were weighed prior to sampling to assess the dose of synthetic GnRH to be administered.

Venous blood (~3,0 ml) was collected in heparinized tubes at 15-min intervals for 45 min before and up to 180 min after an intravenous injection of GnRH (1,7 μg/kg) in 0,9% (w/v) sodium chloride supplied by Hoechst (Frankfurt, Germany). About four weeks before GnRH stimulation each individual was injected with 0,9% sodium chloride only (0,5 ml), the response of each indivdual to sodium chloride was used as a control for reponse to GnRH. Blood samples were centrifuged for 10 min at 1500 **g.** and duplicate aliquots of plasma were stored at –20°C until assayed.

Plasma testosterone levels were determined by radioimmunoassay as described by Van Aarde & Skinner (1986). To denature binding proteins plasma samples (0,1 ml) were incubated for 10 min at 37°C with 0,1 ml sodium hydroxide (0,6 mol dm^{-3}) before extraction with 4,0 ml diethyl ether (Merck, Darmstadt, Germany).

The antiserum used was raised in rabbits against testosterone-3 carboxymethyl-bovine serum albumin. Cross-reaction with naturally occurring steroids was < 0,1% except for dihydrotestosterone for which it was 5,1%. Sensitivity of the assays, defined as twice the standard deviation of the blank values, varied from 30 to 60 pg/ml (mean 40 ± 11,9 pg/ml; n = 14). Non-specific binding averaged 3,8% (n = 14) and specific binding 41,4% (n = 14). Recovery of [1,2,6,7^{3}H] testosterone in 0,1 ml plasma pools was 76,2%. Intra-assay and inter-assay coefficients of variation were 6,9% and 5,3%, respectively.

Luteinizing hormone (LH) levels were determined by

radioimmunoassay as described by Millar & Aehnelt (1977) using an anti-ovine-LH serum (APP-192279) and anti-rabbit gamma globulin as a second antibody. Purified ovine LH (NIADDK-o-LH-1-3; AFPM 95988) was used for iodination and ovine LH (NIADDK-o-LH 5-18) was used as standards.

All means given in the text are followed by one standard deviation. Significance of differences of mean values are based on Student's *t* test.

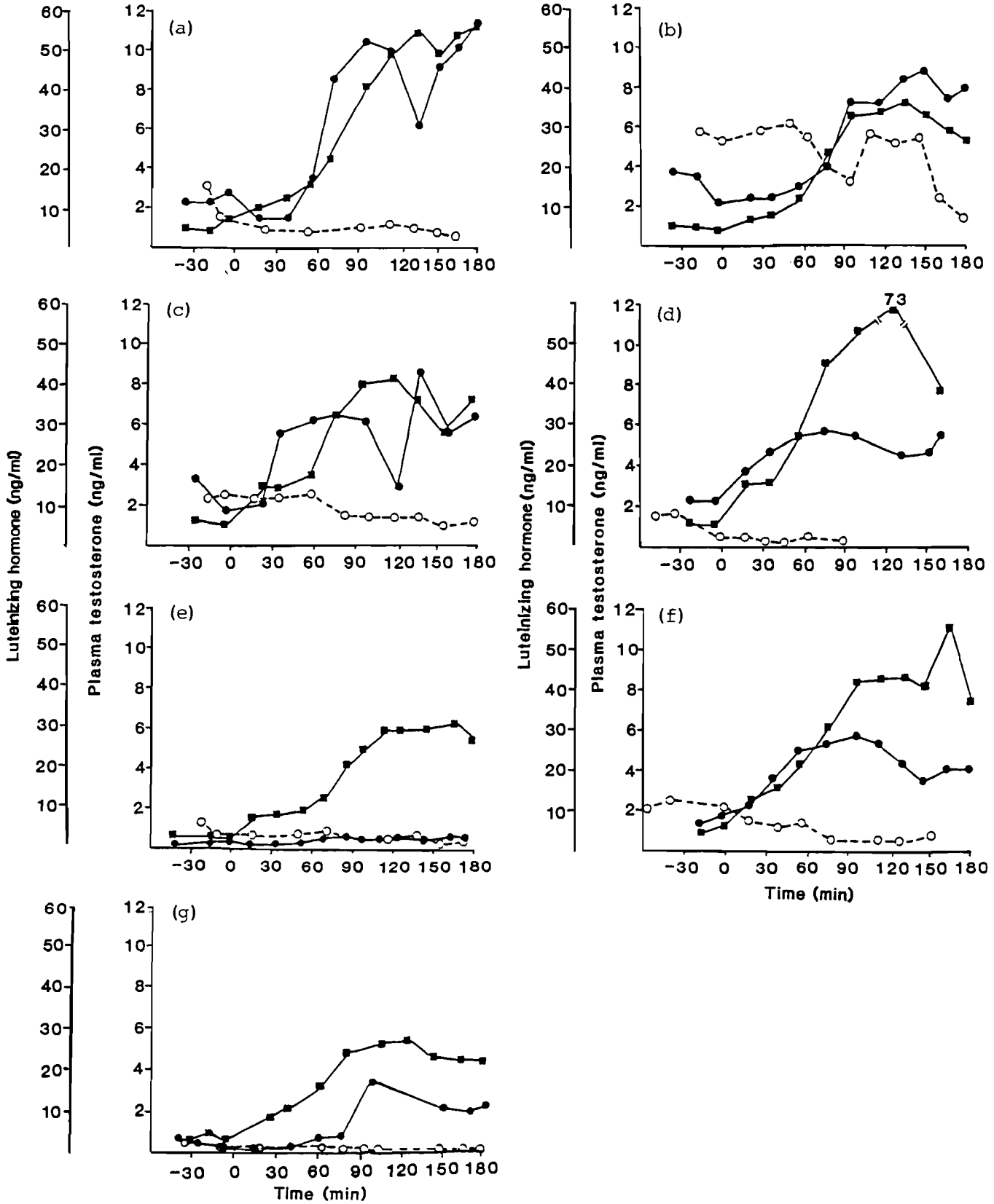

Figure 1a-g Relationships between plasma testosterone (●) and luteinizing hormone (■) concentration in Cape porcupine males stimulated with GnRH and controls (○) (saline) against time. Subadult (g). Anaesthesia was administered ~15 min before the collection of the first blood sample.

Results

Figures 1a–g illustrate temporal changes in circulating testosterone levels before and after saline injections, and testosterone and LH levels before and after GnRH injection. Mean testosterone values before the injection of saline were 2,8 ± 1,56 ng/ml and thereafter 1,21 ± 1,4 ng/ml. These mean values differed significantly ($p < 0,01$). Mean baseline values for testosterone before GnRH stimulation were similar ($p > 0,01$) to those observed before saline injection for all males.

Temporal changes in circulating testosterone levels suggest that five of the six mature males responded to GnRH stimulation, with values in these males, following GnRH stimulation, ranging from 0,2 to 11,2 ng/ml (Figure 1a–d & f). Values peaked about 90 min after stimulation and remained high after the peaks, with a second peak occurring about 180 min after stimulation (Figure 1). Peak values 90 min after GnRH stimulation ranged from 5,8 to 10,5 ng/ml ($\bar{x}$ = 7,4 ± 2,06 ng/ml). In five of the adults, and the immature, testosterone concentrations 90 min after stimulation were significantly higher after GnRH stimulation than after saline treatment ($p < 0,05$). In one adult GnRH stimulation did not result in the increase in testosterone levels observed in other adult males (Figure 1e). Before GnRH or saline injection mean testosterone levels declined from 2,2 ± 1,46 ng/ml (n = 14) to 1,7 ± 1,29 ng/ml (n = 14). This decline was not significant ($p > 0,01$). A significant (r = 0,88; $p < 0,01$) positive, linear relation existed between baseline (before GnRH stimulation) and peak testosterone values (after GnRH stimulation) (Figure 2a).

Immunoreactive LH levels before (5,9 ± 1,78 ng/ml; n = 7) and after saline injection (5,3 ± 1,49 ng/ml; n = 7) were similar. Concentrations before GnRH stimulation varied from 2,3 to 5,9 ng/ml ($\bar{x}$ = 4,00 ± 0,92 ng/ml; n = 7) and did not differ significantly ($p > 0,01$) from values before saline injection. In all males LH values started rising 15 min after GnRH injection and attained peak values ($\bar{x}$ = 44,6 ± 16,8 ng/ml; range 27–73 ng/ml; n = 7) 100 to 120 min after stimulation. Peak values were 10 to 15 times higher than baseline values and a significant (r = 0,83; $p < 0,01$) positive, linear relationship existed between baseline (before GnRH stimulation) and peak LH values (after GnRH stimulation) (Figure 2b). Peak testosterone and peak LH values were not significantly related (r = 0,37; Figure 2c)

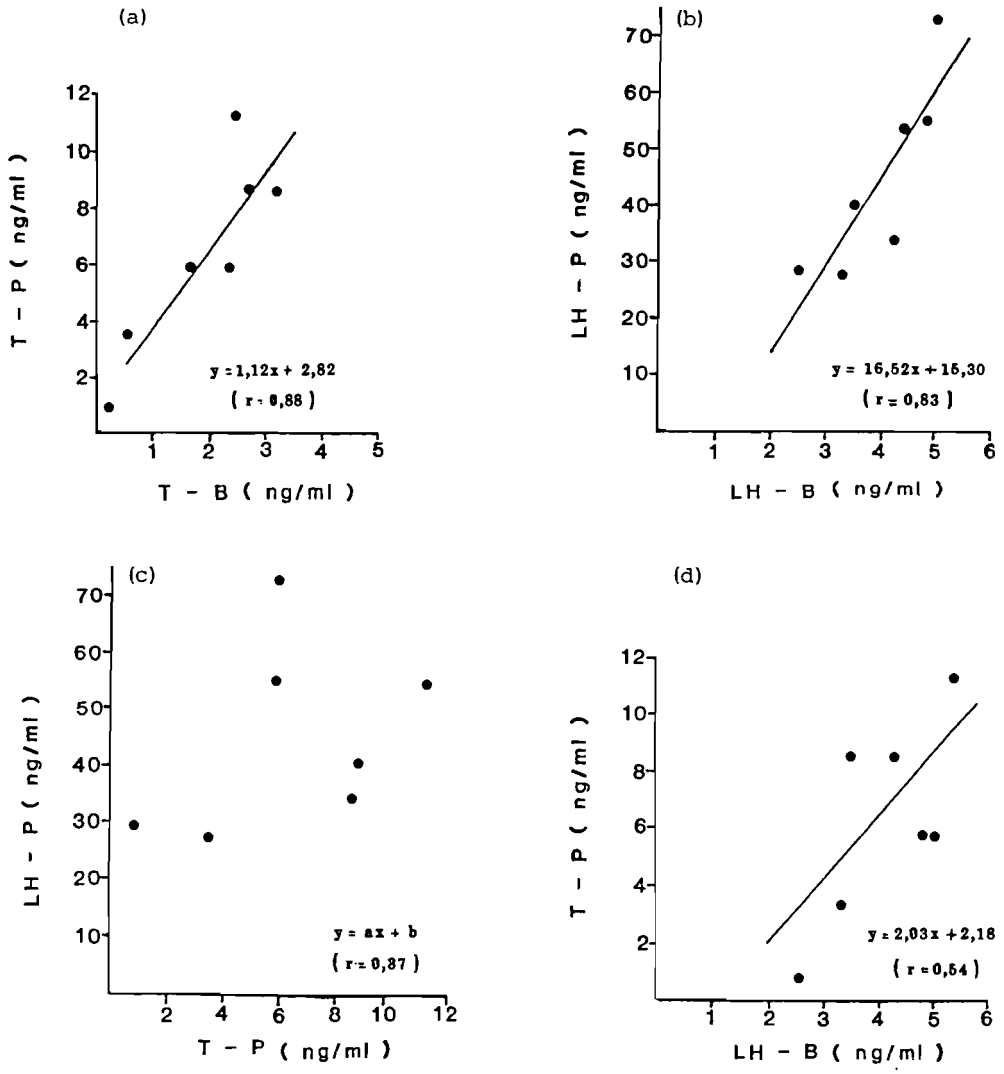

Figure 2 The relationship between (a) basal (T-B) and peak (T-P), testosterone, (b) basal (LH-B) and peak (LH-P) luteinizing hormone, (c) peak testosterone (T-P) and peak luteinizing hormone (LH-P), and (d) basal luteinizing hormone (LH-B) and testosterone peak (T-P) levels in Cape procupine males exposed to GnRH stimulation.

and baseline LH values also were not significantly related to peak testosterone levels ($r = 0,54$; $p > 0,05$) (Figure 2d). In the adult male, in which testosterone levels did not increase after GnRH stimulation, LH levels after stimulation followed a trend similar to that observed in other adults (Figure 1e).

Discussion

All males investigated during the present study failed to show LH and testosterone pulsatility similar to that described for other non-sedated male mammals (see Katongole *et al.* 1971) over the sampling period. This may be ascribed to the effect(s) of anaesthetization and GnRH stimulation on endocrine functions (see Puri, Puri & Kumar 1981). The decline in testosterone levels before GnRH stimulation and the significant differences in testosterone levels before and after control treatment may also be ascribed to the continued effect(s) of anaesthetization as has been described for rams (Illius, Haynes, Lamming, Howles, Fairall & Millar 1983). Baseline plasma testosterone levels reported here (0,20 to 3,1 ng/ml), however, are similar to those recorded in samples obtained from non-sedated, culled, free-ranging adult male Cape porcupines (see Van Aarde & Skinner 1986).

Our results demonstrate that an intravenous injection of ~40 μg GnRH was sufficient to induce a rapid rise in circulating LH and testosterone levels in male Cape porcupines. In most males GnRH stimulation was followed by a gradual increase in testosterone levels and peak levels were three times higher than baseline levels. This agrees with the results obtained by Flavo, Buhl, Reimers, Foxcroft, Dunn & Dziuk (1975) in rams when using 100 μg GnRH. As in rams (Flavo *et al.* 1975), spotted hyaenas *Crocuta crocuta* (Lindeque, Skinner & Millar 1986), and pigs *Sus scrofa* (Pomerantz, Ellendorf, Elsaesser, Konig & Smidt 1974), testosterone peaked about 90 min after GnRH stimulation in five of the six adult male porcupines studied. However, in cheetahs *Acinonyx jubatus*, peaks in testosterone occurred 20 min after stimulation (Wildt, Meltzer, Chakraborty & Bush 1984), in domestic cats *Felis catus*, 60 min thereafter (Goodrowe, Chakraborty & Wildt 1985) and in bulls 2,5 h after stimulation (Kesler & Gaverick 1977). The magnitude and time course of circulating LH (and thus conceivably of testosterone) is known to be affected by the dose of GnRH (see Mongkonpunia, Hafs, Convey, Tucker & Oxender 1975), and the intraspecific differences mentioned here probably result from different dosages.

From the present study it is apparent that in the porcupine the pituitary's response to GnRH stimulation can vary between individuals but that the temporal sequences are very similar. The significant positive relationship between baseline and peak LH values, however, suggests that the pituitary's response to exogenous GnRH is affected by the secretory status thereof. The positive relationship between baseline LH levels and peak testosterone levels, and between baseline and peak testosterone values after GnRH stimulation suggests that the hormonal milieu of the individual also affects its response to exogenous GNRH.

The lack of a testosterone response in one of the adult males after GnRH stimulation, in spite of a positive response of the pituitary (see Figure 1e), may be due to testicular dysfunction. This is supported by the observation that, although housed with a female of proven fertility, this male has not sired an offspring since 1986, 12 months before the experiment began.

The significant linear relationships between baseline testosterone, and LH levels after anaesthesia before GnRH stimulation and peak levels after GnRH stimulation suggest that baseline values determine the peak response to exogenous GnRH. The variability recorded by Van Aarde & Skinner (1986) in free-ranging males may thus be representative of the real situation. However, relationships between values in initial samples and peak values after GnRH stimulation may be affected by other variables such as the specific immobilization agent or the time lapse between sedation and the first sample to be collected.

Gonadotrophin releasing hormone stimulation of anaesthetized porcupines, leads to a rise in testosterone and LH, with peaks occuring at 90 and 120 min respectively after injection. Samples collected 90–120 min after GNRH stimulation are therefore adequate in studies of the pituitary-gonad axis in relation to, for instance, social status, reproductive status, and pubertal development.

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