Gonadotrophin storage patterns in the ewe during the oestrous cycle or after long-term treatment with a GnRH agonist

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Abstract

The storage pattern of gonadotrophins in the ewe pituitary was investigated during the oestrous cycle and after desensitization to GnRH using long-term treatment with a GnRH agonist, buserelin. Oestrous cycles in ewes were synchronized with progestagen sponges. Animals were allocated to two experiments. In the first, ewes were killed 36 h (before the preovulatory surge, n=4), 48 h (end of the preovulatory surge, n=5), 72 h (post-ovulation, n=4) and 240 h (luteal phase, n=3) after sponge removal. In the second experiment, another progestagen sponge was inserted in ewes 84 h after removal of the first sponge. Four ewes were infused continuously with buserelin (50 µg/day) for 15 days before killing. A further four ewes received no buserelin (controls). Pituitaries were collected and processed for immunocytochemistry to detect monohormonal (LH or FSH) and multihormonal (LH/FSH) cells. The percentages of LH or FSH immunoreactive cells in the pituitary were lower at the end of the preovulatory surge (7·4 ± 0·3% and 1·2 ± 0·3% respectively) compared with the other stages (11·4 ± 0·5% and 5·4 ± 0·7% respectively). Analysis of dual immunostaining showed the existence of monohormonal cells for LH and multihormonal cells (LH/FSH). No monohormonal cell for FSH was detected except at the end of the preovulatory surge when a few monohormonal FSH cells appeared (0·1 ± 0·01% of pituitary cells). The percentage of monohormonal LH cells in the pituitary gland was similar in all studied stages of the oestrous cycle, whereas the percentage of multihormonal cells was lower at the end of the surge. In agonist-treated ewes, the percentages of LH or FSH immunoreactive cells (5·3 ± 0·5% and 1·5 ± 0·8% respectively) were decreased compared with controls (9·4 ± 1% and 7·5 ± 1·1% respectively). Analysis of the double immunostaining revealed a few monohormonal FSH cells (0·2 ± 0·01% of pituitary cells) in agonist-treated ewes but not in controls. The percentage of monohormonal LH cells in the pituitary gland increased from 1·9 ± 0·2% in controls to 3·8 ± 0·3% in agonist-treated ewes, whereas multihormonal cells dropped from 7·5 ± 1·1% to 1·3 ± 0·7%. Our data suggest, therefore, that multihormonal cells contribute to gonadotrophin secretion, either during the preovulatory surge of the oestrous cycle or during the ‘flare-up’ effect initially induced by a GnRH agonist. Moreover, the appearance of monohormonal FSH cells in some conditions reflects a differential regulation of LH and FSH.


Introduction

Immunocytochemical studies have shown that the gonadotroph population is very heterogeneous in its ability to store gonadotrophins. In several species including the rat (Nakane 1970, Denef et al. 1978a, Childs et al. 1980) and the pig (Dacheux 1978, Torronteras et al. 1993), gonadotrophs store only one hormone (luteinizing hormone (LH) cells or follicle-stimulating hormone (FSH) cells) or both hormones (LH/FSH cells). Furthermore, subpopulations of gonadotrophs separated on the basis of density and/or size exhibit differences in their relative content of LH and FSH and in their secretory response to gonadotrophin-releasing hormone (GnRH) or androgens (Denef et al. 1978b, Childs et al. 1983, Torronteras et al. 1995). In the rat, large gonadotrophs which are mostly multihormonal respond to GnRH stimulation by increasing both the number of secreting cells and the amount of hormone released per cell (Lloyd & Childs 1988). In contrast, small gonadotrophs which are mainly monohormonal cells have little or no GnRH binding activity (Naor et al. 1982) and do not increase LH release under GnRH stimulation (Tougard 1980, Hyde et al. 1982). However, this subset of small gonadotrophs may become multihormonal during diestrus and proestrus (Childs et al. 1992, 1994) or after GnRH stimulation in vitro (Lloyd & Childs 1988) suggesting that small gonadotrophs respond to GnRH by synthesizing and storing the other gonadotrophin. Taken together, these findings suggest that these subpopulations can reflect several states of a single cell type capable of synthesizing, storing and/or releasing LH and FSH depending on physiological conditions. At a given stage,
the selective modulation of LH or FSH release in vivo may be linked to this heterogeneity of the gonadotrophs. Secretagogues like GnRH may discriminate between subsets of gonadotrophs.

Data concerning the heterogeneity of gonadotrophin storage in the sheep pituitary are sparse. Recently, our laboratory showed the existence of an heterogeneity in the storage of gonadotrophins in the fetal sheep pituitary (Hadj Messaoud-Toumi et al. 1993). In the adult, despite changes in plasma concentrations of LH and FSH being well documented throughout the reproductive life of ewes and particularly during the oestrous cycle, few data are available concerning variations in gonadotrophin storage in different physiological conditions.

The present study had two objectives: in the first experiment, the storage pattern of the gonadotrophins in the pituitary of the ewe was investigated during the oestrous cycle. In the second experiment, the consequences of a pituitary desensitization to GnRH on the pattern of gonadotrophin storage were assessed using a long-term treatment of ewes with a GnRH agonist, buserelin.

Materials and Methods

Animals

The oestrous cycles of cross-bred (Ile de France × Romanov) ewes were synchronized by insertion of vaginal sponges impregnated with synthetic progestagen (40 mg fluorogestone acetate, Intervet, Boxmeer, Holland) during the breeding season. Sponges remained in place for 12 days.

Ewes were assigned to one of two experiments.

Experiment 1

Sixteen ewes were killed at different stages of the oestrous cycle: 36 h (group 1, four ewes), 48 h (group 2, five ewes), 72 h (group 3, four ewes) or 240 h (group 4, three ewes) after sponge removal. Prior to killing, blood samples were collected from the jugular vein at 2 h intervals between 24 h and 36 h for group 1, 32 h and 48 h for group 2, 32 h and 60 h for group 3. For group 4, a single blood sample was collected just prior to killing. Plasma was recovered from these samples and assayed for LH and FSH. Ewes were killed on day 15 of the breeding season. Sponges were removed in place for 12 days.

Ewes were boosted with 50 µg/animal of buserelin. The experiment, the storage pattern of the gonadotrophins in the pituitary of the ewe was investigated during the oestrous cycle. In the second experiment, the consequences of a pituitary desensitization to GnRH on the pattern of gonadotrophin storage were assessed using a long-term treatment of ewes with a GnRH agonist, buserelin.

Experiment 2

In this experiment, another intravaginal progestagen sponge was inserted in eight ewes 84 h after removal of the first sponge. The aim of this second sponge was to maintain a homogeneous steroidal state in the control group. Four ewes were infused continuously with a GnRH agonist, buserelin (50 µg/day, Suprefact, Hoechst, Frankfurt, Germany) using a s.c. implanted osmotic pump (2ML2 Alzet, Phymep, Paris, France) for 15 days according to Brebion & Cognié (1989). On the first day, ewes were boosted with 50 µg/animal of buserelin. The other four ewes received no buserelin treatment and were assigned to the control group. Previous studies have shown that saline treatment had no effect on LH pulsatility compared with non-treated animals (Y Cognié, personal communication). To check the effectiveness of desensitization of the gonadotrophs with buserelin treatment, blood samples were collected via jugular cannulae on day 14 of treatment at 15 min intervals for 12 h. One hour before the end of blood sampling, 250 ng exogenous GnRH (Gonadorelin, Roussel, Paris, France) were injected i.v. In addition, blood samples were collected daily throughout the treatment. Plasma was recovered from samples and assayed for LH and FSH. Ewes were killed on day 15 of buserelin treatment or the equivalent day for control ewes. Anterior pituitaries were removed and treated as described above. Ovaries were macroscopically examined for the diameter of follicles.

Immunocytochemistry

Immunocytochemistry was performed as described previously (Hadj Messaoud-Toumi et al. 1993) with minor modifications.

Monolabelling was used to determine the percentages of LH (mono- and multihormonal) cells or FSH (mono- and multihormonal) cells in the pituitary. The primary antibodies were either a rabbit antiserum to ovine LHβ (oLHβ) (1:1600; obtained in our laboratory) or a horse antiserum to ovine FSHβ (oFSHβ) (1:100). Antibodies were localized with the use of either goat anti-rabbit immunoglobulins conjugated to peroxidase (Diagnostics Pasteur, Marne la Coquette, France) for LH detection or rabbit anti-horse immunoglobulins conjugated to peroxidase (Nordic Immunology, Tilburg, the Netherlands) for FSH detection.

Specificity of the staining for LH or FSH was confirmed since immunostaining was abolished when non-immune rabbit serum or horse serum, respectively, was substituted for the primary antiserum or when the antisera were preabsorbed with 1–100 µg/ml of homologous antigen (oLH CY1083 or oFSH CY1767 II). Preincubation of the antisera with 1–100 µg/ml heterologous antigens (LH or FSH), α-subunit or ovine thyroid-stimulating hormone -12 did not influence the staining.

Double labelling was performed to detect multihormonal cells and determine their proportion among the total gonadotrophs. Briefly, the sections were incubated with both rabbit anti-oLHβ and horse anti-oFSHβ for 2 h.
Antibodies were localized by incubating sections with both goat anti-rabbit immunoglobulins conjugated to fluorescein isothiocyanate and goat anti-horse immunoglobulins conjugated to lissamine rhodamine (Jackson Immunoresearch, West Grove, PA, USA).

Additional controls were made in order to ensure that the goat anti-rabbit immunoglobulins used in the double labelling did not cross-react with the FSH antiserum, that the goat anti-horse immunoglobulin did not cross-react with the LH antiserum, and that the secondary antibodies did not cross-react with each other. For this, sections were either incubated with the oLH antiserum and revealed with anti-horse immunoglobulins or incubated with the oFSH antiserum and revealed with anti-rabbit immunoglobulins. In both cases, no staining appeared. Furthermore, each antiserum was revealed with both secondary antibodies and only the expected staining occurred.

**Quantitative microscopical analysis**

**Monolabelling** Analysis of the percentages of the gonadotrophs was performed using the SAMBA 2005 Image Analyzer (System for Analytical Microscopy in Biological Application, ALCATEL TITN Co., Massy, France) as described by Hadj Messaoud-Toumi et al. (1993). Briefly, light microscope images from 50–80 fields/section were input into the analyser through a ×40 objective and a camera and analysis of stained area vs total area was performed. The percentages of LH or FSH cells among the total pituitary cells corresponded to the ratio stained area/total area. Data were calculated by counting approximately 1500 gonadotrophs from each of three sections per animal stained in an immunocytochemistry assay. Three immunocytochemistry assays were performed per animal.

**Double labelling** Fluorescein and/or rhodamine stained cells were counted by eye under a light microscope and the proportions of each gonadotroph population (monohormonal LH, monohormonal FSH or multihormonal cells) among the overall gonadotrophs (monohormonal LH + monohormonal FSH + multihormonal cells) were determined. In order to express the results as percentage of each gonadotroph population among the total pituitary cells, data were fitted with values obtained in monolabelling.

Analysis of stained area vs total area was performed on some sections after fluorescent labelling and showed similar results compared with peroxidase labelling.

**Hormone assays**

The concentrations of plasma LH were measured in duplicate using RIA according to Pelletier et al. (1968). The results were expressed as nanograms LH CY1051 (equivalent to 2·5 NIH-LH-S1). The minimum detectable concentration for LH was 0·2 ng/ml. Mean intra- and interassay coefficients of variation (CV) were less than 7 and 11% respectively. The concentrations of plasma FSH were measured by RIA using the reagents supplied by NIADDK (Bethesda, MD, USA) and the results were expressed as nanograms of oFSH 19-SIAFP RP2. The minimum detectable concentration for FSH was 0·2 ng/ml. Mean intra-assay and interassay CV were less than 6 and 5% respectively. The cross-reaction with oLH was 0·6%. The concentrations of plasma oestradiol were measured as described previously (Thibier & Saumande 1975). The minimum detectable concentration was 1 pg/ml. Mean intra- and interassay CV were less than 4 and 5% respectively.

**Statistical analysis**

Values are means ± s.e.m. After logarithmic transformation, data were analysed by ANOVA for the effects of oestrous cycle stages or busulferin treatment followed by either the Newman–Keuls test or the Student’s t-test for individual comparisons. The parameters of LH pulse secretion were determined using the Munro pulse analysis programme (Taylor 1987).

**Results**

**Experiment 1**

**Plasma hormone concentrations during the oestrous cycle** Plasma hormone concentrations were determined to verify the timing of the pituitary collection. Data shown in Fig. 1A confirmed that the ewes were killed respectively before (group 1) or at the end (group 2) of the preovulatory LH surge, after ovulation (group 3) and during the luteal phase (group 4). In group 2, one ewe did not exhibit a LH surge before the animal was killed whereas another was just initiating a surge. The data of these two ewes were excluded from the results. For group 3, plasma concentrations determined between 32 h and 60 h after sponge removal demonstrated that the ewes had produced a gonadotrophin surge (data not shown). Plasma concentrations of oestradiol in blood samples recovered at the time of killing gave further evidence for the correct timing (Fig. 1A). Ovarian status for each animal also corresponded to the expected stage.

**Gonadotrophs during the oestrous cycle** Figure 1B shows that the percentages of LH immunoreactive cells (mono- and multihormonal cells) or FSH immunoreactive cells were lower (P<0·005 and P<0·01 respectively) at the end of the surge compared with other periods of the oestrous cycle studied. Analysis of dual immunostaining enabled the composition of the gonadotroph cell population to be described. Figure 2 illustrates the existence of...
multihormonal cells (LH/FSH) and monohormonal cells for LH. In this set of experiments we failed to detect cells containing FSH alone except in group 2 where some monohormonal FSH cells were present (0.1 ± 0.01% of total pituitary cells). Thus, FSH-containing cells were mainly multihormonal cells. The percentage of monohormonal LH cells among the total pituitary cells was similar in all studied stages of the oestrous cycle, whereas the percentage of multihormonal cells was lower (P<0.0005) at the end of the surge (Fig. 3A). When results were expressed as percentages of cells in the gonadotroph population (Fig. 3B), it was noted that the proportion of multihormonal cells was lower (P<0.005) at the end of the surge compared with other periods. In contrast, this proportion tended to be more elevated in the luteal phase compared with the stage preceding the surge (P<0.08).

**Experiment 2**

**Plasma hormone concentrations after GnRH agonist treatment** Plasma concentrations of LH were greater (P<0.0001) in agonist-treated ewes than in control ewes on day 1 of treatment (Table 1). Thereafter, LH concentrations decreased but remained slightly higher than values in controls throughout the treatment (P<0.0001, on day 14). Plasma concentrations of FSH were greater (P<0.0001) in agonist-treated ewes than in control ewes on day 1 of treatment. On day 2 of treatment, FSH concentrations decreased to control levels and remained stable until the end of treatment. On day 14 of GnRH agonist treatment, ewes did not exhibit LH pulses throughout a 12 h period whereas control ewes showed a pulsatile pattern (Fig. 4A; Table 1). Following the injection of GnRH, there was a significant increase in the release of LH and FSH in control ewes but no response in the GnRH agonist-treated ewes (Fig. 4A). However, no follicles larger than 3 mm in diameter were present in the ovaries following treatment with GnRH agonist.

**Gonadotrophs after GnRH agonist treatment** When ewes were treated chronically with buserelin, the percentage of LH-immunoreactive cells (mono- and multihormonal cells) and FSH-immunoreactive cells decreased (P<0.02 and P<0.005 respectively) compared with controls (Fig. 4B). Analysis of the double immuno-staining on the pituitary cells of GnRH agonist-treated ewes showed multihormonal, monohormonal LH and a few monohormonal FSH cells (0.20 ± 0.01% of the pituitary cells). In control ewes, no monohormonal FSH cells were present (0.1 ± 0.01% of total pituitary cells).

Figure 1 (A) Changes in plasma concentrations of LH (●) or FSH (○) during the oestrous cycle. Blood samples were collected at 2 h intervals. Arrows indicate the time of pituitary collection. Boxed inserts show plasma concentrations of oestradiol (pg/ml) for all animals. Values are means ± S.E.M. n=4 for groups 1 and 3, n=3 for groups 2 and 4. (B) Percentages of LH (■) or FSH (□) immunoreactive cells in the pituitary. Immunocytochemistry was performed on pituitaries from ewes killed at the stages indicated by arrows on panel A. Values are means ± S.E.M. n=4 for groups 1 and 3, n=3 for groups 2 and 4. ** P<0.01, *** P<0.005 late follicular phase vs other stages.

Figure 2 Double immunostaining of gonadotroph cells on the same section from ewe pituitary collected before gonadotrophin surge. The section was incubated with both rabbit anti-oLHβ and horse anti-oFSHβ for 2 h. A further 2 h incubation was performed with (A) goat anti-rabbit immunoglobulins conjugated to fluorescein isothiocyanate to reveal LH, and (B) goat anti-horse immunoglobulins conjugated to lissamine rhodamine to reveal FSH. Asterisks indicate monohormonal LH cells. Scale bars represent 18 µm.
detected. The percentage of monohormonal LH cells among the total pituitary cells increased (Fig. 5A; \(P < 0.001\)) in GnRH agonist-treated ewes compared with controls whereas the percentage of multihormonal cells dropped (\(P < 0.001\)). When results were expressed as percentages of cells in the gonadotroph population (Fig. 5B), it was noted that the proportion of monohormonal LH cells increased (\(P < 0.001\)) after GnRH agonist treatment while the proportion of multihormonal cells fell (\(P < 0.001\)).

**Discussion**

The first objective in this study was to investigate changes in the storage pattern of gonadotrophins throughout the oestrous cycle. Percentages of LH cells or FSH cells detected in this study were consistent with data in rats (Childs et al. 1987). A decrease in the percentage of both LH-containing cells and FSH-containing cells (present results) as well as the intensity of the staining (data not shown) occurred at the end of the late follicular phase, following the enhanced gonadotrophin release characterizing the preovulatory surge. These results are consistent with the fall in pituitary concentrations of both LH and FSH associated with the preovulatory surge in the ewe (Leung et al. 1988). Electron microscopy studies on localization of LH secretory granules in sheep gonadotrophs have shown that there is a polarization of secretory granules to the region of the cell next to the vascular system (Currie & McNeilly 1995). During the follicular phase, the percentage of cells with polarized granules increased from 45% at oestrus to 90% in the mid-LH surge. After the LH surge, gonadotrophs were totally devoid of LH granules but LH immunoreactivity was observed in rough endoplasmic reticulum (Currie & McNeilly 1995). These changes are not associated with an increase in LH \(\beta\) mRNA levels suggesting that the enhanced LH secretion during the preovulatory surge is related to a progressive recruitment of gonadotrophs into a releasing state rather than to an increased synthesis of hormone (Currie & McNeilly 1995). Taken together, these observations indicate that the drop presently described in the percentage of immunoreactive gonadotrophs following the high secretory activity reflects the decreased gonadotrophin stores. Ewes in group 3 (post-ovulation stage) had percentages of LH-containing cells similar to pre-surge values. Thus it is suggested that

**Table 1** Changes in LH or FSH concentrations on day 1, 3 or 14 in control ewes (n = 4) or ewes infused for 14 days with a GnRH agonist, buserelin (n = 4). Minipumps delivered buserelin (50 \(\mu\)g/day per ewe) continuously. On the first day, ewes were boosted with 50 \(\mu\)g/ml per animal of buserelin. Blood samples were collected daily. On day 14 of the treatment, blood samples were collected at 15 min intervals for 12 h.

<table>
<thead>
<tr>
<th>Day</th>
<th>LH (ng/ml)</th>
<th>Controls</th>
<th>Agonist treated</th>
<th>FSH (ng/ml)</th>
<th>Controls</th>
<th>Agonist treated</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>0.73 ± 0.05</td>
<td>8.30 ± 0.32***</td>
<td>0.70 ± 0.12</td>
<td>5.12 ± 0.17***</td>
<td></td>
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</tr>
<tr>
<td>3</td>
<td>0.82 ± 0.08</td>
<td>1.56 ± 0.15**</td>
<td>0.84 ± 0.18</td>
<td>0.61 ± 0.11</td>
<td></td>
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</tr>
<tr>
<td>14</td>
<td>0.53 ± 0.02</td>
<td>0.72 ± 0.01***</td>
<td>0.77 ± 0.08</td>
<td>0.61 ± 0.15</td>
<td></td>
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</tr>
<tr>
<td>Pulse frequency on day 14 (number in 12 h)</td>
<td>3.2 ± 1.1</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
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<tr>
<td>Mean pulse amplitude on day 14 (ng/ml)</td>
<td>1.4 ± 0.25</td>
<td>0.00</td>
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**P < 0.005; ***P < 0.0001 vs controls.
the replenishment of the gonadotrophin stores takes place rapidly after the surge, although no modification of the LH mRNA levels was observed at these stages in the ewe (Currie & McNeilly 1995). In the rat, a similar pattern in the percentage of gonadotrophs during the oestrous cycle was described (Childs et al. 1987, 1992). However, these changes are associated with modifications in LHβ mRNA since an increase in the rat LHβ mRNA levels occurred at the time of the gonadotrophin surge followed by a decrease (Zmeili et al. 1986). These differences between rat and sheep may be linked to the shorter timing of the cycle events in rats.

The pattern of the changes in the percentage of FSH-containing cells throughout the oestrous cycle was similar to those of LH-containing cells. However, the decrease between the stage preceding the surge and the end of the surge was much more notable than for LH. This difference could reflect divergences in the mechanisms involved in gonadotrophin secretions. Indeed, the secretion of LH is associated with a regulatory/storage pathway whereas FSH release is mainly associated with a constitutive pathway (McNeilly 1988, Farnworth 1995). High secretory activity alters the cellular FSH content more dramatically than the LH content. Furthermore, when the release of FSH is maximal (during the surge), FSHβ mRNA concentrations decrease (Leung et al. 1988). In our study, the percentage of FSH-containing cells returned to early follicular phase values just after ovulation, at the time of the secondary rise of serum FSH (Pant et al. 1977). This rise follows an increase in FSHβ mRNA levels (Leung et al. 1988) which indicates that intense activities of FSH synthesis and release are positively coupled at this period.
In our study, gonadotrophs found in the ewe pituitary can be either monohormonal (storing LH) or multihormonal (storing LH and FSH). Lloyd & Childs (1988) demonstrated that in the rat monohormonal cells correspond to small cells and multihormonal cells to large cells. However, only a single type of gonadotroph cell has been observed in the ewe (Thomas & Clarke 1997). In the oestrous cycle stages studied, monohormonal FSH cells were not detected except at the end of the surge when only few were detectable. A recent study confirmed the lack of these monohormonal cells in ovariectomized ewes (Thomas & Clarke 1997). In contrast, monohormonal FSH cells were detected throughout the oestrous cycle of the female rat (Childs et al. 1987). They are also present in the pituitary of ovine fetuses (Hadj Messaoud-Toumi et al. 1993). Throughout the oestrous cycle, the pattern of gonadotrophin storage changed in the ewe, as in the rat. More precisely, the ovine multihormonal cell population among the total pituitary cells was lower at the end of the surge compared with the other studied stages, whereas the percentage of monohormonal LH cells among the total pituitary cells did not vary throughout the cycle. These results suggest that multihormonal cells mainly contribute to both LH and FSH release during the preovulatory surge. Because the sensitivity of FSH to the immunostaining or the fixative may not be optimal, cells with a low amount of FSH might not be detected and our results might minimize the percentage of multihormonal cells. However, cells carrying the higher amount of FSH would also contain LH and would be those participating mainly in the hormone release. These data are consistent with previous studies carried out in the rat (Childs et al. 1987, 1992). Those authors proposed a model in which monohormonal cells might translate the other gonadotrophin just before the preovulatory surge in order to add more cells to the secreting population. A similar cell cycle for the gonadotrophs in the ewe might occur but further studies are required. In particular, investigations of pituitaries collected before and during the surge will have to be performed.

Observations of gonadotrophs at the subcellular level showed that in the rat, secretory granules within the same cell have similarly been found to contain one or both gonadotrophins (Childs 1986). However, in ovariectomized ewes, the two hormones are present in separate populations of secretory granules (Thomas & Clarke 1997). This is consistent with our results which showed that some monohormonal FSH cells were detected at the end of the surge suggesting that monohormonal granules in multihormonal cells might exist and allow the selective secretion of LH. This presumes that exocytosis of LH and FSH might be promoted by different second messengers as reported previously by Kile & Nett (1994). Alternatively, these monohormonal FSH cells might emerge from a cell reserve pool and/or from additional cell types contributing to the gonadotroph populations. Support for this hypothesis was presented for the rat in which a subset of cells may express both LHβ, FSHβ and growth hormone (Childs et al. 1994).

The proportion of multihormonal cells among the gonadotrophs tended to decrease between the luteal phase and the stage preceding the surge. This latter stage corresponds to an increase in LH pulsatility and FSH secretion. Since an enhanced release of FSH dramatically affects FSH content compared with LH, a loss of FSH may induce a shift from multihormonal cells to monohormonal LH cells. These data reinforce the hypothesis of multihormonal cells supporting the gonadotrophin release.

The second objective was to investigate the effects of a desensitization to GnRH using an agonist. After 14 days of treatment with GnRH agonist, the pulsatile pattern of LH secretion had disappeared whereas circulating FSH secretion did not seem affected. Moreover, the LH and FSH responses to GnRH were abolished in treated ewes. At the ovarian level, no follicle larger than 3 mm in diameter was present. These results, in agreement with previous reports (Brebion & Cognié 1989, McNeilly et al. 1991), confirm that desensitization of gonadotrophs has occurred after GnRH agonist treatment and is most likely due to down regulation of GnRH receptors and disruption of second messenger system (Clayton et al. 1982, Conn et al. 1987). Both percentages of LH- or FSH-immunoreactive cells were reduced after agonist treatment. The decrease in pituitary gonadotrophin content may result from the ‘flare up’ effect of GnRH agonist which occurs in the first hour of agonist administration and depletes gonadotrophin stores. However, even if the levels of immunodetected pituitary gonadotrophins were reduced, they were not abolished, although GnRH stimulation was suppressed. The lack of LH pulsatility in treated ewes may be attributed to the inability of the pituitary LH to move into a releasable pool in the absence of GnRH stimulation (Janovick & Conn 1993).

Further, the decrease in the percentage of immunoreactive cells was greater for FSH than for LH, as noted at the end of the preovulatory surge. In a previous study, McNeilly et al. (1991) showed a dramatic fall in pituitary LH and a relatively small reduction in pituitary FSH in buserelin-treated ewes compared with non-treated ewes. In their study, buserelin was administered during 44 days which may explain the difference. Moreover, the immunoreactive FSH may not correspond to biologically active forms. Indeed, it has been demonstrated that GnRH increases the release of less acidic isoforms which have a greater biological activity than more acidic forms (Ulloa-Aguirre et al. 1992). Thus, after desensitization of the gonadotrophs, the decrease in the acidic forms might occur and be responsible, at least partly, for the absence of terminal follicular growth in agonist-treated ovaries.

The pattern of gonadotrophin storage changed in agonist-treated ewes compared with control ewes. In
agonist-treated ewes, the percentages of cells containing both LH and FSH among the pituitary cells dropped compared with controls whereas the percentage of monohormonal LH cells increased. This suggests that these multihormonal cells may have participated to the massive secretion of gonadotrophins at the beginning of the treatment. In some of these cells, the ratio of released FSH to FSH content might be elevated and lead to undetectable FSH levels. Hence, these multihormonal cells would contribute to increase the proportion of monohormonal LH cells. Similarly to the end of the preovulatory surge, the presence of some monohormonal FSH cells in agonist-treated ewes might reflect a differential regulation of LH and FSH from these multihormonal cells. Using the reverse haemolytic plaque assay (RHPA) for measurement of secretion by individual pituitary cells, a differential pattern of gonadotrophin release by rat gonadotrophs has been demonstrated with multihormonal gonadotrophs secreting larger amounts of LH and FSH in response to GnRH than monohormonal gonadotrophs (Lloyd & Childs 1988). Moreover, that study gave evidence for a non-parallel release of gonadotrophins from cells storing both hormones. In the sheep, a heterogeneity between the number of LH secreting cells and the number of LH immunoreactive cells following GnRH stimulation was observed using the RHPA (Taragnat & Durand 1993, (C Taragnat, unpublished observations). However, it is not known whether these results reflect a different ability of mono- and multihormonal gonadotrophs to release LH under stimulation. Hence, further studies are required to answer this question.

In conclusion, gonadotrophs of the ewes are mainly composed of multihormonal and monohormonal LH cells. The present data suggest that multihormonal cells contribute to the gonadotrophin secretion, either during the preovulatory surge of the oestrous cycle or during the ‘flare-up’ effect induced by a GnRH agonist. Moreover, the appearance of monohormonal FSH cells at the end of the surge or after GnRH agonist treatment underlines a differential regulation of LH and FSH in some physiological situations.

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