Prolactin as a luteotrophin during late pregnancy in pigs

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The effect of prolonged hyperprolactinaemia on the secretion of LH, progesterone and oestradiol, and its relationship to the maintenance of pregnancy was examined in pigs. Twelve crossbred, pregnant gilts were injected i.m. with 1.5 mg haloperidol kg⁻¹ body weight ($n = 6$) or vehicle ($n = 6$) once a day from day 60 to day 66 of pregnancy. Blood samples were collected at 08:00, 12:00, 16:00, 20:00, 24:00 h from day 60 to day 67 and every 15 min for 4 h (08:00–12:00 h) on days 60, 63 and 66. Plasma concentrations of prolactin were higher ($P < 0.001$) in haloperidol-treated gilts than in control gilts (121.3 ± 4.3 ng ml⁻¹ and 13.6 ± 0.4 ng ml⁻¹, respectively). Hyperprolactinaemia completely inhibited the pulsatile secretion of LH and diminished ($P < 0.001$) basal peripheral concentrations of LH (hyperprolactinaemia, 0.3 ± 0.04 ng ml⁻¹ and control, 0.6 ± 0.005 ng ml⁻¹). Despite the inhibition of LH release in hyperprolactinaemic gilts, plasma concentrations of progesterone were higher ($P < 0.001$) than in the control group (20.8 ± 0.6 and 12.6 ± 0.2 ng ml⁻¹, respectively). Oestradiol concentrations were not different between groups, although oestradiol tended to be higher in hyperprolactinaemic gilts than in the control group throughout the sampling period (29.1 ± 1.9 versus 23.7 ± 1.6 pg ml⁻¹, respectively). Abortion did not occur in any of the gilts. These results are the first to demonstrate that induced hyperprolactinaemia during the second half of pregnancy (days 60–66) will drastically suppress the major porcine luteotrophin but not affect pregnancy maintenance in pigs. It is possible that prolactin has a more important function in the luteotrophic complex as an additional protectant associated with the regulatory mechanism of late pregnancy maintenance than was previously reported.

Introduction

The function of prolactin and the role of the dopaminergic system in the regulation of gonadotrophin secretion in the maintenance of late pregnancy in pigs is still unclear. Kraeling and Davis (1974) reported that hypophysectomy during late pregnancy terminates pregnancy in pigs, but when prolactin was administered after hypophysectomy the pregnancy was maintained (Du Mesnil du Buisson and Denamur, 1969). Moreover, the greatest increase in prolactin-receptor content occurs at about day 60 of pregnancy (Jammes *et al.*, 1985). Prolactin stimulates progesterone synthesis in the corpora lutea by enhancing the uptake of lipoproteins and promoting the utilization of internalized cholesterol for progesterone synthesis (Rajkumar *et al.*, 1985). In addition, prolactin may stimulate membrane receptors for LH or for lipoproteins (Murphy and Rajkumar, 1985). These results suggest the relative importance of prolactin in the maintenance of late pregnancy in pigs. Szafranska and Ziecik (1990) proposed that there is a specific mechanism of 'substitutory compensation' as a protectant for maintenance of late pregnancy. Blocking the action of prolactin (by bromocriptine administration) causes a 10-fold rise in LH from 67 to 72 days of

pregnancy, indicating a strong relationship between LH and prolactin secretion. The resultant hypoprolactinaemia did not result in termination of pregnancy.

Hyperprolactinaemia is frequently involved in the inhibition of gonadal functions, such as in lactational anoestrus, and is commonly associated with infertility. Sortino and Wise (1989), using rat pituitary cells, reported that induction of hyperprolactinaemia *in vitro* decreased LH secretion. Thus, it appears that hyperprolactinaemia may suppress LH secretion during pregnancy, and would result in the termination of pregnancy if there is no additional, specific mechanism of protection for pregnancy.

The objective of this study was to determine the effect of induced hyperprolactinaemia (by blockade of dopamine receptors) on LH, progesterone and oestradiol secretion and how such conditions influence the maintenance of late pregnancy in pigs.

Materials and Methods

Animals and blood sampling sequence

Twelve crossbred gilts mated at second oestrus were assigned to one of two groups: hyperprolactinaemic ($n = 6$) or control ($n = 6$). An indwelling cannula was inserted into the

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jugular vein via the cephalic vein 24–48 h before treatment. Blood samples were collected every 15 min for 4 h (08:00–12:00) and at 16:00, 20:00, 24:00 h on days 60, 63 and 66 or five times a day (08:00, 12:00, 16:00, 20:00, 24:00 h) on day 61, 62, 64, 65 and 67 of pregnancy.

The plasma obtained after centrifugation (1500 g, 4°C, 10 min) from all experimental animals was stored at –20°C until prolactin, LH, progesterone and oestradiol concentrations were measured by radioimmunoassay. Prolactin and LH were determined in all samples. Progesterone and oestradiol were determined in samples taken at intervals of 1 h during the intensive bleeding periods or twice a day.

Treatment

Haloperidol was dissolved in ethanol (less than 0.05% in final dilution for injection) and the solvent was acidified by lactic acid, lightly heated until a clear solution was obtained and finally dissolved in sterile saline. Haloperidol, 1.5 mg kg⁻¹ body weight in 2 ml of vehicle (*n* = 6), was given once a day at 08:05 from day 60 until day 66 of pregnancy. Control gilts (*n* = 6) were given vehicle only at similar times.

Radioimmunoassays

Plasma concentrations of prolactin were determined by the double antibody radioimmunoassay as described by Dusza and Krzymowska (1979) with slight modifications. First antibodies from goats immunized against porcine prolactin (Research Products, Mt Prospect, IL) were used in a titre of 1:50 000. Second antibodies (goat IgG antiserum, Sigma Chemical Co, St Louis, MO), anti-γ-goat from immunized donkeys, were used in a 1:15 dilution and incubation was extended for an additional 24 h. Sensitivity of the assay was 0.12 ng ml⁻¹. Intra- and interassay coefficients of variation were 2.6% and 8.1%, respectively.

Plasma concentrations of LH were determined by the method of double antibody radioimmunoassay as described by Ziecik *et al.* (1978) with the following modifications. Primary antibodies from immunized rabbits against a conjugate of porcine LH with ovalbumin were used (B. Szafranska and A. J. Ziecik, unpublished data). The crossreactions of antiserum used with different antigens were: 0.9% for porcine GH and 0.7% for bovine thyroid-stimulating hormone but 0.0% for porcine FSH, prolactin and hCG. The period of incubation with antiserum was extended from 24 to 48 h. Sensitivity of the assay was 0.08 ng ml⁻¹. Intra- and interassay coefficients of variation were 1.7% and 4.8%, respectively.

Progesterone was determined by direct assay in plasma (Diagnostic Products Co., Los Angeles, CA). Sensitivity of the assay was 0.1 ng ml⁻¹. Intra- and interassay coefficients of variation were 4.6% and 5.0%, respectively.

Oestradiol was determined by using a standard (Hotchkiss *et al.*, 1971) extraction method (500 µl of serum, 4 ml of ethyl ether) with antibodies from immunized rabbits against a conjugate of 17-oestradiol-6-CMO-BSA (B. Szafranska and A. J. Ziecik, unpublished data) and with [2,4,6,7-³H]oestradiol (Du Pont NEN Products, Boston, MA) as tracer. The crossreactions of antiserum used with different antigens were: oestrone

0.58%, and less than 0.01% for 5α-androstane-3,17-dione, androsterone, epiandrosterone and testosterone. Any cross-reactions were determined with 5α-pregnan-3α-ol-20α-one, 5α-pregnan-3β-ol-20-one, progesterone, 4-pregnen-20α-ol-3-one, 4-pregnen-20β-ol-3-one, 5β-androstane-3α-17β-dione, 5β-androstane-3α-17β-diol, 4-androstan-11β-ol-3,17-dione and 5α-androstan-17β-ol-3-one. This antibody has been determined to have an affinity constant by Scatchard plot of 1.0×10^{10} l mol⁻¹. The antiserum was used in a titre of 1:30 000. Recovery of oestradiol in extracted samples was 88% and the sensitivity of the assay was 10 pg per tube. Intra- and interassay coefficients of variation were 3.5% and 5.3%, respectively.

Statistical analysis

Serially collected data were compared by analysis of variance (ANOVA) to establish the overall effects of treatment. Pulse analysis was performed and estimations of the duration and amplitude of LH pulse frequency were determined. Pulse amplitude was characterized as the difference between the mean concentration and the peak in blood LH concentration and pulse frequency as number of pulses in a 4 h period. An LH pulse was defined and analysed as described previously by Lejeune *et al.* (1990).

Results

Prolactin concentration increased within 15–30 min of haloperidol treatment and remained above 65 ng ml⁻¹ during the entire treatment period (Fig. 1a). Concentration of prolactin in hyperprolactinaemic gilts (121.3 ± 4.3 ng ml⁻¹) was higher (*P* < 0.001) than in the control group (13.6 ± 0.4 ng ml⁻¹) from the time of the initial haloperidol treatment until 24 h after the final treatment on day 67. Abortion did not occur in any of the gilts. Prolactin concentrations in haloperidol-treated gilts on the days of frequent sampling (days 60, 63 and 66) are given (Fig. 1b).

Haloperidol treatment strongly inhibited the release of LH (*P* < 0.001). Pulse activity of LH was almost completely inhibited and peripheral LH concentrations were decreased by 50% (0.3 ± 0.04 ng ml⁻¹ compared with 0.6 ± 0.05 ng ml⁻¹ for control group; Fig. 2). During periods of frequent sampling, one peak of LH release was observed (Fig. 3) in hyperprolactinaemic gilts (amplitude 1.65 ng ml⁻¹; duration 20 min). In the control gilts more surges were observed (mean amplitude 1.93 ng ml⁻¹; average duration 32.5 min). On days 63 and 66 of pregnancy, peaks of LH release did not occur in the hyperprolactinaemic group; however, a mean of 1.33 pulses of LH release per period during frequent sampling occurred in the control group (amplitude 1.51 ng ml⁻¹; duration 30 min).

Peripheral plasma concentrations of progesterone (Fig. 4) in hyperprolactinaemic gilts were higher (*P* < 0.001) than in the control group (20.8 ± 0.6 and 12.6 ± 0.2 ng ml⁻¹, respectively) during the entire sampling period. Oestradiol concentrations (Fig. 5) were not different (*P* > 0.05) between groups. Oestradiol concentrations tended to be higher in the hyperprolactinaemic group than in the control group throughout

Fig. 1. Mean concentrations of prolactin (ng ml^{-1}) in haloperidol-treated (▲) ($\text{SEM} = \pm 21.5$) and control (○) ($\text{SEM} = \pm 2.0$) gilts ($n = 6$): (a) from day 60 to day 67 of pregnancy, (b) during frequent sampling periods (days 60, 63 and 66). Arrows indicate time of haloperidol treatment.

Fig. 2. Mean concentrations of LH (ng ml^{-1}) in haloperidol-treated (▲) ($\text{SEM} = \pm 0.06$) and control (○) ($\text{SEM} = \pm 0.21$) gilts ($n = 6$) from day 60 to day 67 of pregnancy. Arrows indicate time of haloperidol treatment.

Fig. 3. Individual profiles of LH concentrations in haloperidol-treated ($\blacktriangle$, $\bullet$) and control ($\triangle$, $\circ$) pregnant gilts ($n = 6$).

Fig. 4. Mean concentrations of progesterone (ng ml^{-1}) in haloperidol-treated ($\blacktriangle$) ($\text{SEM} = \pm 2.25$) and control ($\circ$) ($\text{SEM} = \pm 0.84$) gilts ($n = 6$) from day 60 to day 67 of pregnancy.

the sampling period (29.1 ± 1.9 and $23.7 \pm 1.6 \text{ g ml}^{-1}$, respectively).

Discussion

This study is the first to demonstrate the effect of induced hyperprolactinaemia on maintenance of late pregnancy in pigs. The high concentrations of prolactin observed in hyperprolactinaemic gilts are usual for lactation but not for pregnancy

Fig. 5. Mean concentrations of oestradiol (pg ml^{-1}) in haloperidol-treated ($\bullet$) ($\text{SEM} = \pm 9.38$) and control ($\circ$) ($\text{SEM} = \pm 3.08$) gilts ($n = 6$) from day 60 to day 67 of pregnancy.

and were observed to inhibit LH release during days 60–67 of pregnancy. Dopamine is known to inhibit prolactin: high concentrations of dopamine are present in portal blood and DA receptors (D1 and D2) are found in the anterior and intermediate lobe of the pituitary. The dopamine pathways via tuberoinfundibular dopamine and tuberohypophysial dopamine neurones are involved in regulation of prolactin release (Benker *et al.*, 1990; Ben-Jonathan *et al.*, 1989). Administration of haloperidol, an antagonist of D1 and D2 receptors, blocks the presynaptic and post-synaptic receptors (Imazu *et al.*, 1989).

Induced hyperprolactinaemia decreases the number of rat pituitary cells secreting LH as indicated by the reverse haemolytic plaque assay (Sortino and Wise, 1989). Prolactin may also inhibit LH release via a central action, probably via prolactin–dopamine and dopamine–gonadotrophin interactions. However, the mechanism of direct interaction between gonadotrophs releasing LH and prolactin by intracellular communication or indirectly between the hypophyseal connection of neurone terminals secreting GnRH and dopamine during late pregnancy in pigs is not completely understood. Induced hypoprolactinaemia does not cause abortion (Szafranska and Zieci, 1990), despite a tenfold rise in LH concentration occurring from day 67 to day 72 of pregnancy suggesting a specific mechanism of ‘substitutory protection’ between LH and prolactin for late pregnancy protection. In this earlier study, it was hypothesized that the inhibition of prolactin by dopaminergic bromocriptine probably affected the GnRH-dependent secretion of LH at the hypothalamic level. Gonadotrophin-releasing-hormone-associated-peptide (GAP) and GnRH are found in the secretory granules of nerve terminals from the rat median eminence (Ben-Jonathan *et al.*, 1989). Nevertheless, it has been reported that GAP suppresses the release of prolactin from cultured rat anterior pituitary cells (Vacher *et al.*, 1991), whereas active immunization against GAP increases the concentration of prolactin in serum (Nikolics *et al.*, 1985). These reports have shown only contrasting relationships between prolactin and LH release. However, hyperprolactinaemia in rats increased the turnover of dopamine in the medial basal hypothalamus, decreasing the concentration of GnRH in plasma of hypophysial portal blood (Koike *et al.*, 1991).

Despite the lower concentrations of LH observed in hyperprolactinaemic gilts, plasma progesterone concentrations were higher ($P < 0.001$) in this group. Du Mesnil du Buisson and Denamur (1969) suggested that the corpora lutea of pigs in late

pregnancy requires prolactin for its maintenance. Yangfan *et al.* (1989) confirmed that prolactin is luteotrophic for ageing corpora lutea in pigs, preventing luteal regression immediately after hypophysectomy. The results reported here are supported by findings of Grinwich *et al.* (1983), who demonstrated *in vitro* that short-term incubation of prolactin-treated luteal cells from pregnant pigs killed between days 70 and 85 of pregnancy resulted in an increased progesterone accumulation in the media. Prolactin stimulation of progesterone secretion has been recognized as 'dose dependent' in humans and other species (see Murphy and Rajkumar, 1985). In addition, Wiest *et al.* (1968) suggested that prolactin suppresses the enzymatic degradation of progesterone to its metabolite, 20- α -pregn-4-ene-3-one. However, Jones *et al.* (1983) demonstrated that prolactin induces the synthesis of progesterone as well as that of its precursor, pregnenolone. Rajkumar *et al.* (1984) indicated that prolactin enhances progesterone accumulation by luteal cells induced by high and low density lipoproteins from days 74 to 76 of pregnancy in pigs, probably by increasing the uptake of lipoproteins and promoting the use of internalized cholesterol for progesterone synthesis. Prolactin as well as LH has been shown to stimulate significant increases in progesterone secretion in perfused luteal tissue from pigs, killed on day 80 of pregnancy that had previously (days 67–72) been passively immunized against LH (Szafranska *et al.*, 1992). Thus, our results suggest that prolactin may play a role in the stimulation of progesterone release during this period of pregnancy in pigs.

We observed a tendency for higher concentrations of oestradiol during the entire treatment period in hyperprolactinaemic pigs than in control gilts. Robertson and King (1974) reported that at about days 60–70 of pregnancy in pigs, unconjugated oestrone and oestradiol concentrations begin to increase to the peak values observed before parturition. Moreover, Buttle (1989) reported that in pigs between days 60 and 80 of pregnancy the fetoplacental unit, rather than the ovaries, is the major source of oestrogens. This rise is coincident with the period when it has been suggested that prolactin is the luteotrophin in pigs (Du Mesnil du Buisson and Denamur, 1968, 1969). Hyperprolactinaemia in rats (Koike *et al.*, 1991) suppresses oestrogen-induced LH release by suppressing GnRH release from the hypothalamus. In contrast, administration of oestradiol possibly affects dopamine, probably through the tuberohypophyseal system by increasing turnover and synthesis of dopamine in the median eminence, thus stimulating dopamine release into portal blood (Ben-Jonathan *et al.*, 1989). Oestradiol may act directly on prolactin-releasing factor(s) from the intermediate lobe (Laudon *et al.*, 1990), or indirectly, via hypothalamic neurones terminating in the posterior pituitary (Murai and Ben-Jonathan, 1990). Furthermore, the oestrogenic factor may be 'an enhancer' for luteotrophic function.

In conclusion, prolactin is more involved in the functions of the luteotrophic complex (via a specific mechanism of 'substitutory compensation') during the second half of pregnancy in pigs than has been previously reported, but, presently, this mechanism remains unclear.

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References

- Ben-Jonathan N, Abrogast LA and Hyde JF (1989) Neuroendocrine regulation of prolactin release *Progress in Neurobiology* **33** 399–447
- Benker G, Jaspers C, Hausler G and Reinwein D (1990) Control of prolactin secretion *Klinische Wochenschrift* **68** 1157–1167
- Buttle HL (1989) On the source of oestradiol secretion at mid-pregnancy in pigs *Journal of Reproduction and Fertility Abstract Series 3* Abstract, 48
- Du Mesnil du Buisson F and Denamur R (1969) Mécanismes du contrôle de la fonction luteale chez la truie, la brebis et la vache *Proceedings of the International Congress of Endocrinology Mexico* pp 927–934 Excerpta Medica Foundation, Amsterdam
- Dusza L and Kryzmowska H (1979) Plasma prolactin concentrations during the oestrous cycle of sows *Journal of Reproduction and Fertility* **57** 511–514
- Grinwich DL, McKibbin PE and Murphy BD (1983) Stimulation of progesterone secretion in the pregnant pig corpus luteum *in vitro* by prolactin and lipoproteins. In *Factors Regulating Ovarian Function* pp 123–128 Eds GS Greenwald and PF Terranova. Raven Press, NY
- Hotchkiss J, Atkinson LE and Knobil E (1971) Time course of serum estrogen and luteinizing hormone concentration during the menstrual cycle of the rhesus monkey *Endocrinology* **89** 177–183
- Imazu Y, Kobayashi K and Shohmori (1989) Comparative study of sulpirine and haloperidol on dopamine turnover in the rat brain *Neurochemical Research* **14** 459–464
- Jamnes H, Schirar A and Dijane J (1985) Differential patterns in luteal prolactin and LH receptors during pregnancy on sows and ewes *Journal of Reproduction and Fertility* **73** 27–35
- Jones PBC, Valk CA and Hsueh AJW (1983) Regulation of progestin biosynthetic enzymes in cultured rat granulosa cells: effects of prolactin, β_2 -adrenergic agonist, human chorionic gonadotropin and gonadotropin-releasing-hormone *Biology of Reproduction* **29** 572–585
- Koike K, Miyake A, Aono T, Sakumoto T, Ohmichi M, Yamaguchi M and Tanizawa O (1991) Effect of prolactin on the secretion of hypothalamic GnRH and pituitary gonadotropins *Hormone Research Supplement* **35** 5–12
- Kraeling RR and Davis BJ (1974) Termination of pregnancy by hypophysectomy in the pig *Journal of Reproduction and Fertility* **36** 215–217
- Laudon M, Grossman DA and Ben-Jonathan N (1990) Prolactin-releasing factor: cellular origin in the intermediate lobe of the pituitary *Endocrinology* **126** 3185–3192
- Lejeune H, Bilion P, Charrie A, Fleury MC, Midani MEI, Tourniaire J and Pugeat M (1990) Technology of luteinizing hormone (LH) pulse analysis. In *Recent Progress on GnRH and Gonadal Peptides* pp 113–128 Eds P Bouchard, F Haour, P Frachimont and B Schatz. Elsevier, Paris
- Murai I and Ben-Jonathan N (1990) Acute stimulation of prolactin release by estradiol: mediation by the posterior pituitary *Endocrinology* **126** 3179–3184
- Murphy BD and Rajkumar K (1985) Prolactin as a luteotrophin *Canadian Journal of Physiology and Pharmacology* **63** 257–264
- Nicholich K, Mason AJ, Szony E, Ramachandran J and Seeburg PH (1985) A prolactin-inhibiting factor within the precursor for human gonadotrophin-releasing hormone *Nature* **316** 511–517
- Rajkumar K, McKibbin PE, Buhr MM, Grinwich DL and Murphy BD (1984) Differential effects of the luteotrophic hormones LH and PRL in control of progesterone output from the pregnant pig corpus luteum *in vitro* *Proceedings of the Tenth International Congress on Animal Reproduction and Artificial Insemination* **10** 513–516
- Rajkumar K, Malinek J and Murphy BD (1985) Effect of lipoproteins on progesterone accumulation by luteal cells from the pregnant pig *Steroids* **45** 119–134
- Robertson HA and King GJ (1974) Plasma concentrations of progesterone, oestrone, oestradiol-17 β and of oestrone sulphate in the pig at implantation, during pregnancy and parturition *Journal of Reproduction and Fertility* **40** 133–141

- Sortino MA and Wise PM** (1989) Effect of hyperprolactinemia on LH and prolactin secretion assessed using the reverse hemolytic plaque assay *Biology of Reproduction* **41** 618–625
- Szafranska B and Ziecik AJ** (1990) Effect of bromocriptine administration on maintenance of late pregnancy in the pig *Experimental and Clinical Endocrinology* **96** 321–324
- Szafranska B, Grazul-Bilska AT and Przala J** (1992) Effect of LH and prolactin on steroid secretion by perfused luteal tissue from pregnant gilts with immunoneutralization of LH *Archiva Veterinaria Poland* **32** 7–15
- Vacher P, Mariot P, Dufy-Barbe L, Nikolics K and Seeburg PH** (1991) The gonadotropin-releasing hormone associated peptide reduces calcium entry in prolactin-secreting cells *Endocrinology* **128** 285–294
- Wiest WG, Kidwell WR and Balogh K, Jr** (1968) Progesterone catabolism in the rat ovary: a regulatory mechanism for progestational potency during pregnancy *Endocrinology* **82** 844–859
- Yangfan Li, Molina JR, Klindt J, Bolt DJ and Anderson LL** (1989) Prolactin maintains relaxin and progesterone secretion by aging corpora lutea after hypophysial stalk transection or hypophysectomy in the pig *Endocrinology* **124** 1294–1304
- Ziecik A, Goralska M, Krzymowski T and Pogorzelski K** (1978) Isolation and purification of porcine LH for radioimmunoassay *Bulletin Polish Academy of Science Class II Series Biology* **26** 739–744