Suppression of fetal gonadotrophin concentrations by maternal passive immunization to GnRH in sheep

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The hypothalamo–pituitary–gonadal axis matures during fetal life and may be susceptible to adverse effects. Model systems can be used to understand its physiological role. The aim of this study was to determine whether antibodies to GnRH, administered to the mother, can cross the ovine fetal–placental barrier and suppress fetal gonadotrophin secretion. Maternal passive immunization to GnRH in pregnant Greyface ewes (day 103 of gestation) resulted in GnRH antibody titres of from 1:6000 to 1:9000 after 1 day and a suppression of the pulsatile secretion of fetal LH after 2 days. Fetal FSH concentrations declined gradually over the 11 days of the experiment and were only significantly different from control animals immunized against BSA in male fetuses. The slower decrease in fetal FSH concentrations than in LH concentrations shows that the secretion of FSH, unlike that of LH, is not dependent on short term changes in GnRH release. The lack of a suppressive effect of the maternal GnRH immunoneutralization on female fetal FSH secretion may be due to removal of the negative feedback effect of oestradiol and, possibly, inhibin. There was no sexual dimorphism in the effect of maternal GnRH immunoneutralization on fetal GnRH antibody titres or fetal LH secretion. These findings show that maternal passive immunization against GnRH results in GnRH antibodies crossing the fetal–placental barrier and suppressing fetal LH and FSH secretion in males, but only suppressing LH secretion in the females. Although the lack of effect on FSH secretion in the females needs to be investigated further, the present study provides evidence of a non-invasive procedure for blocking fetal gonadotrophin secretion which may be used to investigate hypothalamo–pituitary–gonadal function during early gestation in sheep.

Introduction

A number of disorders of reproduction in humans, including a reported decline in sperm counts, may be the consequence of alteration of gonadotrophin concentrations in the fetus (Jensen *et al.*, 1995). Sheep models that have been used to examine the fetal pituitary–gonadal axis have shown that inhibition of fetal gonadotrophin secretion, by use of subcutaneous implants of a GnRH agonist, can lead to reduced testicular size and fewer Sertoli cells at birth (Thomas *et al.*, 1994). However, the surgical method of implantation is potentially stressful on the fetus and can be carried out only after mid-gestation when circulating gonadotrophin concentrations are at their highest (Brooks and Thomas, 1995). A method of inhibiting gonadotrophin secretion earlier in gestation is required to investigate mechanisms of reproductive development.

Immunoneutralization of GnRH by injection of antiserum raised against the decapeptide in adult ewes and rams results in an immediate blockade of pulsatile LH secretion (Lincoln and Fraser, 1979; Fraser and McNeilly, 1983). Therefore, maternal passive immunization to GnRH may provide a non-invasive procedure for immunizing the fetus against GnRH and, hence, allow examination of how and when inhibition of fetal gonadotrophin secretion affects the normal ontogeny of the pituitary–gonadal axis. However, this system would rely on the GnRH antibodies crossing the fetal–placental barrier and being bioactive to suppress both LH and FSH.

The anatomy of the fetal–maternal interface, with complete separation of fetal and maternal circulation, provides protective advantages to the conceptus. Exchange of various components is enabled by the trophoblast which acts as a filter limiting the transfer of cells and antibodies from the mother to the fetus. Certain antibodies do gain access across the fetal–placental barrier. In particular, IgG antibodies have been shown to cross the placenta in humans, pigs and mice (Isreal *et al.*, 1995; Jauniaux *et al.*, 1995; Tuo *et al.*, 1996) and there is some evidence that IgG antibodies cross the fetal–placental barrier in sheep (Sanderson *et al.*, 1994). Therefore, an antibody to GnRH

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may be able to gain access to the fetal circulation from the maternal circulation, and subsequently suppress gonadotrophin secretion. To the authors' knowledge, the maternal–fetal transfer of GnRH antibodies has not been tested in any species. In this study, the hypothesis that maternally administered antibodies to GnRH would cross the fetal–placental barrier and suppress circulating fetal gonadotrophin concentrations was tested in sheep.

Materials and Methods

Fifteen cyclic Scottish Greyface ewes (3–5 years old, 53–68 kg body weight) were treated with progestagen-impregnated vaginal pessaries (Chronogest, Intervet Laboratories, Cambridge) to synchronize cycles. After 12 days, the pessaries were withdrawn and the ewes were given an i.v. injection of 300 iu PMSG. After 51 h, the ewes were artificially inseminated with fresh ram semen. On day 98 of gestation (term = 145 days) the jugular vein and carotid artery of the fetuses were cannulated using the method described by Brooks and White (1990). After 3 days recovery, at day 102 of gestation, serial blood samples (0.6–0.8 ml) were taken every 15 min for 6 h from the fetuses to determine pretreatment LH concentrations. Immediately after the serial sampling, a GnRH challenge (100 ng i.v. bolus injection) was given to the fetuses and blood samples were taken 15, 30, 45, 60, 90 and 120 min after challenge.

On day 103 of gestation, indwelling jugular catheters were inserted into the ewes and 4 h later, six ewes were given an i.v. infusion of 250 ml ovine antiserum to BSA and nine ewes were infused with 250 ml ovine antiserum to GnRH. All infusates were administered over 1 h.

On day 104 of gestation, serial blood samples (0.6–0.8 ml) were taken every 15 min for 6 h from the fetuses for LH analysis. Immediately after the serial sampling, a GnRH challenge (100 ng i.v.) was given to the fetuses and blood samples taken 15, 30, 45, 60, 90 and 120 min after challenge.

Daily maternal and fetal blood samples, 1 day before and for 11 days after infusion, were taken for FSH and GnRH antibody titre analysis. Of the nine cannulated fetuses whose mothers were given the antiserum to GnRH, four were male and five were female. Of the six cannulated fetuses whose mothers were given the antiserum to BSA, three were male and three were female.

Hormone assays

Plasma LH and FSH were measured in duplicate by double-antibody radioimmunoassay (McNeilly *et al.*, 1976; Martensz *et al.*, 1976). The limit of detection of the LH assays was 0.29 ± 0.07 ng ml⁻¹ (mean $\pm$ SEM). The intra-assay coefficient of variation was estimated in each LH assay using six replicates of three control samples containing 0.87 ($7.3 \pm 4.3\%$), 1.47 ($8.9 \pm 3.6\%$) and 7.85 ($7.4 \pm 2.1\%$) ng ml⁻¹. The interassay coefficients of variation were 7.6%, 12.4% and 11.2%, respectively, and the effect that this would have had on the detection of LH pulses was avoided by assaying all samples from one animal in the same run. The limit of detection of the FSH assays was 0.47 ± 0.11 ng ml⁻¹ (mean $\pm$ SEM). The intra-assay

Fig. 1. Maternal (■, $n = 9$) and combined male and female fetal (□, $n = 9$) GnRH antibody titres after maternal passive immunization against GnRH in sheep. Values are means $\pm$ SEM.

coefficient of variation was estimated in each FSH assay using six replicates of three control samples containing 0.26 ($5.9 \pm 3.4\%$), 0.47 ($4.2 \pm 1.2\%$) and 2.11 ($6.3 \pm 1.9\%$) ng ml⁻¹. The interassay coefficients of variation were 3.1%, 7.4% and 6.2%, respectively. Estimation of GnRH antibody titre was performed as described by Clarke *et al.* (1978) and was defined as the dilution of plasma binding 33% of a constant amount of ¹²⁵I-labelled GnRH.

Antiserum

The GnRH antiserum used to treat the ewes was a pool obtained from various blood samples taken during the non-breeding season from Ewe 4 immunized against GnRH conjugated to BSA (Fraser *et al.*, 1981). Specificity was directed towards the C-terminal end of GnRH, which results in no significant crossreaction when tested against a variety of peptides including TRH, somatostatin, 1–6 fragment of GnRH and 3–9 fragment of GnRH (Lincoln and Fraser, 1979; Ellis *et al.*, 1983). This antiserum was used subsequently for radioimmunoassay detection of GnRH. Control antiserum was obtained from various blood samples taken during the non-breeding season from a ewe immunized against BSA.

Statistical analysis

One-way analysis of variance was used for comparisons between treatments with no time component. Data containing a time component were tested for time and treatment effects by repeated measures analysis of variance.

Results

GnRH antibody titres

On the first day after maternal antiserum injection, GnRH antibody titres ranged from approximately 1:6000 in fetal plasma to 1:10 000 in maternal plasma (Fig. 1). By 3 days after maternal antiserum injection, both maternal and fetal plasma GnRH antibody titres had increased to approximately

Fig. 2. Effect of maternal passive immunization against GnRH (■, $n = 9$) or BSA (□, $n = 6$) on combined male and female fetal (a) LH pulse frequency, (b) LH pulse amplitude, (c) mean LH concentrations, and (d) LH response to a 100 ng challenge (i.v.) of GnRH in sheep. Values are means $\pm$ SEM. Significantly different: * $P < 0.05$; ** $P < 0.005$.

1:12 000, and remained high until day 11. There was no difference between fetal and maternal GnRH antibody titres throughout the experiment. There was also no difference in the fetal GnRH antibody titres when the results were divided into male and female groups (data not shown).

LH concentrations

There were no differences between the sexes in any of the measured parameters of LH secretion and so data were combined for statistical analysis. After maternal antiserum injection, LH pulse frequency in fetuses whose mothers had been given antiserum to GnRH had decreased by 80% compared with values on day 0 ($P < 0.005$), while there was no difference in the fetuses whose mothers had been given antiserum to BSA (Fig. 2a). Similarly, mean LH concentrations in the anti-GnRH fetuses decreased by 50% compared with values on day 0 ($P < 0.05$), while there was no difference in the anti-BSA fetuses (Fig. 2c). There was no difference by day 2 in the anti-BSA or the anti-GnRH fetuses in LH pulse amplitude (Fig. 2b) or the total amount of LH released in response to a 100 ng challenge of exogenous GnRH (Fig. 2d). There was also a suppression of LH pulse amplitude in the anti-GnRH fetuses, but this effect did not reach significance (Fig. 2b).

FSH concentrations

After injection of maternal antiserum, FSH concentrations in fetuses given both the anti-BSA and anti-GnRH antibodies

Fig. 3. Effect of maternal passive immunization against GnRH (□, $n = 9$) or BSA (■, $n = 6$) on combined male and female fetal FSH concentrations in sheep. Values are means $\pm$ SEM.

decreased, but by 11 days after injection, the concentrations in the anti-GnRH fetuses were about 30% lower than in the fetuses given anti-BSA antibodies (Fig. 3). Repeated measures analysis indicated that after injection of the maternal antiserum there was a modest effect of treatment on mean FSH concentrations ($P = 0.07$), a significant effect of time ($P < 0.05$), but no interaction between treatment and time.

There was a sexual dimorphism in the fetal FSH concentrations in response to maternal GnRH antibody administration (Fig. 4). In males, FSH concentrations in both the anti-BSA and anti-GnRH fetuses declined and, by 11 days after maternal antiserum injection, the concentrations in the anti-GnRH fetuses were about 50% lower than those in the anti-BSA fetuses. Repeated measures analysis indicated that there was a significant effect of treatment on mean FSH concentrations in males ($P = 0.05$), a modest effect of time ($P = 0.09$), but no interaction between treatment and time. In females, repeated measures analysis indicated that there was no effect of treatment or of the interaction between treatment and time on mean FSH concentrations, but a significant effect of time ($P = 0.05$). Repeated measures analysis also indicated that there was no effect of sex, time or the interaction between time and sex on FSH concentrations in the anti-BSA groups, and a highly significant effect of sex ($P < 0.005$), but not of time or the interaction between time and sex on FSH concentrations in the anti-GnRH groups.

Discussion

By cannulating maternal and fetal blood vessels in sheep, we have been able to demonstrate for the first time that maternally administered antiserum against GnRH crosses the fetal-placental barrier and acts on fetal gonadotrophs to suppress fetal gonadotrophin secretion. Titres of GnRH antibodies increased in maternal and fetal plasma in a similar time course and in a similar magnitude after a single maternal injection of GnRH antiserum. An immediate suppression of the pulsatile secretion of fetal LH and a gradual decrease in fetal FSH concentrations were associated with the increase in GnRH

Fig. 4. Sexually dimorphic response of (a) male and (b) female fetal FSH concentrations to maternal passive immunization against GnRH (■, male $n = 4$; female $n = 5$) or BSA (□, male $n = 3$; female $n = 3$) in sheep. Values are means $\pm$ SEM.

antibody titres over the duration of the experiment. Maternal passive immunization to GnRH may provide a useful, non-invasive procedure for blocking fetal gonadotrophin secretion and, hence, allowing intricate studies into the development of the fetal reproductive axis.

The finding that the decrease in fetal FSH secretion in fetuses receiving GnRH antibodies was much slower than the decrease in fetal LH secretion shows that the secretion of FSH, in contrast to secretion of LH, is not dependent on short-term changes in GnRH release. When physiological amounts of GnRH are administered in a pulsatile manner to sheep, it takes several days to modify the secretion of FSH (Lincoln, 1979). Adult ewes actively immunized against GnRH show a clear suppression of FSH secretion, but this effect takes much longer than the suppression of LH secretion in the same experiment (Clarke *et al.*, 1978). Sexual dimorphism in the response of fetal FSH concentrations to GnRH immunoneutralization was also apparent in the present findings. Maternal GnRH immunoneutralization suppressed male, but not female, fetal FSH secretion. It is possible that this may have resulted from an aberration caused by the small sample sizes after sexual segregation. Alternatively, it may have been due to differences in the negative feedback of the gonadal steroids, as experiments in adult ewes have shown that GnRH immunoneutralization suppresses ovarian oestradiol secretion

(McNeilly *et al.*, 1984). Removal of the negative feedback effect of oestradiol, and perhaps inhibin, by GnRH immunoneutralization may play a compensatory role in maintaining plasma FSH concentrations at control values in the female fetuses. However, as our group have previously shown, secretion of the ovarian steroids by fetal ovaries at day 100 of gestation is undetectable (Engelhardt *et al.*, 1995), and Mesiano *et al.* (1991) also reported a lack of effect of castration at days 110–115 of gestation on plasma FSH concentrations in females. Therefore, it is unlikely that differences in gonadal steroid feedback between males and females after GnRH immunoneutralization are involved in this effect. Further studies are needed to investigate the differences in the mechanistic pathways controlling FSH secretion in male and female fetuses.

The natural decrease in plasma FSH concentrations in the control fetuses over the 11 days of the experiment is in agreement with the decline in gonadotrophin secretion observed after mid-gestation in studies in sheep by Brooks and Thomas (1995). This decrease is related to the development of the central nervous system and the sex-steroid feedback systems, and also to an increase in placental steroid production.

The results obtained from the GnRH challenge test were equivocal. There was no difference between the anti-GnRH treated and anti-BSA control fetuses in the LH response to the GnRH challenge. It was expected that the GnRH antibodies present in the fetuses given anti-GnRH antibodies would have blocked the exogenous GnRH. The finding that the LH response in the treated fetuses was similar to that of the control fetuses may be due to the kinetics of the system. The bolus dose of 100 ng GnRH may have been too high for the antibodies present, thus allowing the peptide to couple with the receptors at sufficient concentrations to elicit a normal LH response. In addition, the LH secretory response of the pituitary gland to GnRH may also have been highly sensitive in the fetuses treated with anti-GnRH antibodies, as LH would have accumulated in the gonadotroph cells since synthesis and would have continued to do so throughout the treatment period when release was suppressed. A smaller dose of GnRH may be needed to test this system in future studies.

The success of this initial experiment suggests that this procedure should be developed for possible use in fetal studies. The effect of GnRH immunoneutralization also needs to be tested, for example, at earlier gestational ages, given that in humans the maternofetal transport system for immunoglobulins requires maturation. It has been found that immunoglobulin transfer starts during the middle of gestation and gradually increases in proportion to the gestational age (Stiehm, 1975).

In conclusion, immunoneutralization of maternal GnRH suppresses fetal LH and FSH concentrations in male sheep, and LH concentrations in female sheep. Although the lack of effect on FSH secretion in females needs to be investigated further, with larger sample sizes, this model should provide a valuable, non-invasive method to manipulate hypothalamo-pituitary-gonadal function in the developing fetus. This is of particular importance during early gestation when it is not possible to intervene via current models that involve surgical manipulation.

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