Comparison of bioassay and radioimmunoassay data for study of changes in the pattern of LH secretion from birth to puberty in the heifer*

S. E. Dodson, M. P. Abbott† and W. Haresign

A.F.R.C. Research Group on Hormones and Farm Animal Reproduction, University of Nottingham School of Agriculture, Sutton Bonington, Loughborough, Leics LE12 5RD, U.K. and

†M.R.C. Reproductive Biology Unit, 37 Chalmers Street, Edinburgh EH3 9EW, U.K.

Summary. To define gonadotrophin secretion rates in the prepubertal heifer, 12 Hereford × Friesian heifers were blood-sampled at 15-min intervals for periods of 24 h every 4 weeks from 3 weeks of age until puberty. Radioimmunoassay of plasma LH concentrations showed that, although LH episode frequency increased with age, overall mean LH concentrations and basal LH concentrations decreased between 3 and 15 weeks of age and then increased to 35 weeks of age. The validity of these trends in relation to biological activity of plasma LH was investigated using an in-vitro Leydig cell bioassay. Samples were selected from 24-h profile bleeds of 4 heifers at 3, 7, 11, 15, 19, 27 and 39 weeks of age. No significant differences were found in the patterns of change in overall mean LH concentrations, basal LH concentrations or LH episode amplitude when comparing the estimates obtained by radioimmunoassay with those by bioassay from birth over the prepubertal period. These results indicate that the changes with age observed by radioimmunoassay are representative of changes in biologically active hormone.

Keywords: bioassay; radioimmunoassay; LH; puberty; heifer

Introduction

An initial study in the autumn-born heifer (Dodson *et al.*, 1988) showed that overall mean plasma LH concentrations (estimated by radioimmunoassay) decreased over the period between birth and 15 weeks of age and then increased towards puberty. However, no change in overall mean LH concentration, basal LH concentration, LH episode frequency or LH episode amplitude could be directly associated with the onset of puberty.

Gonadotrophins are known to exist in various forms with differing biological activities, and radioimmunoassay systems may not be able to differentiate between them (Reichert, 1971; Prentice & Ryan, 1975; Wide, 1985). The steroid environment can influence this heterogeneity, thereby controlling the type, as well as the amount, of gonadotrophin released by the pituitary (Bogdanove *et al.*, 1975; Strollo *et al.*, 1981; Wide, 1982). Several studies in the human have reported an increase in the biological activity of LH over the prepubertal period without similar changes in immunological LH activity (Neill *et al.*, 1977; Lucky *et al.*, 1980; Reiter *et al.*, 1982). Furthermore, sudden changes in the nature of the LH molecule are reported to occur at puberty in the rat (Buckingham & Wilson, 1985). In view of these reports, and the apparently high LH concentrations over the neonatal period in the heifer (Dodson *et al.*, 1988), this study was initiated to determine whether the changes in plasma LH concentrations measured by radioimmunoassay in the prepubertal heifer represent changes in the biologically active hormone.

*Reprint requests to Dr W. Haresign.

Materials and Methods

Full details of the initial study, including animals, management and blood sampling procedures, are reported elsewhere (Dodson *et al.*, 1988). Briefly, 12 autumn-born Hereford $\times$ Friesian heifers were blood sampled at 15-min intervals for 24-h periods every 4 weeks from 3 weeks of age until the onset of regular oestrous cycles. All plasma samples were assayed for LH using an homologous radioimmunoassay system.

In this study, selected samples from the initial trial were assayed for LH using an in-vitro bioassay. The number of samples which could be measured by this method was limited by the capacity of the bioassay, and so the study was restricted to 4 heifers. Short periods were chosen from 24-h sampling periods of heifers at 3, 7, 11, 15, 19, 27 and 39 weeks of age which had previously been assayed for LH using a radioimmunoassay. These ages were chosen to provide a detailed account of biological LH activity during the periods over which overall mean plasma LH concentrations, determined by radioimmunoassay, decreased (between 3 and 15 weeks of age), then increased again (from 15 weeks of age), immediately before puberty and finally after the pubertal ovulation. Each period was 3–4 h in duration and was selected to include 1–2 h of basal LH secretion followed by an LH episode.

LH radioimmunoassay. Plasma samples were assayed for LH by the specific homologous double-antibody radioimmunoassay procedure of Webb *et al.* (1977). The limit of sensitivity within this study was 0.33 ng NIH-LH-B9 equiv./ml plasma, and inter- and intra-assay coefficients of variation were 7% and 9%, respectively.

LH bioassay. Plasma concentrations of LH were measured in selected samples using the mouse Leydig cell bioassay described by Lincoln *et al.* (1986). All samples were measured in triplicate, at dilutions of 1:10, 1:20 and 1:40, by incubation with a crude preparation of mouse Leydig cells (~150 000 cells per tube) for 3–4 h at 32°C under a 95% air:5% CO₂ mixture. The resulting medium was assayed for testosterone using a tritium-based radioimmunoassay (Corker & Davidson, 1978). An ovine LH standard, NIH-LH-S18, which showed parallelism with bovine standard plasmas, was used throughout since the bovine standard NIH-LH-B9 was found not to be stable in the bioassay buffers. The limit of sensitivity of the assay using a 1:10 dilution was 0.7 ng NIH-LH-S18 equiv./ml plasma. Both the inter- and intra-assay coefficients of variation were <15%.

Analysis of data. An LH episode was defined by the following criteria, (a) an increase of at least 4 times the coefficient of variation of duplicate pairs above the preceding baseline value, (b) at least 2 points between the peak value and the succeeding trough or baseline and (c) a rate of decline in concentrations after the peak no greater than that allowed by the known half-life of the hormone. The basal LH concentration was defined as the overall mean LH concentration for a profile minus the LH episodes. LH episode amplitude was calculated as the maximum LH concentration attained, irrespective of the basal LH concentration.

Data obtained by radioimmunoassay were analysed by analysis of variance, except LH episode frequency which was tested by analysis of deviance assuming a Poisson distribution.

LH concentrations determined by radioimmunoassay and by bioassay were compared by regression analysis.

Results

LH concentrations determined by radioimmunoassay

A detailed account of these data is presented by Dodson *et al.* (1988). Overall mean LH concentrations showed significant linear ($P < 0.001$) and cubic trends ($P < 0.01$), decreasing from 0.96 ng/ml to 0.76 ng/ml between 3 and 15 weeks of age and then increasing to 1.21 ng/ml by 35 weeks of age. Basal LH concentrations showed significant quadratic and cubic trends (both $P < 0.001$), decreasing from 0.94 ng/ml at 3 weeks of age to 0.55 ng/ml at 15 weeks of age, before increasing to 0.71 ng/ml at 27 weeks of age with no further change up to 35 weeks of age. An episodic pattern of LH secretion developed in all heifers by 7 weeks of age. After the initial rapid increase, LH episode frequency increased gradually from 7 to 35 weeks of age, resulting in significant linear ($P < 0.001$) and quadratic ($P < 0.001$) components within the data. LH episode amplitude increased in a linear manner ($P < 0.001$) from 3 weeks of age.

LH concentrations determined by bioassay

Bioassay data from 2 heifers are illustrated in Fig. 1, superimposed upon the data obtained by radioimmunoassay, and illustrate that the patterns of change in LH concentrations measured by

Fig. 1. Comparison of episodic and basal LH release measured by radioimmunoassay (●) and by bioassay (○) from 2 heifers at 3, 7, 11, 15, 19, 27 and 39 weeks of age. The profiles at 39 weeks of age occurred 4 days after first ovulation (a) and 5 days after first ovulation (b).

bioassay and radioimmunoassay were similar at all ages. The same was true for the other 2 animals studied.

LH values estimated by bioassay and radioimmunoassay were highly correlated ($P < 0.001$), as illustrated in Fig. 2, and the relationship did not alter with age. The equation of best fit for the two animals illustrated in Fig. 2 was:

$$y = 0.396 + 0.517x \quad (P < 0.001, \text{d.f.} = 189; \text{R.S.D.} = 0.429)$$

For the other 2 animals not illustrated the relationship was also significant ($P < 0.001$); the regression coefficient was 0.513 compared to 0.517 and the R.S.D. was 0.458 compared to 0.429, indicating that the relationship between biological and immunological activity was similar.

Fig. 2. Relationship between LH concentrations measured by radioimmunoassay and by bioassay in the samples illustrated in Fig. 1.

Discussion

Since different standard LH preparations were used in the two assay systems, direct comparisons of absolute plasma LH concentrations cannot be made. However, since bovine standard plasmas showed parallelism with the standard LH preparation used in the bioassay system, it is possible to determine qualitative changes in plasma LH and differences in the immunoactive LH to bioactive LH ratio with age.

These results confirm that the LH secreted from birth to the peripubertal period in the heifer is biologically as well as immunologically active, the changes measured by LH radioimmunoassay reflecting changes in biological LH activity. Overall mean plasma LH concentrations, as measured by radioimmunoassay, have been shown to increase gradually from about 15 weeks of age towards puberty in the heifer (Dodson *et al.*, 1988). Dodson *et al.* (1988) concluded that the stimulus for puberty must occur very abruptly since, although the animals were sampled very close to the time of first ovulation, LH episode frequency did not reach the threshold value known to be required to stimulate the final stages of follicular maturation. The results from the present study further demonstrate that the onset of puberty in the heifer cannot be explained on the basis of a change in the bioactive to immunoactive ratio of plasma LH, similar to that seen during puberty in children (Reiter *et al.*, 1982; Marrama *et al.*, 1983). A 15-fold increase in plasma biological LH activity is reported to occur in children before adolescence, but only a 5-fold increase in plasma immunological LH activity (Reiter *et al.*, 1982), thus illustrating that both quantitative and qualitative changes in LH secretion occur at this time in the human.

This study and those of children have assessed biological LH activity using in-vitro bioassays which depend upon the ability of the LH molecule to stimulate testosterone production from dispersed mouse Leydig cell preparations. It is possible, however, that although the LH molecule in the prepubertal heifer is steroidogenically and immunologically active, it may not have the necessary active sites for the induction of ovulation. This has been demonstrated in the rat (Buckingham & Wilson, 1985); plasma LH is immunologically active and biologically active within the mouse Leydig cell bioassay during the prepubertal period, but only becomes active within an ovarian ascorbic acid bioassay at puberty. This latter assay is dependent upon the ability of LH to cause ovulation. If this same situation also exists in the heifer, puberty will occur with no apparent change in immunological

or biological activity measured using the Leydig cell bioassay as was the case in the present study. Further work using an ovarian ascorbic acid bioassay would be required to test this hypothesis.

We thank the A.F.R.C. for financial support and NIAMKDD, Bethesda, U.S.A. for standard LH and FSH preparations.

References

- Bogdanove, E.M., Nolin, J.M. & Campbell, G.T. (1975) Qualitative and quantitative gonadal-pituitary feedback. *Recent Prog. Horm. Res.* **31**, 567–626.
- Buckingham, J.C. & Wilson, C.A. (1985) Peripubertal changes in the nature of LH. *J. Endocr.* **104**, 173–177.
- Corker, C.S. & Davidson, D.W. (1978) The radioimmunoassay of testosterone in various biological fluids without chromatography. *J. Steroid Biochem.* **9**, 373–374.
- Dodson, S.E., McLeod, B.J., Haresign, W., Peters, A.R. & Lamming G.E. (1988) Endocrine changes from birth to puberty in the heifer. *J. Reprod. Fert.* **82**, 527–538.
- Lincoln, G.A., Fraser, H.M. & Abbott, M.P. (1986) Blockade of pulsatile LH, FSH and testosterone secretion in rams by constant infusion of an LHRH agonist. *J. Reprod. Fert.* **77**, 587–597.
- Lucky, A.W., Rich, B.H., Rosenfield, R.L., Fang, V.S. & Roche-Bender, N. (1980) LH bioactivity increases more than immunoreactivity during puberty. *J. Pediatr.* **97**, 205–213.
- Marrama, P., Zaidi, A.A., Montanini, V., Celani, M.F., Cioni, K., Carani, C., Morabito, F., Resentini, M., Bonati, B. & Baraghini, G.F. (1983) Age and sex related variations in biologically active and immunoreactive serum luteinizing hormone. *J. Endocr. Invest.* **6**, 427–433.
- Neill, J.D., Dailey, R.A., Tsou, R.C. & Reichert, L.E., Jr (1977) Immunoreactive LH-like substances in serum of hypophysectomized and prepubertal monkeys: inactive in an *in vitro* LH bioassay. *Endocrinology* **100**, 856–861.
- Prentice, L.G. & Ryan, R.J. (1975) LH and its subunits in human pituitary, serum and urine. *J. clin. Endocr. Metab.* **40**, 303–312.
- Reichert, L.E., Jr (1971) Electrophoretic properties of pituitary gonadotropins as studied by electrofocusing. *Endocrinology* **88**, 1029–1044.
- Reiter, E.O., Beitins, I.Z., Ostrea, T. & Gutai, J.P. (1982) Bioassayable luteinizing hormone during childhood and adolescence and patients with delayed pubertal development. *J. clin. Endocr. Metab.* **54**, 155–161.
- Strollo, F., Harlin, J., Hernandez-Montes, H., Robertson, D.M., Zaidi, A.A. & Diezfalusy, E. (1981) Qualitative and quantitative differences in isoelectric focusing profile of biologically active lutropin in the blood of normally menstruating and post-menopausal women. *Acta endocr., Copenh.* **97**, 166–175.
- Webb, R., Lamming, G.E., Haynes, N.B., Hafs, H.D. & Manns, J.G. (1977) Response of cyclic and postpartum suckled cows to injections of synthetic LH-RH. *J. Reprod. Fert.* **50**, 203–210.
- Wide, L. (1982) Male and female forms of human follicle-stimulating hormone in serum. *J. clin. Endocr. Metab.* **55**, 682–688.
- Wide, L. (1985) Median charge and charge heterogeneity of human pituitary FSH, LH and TSH. I. Zone electrophoresis in agarose suspension. *Acta endocr., Copenh.* **109**, 181–189.

Received 27 May 1987