

Relationships between short-term variations of LH, FSH, prolactin and testosterone in peripheral plasma of prepubertal bulls

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Summary. In 2 prepubertal bulls 10-min blood samples collected during a 24-h period showed that gonadotrophin and testosterone peaks occurred regularly at intervals of 6 h in one animal and 8 h in the other. There was a clear relationship between the LH, FSH and testosterone peaks. The increase of gonadotrophin levels was followed 20 ± 6 (s.d.) min later by an increase of testosterone; the interval between the peak values was 61 ± 9 (s.d.) min. The pattern of prolactin concentrations differed; there were two prolonged elevations rather than regular peaks.

Introduction

Spontaneous pulses of circulating LH, closely followed by pulses of testosterone, have been observed in postpubertal bulls (Katongole, Naftolin & Short, 1971; Thibier & Martin, 1975; Karg *et al.*, 1976). We report here studies of prepubertal bulls in which we have measured, for the first time, FSH and prolactin as well as LH and testosterone concentrations. Prepubertal bulls were chosen as they were expected to give clearer information than would have been obtained from older animals because their basal levels of testosterone are quite low but are nevertheless able to rise in response to LH (Karg *et al.*, 1976).

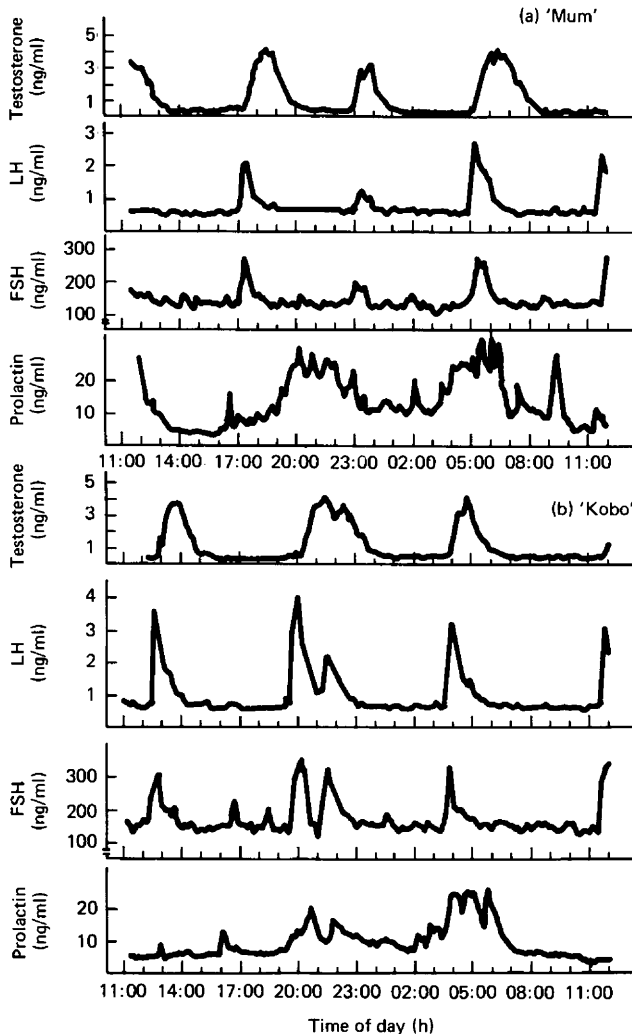
Materials and Methods

Experimental conditions and blood sampling. The experiment was performed at Nairobi (Kenya) during September. The lighting was natural (05:50–17:50 h), maximum temperature was about 23°C, minimum temperature about 10.5°C, and the mean relative humidity was 84%. Two 11-month-old British Friesian bulls were used. They were kept in a stable and fed with grass and alfalfa hay; water was always provided. The age of puberty for bulls of this breed managed in this way is 12–15 months. Additionally, prepuberty was defined according to testosterone values which were measured from birth until 2 years of age. Blood samples (10 ml) were collected, without physical contact with the animals which were in a separate room, via a long indwelling jugular catheter at 10-min intervals for a period of 24 h. Plasma was stored frozen at -20°C until assayed.

Hormone determinations. The concentrations of prolactin, LH and FSH were evaluated radio-immunologically as described by Schams & Karg (1969a), Schams & Karg (1969b) and Schams & Schallenberger (1967) respectively. In the assays for prolactin and LH the antisera showed no cross-reactions with other pituitary hormones. In the FSH assay, a slight cross-reaction with bovine LH and TSH was eliminated by adding a constant amount of LH and TSH to each tube within the assay. Testosterone was determined as originally described by Karg *et al.* (1976). However, specificity was improved by using a highly specific antiserum raised against testosterone-11-hemisuccinate-BSA; there was a negligible cross-reaction with androstanolone and androstendione. Sensitivity of the assays was 0.3 ng/ml for LH and prolactin, 35 ng/ml for FSH and 20 pg/ml for testosterone. Bovine pituitary preparations were used as the reference standards for prolactin (NIH-P-B3, biological activity 24.1 i.u./mg), for FSH (NIH-FSH-B1, biological activity 0.4 times NIH-FSH-S1) and for LH (III-17-BP, biological activity 0.9 times NIH-LH-S1).

Results

The hormonal profiles for the 2 bulls are shown in Text-fig. 1. In both animals distinct variations in concentrations of all 4 hormones were observed throughout the experiment. A very close correlation existed between testosterone, LH and FSH peaks: the increase in LH and FSH levels was followed 20 ± 6 (s.d.) min later by an increase in testosterone values. Gonadotrophin peaks (increasing for LH from 0.5–0.8 ng/ml to peak values between 1.5 and 4.0 ng/ml and for FSH from about 150 ng/ml to peak values of 200–360 ng/ml) were followed 61 ± 9 min later by testosterone peaks (basal levels of 0.2–0.5 ng/ml, maximal concentrations of 3–4 ng/ml). The elevations had a duration of 69 ± 15 min for LH, of 68 ± 17 min for FSH and of 128 ± 31 min for testosterone. Gonadotrophin and testosterone peaks occurred at regular intervals of 365 ± 33 min for bull 'Mum' (Text-fig. 1a) and of 454 ± 15 min for bull 'Kobo' (Text-fig. 1b). In contrast to these hormone profiles, prolactin concentrations were mainly characterized by two distinct elevations, lasting 188 ± 52 min during the beginning and end of the dark period and some smaller spikes lasting only 20–30 min.



Text fig. 1. Concentrations of testosterone, LH, FSH and prolactin in peripheral blood plasma of bulls 'Mum' (a) and 'Kobo' (b) during a 24-h period when blood samples were taken at 10-min intervals.

Discussion

Rhythmic variations of peripheral plasma levels for testosterone and sometimes for LH have already been observed for different species including bulls (Katongole *et al.*, 1971; Sanwal, Sundby & Edquist, 1974; Haynes, Hafs, Waters, Manns & Riley, 1975; Karg *et al.*, 1976; Thibier & Martin, 1975), rams (Katongole, Naftolin & Short, 1974; Sanford, Winter, Palmer & Howland, 1974) and boars (Claus & Giménez, 1977). In agreement with these observations, we have also demonstrated a close relationship between the occurrence of LH peaks and increases in blood testosterone for both bulls in this study.

The interval of 20 min between the start of the LH rise and the start of the testosterone rise is identical with that reported for the ram (Sanford *et al.*, 1974) but the interval of 61 min between gonadotrophin and testosterone peaks calculated in our experiment is longer than that observed for bulls by Katongole *et al.* (1971) and for rams by Sanford *et al.* (1974). One of the most striking features of our study is a clear-cut relationship between FSH (parallel to LH) and testosterone changes but presumably not between prolactin and testosterone. As found for rams (Lincoln, Peet & Cunningham, 1977; Lincoln & Peet, 1977), the peaks of FSH and LH in bulls were also concurrent. Calculations of the half-life for the two gonadotrophins in cattle gave nearly identical values of 35 min for LH (Schams & Karg, 1969c) and of 38 min for FSH (Schallenberger, 1977); however, there was additionally a second component of 127 min for FSH. The decline of the LH and FSH peaks in our study with relatively frequent sampling agrees well with these observations but contrasts with the experiments of Lincoln *et al.* (1977) who used 1 h sampling for rams.

Our results support the current concept of an identical releasing hormone for LH and FSH (Schally *et al.*, 1971). As we are not able to measure inhibin concentrations within our experiment there are no indications for a modulation of FSH secretion by other factors such as inhibin (De Jong & Sharpe, 1976; Steinberger & Steinberger, 1976). The coincidence of LH and FSH release may be related to their synergistic action on the testes. FSH is believed to stimulate the production of an androgen-binding globulin in the Sertoli cells (Hansson *et al.*, 1975), providing a vehicle for testosterone secreted from the Leydig cells under the stimulus of the LH surge.

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References

- CLAUS, R. & GIMÉNEZ, T. (1977) Diurnal rhythm of 5 α -androst-16-en-3-one and testosterone in peripheral plasma of boars. *Acta endocr., Copenh.* **84**, 200–206.
- DE JONG, F.H. & SHARPE, R.M. (1967) The sertoli cell as a possible source of inhibin. *Proc. 5th Int. Congr. Endocrinology, Hamburg*, p. 211. Brühl'sche Universitätsdruckerei, Giessen.
- HANSSON, V., WEDDINGTON, S.C., MCLEAN, W.S., SMITH, A.A., NAYFEH, S.N., FRENCH, F.S. & RITZÉN, E.M. (1975) Regulation of seminiferous tubular function by FSH and androgen. *J. Reprod. Fert.* **44**, 363–375.
- HAYNES, N.B., HAFS, H.D., WATERS, R.J., MANN, J.G. & RILEY, A. (1975) Stimulatory effect of prostaglandin F_{2 α} on the plasma concentration of testosterone in bulls. *J. Endocr.* **66**, 329–338.
- KARG, H., GIMENEZ, T., HARTL, M., HOFFMANN, B., SCHALLENBERGER, E. & SCHAMS D. (1976) Testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) in peripheral plasma of bulls: levels from birth through puberty and short term variations. *Zentbl. Vet. Med. A* **23**, 793–803.
- KATONGOLE, C.B., NAFTOLIN, F. & SHORT, R.V. (1971) Relationship between blood levels of luteinizing hormone and testosterone in bulls, and the effects of sexual stimulation. *J. Endocr.* **50**, 457–466.
- KATONGOLE, C.B., NAFTOLIN, F. & SHORT, R.V. (1974) Seasonal variations in blood luteinizing hormone and testosterone levels in rams. *J. Endocr.* **60**, 101–106.
- LINCOLN, G.A. & PEET, M.J. (1977) Photoperiodic control of gonadotrophin secretion in the ram: a detailed study of the temporal changes in plasma levels of follicle-stimulating hormone, luteinizing hormone and testosterone following an abrupt switch from long to short days. *J. Endocr.* **74**, 355–367.
- LINCOLN, G.A., PEET, M.J. & CUNNINGHAM, R.A. (1977) Seasonal and circadian changes in the episodic

- release of follicle-stimulating hormone, luteinizing hormone and testosterone in rams, exposed to artificial photoperiods. *J. Endocr.* **72**, 337-349.
- SANFORD, L.M., WINTER, J.S.D., PALMER, W.M. & HOWLAND, B.E. (1974) The profile of LH and testosterone secretion in the ram. *Endocrinology* **95**, 627-631.
- SANWAL, P.CH., SUNDBY, A. & EDQUIST, L.-E. (1974) Diurnal variation of peripheral plasma levels of testosterone in bulls measured by a rapid radioimmunoassay procedure. *Acta. vet. scand.* **15**, 90-99.
- SCHALLENBERGER, E. (1977) *Radioimmunologische Folliotropin (FSH)-Bestimmungen beim Rind-methodische Entwicklungsarbeiten und physiologische Ergebnisse*. Thesis med. vet., University of Munich.
- SCHALLY, A.V., ARIMURA, A., KASTIN, A.J., MATSUO, H., BABA, Y., REDDING, T.W., NAIR, R.M.G., DEBELJUK, L. & WHITE, W.F. (1971) Gonadotrophin-releasing hormone: one polypeptide regulates secretion of luteinizing and follicle-stimulating hormones. *Science, N.Y.* **173**, 1036-1038.
- SCHAMS, D. & KARG, H. (1969a) Radioimmunologische Bestimmung von Prolactin im Blutserum vom Rind. *Milchwissenschaft* **24**, 263-265.
- SCHAMS, D. & KARG, H. (1969b) Radioimmunologische LH-Bestimmung im Blutserum vom Rind unter besonderer Berücksichtigung des Brunstzyklus. *Acta endocr., Copenh.* **61**, 96-103.
- SCHAMS, D. & KARG, H. (1969c) Zeitlicher Verlauf und analytische Erfäßbarkeit des endogenen bzw. exogen erhöhten Blutspiegels an Luteinisierungshormon beim Rind. *Zuchthygiene* **4**, 61-65.
- SCHAMS, D. & SCHALLENBERGER, E. (1976) Heterologous radioimmunoassay for bovine follicle-stimulating hormone and its application during the oestrous cycle in cattle. *Acta endocr., Copenh.* **81**, 461-473.
- STEINBERGER, A. & STEINBERGER, E. (1976) Evidence for a FSH-inhibiting Sertoli cell-factor. *Proc. 5th Int. Congr. Endocrinology, Hamburg*, p. 211, Brühl'sche Universitätsdruckerei, Giessen.
- THIBIER, M. & MARTIN, B. (1975) Relation entre les protéines de liaison de la testostérone et les concentrations plasmatiques de testostérone et de LH chez le jeune taurillon. *C. r. hebd. Séanc. Acad. Sci., Paris D* **281**, 827-830.

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