A mathematical model quantifying GnRH-induced LH secretion from gonadotropes

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Blum, J. Joseph, Michael C. Reed, J o Ann J anovick, and P. Michael Conn. A mathematical model quantifying GnRH-induced LH secretion from gonadotropes. Am. J. Physiol. Endocrinol. Metab. 278: E263–E272, 2000.—A mathematical model is developed to investigate the rate of release of luteinizing hormone (LH) from pituitary gonadotropes in response to short pulses of gonadotropin-releasing hormone (GnRH). The model includes binding of the hormone to its receptor, dimerization, interaction with a G protein, production of inositol 1,4,5-trisphosphate, release of Ca2+ from the endoplasmic reticulum, entrance of Ca2+ into the cytosol via voltage-gated membrane channels, pumping of Ca2+ out of the cytosol via membrane and endoplasmic reticulum pumps, and release of LH. Cytosolic Ca2+ dynamics are simplified (i.e., oscillations are not included in the model), and it is assumed that there is only one pool of releasable LH. Despite these other simplifications, the model explains the qualitative features of LH release in response to GnRH pulses of various durations and different concentrations in the presence and absence of external Ca2+.

The secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) by gonadotropes located in the anterior pituitary is stimulated by gonadotropin-releasing hormone (GnRH), a decapetide that is released by the hypothalamus. It was suggested by Conn et al. (5, 7) that dimerization of the GnRH receptors on the surface of the gonadotropes was sufficient to initiate the release of LH, and it has been established that this event occurs in response to agonist (but not antagonist) occupancy of the receptor (17). Mathematical models have been developed to explore some of the consequences of dimerization on the response of the gonadotropes to various concentrations of GnRH and some related peptides (1, 22). Those models focused primarily on the kinetics of receptor binding and dimerization and did not include the interaction of the dimerized receptors with G proteins or the subsequent complex intracellular signaling systems such as the release of inositol 1,4,5-trisphosphate (IP3) and the vesicular and cell membrane Ca2+ dynamics.

Since those early models, our understanding of the signaling systems between GnRH binding and LH release has greatly increased (18, 39). The development of perfusion systems and optical methods to study the changes in cytosolic Ca2+ content of individual gonadotropes has allowed much new data to be obtained on the changes in cytosolic Ca2+ concentration (CAC) and the rates of release of LH in response to short pulses of GnRH. We limit ourselves to modeling the response to short pulses of GnRH, thus allowing us to ignore the effects of changes in gene expression that are known to occur in response to long exposure to GnRH (12, 19). Furthermore, it has become possible to generate pituitary cell lines expressing different concentrations of GnRH receptors (27) and, thus, to obtain data on the effect of receptor number on rates of release of LH and of the change in cytosolic concentrations of IP3, the signaling compound that activates release of Ca2+ from the endoplasmic reticulum (ER) (26). In view of these advances in our understanding of the cell physiology of gonadotropes, we believed that it was appropriate to expand the earlier mathematical models to include the interaction of the dimerized receptors with a G protein in the cell membrane, the consequent release of IP3 and, therefore, of Ca2+ from the Ca2+ stores in the ER (CAER), the opening of Ca2+ channels in the plasma membrane, and the subsequent active transport of Ca2+ back into the ER and into the extracellular fluid.

In this study we have omitted many features of the complex pathway between the binding of GnRH to its receptors and LH release. Furthermore, we focus only on data concerning LH release in response to relatively short pulses of GnRH and the effects of varying GnRH receptor number (GnRHR). In future studies we expect to expand the model to include receptor internalization (14), the biphasic regulation of GnRHR that occurs during long exposure to GnRH (6), and the effects of CAC on the rates of uptake and release of Ca2+ by the ER and the plasma membrane (39).

It is well established that GnRH induces oscillations in CAC in gonadotropes via voltage-gated Ca2+ channels (VGCCs) located in the plasma membrane (39). The initial rise in CAC in response to GnRH is, however, independent of extracellular Ca2+ (CAE) and is due to the rapid release of Ca2+ from the ER. A number of studies have shown that, at low and medium concentrations of GnRH, there is an increase in the
initial spike and the subsequent plateau level of CAC and in the frequency of the subsequent oscillations with increasing concentrations of GnRH (38). At high GnRH concentrations, however, there is no further increase in the initial spike level of CAC, but the frequency does increase. Although there are well-developed models for Ca²⁺ oscillatory behavior in a number of cell types (10, 11), none specifically apply to the CAC oscillations of gonadotropes. Furthermore, most of the published measurements of LH release have been performed over minutes or hours and, thus, represent the LH released over many oscillations. In view of these data and the close correlation between the rise in CAC and the amount of LH released (38), we have chosen to model the CAC (and the CAER) as smooth functions of time that mimic the initial spike and the subsequent average Ca²⁺ levels. Although the rate of CAC oscillations increases with increasing IP₃ concentration, the IP₃ level, on which the GnRH-induced Ca²⁺ responses depend, does not oscillate (37).

Also, considerable evidence indicates that low CAC facilitates and high CAC inhibits the release of Ca²⁺ from the ER (39). The activation of phospholipase C by the G protein(s) after GnRH binds to its receptor stimulates the production of IP₃ and diacylglycerol. Because an inhibitor of diacylglycerol lipase causes dose-dependent inhibition of LH release, without affecting the ability of arachidonic acid to facilitate LH release (2, 34), it appears that arachidonic acid is also involved in the signaling system by which GnRH causes LH release. Present evidence indicates that this is via a Ca²⁺-independent mechanism, and we have not included the diacylglycerol-arachidonic acid pathway in the present model.

Recent studies show that multiple proteins are involved in GnRH signaling (13, 30). Janovick and Conn (16) showed that the G protein involved in the initiation of the GnRH-activated signaling pathway is inhibited by cholera toxin, but not by pertussis toxin, and is, therefore, a Gₛ guanyl nucleotide-binding protein. They also obtained data that indicated two pools of LH in gonadotropes and redistribution of LH by GnRH from a nonreleasable pool to the releasable pool. Because the kinetics of transfer of LH from the nonreleasable pool to the releasable pool have not been studied, we have chosen to treat LH release as occurring from a single pool.

Glossary

Variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
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<tbody>
<tr>
<td>H</td>
<td>GnRH concentration (nM)</td>
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<tr>
<td>R</td>
<td>Free GnRHR concentration (nM)</td>
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<td>HR</td>
<td>Hormone-receptor complex concentration (nM)</td>
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<td>HRRH</td>
<td>Hormone-receptor dimer concentration (nM)</td>
</tr>
<tr>
<td>E</td>
<td>Effector concentration (nM)</td>
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<tr>
<td>IP₃</td>
<td>Inositol 1,4,5-trisphosphate concentration (nM)</td>
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<tr>
<td>CAC</td>
<td>Cytosolic Ca²⁺ concentration (µM)</td>
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<td>CAER</td>
<td>ER Ca²⁺ concentration (µM)</td>
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<tr>
<td>CHO</td>
<td>Fraction of open ER Ca²⁺ channels</td>
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<td>LH</td>
<td>LH concentration (ng)</td>
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Constants

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<tr>
<td>GQ₀</td>
<td>Total G protein concentration (nM)</td>
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<td>ERUL</td>
<td>Resting Ca²⁺ concentration in ER (µM)</td>
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<td>CAE</td>
<td>External Ca²⁺ concentration (µM)</td>
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<td>α</td>
<td>Constant in Eq. 10 for fraction of open ER channels (nM⁻¹)</td>
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<td>β</td>
<td>Constant in Eq. 10 for fraction of open ER channels (min⁻¹)</td>
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Rate Constants

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<td>k₁₀</td>
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MODEL DEVELOPMENT

Our model for the stimulation by GnRH of LH release by pituitary cells is described in three stages. In the first stage, the hormone binds to the receptor, dimers are formed, and the production of effectors and IP₃ occurs. In the second stage, IP₃-regulated channels on the ER allow Ca²⁺ to be released and subsequently pumped back into the ER. In the third stage, the voltage-sensitive cell membrane Ca²⁺ channel, leakage Ca²⁺ channels, the cell membrane Ca²⁺ pump, and the release of LH are described.

Hormone binding to IP₃ formation. We denote by H(t) the concentration of GnRH in the surrounding medium as a function of time, where time t is measured in minutes. The receptor concentration per unit volume will be denoted by R. We assume that the GnRH binds to the receptors via a simple reversible reaction

\[ H + R \rightleftharpoons HR \]  \[ k₁ \]

The bound complex HR reacts reversibly with itself to form dimers, the concentration of which is denoted by HRRH

\[ HR + HR \rightleftharpoons HRRH \]  \[ k₂ \]

A G protein, the concentration of which is denoted by GQ, reacts with the dimer via a reversible reaction to produce an effector, E. In this initial study, as discussed in the introduction, it is assumed that E represents...
only phospholipase C

\[ \frac{d}{dt} \ R = -k_1H \cdot R + k_4HR \]  

(4)

\[ \frac{d}{dt} \ HR = k_1H \cdot R - k_4HR \]  

(5)

\[ \frac{d}{dt} \ HRRH = -k_2HRRH + 2k_3HRRH - 2k_2HR \cdot HR \]  

(6)

\[ \frac{d}{dt} \ GQ = -k_2GQ \cdot HRRH + k_3E \]  

(7)

\[ \frac{d}{dt} \ E = k_3GQ \cdot HRRH - k_3E \]  

(8)

We assume that the production of IP_3 is proportional to the concentration of E. The subsequent metabolism of IP_3 in gonadotropes is complex (26), and the kinetics are unknown. Therefore, we assume simply that IP_3 is converted to inactive metabolites at a rate proportional to its concentration, thus yielding the differential equation

\[ \frac{d}{dt} \ IP_3 = k_5E - k_3JIP_3 \]  

(9)

A reasonable estimate for the number of GnRHRs per gonadotrope is \(10^4\) (4, 23–25, 27). If we assume that there are \(10^5–10^6\) cells/ml in a typical experiment on isolated gonadotropes, the GnRHR concentration would be 1.5–15 pM. Thus we will choose \(R_0 = 0.01\) nM as our "standard" total receptor concentration. We will study the effect of varying \(R_0\) later. The number of G proteins per gonadotrope is not known but, in other cells, is ~10-fold or more greater than the number of receptors (28); we have chosen the total concentration of G protein to be \(GQ_0 = 0.1\) nM. All the variables HR, HRRH, E, and IP_3 have the value 0, and \(R = R_0\) at \(t = 0\).

The standard values of the rate constants in Eqs. 1–9 are listed in the Glossary. Because degradation of GnRH itself has made it difficult to measure the affinity constant for the binding of the naturally occurring ligand to its receptors, a precise value is unavailable. A value of ~0.7 \times 10^9 M^{-1} has been suggested. Studies using a metabolically stable GnRH superagonist that was 40-fold more potent than GnRH gave a value of 2 \times 10^{10} M^{-1}, which when divided by 40 gives 0.5 \times 10^9 M^{-1} (24). The magnitudes of \(k_1\) and \(k_2\) were chosen so that most of the binding occurs within 30 s, and the affinity constant is 0.5 \times 10^9 M^{-1}. The rate constants for dimerization are unknown. We have chosen \(k_2\) and \(k_3\) such that there is a high tendency to dimerize and it occurs quite rapidly. Similarly, we chose the rate constants \(k_3\) and \(k_5\) so that the binding of the dimer to the G protein is rapid and has high affinity.

The rate constants \(k_3\) and \(k_5\) were chosen so that IP_3 approaches its steady-state level in response to H = 0.1 nM in ~2.5 min and to H = 10 nM in ~0.5 min, consistent with the short-pulse data of Morgan et al. (26).

In Figure 1 we show the responses of the variables R, HR, HRRH, and IP_3 to a 5-min pulse of GnRH for H = 0.1, 1, and 10 nM. At H = 0.1 nM, ~95% of the receptors are unoccupied, and thus the amounts of HRRH, E, and IP_3 are also very small. With a 10-fold increase of H to 1 nM, about two-thirds of the receptors are occupied, and much more HRRH, E, and IP_3 is produced. With further 10-fold increase in H, ~96% of the receptors are occupied, and further significant increases in HRRH, E, and IP_3 occur. At this high hormone concentration, the formation of HR is so rapid that HR first peaks (at ~0.1 min) and then declines as HRRH is formed. In all three cases, the concentrations approach their steady-state values in ~1 min and then relax back to their initial values in 2–5 min after the hormone is removed at t = 5 min.

ER Ca^{2+} is stored in the ER and released when IP_3 binds to receptors on the ER membrane. The detailed dynamics of these receptors are a subject of current research (20) and are thought to play a major role in the cytosolic Ca^{2+} oscillations. We do not attempt to model these detailed dynamics but, instead, assume that the fraction of open channels (CHO) has a given time course

\[ \text{CHO}(t) = \frac{\alpha 10^{-3} P_3(t)}{1 + \alpha 10^{-3} P_3(t)} (0.3 + 0.3 \beta t e^{-t/\beta}) \]  

(10)

which depends on IP_3 concentration in a Michaelis-Menten-type saturating fashion. Inside the cells, we measure concentrations in micromolar (instead of nanomolar), which is the reason for the factor 10^{-3} preceding IP_3(t). The fraction in the first factor on the right approaches a maximum of 1 for high IP_3 concentrations. The factor \(\beta t e^{-t/\beta}\) reaches a maximum of 1 when \(t = 1/\beta\). We choose the parameters \(\alpha\) and \(\beta\) of Eq. 10 as

\[ \alpha = 2 \text{nM}^{-1} \quad \text{and} \quad \beta = 4 \text{ min}^{-1} \]

so this factor reaches its maximum at 0.25 min. Therefore, the maximum of CHO (the probability of opening) is 0.6, consistent with the data of Ramos-Franco et al. (29). The time course of CHO(t) is shown in Fig. 2 for the steady-state concentrations of IP_3 = 395, 3,840, and 5,300 nM, which correspond to GnRH concentrations of 0.1, 1, and 10 nM (Fig. 1).
We assume that the release of Ca\(^{2+}\) from the ER is jointly proportional to CHO, to the difference (CAER - CAC), and to an intrinsic rate constant ERR, where

$$
\text{ERR} = k_6 + k_{66} \text{CAC} - k_{666} (\text{CAC})^2
$$

The complicated form of ERR reflects the fact that the release is facilitated by cytosolic Ca\(^{2+}\) at low CAC (via parameter \(k_{66}\)) and inhibited at high CAC (via parameter \(k_{666}\)), as indicated earlier. We assume that Ca\(^{2+}\) is pumped back into the ER at a rate jointly proportional to CAC (via sigmoidal kinetics) and to \((\text{ERUL} - \text{CAER})\), where ERUL is the resting concentration of Ca\(^{2+}\) in the ER. Thus

$$\frac{d}{dt} \text{CAER} = -\text{ERR} \times \text{CHO} \times (\text{CAER} - \text{CAC})$$

$$+ k_{-6} \frac{2(\text{CAC})^2}{0.5 + 2(\text{CAC})^2} \times (\text{ERUL} - \text{CAER})$$

(11)

Although there are no experimental measurements of the ratio of ER volume to cell volume in gonadotropes (or most other cells), a reasonable value is 1/20. Thus the dynamics of CAC (in the absence of the other mechanisms introduced below) would be given by the following equation

$$\frac{d}{dt} \text{CAC} = +(0.05) \text{ERR} \times \text{CHO} \times (\text{CAER} - \text{CAC})$$

$$- (0.05) k_{-6} \frac{2(\text{CAC})^2}{0.5 + 2(\text{CAC})^2} \times (\text{ERUL} - \text{CAER})$$

(12)

In most cells, including gonadotropes, the resting level of cytosolic Ca\(^{2+}\) is 0.05–0.2 µM (11, 38); we have chosen the rate constants in the system so that the equilibrium value (in the absence of H) is 0.1 µM. The resting Ca\(^{2+}\) concentration in the ER is unknown but large, and a reasonable value is ERUL = 40 µM (15, 40).

The rate constant ERR determines the rate of release of Ca\(^{2+}\) from the ER given by the first term on the right of Eq. 11. Initially, CAER = 20 µM and CAC = 0.1 µM, so by choosing \(k_6 = 5 \mu M^{-1} \cdot \text{min}^{-1}\), \(k_{66} = 50 \text{ min}^{-1} \cdot \mu M^{-1}\),

![Fig. 1. Response to a 5-min pulse of gonadotropin-releasing hormone (GnRH). Time variations of R, HR, HRRH, E, and IP3 are shown during and after a 5-min pulse of 3 concentrations of GnRH. A, D, and G: responses to 0.1 nM GnRH; B, E, and H: responses to 1.0 nM GnRH; C, F, and I: responses to 10 nM GnRH. See Glossary for definition of abbreviations for this and subsequent figures.](Image)

![Fig. 2. Probability of IP3-regulated Ca\(^{2+}\) channel opening. Time course is shown in response to a 5-min pulse of GnRH at indicated concentrations.](Image)
and \( k_{666} = 0 \), we would obtain an initial rate of decrease of CAER of

\[
[1 + (10)(0.1)](1)(40 - 0.1) = 80 \text{ \mu M/minute}
\]

if \( CHO = 1 \) (i.e., all the channels were open). The initial rate of increase of CAC would be 1/20th of that, i.e., 4 \( \mu \text{M/minute} \), by our volume hypothesis discussed earlier. These are overestimates, because, in fact, for the first few seconds only a small fraction of the ER channels are open, and the maximum probability of opening is 0.6. With these choices for \( k_6 \) and \( k_{66} \), the initial spike in CAC occurs in <1 min and reaches a maximum value at 0.2–1 \( \mu \text{M} \) (Fig. 3), consistent with experimental data. For this initial study, we ignore the inhibitory effect of high CAC on ERR and take \( k_{866} = 5 \).

The second term on the right of Eq. 11 represents pumping of \( \text{Ca}^{2+} \) back into the ER. By choosing \( k_2 = 5 \) \( \text{min}^{-1} \), the pumping of \( \text{Ca}^{2+} \) back into the ER is sufficiently weak (compared with the release of \( \text{Ca}^{2+} \) from the ER), allowing the initial CAC spike to occur.

\( \text{Ca}^{2+} \) levels and LH release. It is known that the effector E produced from the dimers also activates voltage-sensitive \( \text{Ca}^{2+} \) channels in the cell membrane (35). We let \( \text{CAE} \) denote the (constant) \( \text{Ca}^{2+} \) concentration in the external medium and assume that the rate of \( \text{Ca}^{2+} \) influx through the VGCCs is proportional to \( \text{CAE} - \text{CAC} \). The rate constant for the \( \text{Ca}^{2+} \) influx has the form

\[
VSR = k_8E + k_{866}\text{CAC} - k_{886}(\text{CAC})^2
\]

because there is evidence (35) that the rate is facilitated by low concentrations of CAC and inhibited by high concentrations. We assume that the \( \text{Ca}^{2+} \) pumps in the cell membrane obey second-order Michaelis-Menten kinetics (rate constant \( k_7 \)) and that \( \text{Ca}^{2+} \) leakage from outside to inside (rate constant \( k_9 \)) is a simple first-order process. By addition of these \( \text{Ca}^{2+} \) fluxes to Eq. 12, the following differential equation for CAC is obtained

\[
\frac{d}{dt} \text{CAC} = -(0.05) \text{ERR} * \text{CHO} * (\text{CAER} - \text{CAC})
+ (0.05) k_6 \frac{2(\text{CAC})^2}{0.05 + 2(\text{CAC})^2} * (\text{ERUL} - \text{CAER})
+ VSR * (\text{CAE} - \text{CAC}) - \frac{k_7(\text{CAC})^2}{0.1 + (\text{CAC})^2} + k_9\text{CAE}
\]

Finally, we assume that the rate of release of LH depends on CAC through second-order Michaelis-Menten kinetics

\[
\frac{d}{dt} \text{LH} = \frac{k_{10}(\text{CAC})^2}{2 + (\text{CAC})^2}
\]

We chose second-order kinetics so that, in the absence of GnRH, the low CAC fluctuations about 0.1 \( \mu \text{M} \) would cause a very low baseline LH release (15a). CAE = 1 mM is in the normal range of plasma free \( \text{Ca}^{2+} \) concentration.

The balance between \( k_7 \) and \( k_9 \) was chosen so that the rate of pumping out of the cell equals the rate of leakage into the cell when CAC = 0.1 \( \mu \text{M} \), its resting level. The magnitudes were chosen so that CAC returns to its resting state within a few minutes after the removal of GnRH. The magnitude of \( k_7 \) was chosen to obtain the typically observed elevated CAC values after the initial spike while GnRH is still present. In this initial study we ignore the facilitation and inhibition of the VGCCs by CAC, i.e., \( k_{88} = 0 = k_{866} \). We chose \( k_{10} = 5 \) \( \text{ng/min} \) to be consistent with the observed rates of LH release.

Figure 3A shows the time course of the CAER concentration for 5-min pulses at low, medium, and high concentrations of GnRH. When \( H = 0.1 \text{ nM} \), less than one-half of the \( \text{Ca}^{2+} \) in the ER is released into the cytoplasm, and the time course is slow. A 10-fold increase in the GnRH concentration results in a marked

Fig. 3. Response to a 5-min pulse of GnRH. Time variation of CAER, CAC, and rate of luteinizing hormone (LH) release is shown in response to indicated concentrations of GnRH.
increase in the rate of dumping by the ER, then the CAER concentration remains constant for the duration of the pulse. When \( H = 10 \) nM, the dumping is so fast that the CAER concentration overshoots the equilibrium level. At the end of the 5-min pulse, the dynamics of CAC and CAER are very complicated, because the IP3-sensitive channels are closing (Fig. 2) and the membrane calcium channel is shutting down as E decreases. When the GnRH of the pulse is low, the CAER begins to recover immediately after the pulse is terminated; at medium and high GnRH pulse levels, the recovery is preceded by a small further extrusion of Ca\(^{2+}\) from the ER. The detailed dynamic behavior is affected by the parameter choices and rate kinetics.

Figure 3B shows the CAC as a function of time. When \( H = 0.1 \) nM, there is an initial rise from 0.1 to \( \sim 0.19 \) µM followed by a gradual decrease. At \( H = 1 \) nM, the initial spike occurs more rapidly and goes to a much higher level, then attains a plateau while GnRH is still present. At \( H = 10 \) nM, the initial rate, the peak level, and the plateau level are somewhat higher, but the qualitative behavior remains the same. On termination of the pulse at 5 min, CAC returns to its resting level within \( \sim 5 \) min. In all three cases, the LH production rate follows the CAC. As will be discussed in detail below, this qualitative behavior is similar to that seen in numerous experiments.

The mathematical model consists of Eqs. 4–11, 13, and 14. As we have indicated, many simplifications are inherent in this model and several known processes are ignored. Furthermore, except for Eq. 10, we have chosen first-order, rather than Michaelis-Menten, kinetics. This has the advantage of making the system as simple as possible and reducing the number of parameters. Some of the parameters in the Glossary were chosen by reference to, e.g., measurements of cell volume and receptor number; the others were chosen to obtain model behavior qualitatively similar to experimental results. For convenience, we refer to the set of parameters in the Glossary as standard parameters.

**COMPARISON WITH EXPERIMENTS**

Chang et al. (3) exposed gonadotropes to 10 nM GnRH for 2 min in the presence and absence of extracellular Ca\(^{2+}\). In Fig. 4 we show the rate of LH release predicted by the model and CAC as a function of time in the presence (1,000 µM) and absence (5 µM) of external Ca\(^{2+}\). In the presence of Ca\(^{2+}\), an initial rapid rise in LH release rate is followed by a rapid decline, a brief pause, and then decay to zero in \( \sim 5 \) min, closely resembling the experimental results shown in Fig. 5 of Chang et al. (3). In the absence of Ca\(^{2+}\), the peak level of LH release is considerably lower and there is no pause, also as observed by Chang et al. In both cases, the rate of LH release is closely correlated to cytosolic Ca\(^{2+}\), as expected.

Experiments using 7-min pulses were performed by Stojilkovic et al. (38) at many different GnRH levels. Figure 5 shows the rate of LH release in the model for a wide range of GnRH concentrations. For each GnRH concentration, the rate increases rapidly, reaches a peak, declines rapidly to a plateau level during the pulse, and then declines to resting level within a few minutes. The rate of LH release tracks the CAC (see Fig. 8). The rate of initial rise, the peak height, and the plateau level increase with increasing GnRH concentration. These results are very similar to the graphs shown in Fig. 7 of Stojilkovic et al. (38). However, the LH release by the model does not vary over as wide a GnRH range, as in these experiments. In particular, the model graph at 500 nM GnRH (not shown) differs only slightly from the graph at 50 nM GnRH, whereas Stojilkovic et al. found a small but noticeable difference. Also, Stojilkovic et al. observed a very small release at 0.005 nM GnRH, whereas the model has essentially no release. Thus the present model does not reproduce their results at extremely high or extremely low GnRH concentrations.

Figure 6 shows the LH release peak and plateau levels as a function of GnRH concentration on a logarithmic scale. As in the experiments of Stojilkovic et al. (38), one obtains a sigmoidal saturating curve, and the ratio of plateau level to peak level at high GnRH concentrations is \( \sim 0.3 \), comparable to the experimental...
observation. Figure 7 shows the relationship between ΔLH and ΔCAC at the peak and at the plateau level at the end of the pulse. As in the experimental data, the close relationship between LH release and CAC is clear.

Numerous pulse experiments have compared LH release in the presence and absence of external Ca\(^{2+}\). Figure 8 shows the model LH release rate and model CAC as a function of time for normal external Ca\(^{2+}\) (1,000 µM) and very low external Ca\(^{2+}\) (5 µM). The only difference between Fig. 8 and Fig. 4 is that the duration of the pulse is 2 min in Fig. 4 and 7 min in Fig. 8; this allows the plateau to develop. At low external Ca\(^{2+}\), the LHR peak and the CAC peak are appreciably lower, and both concentrations decline slowly toward zero during the pulse, rather than reach a plateau. These results are comparable to those shown in Fig. 5 of Stojilkovic et al. (38) and Fig. 5 (100 nM GnRH) of Iida et al. (15a).

Fig. 5. LH release rate. Rate of LH release is shown in response to 7-min pulses of GnRH at indicated concentrations.

Fig. 6. Percent maximal LH release rate. Rates of LH release at peak and plateau phases are shown as a function of GnRH concentration.

Fig. 7. Relation between change in LH release rate and change in cytosolic Ca\(^{2+}\) level. ΔLH release rate is plotted as a function of ΔCAC in peak and plateau phase for a 7-min pulse of GnRH.

Fig. 8. Effect of external Ca\(^{2+}\). Time variation of CAC and of rate of LH release is shown in response to a 2-min pulse of GnRH (10 nM) in presence (A, CAE = 1,000 µM) and absence (B, CAE = 5 µM) of external Ca\(^{2+}\).
Recently, it has become possible to vary the number of GnRH receptors on gonadotropes by using an antagonist, NA2D2Na2D2CIPhe-D2Pal-Ser-NMetyr-DLys-Leu-(isp)Lys-Pro-ALANH2 (PAL), that binds to but does not activate the receptor and by creating pituitary cell lines that express different receptor concentrations. Pinter et al. (27) obtained data for LH release in response to the continuous presence of 10 nM GnRH for up to 3 h at various concentrations of PAL (i.e., reduced number of available receptors). Because we model only short-time effects in this initial study, we examined the effect of reduced receptor number on LH release only for the first 15 min. Figure 9 shows that after ~5 min the total LH increases linearly with time, as expected. Decreasing the receptor number in the model decreases the amount of LH produced, as seen experimentally. The percent reduction in LH release is greater than the percent reduction in receptor number, because the rate of dimerization (a 2nd-order reaction) is very sensitive to receptor concentration.

**DISCUSSION**

Although we have ignored much of the complexity of the signaling system between the binding of GnRH to its receptors and release of LH (and FSH) from gonadotropes, the present model nevertheless captures many of the significant short-term features of this system. The shape and time course of LH release in response to GnRH pulses in the model are very similar to those seen experimentally, in the presence and absence of external Ca2+. Furthermore, the behavior of the LH release varies as significant parameters (such as GnRH concentration and receptor number) are changed in ways that are quite similar to experimental observations. The present model allows one to predict, for the first time, the effects of varying concentrations, e.g., external Ca2+ concentration (Fig. 4) or receptor number (Fig. 9), and to compare the predictions with experimental data. When more information becomes available about the kinetic parameters and mechanisms of the internal signaling system, the model can be used to understand the quantitative consequences of its structural organization.

Among the important short-term mechanisms that we have ignored are 1) facilitatory and inhibitory mechanisms for Ca2+ entry, as a result of setting $k_6 = k_8$, and $k_7 = k_9$ equal to zero, 2) the roles of diacylglycerol and arachidonic acid in LH release (3), 3) the roles of multiple G proteins (32), 4) the effect of protein kinase C on Ca2+ channels and on the unmasking of cryptic receptors (31), 5) the mechanisms of degradation of IP3 and/or its conversion to other inositol phosphates that may play a role in the system, 6) receptor endocytosis and recycling, and 7) two pools of LH. Thus the present model demonstrates that the major features of the short-term responses can be understood using only the mechanisms that are included in the model, i.e., binding to the receptor, dimerization, interaction of the dimerized receptor with a G protein, production of an effector that opens the VSCC in the cell membrane and catalyzes the formation of IP3 (which opens the Ca2+ channels in the ER), and the Ca2+-dependent release of LH.

Because we have used such a simple model for the IP3-ER interactions, our model does not exhibit the rapid Ca2+ oscillations seen experimentally in certain GnRH concentration ranges. An important implication is that the Ca2+ oscillations are not necessary to obtain the LH release profiles over periods of >2 min. Although the Ca2+ oscillations are presumably of great importance to avoid damage to the cell, it is their time average, captured in this model, that governs LH release. Korngreen et al. (21) proposed a somewhat similar but less detailed model for biphasic Ca2+ response to signaling.

We plan to use this mathematical and computational model to perform many investigations of short-term behavior. For example, it is known that the time course of CAC in response to endothelin is similar to the response to GnRH with some differences (36, 38). Aside from the obvious changes in $k_1$ and $k_7$, it would be interesting to know whether small changes in subsequent rate constants [e.g., the dimerization or the rate of reaction of the dimerized receptor with the G protein(s)] would explain the observed differences. The model can be easily expanded to include two pools of releasable hormones so that the differences between LH release and FSH release can be compared.

We also plan to expand the model to include mechanisms that affect long-term behavior, such as 1) endocytosis of the dimers and recycling of the receptors (8), 2) synthesis of new receptors and more LH (and FSH) by the nucleus (9, 19), and 3) conversion of a nonreleasable pool of LH to a releasable pool (16). This will enable us to investigate adaptation in multipulse experiments and in response to continuous long-term exposure to GnRH and endothelin.
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