TECHNICAL NOTES

Effect of Carrier and Administration on Luteinizing Hormone Release
By Gonadotropin Releasing Hormone

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ABSTRACT

Ovariectomized ewes in groups of four were administered either saline (I) or 50 µg of gonadotropin releasing hormone (II to VII). Methods of administration and carriers were: II, intramuscular saline; III, intravenous saline; IV, subcutaneous saline; V, subcutaneous gelatin; VI, subcutaneous carboxymethylcellulose; and VII, subcutaneous gelatin capsule. Only subcutaneous administration of gonadotropin releasing hormone in the gelatin capsule (VII) prolonged the interval from treatment to luteinizing hormone peak; however, there were fewer ewes with peak luteinizing hormone concentrations equal to or greater than 100 ng/ml in VII than II, IV, V, and VI. Ewes in V, VI, and VII had luteinizing hormone surges of longer duration than II and III. Ewes in VII had the longest luteinizing hormone surge, which was 7.5 h, even though luteinizing hormone concentrations did not return to pretreatment concentrations by 8 h posttreatment in three of the four ewes.

INTRODUCTION

Synthetic gonadotropin releasing hormone (GnRH) has induced release of luteinizing hormone (LH) in domestic animals during various physiological states (14, 15). In most studies, GnRH has been dissolved in saline and administered by intramuscular or intravenous injection. Following GnRH treatment in ewes, concentrations of LH in plasma increase within 15 min, peak LH is .5 to 2.0 h posttreatment, and concentrations of LH in plasma return to pretreatment concentrations by 4 to 6 hr following treatment (13, 15). This GnRH-induced release of LH is considerably shorter than a preovulatory LH surge, which is about 8 to 10 hr (5, 10). Golter et al. (6), however, reported an LH surge of about 10 h when GnRH was dissolved in 2% carboxymethylcellulose and injected subcutaneously in bulls. Furthermore, in postpartum dairy cows Britt et al. (1) reported that mean peak LH concentrations were 4 h following subcutaneous implantation of a gelatin capsule containing GnRH. Although a variety of carriers and methods of administration have been used, the effect of carrier and method of administration on GnRH-induced LH release has not been examined. This study was to evaluate the effect of various carriers and methods of administration on GnRH-induced LH release in ovariectomized ewes.

MATERIALS AND METHODS

Twenty-eight mature ewes ovariectomized in September, 1977, were evenly assigned to one of seven groups. In November, 1977, ewes in II to VII were administered 50 µg of GnRH, whereas ewes in Group I received an injection of saline (.9% with .9% benzyl alcohol). The methods of administration and carriers of GnRH were: II, intramuscular saline; III, intravenous saline; IV, subcutaneous saline; V, subcutaneous 2% gelatin; VI, subcutaneous 2% carboxymethylcellulose; and VII, subcutaneous gelatin capsule. For II, III, and IV, GnRH was dissolved in .9% saline with .9% benzyl alcohol and stored at 4°C until used. For GnRH in the 2% gelatin carrier, gelatin powder for a 2% solution was added to saline (.9% with .9% benzyl alcohol) and stored at 4°C until used. For GnRH in the 2% gelatin carrier, gelatin powder for a 2% solution was added to saline (.9% with .9% benzyl alcohol) and stirred continuously over heat until the gelatin powder was dissolved. The mixture was allowed to cool to approximately 32°C at which time the GnRH was added. After thorough mixing, 1 ml of the mixture was aspirated into syringes and stored at 4°C until used. The GnRH in the 2% carboxymethylcellulose carrier was prepared the same as described for 2% gelatin except carboxymethyl-
cellulose powder was used. All injections (II to VI) had 50 μg GnRH/ml solution. For VII, 50 μg of GnRH was dissolved in 10 μl of saline (.9% with .9% benzyl alcohol), pipetted into one end of a Number 00 gelatin capsule, quick frozen, the remaining end of the capsule replaced and stored at -20°C until used.

Blood was collected via jugular venipuncture into heparinized vacutainers immediately prior to treatment, at .5-h intervals for 4 h and at 5.0, 6.0, 7.0, and 8.0 h posttreatment. Following collection, blood was chilled on ice water until centrifugation at 10,000 x g for 10 min at 4°C. Plasma was decanted and stored at -20°C until assayed.

LH Assay

The LH in plasma was quantified by double antibody radioimmunoassay as reported by Niswender et al. (12) except 125I was the radioactive label. The anti-ovine LH antiserum (GDN #15), diluted 1/40,000, exhibited a mean binding of 44%. Plasma samples were assayed in duplicate 100 μl volumes. The NIH-LH-S 16 ovine LH was used as the ovine standard at a range of .1 to 10 ng per tube (92.9% bound to 14.5% bound, respectively). The .1 ng/tube (92.9% bound) point of the standard curve was greater (P<.05) than 0 ng/tube (100% bound). When various volumes (50, 100, and 200 μl) of pooled sheep plasma were assayed, LH's were 1.5, 1.7, and 1.3 ng/ml, respectively. The intra-assay coefficient of variation was 6.2%. Standard curves showed intra-asay coefficients of variation of 2.1% at the lowest concentration of the curve to 5.3% at the highest concentration of the curve.

Data Analysis

Following treatment (0 to 8 h), LH data from all groups were analyzed by split plot analysis of variance (4). The interval from treatment to LH peak and the duration of LH surge were analyzed by analysis of variance (17). The duration of the LH surge was defined as the interval LH concentrations remained above pretreatment concentrations. A multiple range test was used for identification of differences between means (9). Concentrations of LH in plasma from the peak to the return to pretreatment content were analyzed also by linear regression and analysis of covariance (16). A Chi-square test described by Fisher and Yates (2) was used to test differences in enumerative data.

RESULTS

Although concentrations of LH in plasma fluctuated in the control ewes, increase in plasma LH was not sustained. The GnRH induced release of LH in all treated ewes. Even though the mean concentration of LH in plasma in III was less (P<.05) than in II, IV, V, and VI, all groups administered GnRH had higher (P<.05) concentrations of LH than I (control) (Table 1).

Concentrations of LH in plasma increased by .5 h posttreatment in all but two ewes which were in VII. In those two ewes, concentrations of plasma LH were higher than pretreatment concentrations by 1 h posttreatment. The mean interval from GnRH treatment to peak LH concentrations was .8 to 1.6 h for II to VI (P>.05) (Table 1). The interval from GnRH treatment to LH peak in VII, however, was 2.8 ± .5 h and was longer (P<.05) than for II to VI. In II, IV, V, and VI, at least three of the four ewes in each group had an LH peak equal to or greater than 100 ng/ml, whereas only two ewes in III and one ewe in VII had peak LH concentrations equal to or greater than 100 ng/ml (Table 1). There were fewer (P<.05) ewes with peak LH concentrations equal to or greater than 100 ng/ml in VII than II, IV, V, and VI.

Both carrier and method of administration affected duration of GnRH induced LH release (Table 1). Although subcutaneous injection of GnRH in saline (IV) induced an LH surge 1.7 h longer (P<.05) than the LH surge following intravenous GnRH treatment in saline (III), neither was different (P<.05) from the LH surge induced by intramuscular injection of GnRH in saline (II). Dissolving GnRH in gelatin (V) or carboxymethylcellulose (VI) and injecting subcutaneously did not prolong (P>.05) the LH surge as compared to dissolving in saline and injecting subcutaneously (IV). However, the LH surge induced by GnRH dissolved in gelatin or carboxymethylcellulose and injected subcutaneously was longer (P<.05) than the LH surge induced by GnRH in saline and injected intravenously or intramuscularly. Subcutaneous administration of GnRH in a
TABLE 1. Number of ewes with peak LH concentrations > 100 ng/ml, interval from treatment to LH peak, duration of the LH surge, and mean LH release in ovariectomized ewes administered GnRH.

<table>
<thead>
<tr>
<th>Treatment group&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Carrier vehicle</th>
<th>Method of administration</th>
<th>Number with and LH peak ≥ 100 ng/ml</th>
<th>Time to LH peak (h)</th>
<th>Duration of LH surge&lt;sup&gt;c&lt;/sup&gt; (h)</th>
<th>Mean LH release (ng/ml)</th>
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<tr>
<td>I&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Saline intramuscular</td>
<td></td>
<td>0</td>
<td>.8&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5.1&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>8.6&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>II</td>
<td>Saline intravenous</td>
<td></td>
<td>2&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>.8&lt;sup&gt;e&lt;/sup&gt;</td>
<td>4.1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>45.7&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>III</td>
<td>Saline Subcutaneous</td>
<td></td>
<td>3&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.6&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5.8&lt;sup&gt;fg&lt;/sup&gt;</td>
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<td>IV</td>
<td>Gelatin Subcutaneous</td>
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<td>54.2&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
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<td>V</td>
<td>Carboxymethyl-cellulose Subcutaneous</td>
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<td>Gelatin capsule Subcutaneous</td>
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<td>2.8&lt;sup&gt;f&lt;/sup&gt;</td>
<td>7.5&lt;sup&gt;h&lt;/sup&gt;</td>
<td>41.0&lt;sup&gt;ef&lt;/sup&gt;</td>
</tr>
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</table>

<sup>a</sup>Four ewes per group.  
<sup>b</sup>Standard error.  
<sup>c</sup>Interval that LH concentrations remained above pretreatment concentrations.  
<sup>d</sup>Control ewes.  
<sup>e</sup>,<sup>f</sup>,<sup>g</sup>,<sup>h</sup>Means with a different superscript are significant (P<.05).  
<sup>i</sup>Figures in parentheses are the number of ewes in which LH concentrations did not return to pretreatment concentrations.
gelatin capsule (VII) induced the longest LH surge. Even though the duration LH surge for ewes in VII was not different (P>.05) from V and VI, in three of the four ewes in VII, LH concentrations did not return to pretreatment concentrations. The linear decrease in LH from peak concentrations to the return to pretreatment concentrations is in Figure 1. Only VI and VII had different regression slopes (P<.01) from II and III.

DISCUSSION

The preovulatory LH surge in the ewe is characterized by an LH release of 8 to 10 h (5, 11). Although peak concentrations of LH induced by exogenous GnRH are similar to the preovulatory LH surge, the duration of the LH release is only about half the duration during the preovulatory LH surge (13, 15). Route of administration and carrier as in this study and dosage as reported by Kinder et al. (8) appear to affect duration of LH release induced by exogenous GnRH. Furthermore, the dosage of GnRH and the total amount of LH released by exogenous GnRH appear to be associated with GnRH induced ovulation in anestrous ewes (3, 8, 13). In cattle, Britt et al. (1) showed that subcutaneous administration of GnRH in a gelatin capsule induced ovulation in all postpartum dairy cows treated whereas others (7, 18) showed a 50% ovulation response when postpartum dairy cows were administered intramuscularly the same dosage of GnRH in saline. Subcutaneous administration of GnRH in carboxymethylcellulose or in the gelatin capsule appears to prolong effectively LH release. Therefore, future experiments are warranted to compare carrier and method of administration on ovulation responses to GnRH in anestrous ewes and postpartum cows.

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REFERENCES


