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Investigations of Ultradian and Circadian Rhythms in the Concentration of Cortisol and Prolactin in the Plasma of Dairy Cattle

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Abstract

This experiment analysed the circadian and ultradian pattern of secretion of cortisol and prolactin in cattle, and from this a meaningful sampling technique for these hormones has been suggested.

Two Friesian and two Jersey heifers, non-pregnant and 18 months of age, were bled (2 ml blood) every 10 min for 24 h via an indwelling jugular cannula inserted 6 h before sampling commenced.

The concentration of cortisol in plasma showed an ultradian rhythm with an amplitude of approximately 30 ng/ml and a frequency of about 0·6 cycles/h. Elimination of the ultradian component of variation, by taking the mean of the rolling means (last three values) for individual heifers, indicated a diurnal variation in cortisol concentration which was high between midnight and mid-morning and low in the afternoon.

There were frequent sporadic 'bursts' of prolactin secretion but these were inconsistent. There was no evidence of diurnal variation in prolactin concentration, or of a temporal relationship between cortisol and prolactin secretion.

Introduction

Prolactin and glucocorticoids are believed to be intimately involved in the initiation and maintenance of lactation (Cowie and Tindal 1971). Supporting evidence for this comes from studies in which the concentration of these hormones in circulation were monitored at lactogenesis and in response to milking, during lactation. It is therefore surprising that in the literature there is contradiction about basal patterns of secretion of these hormones in the dairy cow.

Several authors report that the concentration of cortisol (Macadam and Eberhart 1972) and prolactin (Schams and Karg 1974; Koprowski *et al.* 1972; Karg *et al.* 1973) is high at night and early morning similar to the human (Sassin *et al.* 1972). Others have found no, or variable, changes in the daily secretion of these hormones (cortisol—Hudson *et al.* 1972; Wagner and Oxenreider 1972; prolactin—Wetteman and Tucker 1974).

These contradictions may reflect the influence of various factors which are known to effect the secretion of prolactin and cortisol. Apart from responding to milking/suckling (Johke 1969; Koprowski and Tucker 1973), the secretion of both hormones, in ruminants, is influenced by stress (Raud *et al.* 1971; Fulkerson and Jamieson, personal communications), temperature (Christison and Johnson 1972; Wetteman and Tucker 1974) and reproductive state (Edgerton and Hafs 1973; see Karg *et al.* 1973). In addition, prolactin secretion changes in response to daylength or season

(Schams and Reinhardt 1974); oestrus (Raud *et al.* 1971) and administration of various hormones (oestrogen—Fell *et al.* 1972; prostaglandins—Gray *et al.* 1979). The influence of these factors should be taken into account in any attempt to obtain meaningful basal values for these hormones. In addition, spot sampling to measure cortisol and prolactin levels in cattle could be misleading if cattle show the same wide ultradian and circadian patterns of secretion as have been seen in the sheep.

This experiment analysed the patterns of basal secretion of these two hormones in order to form a basis for assessing their quantitative role in lactation.

Materials and Methods

Experimental Procedure

Four 18-month-old, non-pregnant heifers, two Jerseys and two Friesians, were tethered in cubicles in a large shed from September 1978 and fed *ad libitum* a ration containing pasture hay and grain. After 3 weeks in this environment, a 2-ml blood sample was collected from each heifer every 10 min for 24 h from an indwelling jugular cannula (Disposable Infusion Sets, Terumo Corporation, Tokyo) inserted at least 6 h previously. The cannulae were flushed with heparinized saline after each blood sample to keep them patent. The blood samples were immediately centrifuged and the plasma stored at −16°C until analysed.

As the animals were restrained in cubicles, they could be bled at night without illumination and with minimum of disturbance. Sampling did not interfere with feeding or rumination.

On the day of blood sampling, dusk was at 1850 and dawn at 0640 h and the minimum and maximum dry bulb temperatures were 14 and 19°C, respectively. Oestrous behaviour had previously been monitored and all heifers were known to be in the luteal phase of the oestrous cycle when samples of blood were taken.

Determination of Hormone Concentration in Plasma

Cortisol was estimated, directly without extraction, by radioimmunoassay using the method described by Endocrine Sciences, Tarzana, California 91356, U.S.A. The coefficient of variation for within and between assays was 2·59 and 9·00% respectively at 30 ng/ml, with a sensitivity of 3 ng/ml.

Prolactin was determined by solid-phase radioimmunoassay by the method described by Wallace and Bassett (1970). The sensitivity of the assay was 1 ng/ml. The between-assay precision was found by assaying three standard samples in several assays which were found to be 254·00±8·75 (n = 9), 3·94±0·08 (n = 12), 8·24±0·12 (n = 14) (mean±S.E.) ng/ml prolactin with corresponding coefficients of variation of 10, 7 and 5% respectively.

Results

The profile of cortisol in plasma, over 24 h for individual heifers showed an ultradian rhythm (Figs 1*a* and 1*b* are for the Jerseys and Figs 1*c* and 1*d* are for the Friesians). The frequency of the secretory episodes was approximately 0·6 cycles/h but at some periods, particularly in the afternoon, no ultradian rhythm was descernable. Mean value for cortisol was approximately 25 ng/ml.

The existence of a diurnal rhythm in plasma cortisol becomes apparent after the rolling mean of individual heifers (previous three values), is averaged over the four heifers (Fig. 1*e*). Concentrations of cortisol were high from midnight to mid-morning, and lowest in the afternoon.

In contrast to cortisol, the fluctuations in plasma prolactin were erratic and there is no evidence of ultradian or diurnal rhythm (Figs 1*a*–1*e*). The mean value of prolactin was approximately 35 ng/ml.

There was no temporal relationship between prolactin and cortisol.

Discussion

The profile of cortisol in the plasma of heifers is similar to that previously reported in sheep (Fulkerson and Tang 1979) and primates (Hellman *et al.* 1970; Holaday *et al.* 1977), although the fluctuations were less marked. The episodic 'bursts' of cortisol in each heifer appeared to be 'in phase' initially, with the commencement of

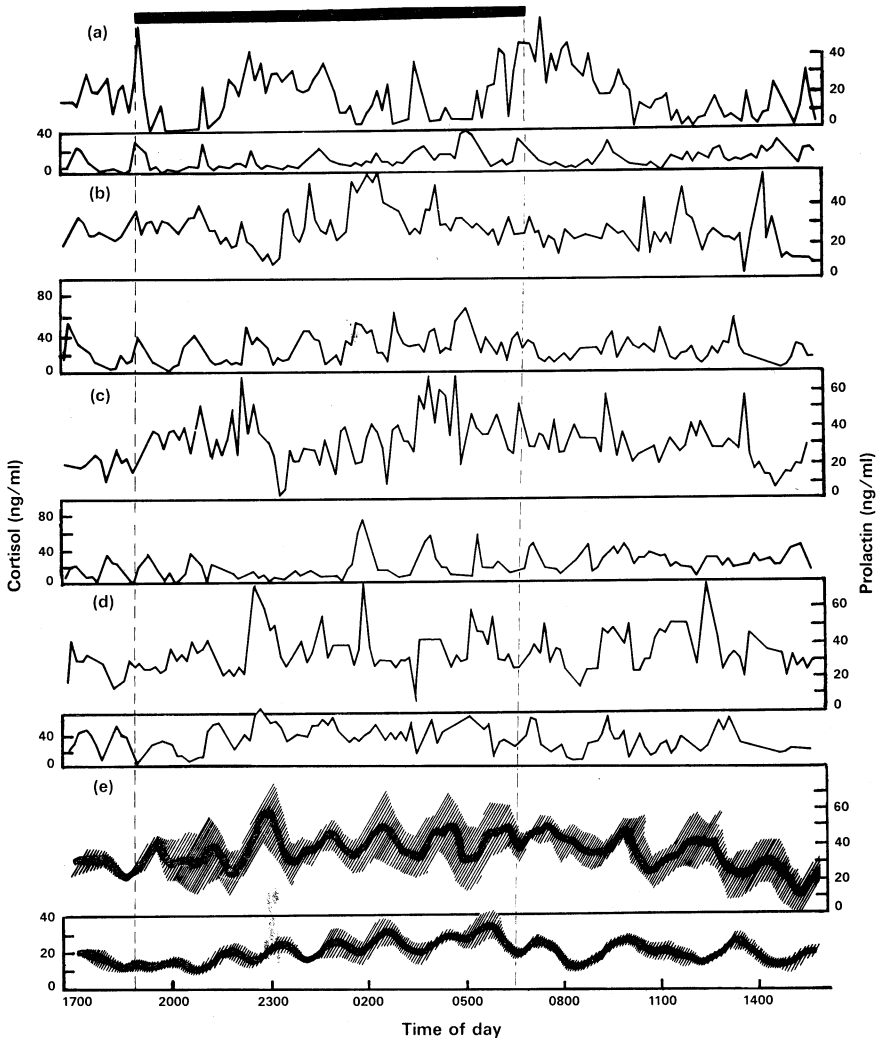

Fig. 1. Concentration (ng/ml) of prolactin and cortisol in the plasma of individual heifers (*a*, *b*, *c* and *d*) and the rolling mean for all heifers (*e*) together with the standard error which is indicated by shading. The period of darkness is shown by a bar at the top of the figure.

blood sampling as its starting point. A similar phenomenon has been observed in sheep (Fulkerson 1978). Such a pattern of secretion would be expected if cortisol were controlled by positive stimulus (ACTH)/negative feedback (of cortisol onto ACTH release).

There was a mild diurnal rhythm in cortisol secretion, with high values during darkness and the lowest values in the afternoon, and this confirms earlier studies by

Macadam and Eberhart (1972). In contrast, Wagner and Oxenreider (1972) found the lowest concentration of cortisol between 1800 and 0200 h in lactating cows although variation within animals over time was not indicated.

The changes in prolactin in circulation indicate irregular pulses in secretion with no consistent changes through the day. This contrasts with the marked diurnal changes observed in the bovine by some workers (Koprowski *et al.* 1972; Karg *et al.* 1973). However, the mean concentration in these studies tended to be higher than in ours indicating blood samples were probably taken over the summer months (long days), although the season was not specified by the authors. There is some evidence that the degree of diurnal variation in prolactin is related to season (Lincoln *et al.* 1978).

In the present experiment the influence of reproductive state (including oestrus), stress and temperature on the secretion of cortisol and prolactin were minimized while blood sampling was sufficiently frequent to detect episodic release of these hormones. We can therefore be confident that the pattern of change of these hormones in blood reported here are normal basal values.

The type of fluctuations observed in the concentration of prolactin and cortisol suggests that attention must be given to experimental design and sampling procedure. Meaningful basal levels, for cortisol in particular, can only be obtained if samples are taken at intervals of 10–20 min or if very large numbers of samples are taken at more widely spaced intervals. Conversely, results relating to changes of these hormones in circulation should be interpreted with caution.

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