The temporal relationship between plasma levels of FSH and LH in the ram

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Summary. Blood samples were collected from adult Soay rams at frequent intervals during the regressed, developing and active phases of the sexual cycle, or after i.v. injections of synthetic LH-RH or standard preparations of ovine FSH and LH, and were assayed for FSH, LH and testosterone.

The highest FSH and LH levels occurred during the developing stage of the cycle, and the highest testosterone levels during the active stage. At each phase there were conspicuous short-term fluctuations in the concentrations of all three hormones, and episodic peaks of plasma LH were associated with increases in both FSH and testosterone. Synthetic LH-RH (5 µg) induced a rise in the plasma values of FSH and LH at all stages and the magnitude and duration of the response could be predicted from a knowledge of the endogenous short-term fluctuations. When 50–250 ng LH-RH were given i.v. plasma LH increased in a way similar to that occurring spontaneously. The ratio of FSH:LH released after LH-RH changed with the stage of the sexual cycle, but the clearance rate of the two hormones was not affected.

These findings are consistent with the control of FSH and LH by a single releasing hormone which is secreted in pulses. The different temporal patterns in the circulating FSH and LH result from differences in secretion and clearance.

Introduction

LH-RH induces the release of FSH and LH in the ram (Hopkinson, Pant & Fitzpatrick, 1974) and there is some evidence that in the natural situation this single releasing hormone controls the secretion of both gonadotrophins (Lincoln & Peet, 1977). Modulation of the action of LH-RH at the level of the pituitary may account for the differences that exist between FSH and LH secretion during such reproductive events as puberty (Lee *et al.*, 1976) and the seasonal sexual cycle (Lincoln, Peet & Cunningham, 1977), although the existence of other hypothalamic peptides which differentially control the two gonadotrophins remains a possibility.

The present study describes the natural secretory pattern of FSH and LH in rams during three stages of the sexual cycle.

Materials and Methods

Nine adult Soay rams were housed under artificial lighting conditions of alternating 4-month periods of long days (16 h light: 8 h darkness; 16L:8D) and short days (8L:16D) which resulted in a series of 'seasonal' cycles in testicular activity at intervals of 32 weeks (Lincoln & Davidson, 1977). Routine measurements of testicular size made it possible to distinguish three stages in the sexual cycle (Lincoln, 1976), namely 'regressed' (testes fully involuted), 'developing' (testes growing rapidly) and 'active' (testes fully enlarged), and the experiments were performed at these three stages.

Serial blood sampling. All rams were bled from an indwelling jugular catheter (Lincoln *et al.*, 1977) at 15- or 20-min intervals for 8 h at the 'regressed' (sampled on two occasions in separate cycles), 'developing' and 'active' stages of the sexual cycle. Collections were begun at 08:00–09:00 h in the daylight phase of the 24-h period, 'lights on' occurring at 08:00 h.

LH-RH tests. At the end of the serial blood sampling periods, each animal was given a single i.v.

injection of 5 µg synthetic LH-RH (I.C.I.) via the jugular cannula. Blood samples were collected at 0, 10, 20, 30, 45, 60, 75, 90, 105 and 120 min after the injection.

On a separate occasion during the 'developing' stage of the sexual cycle the rams (groups of 4) were given a single i.v. injection of 10, 50, 250 or 1000 ng synthetic LH-RH, and blood samples collected by vacutainer at -10, 0, 15, 30, 50, 75 and 120 min relative to the timing of the LH-RH injection.

LH/FSH clearance study. At two stages of the sexual cycle ('regressed' and 'active') all rams received single i.v. injections of 200 µg FSH (NIH-FSH S9) and 40 µg ovine LH (NIH-LH-S19) 1 week apart. Blood samples were collected with vacutainers at 0, 15, 45, 75, 120, 180, 240, 300 and 360 min after the FSH injection and 0, 15, 30, 45, 60, 75, 90 and 150 min after the LH injection. Metabolic clearance rate (MCR) for both hormones was calculated from the area under the decay curve (Tait, 1963; Rizkallah, Gulpide & Vande Wiele, 1969).

In all experiments the blood plasma was separated and stored at -20°C until the levels of FSH and LH were determined by radioimmunoassay. The FSH assay (Lincoln *et al.*, 1977) showed a significant cross-reaction with ovine LH (8.5%) but minimal cross-reactions with other pituitary hormones, while the LH assay (Scaramuzzi, Caldwell & Moor, 1970) showed a 4% cross-reaction with ovine FSH (Martensz, Baird, Scaramuzzi & Van Look, 1976) but was otherwise specific. All samples were assayed in duplicate except for the serial samples in which FSH measurements were performed in quadruplicate. For the LH assay, the sensitivity was 0.05–0.075 ng/tube and the intra- and inter-assay coefficients of variation were 8.1 and 12.0% respectively: for the FSH assay, the corresponding values were 0.8–1.8 ng/tube and 4.9 and 6.4%. Most of the blood samples from the study were also assayed for testosterone by the method described by Corker & Davidson (1977).

Results

Serial blood samples

The plasma levels of FSH, LH and testosterone changed markedly with the stage of the sexual cycle: gonadotrophin levels were highest in the 'developing' and 'active' stages (Text-fig. 1). Short-term fluctuations characteristic of episodic secretion were apparent at each time, and analysis of the plasma profiles for all animals revealed that the variations for FSH and LH were temporally linked (Text-fig. 2). For example, at the time of each plasma LH peak there was a rise in plasma concentrations of FSH. The peak values of FSH were not always precisely synchronized with those of LH and could be delayed (e.g. 'active' stage, Text-figs 1 and 2), or absent although there was an overall elevation in the plasma FSH levels lasting over an hour (e.g. 'developing' stage, Text-figs 1 and 2). At the time of maximum FSH secretion there were often marked fluctuations in plasma FSH which were independent of episodic LH peaks (Text-fig. 1).

All increases in plasma levels of testosterone were associated with increases in plasma LH, peak values occurring 40 min ('active' stage) to 160 min ('regressed' stage) after the LH peak. The magnitude of the testosterone change varied dramatically with the stage of the cycle, being greatest at the 'active stage' and least at the 'regressed' stage (Table 1).

LH-RH tests

The injection of 5 µg synthetic LH-RH stimulated an increase in the plasma concentrations of FSH and LH at all 3 stages of the sexual cycle, although the magnitude and duration of the response varied (Text-fig. 3). The ratio of FSH:LH release, as calculated from the response to exogenous LH-RH, also changed with the stage of the cycle (Table 2): the relatively larger increase in FSH occurring in the 'developing' and 'active' stages resulted in the FSH:LH ratios in these stages being significantly different from those in the 'regressed' stage ($P < 0.01$, Student's *t* test). Gonadotrophin secretion was most responsive to LH-RH in the 'developing' stage, and this was also the time when endogenous gonadotrophin secretion was maximal; there was a correlation between the magnitude of the response to LH-RH and the magnitude of the episodic FSH and LH fluctuations (compare Text-figs 2 and 3).

Table 1. Plasma levels (mean $\pm$ s.e.m.) of LH and testosterone (ng/ml) after a natural or induced rise in circulating LH levels at 3 stages of the sexual cycle of the Soay ram (see text)

Origin and measure of LH stimulus and testosterone response	Stage of sexual cycle		
	'Regressed'	'Developing'	'Active'
Spontaneous episodic release			
Plasma LH increase	4.3 $\pm$ 0.8	5.1 $\pm$ 0.7	1.1 $\pm$ 0.2
Plasma testosterone increase	2.7 $\pm$ 0.6	5.2 $\pm$ 0.9	14.8 $\pm$ 3.5
Time to peak testosterone (min)	50 $\pm$ 5	41 $\pm$ 5	33 $\pm$ 6
LH injection (40 μg NIH-LH-S19)			
Plasma LH increase	14.1 $\pm$ 0.7	—	14.5 $\pm$ 0.7
Plasma testosterone increase	2.0 $\pm$ 0.2	—	23.9 $\pm$ 3.7
Time to peak testosterone (min)	72 $\pm$ 6	—	48 $\pm$ 6
LH-RH injection (5 μg synthetic)			
Plasma LH increase	14.0 $\pm$ 1.7	18.0 $\pm$ 3.8	7.2 $\pm$ 1.0
Plasma testosterone increase	3.0 $\pm$ 0.3	4.3 $\pm$ 0.8	12.9 $\pm$ 3.1
Time to peak testosterone (min)	>120	77 $\pm$ 10	60 $\pm$ 4

Text-fig. 1. Plasma levels of FSH (○), LH (●) and testosterone (—) in blood samples collected from one Soay ram (S6) sampled at 15- or 20-min intervals for 8 h at 3 stages of the sexual cycle (see text). The LH peaks are numbered consecutively for each study period (▼, solid arrows) and the corresponding peaks of FSH (▽, open arrows) and testosterone (*, asterisks) are shown.

Text-fig. 2. Plasma levels (mean $\pm$ s.e.m.) of FSH and LH in serial blood samples of 9 Soay rams bled at 15- or 20-min intervals at 3 stages of the sexual cycle (see text). Hormone profiles for all animals were scanned for LH peaks occurring between 1 h after start of sampling and 2 h before the end of sampling; all profiles were then synchronized about the plasma LH peak (0 h) and the mean values of LH and FSH were determined. The values are expressed as an incremental increase from the mean ($\pm$ s.e.m.) pre-peak values, which were 10.4 ± 1.4 , 46.3 ± 8.3 and 32.4 ± 2.1 ng FSH/ml and 0.50 ± 0.05 , 0.60 ± 0.03 and 0.70 ± 0.04 ng LH/ml for the 'regressed' ($n = 7$), 'developing' ($n = 7$) and 'active' ($n = 15$) stages respectively.

Table 2. Plasma FSH:LH ratios in blood samples collected from 9 rams at 3 stages of the sexual cycle (see text) following an i.v. injection of 5 μ g LH-RH or at the time of an episodic LH peak

	Stage of sexual cycle		
	'Regressed'	'Developing'	'Active'
Incremental increase*			
Induced release	1:2.2	1:1.2	1:1.0
Spontaneous episodic release	1:2.0	1:0.4	1:0.4
Area beneath curve†			
Induced release	1:2.2	1:1.2	1:1.3
Spontaneous episodic release	1:1.4	1:0.3	1:0.3

* Calculated from the maximum levels of FSH and LH achieved minus the levels before treatment or the episodic peak.

† Calculated from the area beneath the curve between 0 and 120 min after response.

In addition, the change in the FSH:LH ratio after exogenous LH-RH was similar to that at a spontaneous episodic release (Table 2). The changes in the testosterone concentrations following LH-RH were also correlated with those occurring spontaneously (Table 1).

The rise in plasma LH levels after LH-RH was dose-related and 50–250 ng LH-RH produced LH peaks within the normal physiological range for the ram (Text-fig. 4).

Text-fig. 3. Plasma levels (mean $\pm$ s.e.m.) of FSH and LH at 3 stages of the sexual cycle (see text) in 9 Soay rams given an i.v. injection of 5 μ g synthetic LH-RH (arrows). The values are expressed as incremental increases from the mean ($\pm$ s.e.m.) pre-injection levels, the values of which were 9.3 ± 0.7 , 39.0 ± 0.6 and 21.8 ± 0.3 ng FSH/ml and 0.37 ± 0.62 , 2.47 ± 0.94 and 1.13 ± 0.53 ng LH/ml for the 'regressed' ($n = 9$), 'developing' ($n = 9$) and 'active' ($n = 9$) stages, respectively.

FSH/LH clearance study

The mean ($\pm$ s.e.m.) MCR of standard preparations of ovine FSH and LH was similar in the 'regressed' and 'active' stages of the sexual cycle of the rams, being respectively 50.8 ± 3.7 and 50.7 ± 2.5 l/day for FSH and 89.7 ± 4.8 and 91.2 ± 3.6 l/day for LH.

Discussion

In practice the 8.5% cross-reaction of the FSH assay with ovine LH has little influence on FSH values since the levels of LH are generally only about 5% of those of FSH. The effect of an injection of 40 μ g LH (NIH-LH-S19), which increased circulating levels of LH from 0.6 to 15.0 ng/ml, could not be detected by the FSH assay, although the induced rise in LH was greater than any that occurred spontaneously. Conversely, the 4% interference of FSH in the LH assay seems of minor importance because the observed changes in the plasma concentrations of the two hormones seldom occurred in parallel (Text-figs 1 and 2). However, temporal relationship between the fluctuations in plasma levels of FSH and LH was apparent and could not be explained merely in terms of cross-reactions of the assays. The most logical explanation of this relationship is that it reflects the common control of FSH and LH secretion by LH-RH. The episodic peaks of plasma LH reflect episodic surges of LH-RH secretion from the hypothalamic neurones, and this stimulus also promotes FSH secretion. The close

Text-fig. 4. Plasma levels (mean $\pm$ s.e.m.) of LH in groups of 4 Soay rams at the 'developing' stage of the sexual cycle after an i.v. injection of 10, 50, 250 or 1000 ng synthetic LH-RH. The amplitudes of the naturally occurring episodic peaks of plasma LH (see Text-fig. 2) during the 'regressed' (R), 'developing' (D) and 'active' (A) stages of the sexual cycle are shown in the histograms.

similarity between the nature of the episodic increases in gonadotrophins at the 3 stages of the sexual cycle, and the response to synthetic LH-RH is consistent with this hypothesis.

A dose of 5 μ g synthetic LH-RH induced an increase in plasma levels of LH greater in magnitude and duration than any spontaneous increase in levels of LH occurring naturally; 50–250 ng provided a more physiological response. Since this quantity of LH-RH was administered peripherally into the jugular vein it follows that the concentration reaching the pituitary will be much reduced, and a peak concentration of 25–125 pg/ml would be predicted, assuming even mixing in a total blood volume of 2 l for the Soay ram (average body weight 33 kg). This low level of LH-RH may be taken as a very approximate estimate of the concentration of LH-RH achieved naturally at the time of each phase of episodic secretion. These pulses of release are presumably of short duration, but must represent the combined secretion of a large number of hypothalamic neurones discharging LH-RH simultaneously.

When the ram develops from the sexually regressed to the active stage of the seasonal cycle there is an increase in endogenous LH-RH secretion which is apparently due to an increase in frequency of episodic LH-RH release (Katongole, Naftolin & Short, 1974; Lincoln & Peet, 1977). Because LH-RH controls synthesis of FSH and LH as well as release (Schally *et al.*, 1971), it follows that pituitary synthetic activity will change over this period and this will affect the pattern of gonadotrophin release after LH-RH, causing a more prolonged secretion in the sexually active animals (Lincoln, 1976, 1977). The profile of hormones in the blood plasma of the ram at any stage therefore reflects both the short-term (response to each LH-RH pulse) and the long-term (response to the previous LH-RH pulses) effects of endogenous LH-RH secretion.

While the control by LH-RH explains the short-term relationship between the changes in the blood levels of FSH and LH, some explanation is required for the gross differences in the behaviour of the two gonadotrophins. From the results shown in Text-fig. 1 it is clear that fluctuations in FSH levels can occur independently of those of LH, and this could be taken as evidence for a separate hypothalamic control of FSH secretion. However, the simplest explanation is that there is just one

hypothalamic releasing hormone for gonadotrophin control, and the differences in the plasma profiles for FSH and LH result from the secretory behaviour of the anterior pituitary cells, compounded by a slower clearance of FSH from the blood after secretion. FSH is cleared about half as quickly as LH and therefore the short-term fluctuations in FSH are less spectacular than those for LH, although the overall increase in circulating hormone levels at times of hypersecretion (e.g. 'developing' stage) is much greater for FSH.

The temporal pattern of FSH secretion differs from that of LH after LH-RH (Text-figs 2 and 3) and this is reflected in the plasma profiles at all stages of the sexual cycle. In particular, FSH secretion increases less dramatically than LH secretion after LH-RH, but the release goes on for longer. This long-term 'leakage' by the pituitary gonadotrophs occurs to some extent for LH when the pituitary is synthetically active (Lincoln, 1977), but is much more pronounced for FSH; the effect could explain the changes in FSH levels which occur, apparently independently of those for LH, during the 'developing' stage at a time of heightened pituitary activity.

While the pituitary appears to have an intrinsic capacity to secrete FSH and LH, differently in response to LH-RH, there is the possibility that testicular steroids or inhibin also influence the pattern of secretion. Testosterone is said to selectively inhibit LH secretion (Hopkinson *et al.*, 1974) while the putative inhibin preferentially reduces FSH secretion (Franchimont, Chari & Demoulin, 1975). The present results add little to these claims, however, since the steroidogenic and spermatogenic functions of the testes in the ram change in parallel. The reduction of LH and FSH secretion in the sexually active phase could be the result of the negative feedback effects of any products from the testes.

In conclusion, it is apparent that changes in endogenous LH-RH secretion can explain the changes in FSH and LH secretion in the ram. The behaviour of the two gonadotrophins in the circulation results from the differences in secretion in response to LH-RH and differences in the rate of clearance from the blood.

References

- CORKER, C.S. & DAVIDSON, D.W. (1977) The radioimmunoassay of testosterone in various biological fluids without chromatography. *Steroid Biochem.* (in press).
- FRANCHIMONT, P., CHARI, S. & DEMOULIN, A. (1975) Hypothalamus-pituitary-testis interaction. *J. Reprod. Fert.* **44**, 335–350.
- HOPKINSON, C.R.N., PANT, H.C. & FITZPATRICK, R.J. (1974) Release of LH and FSH in the normal intact ram by synthetic LH-RF and the effect of pre-treatment with testosterone propionate. *J. Reprod. Fert.* **39**, 135–139.
- KATONGOLE, C.B., NAFTOLIN, F. & SHORT, R.V. (1974) Seasonal variation in blood luteinizing hormone and testosterone levels in rams. *J. Endocr.* **60**, 101–106.
- LINCOLN, G.A. (1976) Seasonal variation in the episodic secretion of luteinizing hormone and testosterone in the ram. *J. Endocr.* **69**, 213–226.
- LINCOLN, G.A. (1977) Changes in pituitary responsiveness to luteinizing hormone-releasing hormone in rams exposed to artificial photoperiods. *J. Endocr.* **73**, 519–527.
- LINCOLN, G.A. & DAVIDSON, W. (1977) The relationship between sexual and aggressive behaviour, and pituitary and testicular activity during the seasonal sexual cycle of rams, and the influence of photoperiod. *J. Reprod. Fert.* **49**, 267–276.
- 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.
- LEE, V.W.K., CUMMING, I.A., DE KRETZER, D.M., FINDLAY, J.K., HUDSON, B. & KEOGH, E.J. (1976) Regulation of gonadotrophin secretion in rams from birth to sexual maturity. *J. Reprod. Fert.* **46**, 1–6.
- MARTENSZ, N.D., BAIRD, D.T., SCARAMUZZI, R.J. & VAN LOOK, P.F.A. (1976) Androstenedione and the control of luteinizing hormone in the ewe during anoestrus. *J. Endocr.* **69**, 227–237.
- RIZKALLAH, T., GURPIDE, E. & VANDE WIELE, R.L. (1969) Metabolism of HCG in man. *J. clin. Endocr. Metab.* **29**, 92–100.
- SCARAMUZZI, R.J., CALDWELL, B.V. & MOOR, R.M. (1970) Radioimmunoassay of LH and oestrogen during the oestrous cycle in the ewe. *Biol. Reprod.* **3**, 110–119.
- SCHALLY, A.V., ARIMURA, A., KASTIN, A.J., MATSUO, H., BABA, Y., REDDING, T.W., NAIR, R.M.G., DEBELJUK, L. & WHITE, W.E. (1971) Gonadotrophin-releasing hormone: one polypeptide regulates secretion of luteinizing and follicle stimulating hormone. *Science*, **N.Y.** **173**, 1036–1038.
- TAIT, J.F. (1963) Review. The use of isotopic steroids for the measurement of production rates *in vivo*. *J. clin. Endocr. Metab.* **23**, 1285–1297.

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