EXPERIMENTAL STUDY

The secretory patterns of growth hormone in pregnant and hysterectomized ewes

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Abstract

This work was undertaken to determine the secretory patterns of GH during pregnancy, and to evaluate the effect, if any, of hysterectomy during early pregnancy on subsequent secretion of GH in ewes. The concentrations of GH were determined in the plasma of jugular blood samples collected at 15-min intervals during a 6-h period on days 20, 40, 60, 80, 100 and 120 post-mating, and three times per week between days 29 and 120 post-mating from 5 pregnant ewes and from 5 ewes from which the gravid uterus was removed on day 30 post-mating. A pulse analysis program (Pulsar) was used to analyse the secretory patterns of GH in individual profiles of the serial sampling period. In the two groups of ewes, peripheral concentrations of GH fluctuated in an episodic manner during the frequent blood sampling of any stage of the post-mating period examined. The overall GH concentrations, the basal GH concentrations, the frequency and the amplitude of GH pulses remained fairly stable between days 20 and 120 post-mating in the two groups of ewes. The parameters of GH secretion were not different between the two groups of ewes. The secretory patterns of GH, as determined in plasma of blood collected three times per week between days 29 and 120 post-mating were also not different between the two groups of ewes.

In conclusion, results of this study show that (i) the pulsatile secretion of GH does not change as pregnancy advances, and (ii) hysterectomy performed during early pregnancy does not subsequently affect the secretory patterns of GH. These findings suggest that the gravid uterus and/or the fetoplacental unit secretory products are unlikely to be involved in the control of GH secretion during pregnancy in the ewe.

European Journal of Endocrinology 141 83–89

Introduction

In mammals, the detailed information on ovarian follicular development throughout pregnancy comes from studies in cattle and sheep (1). These investigations have shown that follicular growth decreases progressively with advancing pregnancy, and the largest follicles never exceed a diameter larger than 3 mm and 2 mm during the last month of pregnancy in ovaries of cattle and sheep respectively. In the ewe, hysterectomy performed on day 30 of pregnancy allows the maintenance of corpus luteum (CL) structures and progesterone secretion (2, 3) at least until day 120 post-mating and subsequently enhances follicular growth, which is characterized by the presence on day 120 post-mating of healthy follicles larger than 3 mm (2), without changes in follicle-stimulating hormone (FSH) and luteinizing hormone (LH) secretion (3). These findings suggest that the gravid uterus and/or the fetoplacental unit exert a suppressing action at the ovarian level, thereby preventing the development of large

healthy follicles during late pregnancy. Although the pivotal role of FSH and LH in the regulation of ovarian follicular development and function is well established in mammalian species, growth hormone (GH) may be involved in these processes. *In vitro* studies demonstrate that GH enhances gonadotrophin-induced differentiation of rat granulosa cells (4, 5) and stimulates steroidogenesis by rat (4) and human (6) granulosa cells and by perfused rabbit ovaries (7). *In vivo* treatment with GH has been shown to amplify the response of the human ovaries to gonadotrophin stimulation (8, 9). *In vivo* treatment with GH has also been shown to increase the number of follicles of 2–5 mm in heifers (10), the number of follicles of 6–15 mm in lactating cows, the size of the second largest follicle in both lactating and non-lactating cows (11) and the number of follicles of 2–3 mm in cyclic ewes (12). In hypophysectomized ewes, gonadotrophin treatment fails to stimulate follicular growth and ovulation (13) unless GH is also administered (14). During the ovine oestrous cycle, both pituitary GH mRNA and GH release peak near the time

of ovulation, suggesting a role for GH in ovarian follicular development and maturation (15).

Despite the general acceptance of the importance of GH in ovarian follicular development, the secretory patterns of GH during pregnancy have not been characterized by frequent blood sampling suitable for the determination of pulse parameters. These GH secretory patterns may be of utmost importance for our understanding of changes in ovarian follicular dynamics during pregnancy. Available data suggest that hysterectomy and uterine extract influence the synthesis and release of GH in the rat (16–18). Therefore, the aims of the present study were to determine: (i) the secretory patterns of GH during pregnancy and (ii) the effect, if any, of hysterectomy during early pregnancy on subsequent secretion of GH in ewes.

Materials and methods

Animals and management

Ewes of the Préalpes-du-Sud breed were used in this study. Oestrus was synchronized using a 14-day treatment with intravaginal pessaries containing 40 mg fluorogestone acetate (Intervet, Angers, France). After removal of the pessaries, the ewes were given 400 IU pregnant mares' serum gonadotrophin (Intervet) and were mated with fertile rams. The ewes were housed indoors under conditions of natural daylength and temperature. They were provided daily with a diet of hay, straw and concentrates and had free access to mineral licks and water.

Experimental design

On day 30 of pregnancy, 5 ewes per group were assigned at random to be either hysterectomized (Group H) or control sham-operated (Group C). General anaesthesia was induced by intravenous injection of pentobarbitone (Sanofi, Paris, France) and thiopentone (Abbott, Aubervilliers, France) and maintained by constant inhalation of a mixture of halothane and oxygen. The reproductive tract was exteriorized through a midventral incision and the CL of each ewe were marked with Indian ink for subsequent identification. Hysterectomy was performed as described previously (2). The ewes that underwent sham operation were laparotomized and the uterus and ovaries were exteriorized and manipulated. All ewes were treated with penicillin (10^6 IU per day) for 3 consecutive days after surgery. For the determination of pulsatile GH secretion, blood samples (3 ml) were collected at 15-min intervals during a 6-h period beginning at 0900 h on days 20, 40, 60, 80, 100 and 120 post-mating. Blood samples were also collected three times per week (on Monday, Wednesday and Friday) between days 29 and 120 post-mating. Blood samples were taken from the jugular vein into evacuated heparinized tubes. After centrifugation

(4000 r.p.m. for 30 min) plasma was separated and stored at -20°C until assayed for GH.

Assay of GH

Concentrations of GH were measured using a double-antibody radioimmunoassay (19). The assay sensitivity was 0.6 ng/ml. The intra- and interassay coefficients of variation were $<10\%$.

Data analysis

The Pulsar Algorithmic Program (20) for the study of pulsatile hormone secretion was used to calculate the basal concentrations, and the frequency and the amplitude of episodic GH release (pulses) in individual profiles of the serial sampling period. The G values, or number of assay coefficients of variation by which a pulse must exceed the base line were: $G(1) = 3.80$, $G(2) = 2.60$, $G(3) = 1.92$, $G(4) = 1.46$ and $G(5) = 1.13$ for pulses with 1–5 consecutive elevated points respectively. Data were subjected to two-way analysis of variance for repeated measurements using general models procedures (21). A logarithmic transformation was applied to the data to equitize variance. The number of pulses + 0.5 was subjected to square-root transformation before analysis. The median amplitude of GH pulses detected within the individual profile of each ewe was calculated, and the non parametric test of Mann and Whitney was used to compare the median of the amplitude of GH pulses between the two groups of ewes.

Results

The number of CL per ewe recorded on the day of surgery (day 30 post-mating) was confirmed at laparotomy performed on day 130 post-mating. All the ewes had 1 or 2 CL. Individual profiles of GH concentrations in plasma of jugular blood samples collected at 15-min intervals during a 6-h period on days 20, 40, 60, 80, 100 and 120 post-mating from the 5 pregnant and the 5 hysterectomized ewes are shown in Figs 1 and 2 respectively. In the two groups of ewes, the concentrations of GH fluctuated in an episodic manner. The characteristics of pulsatile GH secretion are shown in Table 1. The overall GH concentrations, the basal GH concentrations, the frequency and the amplitude of GH pulses remained fairly stable between days 20 and 120 post-mating in the two groups of ewes. None of the parameters of GH secretion were significantly different between the two groups of ewes (treatment effect, $P > 0.05$; treatment by time interaction, $P > 0.05$). Individual profiles of GH concentrations in plasma of jugular blood samples collected three times per week between days 29 and 120 post-mating from the 5 pregnant and the 5 hysterectomized ewes are shown in Fig. 3. The overall secretory pattern of GH

Figure 1 Profiles of GH concentrations in plasma of jugular blood samples collected every 15 min during a 6-h period on days 20, 40, 60, 80, 100 and 120 post-mating from 5 sham-operated pregnant ewes. Sham operation was carried out on day 30 post-mating.

was not different between the two groups of ewes. In the two groups of ewes, occasional GH secretory spikes of varying magnitude were observed. Relatively low and constant basal GH concentrations were maintained between days 29 and 120 post-mating in the two groups of ewes.

Discussion

Only limited information is available regarding the secretion of GH in pregnant sheep. Previous studies on the secretion of GH in the ewe have been restricted to measurement of GH in only one blood sample collected at irregular and at infrequent intervals during the last two trimesters (22) or the last trimester (23) of pregnancy. Others (24) measured GH in blood samples taken every hour for a 24-hour period during late pregnancy only.

Since GH is secreted episodically in mammalian species, including sheep (25), it is generally agreed that GH secretion must be studied by repeated measurement over several hours. In the present study, the secretion of GH has been well characterized by intensive blood sampling (every 15 min for a 6-h period) over relatively frequent and regular intervals throughout pregnancy. We showed that the secretion of GH is pulsatile during pregnancy in the ewe. By using frequent blood sampling, at 15-min (26) or at 5-min (27) intervals, the pulsatile secretion of GH has also been demonstrated during late pregnancy in the rat. The mean concentrations of GH and the frequency and the amplitude of GH pulses remained fairly stable between day 20 and day 120 of pregnancy (present study). These results on peripheral concentrations of GH are similar to those reported by others (28) who showed no change in

Figure 2 Profiles of GH concentrations in plasma of jugular blood samples collected every 15 min during a 6-h period on days 20, 40, 60, 80, 100 and 120 post-mating from 5 hysterectomized ewes. Surgical removal of the gravid uterus was carried out on day 30 post-mating.

Table 1 Characteristics of GH secretion in 5 pregnant ewes (Group C) and in 5 hysterectomized ewes (Group H). Hysterectomy was carried out on day 30 of pregnancy. Values for GH concentration, basal GH concentration and pulse frequency are means $\pm$ S.E.M. Values for pulse amplitude are median (0.95 confidence interval).

Parameter	Day post-mating					
	20	40	60	80	100	120
Group C						
GH conc. (ng/ml)	2.9 $\pm$ 0.5	3.1 $\pm$ 0.4	2.7 $\pm$ 0.4	2.5 $\pm$ 0.5	2.1 $\pm$ 0.5	2.4 $\pm$ 0.6
Basal GH conc. (ng/ml)	2.5 $\pm$ 0.5	2.7 $\pm$ 0.5	2.2 $\pm$ 0.4	2.3 $\pm$ 0.5	1.7 $\pm$ 0.4	2.1 $\pm$ 0.6
Pulse frequency/6 h	3.8 $\pm$ 0.2	3.4 $\pm$ 0.5	4.0 $\pm$ 0.8	3.6 $\pm$ 0.7	4.6 $\pm$ 0.7	4.0 $\pm$ 0.3
Pulse amplitude (ng/ml)	1.6 (0.8–2.4)	1.8 (1.5–2.9)	1.0 (0.5–2.2)	0.9 (0.7–1.4)	1.0 (0.7–2.7)	1.3 (0.6–1.6)
Group H						
GH conc. (ng/ml)	2.0 $\pm$ 0.3	3.9 $\pm$ 0.4	2.1 $\pm$ 0.4	2.4 $\pm$ 0.5	1.9 $\pm$ 0.3	1.4 $\pm$ 0.2
Basal GH conc. (ng/ml)	1.8 $\pm$ 0.3	2.9 $\pm$ 0.4	1.6 $\pm$ 0.3	2.1 $\pm$ 0.4	1.7 $\pm$ 0.3	1.3 $\pm$ 0.3
Pulse frequency/6 h	3.2 $\pm$ 0.5	5.2 $\pm$ 0.4	4.0 $\pm$ 0.6	4.2 $\pm$ 0.7	3.8 $\pm$ 0.5	2.8 $\pm$ 0.9
Pulse amplitude (ng/ml)	0.8 (0.3–1.3)	2.3 (1.6–4.5)	1.2 (1.0–3.0)	0.9 (0.5–1.7)	1.2 (0.7–1.3)	0.6 (0.4–1.2)

Figure 3 Profiles of GH concentrations in plasma of jugular blood collected three times per week between days 29 and 120 post-mating from 5 sham-operated pregnant ewes (Group C) and from 5 hysterectomized ewes (Group H). Sham operation (S) or surgical removal of the gravid uterus (H) was carried out on day 30 post-mating.

the pituitary content of GH with advancing pregnancy. In women, unlike the rat and the ewe, the secretion of pituitary GH progressively declines as pregnancy advances, and is abolished during late pregnancy (29, 30). In women, the placenta produces an alternative form of GH (31) which appears in the circulation at mid-pregnancy and increases thereafter up to term (29, 30). The placental GH variant is secreted continuously without pulses during late pregnancy in women (30). Placental removal by caesarean section during late pregnancy in women results in a rapid fall in peripheral GH concentrations, indicating that high levels of GH are derived from the placenta (32). In contrast, the lack of effect of hysterectomy performed on day 30 of pregnancy on subsequent secretion of GH (present study) indicates that circulating GH during pregnancy in the ewe is derived from the pituitary. A pituitary origin of GH secretion in the pregnant rat is supported by the fact that hypophysectomy results in undetectable levels of GH (27).

The present study is the first report describing plasma concentrations of GH in ewes hysterectomized during early pregnancy. Hysterectomy performed on day 30 of pregnancy had no subsequent effect on the patterns

of GH secretion, as demonstrated by the absence of differences between pregnant and hysterectomized ewes in plasma concentrations of GH. Thus, the gravid uterus and/or the feto-placental unit secretory products are unlikely to be involved in the control of GH secretion during pregnancy in the ewe. Dlamini *et al.* (33) showed that plasma concentrations of GH during late pregnancy (days 99–114) in gilts are similar to those measured at equivalent stages in unmated gilts hysterectomized on days 6–8 after estrus. Kornalijslijper *et al.* (34) showed that plasma concentrations of GH during pregnancy in goats are similar to those measured at equivalent stages in goats hysterectomized on days 32–58 of pregnancy.

On day 120 (2) and day 50 (35) after mating, the ovaries of ewes from which the gravid uteri were removed on day 30 of pregnancy contain healthy follicles >3 mm, while those of pregnant ewes do not. Since peripheral concentrations of FSH (3) and GH (present study) do not change after hysterectomy performed on day 30 of pregnancy, and are similar in pregnant and hysterectomized ewes between days 20 and 120 post-mating, the inhibitory effects of pregnancy on antral follicular development are probably not

mediated through changes in the secretion of these pituitary hormones. Evidence is available to suggest that non-steroidal factor(s) from the sheep placenta of late pregnancy inhibit directly, at the ovarian level, aromatase activity (36) and follicular growth (37).

There is evidence to indicate that the mRNA for GH receptor is abundantly localized in granulosa cells of rat (38) and ewe (39) ovarian follicles. The failure of antral follicles in hypophysectomized ewes to grow in response to exogenous gonadotrophins (13) is attributed to a reduction of mRNA for GH receptor in the membrana granulosa of follicles (39). Cattle with a GH receptor deficiency fail to maintain follicular development during the oestrous cycle (40). Thus, other factors that could also contribute to the inhibition of antral follicular growth during late pregnancy are insufficient follicular receptors for GH, that render the follicles refractory to endogenous stimulation. It remains to be elucidated if changes in ovarian follicular development during pregnancy are accompanied by variations in the follicular expression of GH receptor.

In conclusion, results of the present study show that (i) the pulsatile secretion of GH does not change as pregnancy advances, and (ii) hysterectomy performed on day 30 of pregnancy does not subsequently affect the secretory patterns of GH. Taken together, these findings suggest the gravid uterus and/or the fetoplacental unit secretory products are unlikely to be involved in the control of GH secretion during pregnancy in the ewe.

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