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Plasma concentrations of dimeric inhibin and oestradiol in heifers undergoing superovulation with eCG or FSH

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Introduction

The secretion of FSH in female mammals is generally accepted to be regulated by ovarian oestradiol and inhibin (reviewed by McNeilly, 1988; Price, 1991). While the patterns of circulating oestradiol concentrations in cattle are well documented, those of inhibin are not. Most described assays for inhibin use antibodies raised against the α subunit and, as many forms of free or precursor α-inhibin subunit are found in bovine follicular fluid (Knight *et al.*, 1989), these assays are not valid in this species.

The development of a two-site enzyme linked immunosorbent assay (ELISA), which recognizes only the α–β dimer (the biologically active form of inhibin), has permitted the specific measurement of the inhibin dimer in human and bovine follicular fluid, and in human plasma (Groome *et al.*, 1994). We report here, for the first time, the adaptation of this ELISA for use in bovine plasma, and the detection of dimeric inhibin in cattle undergoing treatment for superovulation.

Materials and Methods

Friesian–Holstein heifers underwent standard superovulation cycles with a single i.m. injection of 2500 i.u. equine chorionic gonadotrophin (eCG) (Folligon: Intervet Canada Inc., West Hill, Ontario; $n = 5$), or with a commercial FSH preparation (Folltropin-V: Vetrepharm Inc., London, Ontario) given at 12 h intervals for four consecutive days ($n = 5$), while five heifers, which were not stimulated, served as controls. Treatments began on day 9 of a synchronized cycle, and luteolysis was induced in all heifers on day 12, by an injection of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$).

Blood samples were taken every 2 h for 60 h from the $PGF_{2\alpha}$ injection for the detection of the LH surge, and additional samples were taken every 6 h during this time for oestradiol and dimeric inhibin assay. Ovaries were collected at slaughter (5 days after the $PGF_{2\alpha}$ injection), and the ovulation rate was determined by counting the number of corpora lutea.

Concentrations of LH were estimated in a single assay as described by Price *et al.* (1987). The sensitivity was 0.2 ng ml^{-1} and the intra-assay coefficient of variation (CV) was 9%. Concentrations of oestradiol were estimated with the assay described by Bélanger *et al.* (1990) after extraction of samples (1 ml) with 10 ml 11:2 petroleum ether:ethyl acetate (mean recovery of 78%). All samples were run in a single assay, with an intra-assay CV of 12% and a sensitivity equivalent to 3.5 pg ml^{-1}.

Dimeric inhibin was quantified essentially as described by Groome *et al.* (1994). Recombinant human 32 kDa inhibin was used as the assay standard, and was diluted in a pool of plasma from ovariectomized cows. Samples and standards (150 µl) were incubated with 15 µl 10% hydrogen peroxide for 30 min, then diluted with 150 µl assay diluent. Aliquots (100 µl) were assayed in duplicate with an intra-assay CV of 6%. The interassay CV was 11% and the sensitivity (defined as the smallest quantity which could be statistically distinguished from the zero control) was 0.1 pg per well, equivalent to 2 pg ml^{-1}. Plasma samples from superovulated cows showed parallelism with the standard curve, and samples from

ovariectomized cows showed no detectable inhibin concentrations. The mean recovery of 0.2–1.3 pg recombinant inhibin in plasma from a luteal-phase cow was 110%.

The data were aligned to the time of the LH surge, and log hormone concentrations were analysed by repeated measures ANOVA, and examined for correlations with ovulation rate.

Results

Dimeric inhibin was detected in all samples from cattle undergoing superovulation, whereas inhibin was not detectable in any sample from nontreated follicular phase cows. Mean concentrations for the two treatment groups are given in Fig. 1. Repeated measures analysis of variance of log-transformed data revealed significant effects of cow and of time ($P < 0.001$). During the period before the LH surge (which occurred approximately 39 h after $PGF_{2\alpha}$ injection for both superovulated groups), dimeric inhibin concentrations were significantly higher in FSH- than in eCG-treated animals ($P < 0.05$).

Oestradiol concentrations in superovulated heifers were significantly affected by cow and by time ($P < 0.001$; Fig. 1), and there was a time by treatment interaction ($P < 0.06$), although there was no overall effect of treatment ($P = 0.15$). Mean oestradiol concentrations in nontreated controls were 5.0 ± 0.5 pg ml^{-1}.

For animals stimulated with eCG, there was a significant correlation between ovulation rate and dimeric inhibin concentrations during the period −18 to +12 h from the LH surge ($r = 0.94$; $P < 0.02$). The data were also expressed as a function of time after injection of prostaglandin; there was a significant correlation between ovulation rate and dimeric inhibin concentrations from 6 h to 36 h after $PGF_{2\alpha}$ injection ($r = 0.95$; $P < 0.02$), and a correlation between ovulation rate and oestradiol concentrations which approached significance ($r = 0.87$; $P = 0.053$). No significant correlations were detected for animals stimulated with FSH.

Discussion

This is the first report of dimeric inhibin concentrations in the blood plasma of cattle. Values varied markedly between animals undergoing superovulatory treatments, and were higher in animals stimulated with FSH than in animals stimulated with eCG. The different nature and doses of the preparations used mean that we cannot suggest which is the more potent stimulator of dimeric inhibin concentrations. The treatment giving the higher inhibin concentrations resulted in lower oestradiol concentrations, suggesting that the mechanisms controlling plasma oestradiol and inhibin concentrations may differ.

Both oestradiol and dimeric inhibin concentrations fell significantly after the preovulatory LH surge. This has been well documented for oestradiol (for example, by Callesen *et al.*, 1990), and the data for dimeric inhibin are in agreement with those for bioactive inhibin following treatment with eCG (Kaneko *et al.*, 1992). In contrast, total α-inhibin immunoactivity remained high for 7 days after the LH surge in the last study (Kaneko *et al.*, 1992). These data illustrate the danger of measuring total α-inhibin immunoreactivity in cattle in view of the high concentrations of free and precursor α-inhibin subunits found in biological fluids from a number of species (Bicsak *et al.*, 1988; Knight *et al.*, 1989, 1992). In sheep, there are no consistent changes in total α-inhibin immunoactivity between follicular and early luteal phases (Engelhardt *et al.*, 1993) although, again, these data may be confounded by the presence of nondimeric forms of inhibin.

Plasma dimeric inhibin was not detectable in nonstimulated heifers in the present study, and bioactive inhibin was not detected in control cows in the study of Kaneko *et al.* (1992). This may be due to insufficient sensitivity of the assay or the masking of epitopes by circulating binding proteins. The effects of binding proteins are likely to be minimal in the present assay configuration, as the recovery of even 0.2 pg recombinant inhibin from plasma was close to 100%. Preliminary experiments indicated that pretreatment with urea or transient acidification had negligible effects on the measured inhibin content of these plasma samples (C. A. Price, unpublished). The current assay sensitivity averaged 2 pg ml^{-1}. On the basis of the human recombinant 32 kDa standard, this represents a circulating concentration of less than 0.1 pmol l^{-1}, which is approximately 200 times lower than the mean

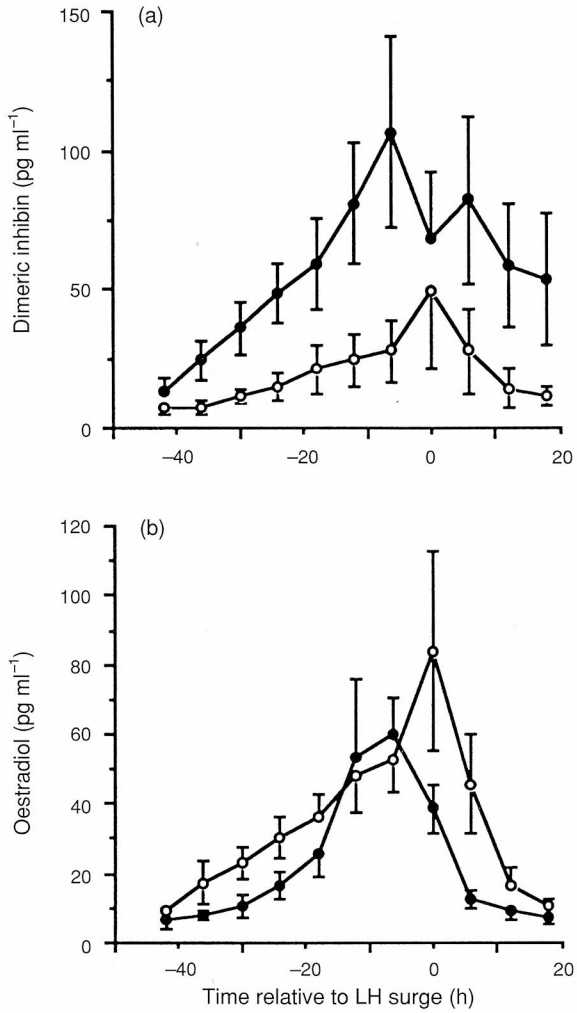

Fig. 1. Mean (± SEM) dimeric inhibin (a) and oestradiol (b) concentrations in plasma from cattle undergoing superovulation with equine chorionic gonadotrophin (eCG) (○) or FSH (●). Jugular blood samples were taken at intervals of 6 h for 60 h beginning at the initiation of luteolysis. Data are aligned to the LH surge (determined in additional blood samples taken every 2 h).

circulating concentration of oestradiol (although it should be noted that the assay may not recognize bovine inhibin as well as the human molecule; Price *et al.*, 1995). Oestradiol is probably a more potent inhibitor of FSH secretion than is inhibin (compare Henderson *et al.*, 1989, with Knight *et al.*, 1992) so, given the present data, it is difficult to envisage the physiological role for dimeric inhibin in the regulation of FSH secretion in cattle.

A strong correlation was observed between ovulation rate and preovulatory dimeric inhibin concentrations in animals superovulated with eCG, irrespective of whether the data were aligned to the time of the LH surge or the injection of $PGF_{2\alpha}$. In agreement with previous reports (for example, Callesen *et al.*, 1990), ovulation rate was closely related to oestradiol concentrations in eCG-treated animals, although only when the data were expressed relative to $PGF_{2\alpha}$ injection. Clearly, if hormone measurements are to be useful as predictors of the ovulatory response to eCG, it would be more practical

to express the data relative to the injection of $PGF_{2\alpha}$ rather than the LH surge. Large scale trials are required to verify these observations.

In conclusion, a sandwich ELISA has been devised that detects dimeric inhibin in the peripheral blood of cattle undergoing superovulation with FSH or eCG. Before the LH surge, inhibin concentrations were significantly higher in FSH- compared with eCG-stimulated cattle, whereas oestradiol concentrations tended to be higher during treatment with eCG than during treatment with FSH. These data suggest that the mechanisms regulating plasma oestradiol and dimeric inhibin concentrations differ. Inhibin concentrations were undetectable (less than 2 pg ml^{-1} or 0.1 pmol l^{-1}) in nonstimulated cattle. Preovulatory dimeric inhibin concentration may be a useful predictor of the ovulatory response in eCG-treated cows.

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