Relationship between pituitary responsiveness to Gn-RH and number of Gn-RH-binding sites in pituitary glands of beef cows

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Summary. Changes in the ability of Gn-RH to induce gonadotrophin release with time after synchronization of oestrus was determined in 4 groups of 6 cows each. Cows were given Gn-RH at 40-min intervals for 6 h beginning at -24, 0, 18 or 36 h (time 0 = removal of progestagen implant). Changes in concentration (ng/ml) of serum LH after Gn-RH averaged 2-9, 6-2, 6-4 and 33-4, whereas serum FSH averaged 25-7, 35-8, 35-8 and 97-3. Thus the responsiveness of the pituitary to Gn-RH had increased by 36 h after implant removal. Other groups of cows subjected to the same synchronization scheme were slaughtered at 0 h, 24 h or at various times after onset of oestrous behaviour. Gn-RH binding to crude pituitary membrane preparations was assessed. There was no apparent change in the affinity constant of Gn-RH-binding sites with time after synchronization. The number of Gn-RH-binding sites remained unchanged until the period of oestrus when a significant decline with time was detected. We conclude that the increase in pituitary responsiveness to Gn-RH that occurs before the preovulatory gonadotrophin surge was not directly associated with changes in number or affinity of pituitary Gn-RH-binding sites in crude pituitary membrane preparations.

Introduction

The quantities of LH and FSH released from the anterior pituitary gland after a single dose of Gn-RH vary during different stages of the ovulatory cycle in rats (Aiyer, Fink & Greig, 1974), hamsters (Arimura, Debeljuk & Schally, 1972), sheep (Reeves, Arimura & Schally, 1971) and women (Yen, Vandenberg, Rebar & Ehara, 1972). In each of these reports, Gn-RH-induced LH and FSH release was greatest just before the preovulatory gonadotrophin surges. This pro-oestrous increase in responsiveness of the pituitary gland to Gn-RH was also observed in dairy heifers in which oestrus was synchronized with PGF-2α (Convey, Beal, Seguin, Tannen & Lin, 1976). This increase in responsiveness may be a prerequisite for the development of gonadotrophin surges.

Gn-RH is a peptide hormone and therefore is believed to bind to specific binding sites in the plasma membrane of the gonadotrophs, thereby initiating intracellular changes which result in synthesis and release of LH and FSH. Changes in responsiveness of the pituitary gland to Gn-RH may be due to changes in numbers of Gn-RH binding sites. So far, during the time of increased responsiveness of the anterior pituitary gland to Gn-RH either no change or a decline in numbers of Gn-RH-binding sites was observed in rats (Savoy-Moore, Schwartz, Duncan & Marshall, 1980;
Clayton, Solano, Garcia-Vela, Dufau & Catt, 1980; Reeves, Tarnavsky & Platt, 1982) and ewes (Wagner, Adams & Nett, 1979; Crowder & Nett, 1982). The experiments described herein evaluate the relationship between the ability of the pituitary gland to release gonadotrophins in response to Gn-RH and total number of Gn-RH-binding sites in pituitary glands of beef cows.

**Materials and Methods**

**Hormones**

Gn-RH and its analogue, d-Ala⁶-des-Gly¹⁰-Gn-RH ethylamide (Gn-RH-A), were obtained from Beckman (Palo Alto, CA, U.S.A.) and Peninsula Laboratories (San Carlos, CA, U.S.A.), respectively.

**Preparation of crude membrane fraction from pituitary glands**

Pituitary glands were processed as previously described by Savoy-Moore *et al.* (1980) with modifications. Each anterior pituitary gland was bisected and then homogenized in 30 ml Tris–HCl buffer (pH 7.7, 10 mM-Tris with 1 mM-dithiothreitol) using an Omni Mixer (50 ml container; Sorvall, Norwalk, CT, U.S.A.) at maximal speed for 2 min. The resulting slurry was further homogenized with a hand-driven tissue grinder (glass to glass Pyrex 7726, 0-15 mm clearance, 10 strokes). The homogenate was centrifuged at 300 g for 5 min, and the supernatant fluid was centrifuged at 10 800 g for 20 min. The resulting pellet consisted of a membrane-rich upper layer and a lower layer of secretory granules (Clayton, Shakespear & Marshall, 1978). Because the Gn-RH-binding sites in membranes were our primary interest, we selectively recovered the loosely packed membrane portion of the pellet. This procedure was repeated twice after the membrane portion of the pellet was washed with buffer and centrifuged. This crude membrane fraction was then resuspended in 6 ml Tris–HCl buffer per g anterior pituitary gland (wet weight) to provide 300–500 µg membrane protein in 100 µl volume for use in the binding assay.

**Gn-RH-A iodination and validation of Gn-RH binding assay**

¹²⁵I-labelled Gn-RH-A was prepared by the lactoperoxidase–glucose oxidase method described by Clayton, Shakespear, Duncan & Marshall (1979). The specific activity of the ¹²⁵I-labelled Gn-RH-A was estimated by plotting quantity of ¹²⁵I-labelled Gn-RH-A specifically bound in the presence of various concentrations of unlabelled or labelled Gn-RH-A. Specific activity, calculated as described in the legend of Text-fig. 1, ranged from 1000 to 1350 µCi/µg. The maximal percentage of specific binding of ¹²⁵I-labelled Gn-RH-A to an excess of membrane protein represents that portion of intact ¹²⁵I-labelled Gn-RH-A that could be recognized by the binding sites. This maximal value varied with iodinations and ranged from 27 to 42%. Total radioactivity added to each assay tube was corrected for variation in maximal specific binding before Scatchard analyses.

For validation of the binding assay, fresh bovine pituitary glands were obtained from a local abattoir and processed as described above unless otherwise stated. The time course of binding reaction was estimated by incubating crude membrane preparation with 20 000 c.p.m. (15 pg) of ¹²⁵I-labelled Gn-RH-A in the presence of 0 or 1 µg unlabelled Gn-RH-A for variable periods ranging from 10 to 360 min. By 60 min, maximal binding was achieved. To determine temperature dependency, the binding assay was conducted similarly but incubation temperature was varied, i.e. 4, 17 or 37°C for 60 min. It was found that maximal binding was obtained at an incubation temperature of 4°C. Very little binding was observed at 17 or 37°C. Hence all incubations were at 4°C for 60 min hereafter, unless otherwise stated.

The number of Gn-RH binding sites in crude membrane preparations was limited as depicted in Text-fig. 2. The amount of ¹²⁵I-labelled Gn-RH-A in excess of 10⁶ c.p.m. did not increase.
Text-fig. 1. Estimation of the specific activity of $^{125}$I-labelled Gn-RH-A (D-Ala$^6$-des-Gly$^{10}$-Gn-RH ethylamide). $\times \cdots \times$ % specific binding of $^{125}$I-labelled Gn-RH-A in the presence of increasing amounts of unlabelled Gn-RH-A; $\bullet \cdots \bullet$ % specific binding in the presence of increasing amounts of $^{125}$I-labelled Gn-RH-A. Specific activity (sp. act.) was calculated by dividing the c.p.m. used at 50% of the maximal specific binding by the quantity of unlabelled Gn-RH-A that displaced half of the $^{125}$I-labelled Gn-RH-A bound.

\[
\% \text{ Specific binding} = \frac{\text{Total binding (c.p.m.)} - \text{non-specific binding (c.p.m.)}}{\text{$^{125}$I-labelled Gn-RH-A added (c.p.m.)}} \times 100\%
\]

Text-fig. 2. Specific binding of $^{125}$I-labelled Gn-RH-A to a crude membrane fraction prepared from bovine anterior pituitary glands, expressed as a function of $^{125}$I-labelled Gn-RH-A concentration. Specific binding is the difference between total binding and non-specific binding.

Specific binding to a constant amount of membrane preparation. Thus, the specific binding of $^{125}$I-labelled Gn-RH-A to crude membrane preparation was a saturable phenomenon.

Scatchard analyses of binding assays using crude membrane fractions of pituitary glands from 4 ovariectomized cows and 3 intact cows (early luteal phase) gave similar estimates of affinity.
Text-fig. 3. Scatchard analysis of $^{125}$I-labelled Gn-RH binding to two concentrations of crude membrane fraction prepared from bovine anterior pituitary glands (224 and 735 mg). Each analysis consisted of 3 replicates of 5 concentrations of unlabelled Gn-RH-A.

Table 1. Number and affinity of Gn-RH-binding sites in bovine anterior pituitary glands from 3 intact cows and 4 ovariectomized cows.

<table>
<thead>
<tr>
<th></th>
<th>Affinity constant ($\times 10^{10}$ M$^{-1}$)</th>
<th>Binding sites (fmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact cows</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.9</td>
<td>47.0</td>
</tr>
<tr>
<td>2</td>
<td>1.2</td>
<td>37.3</td>
</tr>
<tr>
<td>3</td>
<td>1.0</td>
<td>35.8</td>
</tr>
<tr>
<td>Mean ± s.e.m.</td>
<td>1.0 ± 0.09</td>
<td>40.0 ± 3.51</td>
</tr>
<tr>
<td>Ovariectomized cows</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.9</td>
<td>89.6</td>
</tr>
<tr>
<td>2</td>
<td>1.3</td>
<td>59.3</td>
</tr>
<tr>
<td>3</td>
<td>1.0</td>
<td>100.7</td>
</tr>
<tr>
<td>4</td>
<td>1.0</td>
<td>81.2</td>
</tr>
<tr>
<td>Mean ± s.e.m.</td>
<td>1.1 ± 0.09</td>
<td>82.7 ± 8.76*</td>
</tr>
</tbody>
</table>

* Value significantly higher ($P \approx 0.01$) than that of intact cows.

constant (average 1.0 ± 0.1 × 10$^{-10}$ M$^{-1}$; Table 1). However, the number of Gn-RH-binding sites in the pituitary glands of these 4 ovariectomized cows was higher ($P = 0.01$) than that for the 3 intact cows (82.7 compared with 40.0 fmol/mg protein respectively). Affinity constants obtained from Scatchard analyses of binding using 224 and 735 µg crude membrane preparation of pituitary glands from the local abattoir were similar (1.2 and 1.4 × 10$^{10}$ M$^{-1}$, respectively; Text-fig. 3). Furthermore, comparable numbers of Gn-RH binding sites (121.6 and 125.9 fmol, respectively) per mg protein were found. These values were higher than the aforementioned values in this manuscript. We believe that this difference is due to the undefined reproductive status of pituitary glands of cattle from the local abattoir. In fact, consistently higher numbers of Gn-RH-binding sites were obtained from Scatchard analyses of binding assays performed after each Gn-RH-A iodination using this source of pituitary glands.

Hormone specificity of binding of $^{125}$I-labelled Gn-RH-A to a crude membrane preparation of
antior pituitary glands was determined by incubating 1 µg bovine TSH, bovine FSH, bovine LH, ovine LH, ovine FSH, somatostatin, TRH, ACTH, oxytocin, synthetic Gn-RH, and increasing amounts of unlabelled Gn-RH-A with a constant amount of 125I-labelled Gn-RH-A (20,000 c.p.m./tube). Only synthetic Gn-RH and Gn-RH-A inhibited the binding of radioactive Gn-RH-A.

To determine whether Gn-RH-A binding is specific for membranes from anterior pituitary glands, binding was tested using the crude membrane fraction of various tissues prepared as described for anterior pituitary glands. There was little if any binding of 125I-labelled Gn-RH-A to crude membrane fractions of bovine pancreas, lung, renal cortex, corpus luteum, cerebral cortex and hypothalami from 3 cows (0–6% of that observed with crude membrane fraction of pituitary glands on per mg protein basis). We did, however, detect significant Gn-RH-A binding to liver and the adrenal glands (12 and 11%, respectively).

Gn-RH binding assay

The assay reaction was carried out in a total volume of 500 µl assay buffer consisting of 10 mM-Tris–HCl with 1 mM-dithiothreitol and 0.5% bovine serum albumin. 125I-labelled Gn-RH-A (15 pg) was incubated with increasing amounts of unlabelled Gn-RH-A and 300–500 µg crude membrane preparation in polypropylene tubes at 4°C for 60 min. Non-specific binding was assessed in tubes containing 1 µg unlabelled Gn-RH-A. At the end of incubation, 3 ml assay buffer were added to each tube and all were centrifuged at 27,000 g for 20 min. The supernatant fluid was decanted and the pellet counted in a gamma spectrometer with a counting efficiency of 86%.

Experiments

Beef cows showing normal oestrous cycles were used. Oestrus was synchronized using the following treatment scheme (Staigmiller, England, Webb, Short & Bellows, 1982). A synthetic progestagen (SC21009: Searle Co., Chicago, IL, U.S.A.) was implanted into an ear of each cow and left in place for 7 days. At 24 h before removal of the implant, 25 mg prostaglandin F-2α (Upjohn Co., Kalamazoo, MI, U.S.A.) were given intramuscularly to induce regression of any existing corpora lutea. The time when the progestagen implant was removed was designated as time zero. Based on considerable experience with this synchronization regimen, we expected cows so treated to exhibit oestrous behaviour from about 36 h. Pro-oestrus was therefore the time period from removal of progestagen implants to onset of oestrus.

Pituitary responsiveness to Gn-RH during pro-oestrus. Beef cows, 6 in each of 4 groups, were randomly assigned to be given 9 injections of Gn-RH (500 ng/injection) via a jugular cannula at 40-min intervals beginning at -24, 0, 18 or 36 h in relation to implant removal. Blood was obtained via the same cannula at 10-min intervals beginning 10 min before the first Gn-RH injection and ending 40 min after the 9th injection of GnRH.

Pituitary binding of GnRH during pro-oestrus/oestrus. Beef cows (N = 31) were randomly assigned to be slaughtered at 0 (N = 8) or 24 h (N = 8) after removal of the progestagen implant, or at times after first detection of oestrous behaviour (N = 15). Onset of oestrus was detected with the aid of a sterile bull fitted with a grease marking harness and was defined as the first time a cow would stand to be mounted by the bull. Blood was collected at 4 and 2 h before, and at the time of, slaughter for cows slaughtered at 0 and 24 h. Cows in the third group were observed for signs of oestrous behaviour at 30-min intervals beginning at 30 h. They were bled at 6-h intervals, beginning at 24 h, then at 2-h intervals after oestrous behaviour was first observed. The times of slaughter were between 0-5 and 11-8 h after first detection of oestrous behaviour and onset of preovulatory gonadotrophin surges (or 43-0 and 78-8 h after removal of the progestagen implant).

Pituitary glands were collected and placed in Tris–HCl buffer within 15 min after the donor was killed. Anterior pituitary glands were dissected, weighed and homogenized as described above.
Portions of this homogenate were stored at -70°C until assayed for LH and FSH content. A crude membrane fraction was prepared from the remaining homogenate as described. Binding assays were carried out for individual animals and Scatchard analyses were performed from data so obtained. Numbers of binding sites, determined by Scatchard analyses, are expressed as fmol/mg membrane protein. Protein was assayed as described by Lowry, Rosebrough, Farr & Randall (1951) using bovine serum albumin as standard.

Infundibular stalks were also dissected, weighed and stored at -70°C until measurement of Gn-RH content. We have previously demonstrated that 96% of Gn-RH in the hypothalamus resides in the pituitary stalk in cattle (Estes, Padmanabhan & Convey, 1977).

Radioimmunoassays

Blood was stored for 4 h at room temperature, then at 4°C for an additional 12 h before centrifugation to obtain serum. Concentrations of LH and FSH in serum samples from each experiment were determined each in a single assay using double-antibody procedures previously validated in this laboratory (Convey et al., 1976; Carruthers, Convey, Kesner, Hafs & Cheng, 1980). The standard preparations of LH and FSH were NIH-LH-B8 and NIH-FSH-B1, respectively. Within-assay coefficient of variation averaged 7.7 ± 0.6% for LH and 16.2 ± 0.6% for FSH and sensitivities of LH and FSH assays were 0.125 and 2.5 ng/tube, respectively. Serum oestradiol was measured by radioimmunoassay as previously reported (Carruthers & Hafs, 1980). Inter- and intra-assay coefficients of variation determined from 6 assays were 6.8% and 11.1% for a pool of serum from ovariectomized cows to which 5 pg oestradiol/ml was added. The sensitivity of the oestradiol assay was 0.8 pg/tube.

Gn-RH in infundibular stalks was extracted with 2 N-acetic acid and measured in a single assay using an antibody validated by Nett, Akbar, Niswender, Hedlund & White (1973) and procedures described by Estes et al. (1977). Within assay coefficient of variation was 10.0% and the sensitivity of the Gn-RH assay was 3.0 pg/tube.

Statistical analyses

All data were tested for heterogeneity of variances amongst groups using Bartlett’s test (Gill, 1978). When heterogeneity was found, data were subjected to logarithmic or square-root transformation before analysis by one-way analysis of variance. Specific comparisons of means were conducted using Bonferroni’s t test or Dunnett’s t test (Gill, 1978).

Results

Pituitary responsiveness of Gn-RH during pro-oestrous

Mean serum concentrations of LH and FSH in animals given multiple injections of Gn-RH are shown in Text-fig. 4. Changes in concentrations of LH and FSH in serum in response to Gn-RH, expressed as difference (Δ) between mean pretreatment values and the single maximal gonadotrophin concentrations obtained during all Gn-RH injections were used in the statistical analysis. Before the start of Gn-RH-treatment, LH and FSH concentrations in serum were not different (P > 0.20) amongst groups, and were 1.8 ± 0.14 and 56.3 ± 3.23 ng/ml, respectively. Δ LH and Δ FSH concentrations (ng/ml serum) of those beef cows given Gn-RH beginning -24, 0, and 18 h after implant removal were small and relatively stable, averaging 2.9, 6.2 and 6.4 for LH, and 25.7, 35.8 and 35.8 for FSH, respectively. However, animals given Gn-RH beginning at 36 h had a greater change (Δ LH = 33.4 ± 6.1 ng/ml and Δ FSH = 97.3 ± 15.8 ng/ml) (P < 0.005 compared with the other 3 groups).

Although there was no change in the concentrations of oestradiol in serum collected during each
Gn-RH injection period, a linear increase \((P < 0.05)\) in mean treatment concentrations of serum oestradiol from 5.5 to 8.9 ng/ml was detected between \(-24\) and 36 h.

**Pituitary binding of Gn-RH during pro-oestrus/oestrus**

Concentrations of LH and FSH in serum of all three treatment groups are presented in Text-fig. 5. Data from the group slaughtered after onset of the preovulatory gonadotrophin surges were normalized to the time of peak gonadotrophin surges. In all cases, maximal FSH concentration in serum coincided with that of LH. Preovulatory LH and FSH surges in these cattle resembled those of normally cyclic cows in terms of magnitude, duration and general shape (Chenault, Thatcher, Kalra, Abrams & Wilcox, 1975; Dobson, 1978; Rahe, Owens, Fleeger, Newton & Harms, 1980). The time of onset of oestrus occurred at an average of \(-3.5 \pm 0.7\) h before peak gonadotrophin surges.

Concentration of oestradiol was measured in the last sample obtained from each animal.
Text-fig. 5. LH and FSH in serum collected during the pro-oestrous/oestrous period after synchronization of oestrus. Values from animals killed at 0 and 24 h are means ± s.e.m. of samples collected at −4, −2 and 0 h before slaughter. Data obtained during the preovulatory gonadotrophin surges are normalized to the time of peak of LH and FSH values (from −4 to +6 h). Values are mean ± s.e.m. (when larger than the datum symbol) for the no. of cows indicated in parentheses.

Text-fig. 6. Number of Gn-RH-binding sites and concentrations of gonadotrophins in bovine anterior pituitary glands during pro-oestrous/oestrous. Values from animals killed at 0 and 24 h are means ± s.e.m. Data from animals killed during the oestrous period are plotted individually. Onset of oestrus occurred −3.5 ± 0.7 h before the peak gonadotrophin surges.
slaughtered at 0 or 24 h, and also in samples obtained after first detection of oestrous behaviour. During the period of oestrus, mean oestradiol concentrations in serum were higher \((P < 0.05)\) than in cows killed at 0 and 24 h \((8.6 \pm 0.6\) compared with \(4.7 \pm 0.7\) pg/ml).

Neither Gn-RH content in the infundibular stalk nor the affinity constant of Gn-RH-binding sites were different \((P > 0.25)\) amongst all groups, averaging \(1.4 \pm 0.10\) Ng Gn-RH/mg stalk and \(1.2 \pm 0.04 \times 10^{10} \text{M}^{-1}\), respectively (data not shown). In addition, numbers of Gn-RH-binding sites and pituitary contents of LH and FSH remained constant 0 and 24 h after implant removal (Text-fig. 6). However, a significant decrease in the number of Gn-RH-binding sites from the time of onset of oestrus was detected. This decline was concurrent with the decreasing pituitary contents of LH and FSH.

**Discussion**

The objective of this research was to determine whether the pro-oestrous/oestrous increase in the ability of the pituitary gland to release gonadotrophins in response to Gn-RH in cattle was associated with changes in number and/or affinity of Gn-RH binding sites of the pituitary gland. To be able to predict time of oestrus with reasonable accuracy, we chose to use a scheme for synchronization of oestrus. Because we did not know whether pituitary responsiveness to Gn-RH would increase before oestrus in cattle so treated, we deemed it necessary to measure change in responsiveness during the pro-oestrous period after this synchronization treatment. In the present study, the quantity of LH and FSH released in response to Gn-RH increased several fold by 36 h after completion of the synchronization schedule, indicating that the responsiveness of the pituitary gland is increased before the time of expected preovulatory surges of gonadotrophins. This result is in agreement with a similar report by Convey *et al.* (1976) in which oestrus was synchronized in dairy heifers by using PGF-2\(\alpha\) alone.

If the increase in the ability of the pituitary gland to release gonadotrophins was directly associated with Gn-RH-binding sites, one would expect to detect changes in the number and/or affinity of Gn-RH-binding sites before or concurrent with the increase in pituitary responsiveness to Gn-RH. No changes in the number or affinity of Gn-RH-binding sites were detected up to the time of oestrus. Although it is possible that we did not assess Gn-RH-binding sites at the appropriate time, we feel that this is unlikely in view of data from equivalent studies of rats, hamsters and ewes. For example, increased responsiveness of the pituitary gland to Gn-RH occurs during pro-oestrus in rats (Aiyer *et al.*, 1974) and hamsters (Arimura *et al.*, 1972) and at oestrus in ewes (Reeves *et al.*, 1971), although no change in number and affinity of Gn-RH-binding sites in the pituitary glands was observed on the morning of pro-oestrus in rats (Savoy-Moore *et al.*, 1980; Clayton *et al.*, 1980) and hamsters (Adams & Spies, 1981), or in ewes before, during and after oestradiol-induced surges of gonadotrophins (Wagner *et al.*, 1979). The only time during the oestrous cycle when the number of Gn-RH-binding sites increases in rats is between the days of metoestrus and di-oestrus, a time when responsiveness of the pituitary gland to Gn-RH is quite low relative to that at pro-oestrus (Aiyer *et al.*, 1974).

During the period of oestrous behaviour and preovulatory gonadotrophin surges, we observed a gradual decline in the number of Gn-RH-binding sites that occurred in synchrony with depletions of pituitary contents of LH and FSH. Again this is in agreement with data obtained from rats and hamsters. Marked decreases in numbers of Gn-RH-binding sites were observed in the late afternoon of pro-oestrus coincident with the LH surge (rat: Savoy-Moore *et al.*, 1980; Clayton *et al.*, 1980) or on the day of oestrus (hamster: Adams & Spies, 1981; rat: Reeves *et al.*, 1982). The detection of decreased Gn-RH-binding sites during oestrus in the present study in conjunction with the observation of higher numbers of Gn-RH-binding sites in ovariectomized cows when compared with intact cows (see 'Materials and Methods') provided evidence that this assay procedure was able to detect differences in the numbers of Gn-RH-binding sites in the anterior pituitary gland when differences existed. Therefore, the failure to detect any change in the
number or affinity of the binding sites concurrent with increased responsiveness of the pituitary glands to Gn-RH in the present study provides evidence that the change in the responsiveness of the pituitary gland during pro-oestrus may not be directly related to a concomitant change in number or affinity of the Gn-RH-binding sites in the gland.

There are many possible cellular changes that could result in a prompt increase in the responsiveness of the pituitary gland to Gn-RH. One could be an increase in the activity at site(s) of control subsequent to the formation of Gn-RH-binding site complexes. For example, maturation of a second messenger system or a calcium-dependent process may govern the eventual increase in the responsiveness of the anterior pituitary gland to Gn-RH. Cyclic AMP (Borgeat et al., 1972) or cyclic GMP (Naor, Fawcett & McCann, 1978) has been suggested as the second messenger of Gn-RH action in stimulating gonadotrophin release. However, the influx of calcium after Gn-RH administration may be important for the release of LH (Conn, Rogers & Sandhu, 1979). Any increase in concentration of cAMP, cGMP or calcium intracellularly could eventually lead to increased release of both LH and FSH from the pituitary glands in response to exogenous Gn-RH.

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