Determination of luteinizing hormone (LH) response to different doses of lecirelin acetate (a GnRH analogue) in Tuj ewe-lambs

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SUMMARY

The aim of the present study was to determine the LH response to different levels of lecirelin acetate (a GnRH agonist) in Tuj ewe-lambs. Twenty lambs, aged 5-months-old, were randomly allocated into four groups of five. Lecirelin acetate was intravenously administered to lambs from groups 1, 2, 3, and 4 at 0.1, 0.2, 0.5 and 1.0 µg per kg BW, respectively (Administration I). Blood samples were collected from the jugular vein 30 minutes before and at 30 min intervals for 300 minutes after the administration to determine the plasma LH concentrations. The administration was repeated 72 h later, to determine the replenishment of pituitary stores of LH in the same lambs (Administration II). Following both administrations, LH response was dose-dependent and the doses of 0.5 and 1.0 µg per kg resulted in a significantly greater response than the dose of 0.1 and 0.2 µg/kg (P lower than 0.05). On the other hand, LH release characteristics for all dosage groups were similar between the first and second administrations (P greater than 0.05). These data suggest that (1) LH stores were replenished within 72 hours after lecirelin acetate administration and that (2) highest LH responses were obtained at 0.5 and 1.0 µg per kg dose levels in Tuj ewe-lambs.

Keywords: Lecirelin, LH, GnRH, Lamb, dose response.

Introduction

The importance of gonadotropin releasing hormone (GnRH) for generation of the preovulatory surge of luteinizing hormone (LH), and thus ovulation, is fully recognized [17, 18, 25]. GnRH binds with high affinity to the GnRH receptor (GnRH-R) on the cell surface of pituitary gonadotrophs. Therein, it activates intracellular signal transduction pathways to affect both the synthesis and intermittent release of the gonadotropins [23]. The density of GnRH-R on gonadotropes determines their ability to respond to GnRH [30]. This density reaches the highest level just before ovulation and this might be important for complete expression of the preovulatory surge of LH. Regulation of GnRH-R gene expression is influenced by multiple factors including gonadal steroid hormones and, perhaps most importantly, GnRH itself [27]. GnRH stimulation elicits an LH response that is measurable in the peripheral circulation within minutes. The LH response profile, however, is dependent on the dosage of GnRH given and the method of administration [16, 26]. During and/or following a complete preovulatory LH surge, the gonadotrophs deplete their LH stores, substantially, after which the replenishment of storage granules containing LH takes place sometimes between 24-48 h [8] or 72 h [29] post-surge.

GnRH agonists are peptides that are similar to GnRH but they are modified at the sites of enzymatic degradation of
Lecirelin acetate, a synthetic hypothalamic hormone of prolonged action, is a superanologue of GnRH and is obtained by the modification of gonadorelin’s structure. The difference of lecirelin acetate from native GnRH is that it is a nanopeptide and that glycine at position 6 was replaced with an ethylamino group. To our knowledge, their efficacy and effective dose(s) in sheep have not been reported so far. Therefore, the aim of the present study was to determine the LH response to different lecirelin acetate doses in ewe-lambs of Tuj breed.

Statistical analysis

Data were analyzed using General Linear Model (GLM) procedures. Independent variables were the administrations and dose groups. Dependent variables analyzed were the time of LH peak (ToP), peak value (PV), duration of surge (DoS) and area under the LH curve (AUC). Tukey’s multiple comparison test was used to compare the means when significant differences were detected (P < 0.05). However, data obtained in the first and second GnRH administrations were analyzed by “Paired t” test. The LH surges were identified in individual lambs after plotting their LH concentrations over time. The onset of the LH surge has been described by CARATY and SKINNER [7]. Briefly, the onset of a surge was determined as 3 SD rises in consecutive two points leading to surge. The end of a surge was also described as 3 SD decreases in consecutive two points leading to baseline (when detectable). However, as the present data available (for baseline level) were insufficient (e.g. not allowing for appropriate SD calculation), some modification had to be made herein, as follows: Instead of SD, the difference between the two consecutive points (as drawn on a graph for each individual; data not shown) was considered when it was 30% as the minimal difference. The DoS (as minutes) was then calculated from the difference between the time of onset and end of the surge. All the statistical analyses and calculations were carried out by using SAS Statistical Software (SAS 9.1.3, SAS Inc., Cary NC., 2007).

Results

The results showed that for different GnRH-dose groups (of 0.1, 0.2, 0.5, and 1.0 µg/kg BW), there was no significant difference between the first (Administration I) and second administrations (Administration II) for the ToP, PV, DoS and AUC of LH release, except for the ToP and DoS values at 0.1 µg/kg dose (Table 1).
I and II, respectively. The LH releases following the injection of different doses of lecirelin acetate were significantly different (P < 0.05) within each of the Administrations I and II.

Figures 2a and 2b represent the relationship between the GnRH dose and AUC for the Administrations I and II, respectively. There was no significant difference either between 0.1 and 0.2 µg/kg or between 0.5 and 1.0 µg/kg doses for the AUC. By contrast, the latter two higher doses (of 0.5 and 1.0 µg/kg) resulted in a significantly greater (P < 0.05) response than those of the former two lower doses (of 0.1 and 0.2 µg/kg) for the AUC within each Administrations I and II. The baseline for LH could not be calculated from the data obtained at -30 and zero minutes. This was because their values were under the detectable limit of the assay used throughout the study.

### Table 1: LH parameters for different levels of lecirelin acetate dose in the first and second administrations (least squares means ± standard error of means).

<table>
<thead>
<tr>
<th>Dose of Lecirelin</th>
<th>Time of peak value (minute)</th>
<th>Peak value (ng/ml)</th>
<th>Duration of surge (minute)</th>
<th>Area under the LH curve [(ng/ml)*min]</th>
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<tr>
<td>0.1 µg/kg</td>
<td>54.0 ± 13.3 a</td>
<td>96.0 ± 13.3 a</td>
<td>204.0 ± 11.2 a</td>
<td>935.0 ± 358.0 a</td>
</tr>
<tr>
<td>0.2 µg/kg</td>
<td>66.0 ± 13.3 a</td>
<td>102.0 ± 13.3 a</td>
<td>258.0 ± 11.2 a</td>
<td>1488.0 ± 358.0 a</td>
</tr>
<tr>
<td>0.5 µg/kg</td>
<td>102.0 ± 13.3 a b</td>
<td>126.0 ± 13.3 a</td>
<td>276.0 ± 11.2 a</td>
<td>2724.2 ± 358.0 a</td>
</tr>
<tr>
<td>1.0 µg/kg</td>
<td>120.0 ± 14.8 c</td>
<td>114.0 ± 13.3 a</td>
<td>276.0 ± 11.2 a</td>
<td>2942.2 ± 358.0 b</td>
</tr>
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a-c: Means with different superscripts in a column are significantly different (P < 0.05); ToP: Time of peak, PV: Peak value, DoS: Duration of surge, AUC: Area under the LH curve, I: Administration I, II: Administration II. There were no differences between the administrations except for the ToP and DoS at the level of 0.1 µg/kg dose.
Discussion

The main objective of the present study was to investigate the LH response to different doses of lecirelin acetate (GnRH analogue) in Tuj ewe-lambs. For this purpose, the pituitary LH response was selected since the plasma LH concentration offers all the ideal properties for a surrogate. It is graded, sensitive and continuous in nature and it is an objectively measurable end-point.

The present results showed that the pituitary LH response to lecirelin acetate is dose dependent in ewe-lambs. These results, in agreement with previous reports [4, 9, 12, 18, 21], confirm that pituitary LH responses increase with higher doses of GnRH (fig. 1a, b). When the relationships of dose-effect for GnRH were analyzed, the dose of 0.5 and 1.0 µg/kg resulted in a significantly greater response than the dose of 0.1 and 0.2 µg/kg did, but the dose of 0.5 and 1.0 µg/kg were not significantly different within the first and second administrations. It has been suggested that the storage of releasable LH is in two pools. The first readily releasable pool, located near the cell surface, is responsible for the initial phase of LH secretion following the GnRH exposure. The second pool, releasable one, requires either mobilization from deeper sources within the cell or minor processing prior to the release during the second phase of the LH response. According to the two-pool theory [5, 33], a low dose of GnRH only stimulates LH release from the readily releasable pool, and this reflects both the responsiveness of pituitary and the dosage of GnRH administered. In contrast, higher doses of GnRH stimulate the release of all readily releasable LH, with subsequent LH being released from the releasable pool. The amplitude of the first phase of LH release, therefore, increases with the dosage of GnRH until it reflects depletion of the readily releasable pool; thereafter it remains constant irrespective of the dosage of GnRH administered. The size of the second phase of LH release however, increases as the dosage of GnRH increases, until the maximal LH response is achieved. The size of the first phase of the LH release, therefore, provides a measure of the size of the readily releasable pool of LH, while the maximal size of the second phase is a measure of the size of the releasable pool of LH stored within the pituitary gland. In our study, the administrations of 0.1 and 0.2 µg/kg doses of GnRH resulted in the LH release from the readily releasable pool. Meanwhile, the 0.5 and 1.0 µg/kg doses resulted from the release of all readily releasable LH, with LH subsequently being released from the releasable pool. However, LH response to 0.5 and 1.0 µg/kg doses of GnRH were similar in both occasions of administrations. Considering figures 2a and 2b, representing the LH response of the two administrations, there was, in general, a linear relationship between the dose of lecirelin acetate administered and pituitary response. The relationship was linear for the doses of 0.1, 0.2, and 0.5 µg/kg, but then it turned into a plateau phase between the 0.5 and 1.0 µg/kg doses. This may be due to the depletion of pituitary LH stores and desensitization of the gland. Indeed, the evidence suggests that the numbers of GnRH receptors are decreased at the end of the LH surge [10] following the natural GnRH surge. Therefore, it has been suggested that high GnRH doses might lead to the desensitization of pituitary gland by down-regulation of its own receptors [6]. Likewise, the number of GnRH receptors correlates with the sensitivity of the gland to the GnRH [23].

Considering the early studies of GnRH induced LH release in lambs, the present results are in agreement with those of EVANS et al. [12]. They investigated the effect of different doses of GnRH on the pituitary cells of ram-lambs and found that the mean LH responses of pituitary cells increased as the dose of GnRH increased. Furthermore, the PV of LH for 0.5 µg/kg lecirelin acetate dose used herein correlates well with those of MANN and HARESIGN [19], using 250 µg GnRH in anoestrous ewes (18.6 vs. 18.8 ng/ml, respectively).

In general, data from the ToP, PV, DoS, and AUC of LH release for the pairs of identical GnRH dose groups did not substantially differ between the first and second administrations. The exception to this was that the values of ToP and DoS at 0.1 µg/kg dose were even markedly higher in the second administration. This situation may indicate that the pituitary LH stores released following the first administration were replenished by the time of the second administration. Indeed, TARAGNAT et al. [29] have reported that the replenishment of gonadotropin stores takes place rapidly (approximately 72 h) following the surge. Likewise, CRAWFORD et al. [8] noted that the gonadotrophs are capable of storing copious amounts of both LH and FSH. During a complete preovulatory LH surge, the gonadotrophs deplete their LH stores substantially, followed by the initial signs of replenishment of storage granules containing LH sometime between 24-48 h post-surge.

In conclusion, the present study demonstrated that the release of LH increased following the administration of lecirelin acetate in a dose-dependent manner. There was a relationship between the lecirelin acetate dose administered and the amplitude and area under the curve of lecirelin-induced LH surge. According to these findings, 1.0 µg/kg dose of lecirelin acetate did not give higher LH release compared to the 0.5 µg/kg dose. Hence, lecirelin acetate (at 0.5 µg/kg BW dose) may be sufficient, as a GnRH agonist, to obtain an exogenously induced LH surge in ewe-lambs. Whether the latter dose is also effective for successful ovulation and whether the response of the pituitary gland in ewes is different between seasons still remain a question for future investigations.

References


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