Plasma concentrations of testosterone and LH in the male dog*

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Summary. Blood samples were withdrawn every 20 min from 3 conscious intact and 2 castrated mature males during non-consecutive periods of 12 h during the light and dark phases of the lighting schedule (intact dogs) and of 11 h during the light period (castrated dogs). In the intact dogs testosterone concentrations ranged from 0.4 to 6.0 ng/ml over the 24-h period. LH concentrations varied from 0.2 to 12.0 ng/ml. In all animals, LH peaks were clearly followed, after about 50 min, by corresponding testosterone peaks, but no diurnal rhythm could be established. LH concentrations in the castrated dogs were high (9.8 ± 2.7 (s.e.m.) ng/ml), and still showed an episodic pattern in spite of the undetectable plasma testosterone levels.

Introduction

A limited number of studies exist in the literature concerning the mechanisms regulating the neuro-endocrine axis of the male dog (Jones & Boyns, 1974, 1976; Jones, Baker, Fahmy & Boyns, 1976). Because of its size and blood volume, the dog is potentially a suitable research model for studies requiring repeated blood sampling over extended periods of time. Moreover, it is docile, easy to handle and a good surgical risk. The present investigation was undertaken as a prelude to further studies of the hypothalamo–pituitary–testicular axis in dogs and the objectives of the study were (a) to examine whether 24-h variations of plasma LH and testosterone concentrations exist in the dog; (2) to examine the relationship between LH and testosterone; and (3) to determine the effects of castration on LH release.

Materials and Methods

The dogs used were mature, healthy mongrels weighing 21–27 kg. They were housed individually in the SIU-C Vivarium in an artificial 12 h light: 12 h dark (lights on 07:00 h, C.D.T.) lighting schedule; bitches were not present in the same building. Water and dog chow were constantly available.

Surgical procedures

Dogs were fasted for 24 h before surgery. Anaesthesia was induced intravenously with sodium pentobarbital (1 g/ml; Holmes Serum Co., Inc., Springfield, Illinois) at a dose of 1 ml/2.3 kg body weight. Under sterile conditions, the jugular vein was cannulated with a sterile, premeasured length of Silastic tubing (i.d. 4.8 mm, o.d. 7.9 mm: Dow Corning Corp., Midland, Michigan) which was externalized at the back of the neck and secured by means of an encircling Teflon disc (5 cm diam.) and then sutured onto the skin. The incision was treated with antibacterial powder (Furacin: Eaton

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Veterinary Laboratories, Norwich, New York) and then sutured. Castrations were effected via a scrotal incision. Post-operative care consisted of cleansing and dressing of surgical wounds and intramuscular injections of 2 ml Flo-Cillin (300 000 u/ml: Bristol Laboratories, Syracuse, New York) for at least 4 days. Dogs were given a minimum of 7 days recuperation time from surgery before experimental manipulation and no post-operative difficulties were experienced. Patency of the cannulae was maintained throughout the study by flushing with heparinized saline every 2–3 days. With these precautions dogs and indwelling cannulae were responsive to handling for up to 2 months.

Blood sampling

Animals were moved to appropriate rooms at least 1 h before initiation of blood collection and housed individually with free access to food and water throughout the sampling periods. Blood samples were taken at 20-min intervals from 3 intact dogs throughout the 12 h of the light period (07:00–19:00 h) and, 2–3 weeks later, throughout the 12 h of the dark period (19:00–07:00 h). A red light was used (25 W) during blood sampling and care was taken not to expose the dogs to any other light source. Two castrated dogs were sampled during 11 h of a light period (07:00–18:00 h). The sampling protocol consisted of an initial withdrawal of 4 ml blood from the cannula into a heparinized syringe; this was the 'residual' sample. The next sample constituted the assayable fraction (2.5 ml), and the 'residual' sample, followed by 1.5 ml heparinized saline, was returned to the animal via the cannula. Blood samples were kept on ice until centrifugation. Plasma from each sample was divided into 2 aliquots and stored at -20°C until assayed.

Testosterone assay

The double-antibody radioimmunoassay used was essentially that of Micromedix Diagnostics, Inc. (Fort Collins, Colorado) with the following modifications. The first antibody (S-250) raised in sheep to testosterone-11-BSA, was obtained from Dr G. D. Niswender and diluted 1:250 000 in 1:400 normal sheep serum (NSS). Antisheep gamma globulin (ASGG), prepared in rabbits and obtained from Miles-Yeda, Ltd (Elkhart, Indiana), had a potency of 4 mg/ml and was used at a final dilution of 1:35 in phosphate-buffered saline (PBS). These antibody dilutions bound approximately 50% of testosterone labelled with ^{125}I at C-11 (Micromedix Diagnostics, Inc.) in the absence of unlabelled testosterone. Two 100 μl aliquots of plasma for each sample were extracted in disposable culture tubes with 4 ml benzene:hexane (1:2 v/v) distilled in glass as described by Frankel, Moch, Wright & Kamel (1975). Assay tubes containing solvent were evaporated under filtered air in a water bath kept at 40°C . On Day 1 of the assay, 200 μl 0.1% PBS-gelatine, 100 μl first antibody and 100 μl ^{125}I -labelled testosterone were added to all tubes. The tubes were incubated at 4°C for at least 12 h. Then 200 μl ASGG were added to all tubes except those containing the labelled testosterone alone. After further incubation for 24 h all tubes were centrifuged, decanted, and counted for activity in a Packard Gamma Spectrometer (Model 5230) for 4 min. Control tubes containing NSS in place of the first antibody were used to assess non-specific binding.

The upper and lower limits of the standard curve were, respectively, 0.01 ng (91% bound) and 1.0 ng (29% bound). Extraction and water blanks gave an average of 31.6 ± 2.7 pg (s.e.m.) testosterone. Bitch plasma assayed alone showed an average of 80.5 ± 9.5 pg testosterone/100 μl and addition of 100 pg testosterone to 100 μl bitch plasma revealed an average of 229.5 ± 11.1 pg testosterone; the average recovery of testosterone was therefore determined to be 117.3 pg. When [1,2,6,7- ^3H]testosterone (New England Nuclear, Boston, Massachusetts) was added to 100 μl dog plasma, the mean extraction recovery of the tracer was 92%. The average interassay coefficient of variation (C.V.) was 13.8%. Standard curves from 5 assays showed a C.V. of 2% at the lowest concentration on the curve to 7% at the highest point. Cross-reactivity of 1.0 ng dihydrotestosterone (DHT), 5 α -androst-3 α ,17 β -diol and 5 α -androst-3 β ,17 β -diol with the S-250 antibody was 33%, 10% and 5% respectively. When various volumes (50, 100 and 200 μl) of plasma from the same dog were assayed, the testosterone values obtained were 4.8, 4.3 and 4.1 ng/ml.

Chromatographic separation of testosterone from DHT in dog plasma was accomplished by the

procedure outlined by Falvo & Nalbandov (1974) to establish whether a preliminary chromatographic step was necessary for obtaining reliable estimates of testosterone concentrations. Since there was a high correlation between chromatographed and unchromatographed samples, chromatography was omitted for subsequent testosterone determinations.

LH assay

Plasma LH was measured by the double-antibody radioimmunoassay of Niswender, Reichert, Midgley & Nalbandov (1969), with some modifications. Ovine LH (LER-1056-62) was iodinated for 2 min with 1 mCi ^{125}I (New England Nuclear) in 0.1 M-NaOH at a volume of 1 μl according to the method of Greenwood, Hunter & Glover (1963). The first antibody was No. 15 of G. D. Niswender which has been shown to be specific for ovine LH (Niswender, Midgley & Reichert, 1968) and validated for use in canine LH assays by Smith & McDonald (1974). Plasma samples were assayed in duplicate at 200 μl volumes for intact animals and at 100 μl volumes for castrates. LER-1685-1 was used as the canine standard at a range of 0.2 to 4.0 ng (93% bound to 18% bound, respectively). The interassay C.V. was 11%. All statistical analysis was performed by *t* test.

Results

The profiles of the peripheral plasma concentrations of testosterone and LH over a 24 h (12 h light, and a separate 12 h dark) period in the intact dogs are shown in Text-fig. 1, and the ranges and mean values are given in Table 1.

Table 1 also indicates the number of testosterone and LH peaks and the peak intervals between the various hormones. A peak was defined as an elevation in which the percentage change from nadir to maximum was at least 25%. No general pattern of peak number was seen during the light and dark sampling periods. However, the intervals between the appearance of an LH peak and a corresponding testosterone peak were relatively constant for the light (57.3 ± 8.9 min, $n = 15$) and dark (50.5 ± 2.8 min, $n = 19$) sampling periods. The interval between peaks of LH was 103.4 ± 14.2 min ($n = 17$)

Table 1. Characteristics of the plasma testosterone and LH concentrations in 3 intact male dogs during a 12 h light (07:00–19:00 h) and a non-consecutive 12 h dark (19:00–07:00 h) period

	Dog					
	B.Y.		A.K.		O.Y.	
	Light	Dark	Light	Dark	Light	Dark
Testosterone						
Conc. (ng/ml)						
Range	0.4–2.3	1.0–5.7	1.0–5.6	0.4–2.6	1.6–4.2	0.7–6.2
Mean $\pm$ s.e.m.	1.0 ± 0.9	2.3 ± 0.2	2.8 ± 0.2	1.8 ± 0.1	2.9 ± 0.1	2.3 ± 0.2
No. of peaks	4	8	5	5	8	10
Interval (mean $\pm$ s.e.m.) between peaks (min)	126 ± 24	100 ± 17	135 ± 29	128 ± 17	91 ± 15	64 ± 8
LH						
Conc. (ng/ml)						
Range	0.2–4.8	0.2–9.8	0.2–10.4	0.2–10.2	0.8–12.0	1.0–10.6
Mean $\pm$ s.e.m.	1.3 ± 0.2	3.3 ± 0.4	3.1 ± 0.6	3.5 ± 0.5	5.7 ± 0.5	4.5 ± 0.4
No. of peaks	4	10	5	6	10	8
Interval (mean $\pm$ s.e.m.) between peaks (min)	140 ± 42	80 ± 9	130 ± 23	106 ± 18	75 ± 13	80 ± 16
Interval (mean $\pm$ s.e.m.) to next testosterone peak (min)	65 ± 9	54 ± 4	40 ± 6	40 ± 0	66 ± 20	54 ± 6

Text-fig. 2. LH concentrations in the 2 castrated dogs during 11 h of the light period.

in the light period and 86.9 ± 8.2 min ($n = 23$) for the dark period, while the corresponding ones between testosterone peaks were 111.4 ± 12 min. ($n = 15$) and 90.0 ± 9.2 min ($n = 21$).

Text-figure 2 shows the profile of peripheral plasma LH in the 2 castrated dogs. The mean values ($\pm$ s.e.m.) were 7.1 ± 0.4 (2.8–12.2) ng/ml for B.L. and 12.5 ± 0.6 (4.0–19.4) ng/ml for O.M. The 5 intervals between the 6 LH peaks observed in B.L. averaged 112 ± 17 min; the 7 intervals for 8 peaks in O.M. had a mean of 69 ± 9 min. The peripheral plasma concentrations of testosterone were below the sensitivity of the assay. Comparison of the LH plasma concentrations of the 2 castrated dogs with those of the intact dogs during the same 11-h sampling period showed that both castrates had higher LH concentrations than the intact dogs ($P < 0.05$).

Discussion

The mean testosterone concentrations found in this study are in agreement with previous determinations for the dog (Folman, Haltmeyer & Eik-Nes, 1972; Tremblay *et al.*, 1972), whereas the mean LH concentrations were lower than those reported for beagle bitches (Mellin, Orczyk, Hichens & Behrman, 1976). The results of our study suggest that LH release in the male dog stimulates the testes to secrete testosterone as reported for the bull (Katongole, Naftolin & Short, 1971), eugonadal men sampled during the day (Naftolin, Judd & Yen, 1973), and the ram (Sanford, Winter, Palmer & Howland, 1974). As in these studies, peaks of LH in the 3 intact dogs were clearly followed by testosterone peaks. The approximate 50-min interval between corresponding LH and testosterone peaks

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Text-fig. 1. LH (●) and testosterone (○) concentrations in intact male dogs during sampling for 12 h during the light period (07:00–19:00h) and for 12 h during a non-consecutive dark period (19:00–07:00 h). LH values are expressed in terms of ng LER-1685-1/ml; LER-1685-1 has an LH activity of 0.025 units NIH-LH-S1/mg.

is similar to that reported for men (Judd, Parker, Rakoff, Hopper & Yen, 1974) and in anaesthetized dogs given an injection of hCG into the spermatic artery (Eik-Nes, 1971). The interval between injection of LH-RH and maximal testosterone rises in dogs is also similar—40 min (Jones *et al.*, 1976).

However, while our data do show a fluctuation of testosterone and LH in the peripheral plasma of the intact dog over a 24 h period, no diurnal rhythm could be established. The existence of diurnal cycles of plasma LH and testosterone in the male is a controversial topic with evidence both for and against such patterns, particularly for testosterone. In the monkey (Goodman, Hotchkiss, Karsch & Knobil, 1974), man (Rowe *et al.*, 1974), rat (Kinson & Lieu, 1972) and miniature pig (Ellendorf *et al.*, 1975), a diurnal rhythm of testosterone secretion has been detected, whereas no such pattern was found in the rabbit (Rowe, Hopkinson, Shenton & Glover, 1975; Moor & YoungLai, 1975). It is generally agreed that a diurnal cycle for plasma LH is not present in man (Odell, Ross & Rayford, 1967; Peterson, Midgley & Jaffe, 1968; Faiman & Winter, 1971), except for a single report by Saxena, Leyendecker, Chen, Gandy & Peterson (1969). Data also exist to show an inconsistent testosterone–LH relationship in the ram (Falvo *et al.*, 1975), boar (Pomerantz, Ellendorf, Elsaesser, König & Smidt, 1974) and rhesus monkey (Goodman *et al.*, 1974). Much ambiguity exists concerning these hormonal relationships in the rat (Bartke, Steele, Musto & Caldwell, 1973).

The explanation of the hormonal fluctuations in the present study and previous ones can be attributed to a number of factors, e.g. half-life, clearance and enzyme conversion of LH during 24 h, or changes in Leydig cell sensitivity, alone or in synergism. Johnson (1974) demonstrated that when prolactin and LH were simultaneously administered to rats 1 h after the start of the dark period the increases in testicular and accessory sex gland weights were maximal. The data were considered to be suggestive of a rhythm in the sensitivity of the steroidogenic mechanism of the testis to pituitary hormone stimulation. Prolactin may also be involved in the responsiveness of dog Leydig cells. The possible involvement of FSH in the mechanics of testosterone secretion cannot be excluded. Administration of FSH in the presence of basal LH secretion in the dog resulted in stimulation of testosterone production (Eik-Nes, 1962), and a cyclic pattern of FSH coinciding with episodes of testosterone secretion has been reported for man (Faiman & Winter, 1971). Evidence for or against these possibilities awaits further investigation.

As expected, the mean concentration of plasma LH was higher in the 2 castrated dogs than in the intact animals, but in spite of the undetectable plasma testosterone concentrations castration did not alter the rhythm of LH secretion: the mean interval between LH peaks was similar to that of intact dogs during the same light period. These findings are in agreement with those reported for the rat (Gay & Sheth, 1972) and ovariectomized monkey (Dierschke, Bhattacharya, Atkinson & Knobil, 1970). Our results indicate that there is CNS-mediated control of LH secretion as well as the negative feedback effect of testosterone under normal physiological conditions. Work is now in progress to determine which testicular steroid(s) is responsible for the feedback control of LH in the male dog.

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