Modeling the Male Reproductive Endocrine Axis: Potential Role for a Delay Mechanism in the Inhibitory Action of Gonadal Steroids on GnRH Pulse Frequency

Teuku R. Ferasyi, P. Hugh R. Barrett, Dominique Blache, and Graeme B. Martin

University of Western Australia Institute of Agriculture and School of Animal Biology (T.R.F., D.B., G.B.M.), School of Medicine and Pharmacology (P.H.R.B.), and Faculty of Engineering, Computing, and Mathematics (P.H.R.B.), The University of Western Australia, Crawley 6009, Australia

We developed a compartmental model so we could test mechanistic concepts in the control of the male reproductive endocrine axis. Using SAAM II computer software and a bank of experimental data from male sheep, we began by modeling GnRH-LH feed-forward and LH-T feedback. A key assumption was that the primary control signal comes from a hypothetical neural network (the PULSAR) that emits a digital (pulsatile) signal of variable frequency that drives GnRH secretion in square wave-like pulses. This model produced endocrine profiles that matched experimental observations for the testis-intact animal and for changes in GnRH pulse frequency after castration and T replacement. In the second stage of the model development, we introduced a delay in the negative feedback caused by the aromatization of T to estradiol at the brain level, a concept supported by empirical observations. The simulations showed how changes in the process of aromatization could affect the response of the pulsatile signal to inhibition by steroid feedback. The sensitivity of the PULSAR to estradiol was a critical factor, but the most striking observation was the effect of time delays. With longer delays, there was a reduction in the rate of aromatization and therefore a decrease in local estradiol concentrations, and the outcome was multiple-pulse events in the secretion of GnRH/LH, reflecting experimental observations. In conclusion, our model successfully emulates the GnRH-LH-T-GnRH loop, accommodates a pivotal role for central aromatization in negative feedback, and suggests that time delays in negative feedback are an important aspect of the control of GnRH pulse frequency. **(*Endocrinology* 157: 2080–2092, 2016)**

The reproductive endocrine system essentially comprises a homeostatic loop with two aspects, stimulatory feed-forward from the central nervous system to the anterior pituitary gland to the gonads, and inhibitory feedback from the gonads to the central nervous system and the pituitary gland. Over the last 3 decades, the sheep has proven to be a valuable model in revealing detail of the processes involved. Thus, we know that the major participants in feed-forward are GnRH produced by neurons in the preoptic-hypothalamic continuum (1, 2) and the pituitary gonadotropins, LH, and FSH, whereas the major participants in feedback are gonadal inhibin and sex steroids. A key feature of the system is a hypothetical pulsar in the preoptic-hypothalamic continuum that emits a digital signal of variable frequency that drives the secretion of GnRH (3).

The origin of the pulsatile signal, a puzzle for decades, has perhaps become clearer in recent years with the discovery of kisspeptin neurons that are autoregulated by neurokinin B and dynorphin (the KNDy neurons) and the suggestion that these neurons alone might comprise the GnRH pulse generator (for review see reference 4). The resultant pulses of GnRH induce pulses of LH and, in turn, the LH pulses induce pulses of gonadal steroid so that, in the male sheep, T secretion is pulsatile (5). T completes the homeostatic loop by exerting negative feedback on the hypothalamic systems that control GnRH secretion, thus decreasing the GnRH/LH pulse frequency

ISSN Print 0013-7227 ISSN Online 1945-7170
Printed in USA

Received October 29, 2015. Accepted February 10, 2016.
First Published Online February 24, 2016

Abbreviations: CV, coefficient of variation; TE, T concentration.

(6, 7, 8). In general, there is a strong 1:1:1 relationship for the pulses of GnRH, LH, and T (5, 9, 10), especially for the male sheep under normal physiological conditions, a major advantage for the present study. Indeed, pulsatile endocrine signals are robust: the same LH signal is seen in both of the major veins draining the pituitary end of the body and in the mammary and testicular veins, despite the redistribution and mixing of blood between the sites (11, 12). In other situations, the 1:1:1 relationship might not be as clear because of technical limitations, differences among species in their endocrine control systems, differences among the neuroendocrine, pituitary and gonadal tissues in their ability to respond rapidly to endocrine stimuli, or extreme physiological situations (10).

Investigations into these systems have typically been based on the classical endocrinology model: gonadectomized animals given sc implants that release sex steroids at a constant rate. These studies have greatly advanced our knowledge of the male reproductive endocrine axis and revealed many interactions among the various elements, such as the dose-response relationship between T concentration and GnRH/LH pulse frequency (13, 14). However, the constant supply of T is a major weakness because it imposes a steady state that does not reflect two intrinsically dynamic aspects of the homeostatic loop: first, in the gonad-intact animal, T is released in a pulsatile manner and the interval between pulses varies within hours (5, 15, 16); second, feedback loops may tend toward equilibrium, but, in fact, the balance of feed-forward and feedback fluctuates with both GnRH pulse interval and T concentration, and it must also change profoundly in response to internal and external stimuli, such as photoperiod, or it would not be possible to have simultaneous increases in both GnRH pulse frequency and the concentration of T at the start of the breeding season (17). Seasonal changes in the homeostatic balance take months to unfold (7, 18, 19, 20), but much shorter temporal delays are also possible: hours for responses to a change in nutrition (21) or minutes for responses to sociosexual signals (for review see reference 3). Moreover, these internal and external factors interact with each other, so studying one in isolation from the others gives us an incomplete picture of the processes that control the reproductive endocrine axis (22).

The dynamics of the feedback loop are difficult to study in experiments with animal models because of the need to measure minute-by-minute changes in GnRH, LH, and T secretion (to detect pulses) over long periods of time (to determine pulse frequency). Moreover, even under tightly controlled conditions, the feedback loop is readily disturbed by internal and external factors, as described above as well as by the experimental procedures themselves (23). In fact, a fully balanced study designed to assess a real-world situation for male sheep might require a factorial design (three environmental factors, two genotypes, with/without steroid), at least 48 animals, and blood sampling every 20 minutes for 48 hours. An obvious alternative is mathematical modeling. We have chosen a compartmental model because it offered two major advantages: first, compartmental models can be used to test interactions among compartments that are mediated by control signals (24, 25, 26), the normal process of communication in the reproductive endocrine system; second, compartmental models can be used to test the impact of hypothetical dynamic aspects of a control system, such as time delays (24).

We began with a simple model of a feed-forward-feedback loop for GnRH-LH-T, with negative feedback exerted only through T acting on the pulsar. This model differs from that of Cartwright and Husain (27), who assumed short-loop negative feedback on GnRH by LH as well as T. It also differs from the model of Keenan and Veldhuis (28), who included T-negative feedback on the pituitary gland as well as the hypothalamus. The previous models were based on male human data, whereas ours was based on the wealth of data available for male sheep, in which T feedback is exerted mainly at cerebral sites to increase GnRH pulse interval (6, 8, 29).

We then introduced a novel direction in our modeling by including a delay in negative feedback caused by the conversion of T to estradiol by aromatase at the brain level, a decision justified by the following observations in male sheep: 1) experimental evidence that time delays play a role in determining GnRH pulse interval (30); 2) aromatase activity can be measured in several brain regions (31, 32); intracerebral administration of aromatase inhibitor increases LH pulse frequency (33); and 4) estradiol reduces the frequency of GnRH pulses through an action at brain level (29). The concept is also applicable beyond the male sheep model: for example, in male rats, aromatase activity has been observed in several brain regions (34, 35), and in male hamsters, brain aromatase activity changes with photoperiod in a manner that is T-independent and seems to better explain seasonal changes in the equilibrium of the reproductive endocrine axis than changes in steroid receptors (36). We therefore tested whether controlled changes in the rate of conversion of T to estradiol would explain the dynamic properties of the feedback inhibition of GnRH secretion.

Materials and Methods

Stage 1: the GnRH-LH-T-GnRH loop

GnRH-LH positive feed-forward

Model development began with the building of two positive feed-forward models, one for GnRH-LH and one for GnRH-

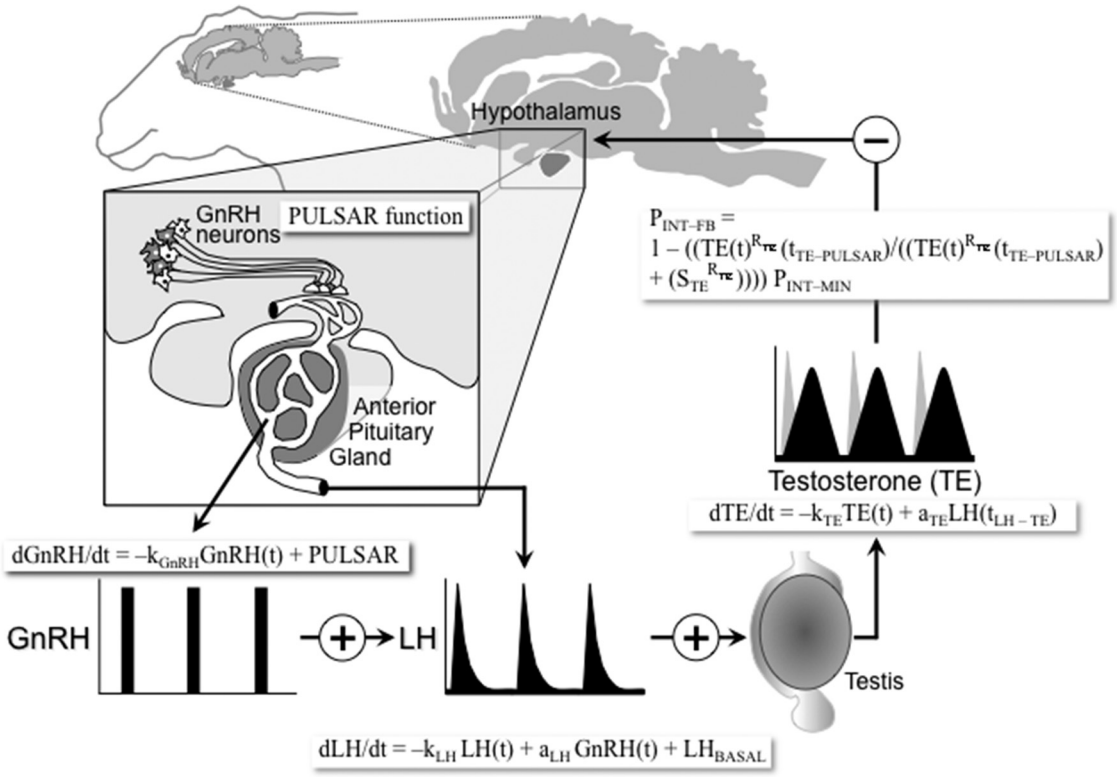

Figure 1. Schematic representation of a model of pulsatile secretion for GnRH, LH, and T in the male sheep. A hypothetical regulator, the pulsar (32), or GnRH pulse generator, elicits the secretion of GnRH pulses that are transposed into LH pulses on a one-to-one basis by the anterior pituitary gland. In turn, LH pulses stimulate the testis to release T pulses, also on a one-to-one basis. The homeostatic loop is closed by negative feedback that reduces the frequency of the pulsar signal, thus increasing the interval between GnRH pulses. The arrows represent the signaling processes between compartments, with a plus sign for positive feed-forward (from GnRH to LH and from LH to T) and the minus sign for negative feedback. For the sake of simplicity, GnRH secretion is modeled as 4-minute square waves (37). Two alternative negative feedback loops have been modeled for stage 1 in the modeling, T acts directly on the pulsar; for stage 2, T is aromatized to estradiol (31, 32) and, after a time delay, estradiol acts directly on the pulsar. Also, for the sake of simplicity, the possibilities of short-loop feedback by LH at pituitary level (38) and ultrashort-loop feedback by GnRH on the pulsar (39, 40) have been omitted.

LH-T, represented schematically in Figure 1. The GnRH-LH positive feed-forward consisted of the following two differential equations:

$$dGnRH/dt = -k_{GnRH}GnRH(t) + PULSAR \quad (1)$$

$$dLH/dt = -k_{LH}LH(t) + a_{LH}GnRH(t) + LH_{BASAL} \quad (2)$$

Change in GnRH mass (equation 1) is a function of PULSAR (described in the appendix) and k_{GnRH}. In brief, PULSAR was defined as a signal duration of 4 minutes, nominally at 100 pg/min, reflecting GnRH secretion in square wave-like pulses, as observed in sheep by Moenter et al (37). The pulses could be achieved only by assuming a high fractional rate constant for GnRH, k_{GnRH}, set at the value of 42 minutes^{-1}. Change in LH concentration (equation 2) was a function of LH catabolism, GnRH mass, and basal LH secretion (LH_{BASAL}), which is independent of GnRH mass. The fractional rate constant for LH (k_{LH}) was initially set at 0.002 minutes^{-1} based on a previous model developed by Heinze et al (41).

The GnRH-LH positive feed-forward model was fitted to the experimental data of Blache et al (30) to obtain values for the unknown parameters, LH_{BASAL}, k_{LH} and a_{LH}.

GnRH-LH-T positive feed-forward

An additional compartment was added to the model to describe the change in T concentration (equation 3, shown below) as a function of LH concentration and the fractional rate constant of T (k_{TE}). Previous studies have shown that the interval between the peak of the LH pulse and the peak of the subsequent T pulse can range from 35 to 160 minutes (5, 20, 30, 42, 43, 44). To study this, we tested the model in the presence and absence of a delay (t_{LH-TE}):

$$dTE/dt = -k_{TE}TE(t) + a_{TE}LH(t_{LH-TE}) \quad (3)$$

Models with and without the delay were fitted to experimental data from Blache et al (30). In the fitting process, values for the parameters in equations 1 and 2 were fixed from the previously fitted GnRH-LH positive feed-forward model.

Testosterone negative feedback

To model the negative feedback loop (Figure 1), it was assumed that T inhibits the secretion of GnRH by increasing the interval between the pulses emitted by the PULSAR function. The inhibitory action of T is described by equation 4 as follows:

$$P_{INT-FB} = (1-((TE(t)^{R_{TE}}(t_{TE-PULSAR}))/((TE(t)^{R_{TE}}(t_{TE-PULSAR}) + (S_{TE}^{R_{TE}}))))P_{INT-MIN} \quad (4)$$

This equation works by obtaining the difference between the maximum possible value (a value of 1) and the actual value, which is the result of interaction between the following: 1) T

concentration (TE); 2) the effect of the time delay in T negative feedback ($t_{TE\text{-}PULSAR}$); and 3) the sensitivity of PULSAR to T negative feedback (S_{TE}), itself controlled by the threshold for the response of PULSAR to T (R_{TE}). The difference was multiplied by a factor limiting minimum pulse interval ($P_{INT\text{-}MIN}$). For equation 4, a combination of values for S_{TE} and R_{TE} was chosen to allow the model to describe the response by PULSAR to a specific level of T. In developing the model, the initial values chosen were 700 ng/min for S_{TE} and 4 (dimensionless) for R_{TE}. As a consequence, there was a threshold T concentration, approximately 1.5 ng/ml, that determined whether PULSAR would initiate. This value is similar to that measured in testis-intact rams (6, 13, 15). These conditions resulted in a frequency of five GnRH pulses per 24 hours in the testis-intact state, in which all components of the system are present. This frequency was chosen as one of the baseline values for further simulations because it is typical of that observed in testis-intact male Merino sheep under controlled conditions, including a maintenance diet (45), and reflects a strong inhibitory effect of T.

A factor-limiting minimum pulse interval, $P_{INT\text{-}MIN}$, was set at 0.5. This value was chosen because it allowed the model to achieve the expected lowest interval between GnRH/LH pulses during the castration state of 40 minutes (maximum frequency of 36 pulses per 24 h), again matching experimental observations (14).

Testing how the model demonstrates negative feedback

In simulations, the parameters for GnRH, LH, and TE were derived from the fits of the GnRH-LH model and the GnRH-LH-TE positive feed-forward model, as described above. The simulations were run with and without a time delay in T negative feedback ($t_{TE\text{-}PULSAR}$). Initially, the model was tested without the time delay to obtain a base from which further simulations could begin. This initial model was considered operational when it could simulate the following three situations: 1) a pulse interval of 6 hours (five pulses per 24 hours) in the testis-intact state; 2) a minimum pulse interval of 40 minutes (36 pulses per 24 hours) when T concentration was set to zero in the castration state; and 3) a dose-response relationship between T concentration and GnRH pulse interval, simulated by infusing two levels of exogenous T during the castration state (emulating the steady state experimental model).

When the model could simulate these conditions, it was used to examine the effect of time delays in negative feedback. To do this, values for $t_{TE\text{-}PULSAR}$ in the range of 1–120 minutes were tested. The maximum value of 120 minutes was selected on the basis of experimental observations by Blache et al (30). In addition, because model solutions were also sensitive to changes in S_{TE}, different values of this parameter were tested.

Stage 2: addition of a time delay in feedback

An additional structure was added to describe the aromatization of T to estradiol at the brain level, with estradiol negative feedback replacing the direct T negative feedback used in stage 1 (Figure 1).

$$dOE/dt = -k_{OE}\mathrm{OE(t)} + k_{AO}\mathrm{TE(t)}(t_{AA-OE}) \quad (5)$$

$$P_{INT-FB} = (1-((500\ \mathrm{OE(t)}^{R_{OE}}(t_{OE-PULSAR}))/((500\ \mathrm{OE(t)}^{R_{OE}}(t_{OE-PULSAR}) + (S_{OE}{}^{R_{OE}})))))P_{INT-MIN} \quad (6)$$

Estradiol production was assumed to be a first-order process dependent on the concentration of T (with the rate constant k_{AO}) because it is an irreversible mechanism of mass transfer with a time delay ($t_{AA\text{-}OE}$). The fractional rate constant of estradiol (k_{OE}), set at 0.021 minutes^{-1}, was calculated using sheep data (46). As a consequence of these new equations, changes in the output of pulse interval depended on estradiol concentration, the time delay in estradiol negative feedback ($t_{OE\text{-}PG}$), and the sensitivity of PULSAR to estradiol (S_{OE} replacing S_{TE}). The Hill coefficient was also changed (R_{OE} replacing R_{TE}).

In equation 6, estradiol was assumed to be approximately 500 times more potent (on a mass basis) than T, based on data comparing the concentrations of estradiol and T required to reduce LH pulse frequency (by inference GnRH pulse frequency) in male Merino sheep (29). In initial simulations with the stage 2 model, the value for S_{OE} was set at 700 ng and the value for R_{OE} was set at 4 (dimensionless), as for stage 1. The value for k_{AO} was altered until the model produced a frequency of five pulses per 24 hours, a target that provided the same starting point as for stage 1.

Model testing

The model was tested by simulating changes in the delay in T aromatization and changes in the sensitivity of the GnRH pulse generator to estradiol inhibition (S_{OE}). Delays of 100, 200, and 400 minutes were tested. The lower value was selected on the basis of experimental observations by Blache et al (30), suggesting a delay of 120 minutes between change in circulating T concentration and changes in GnRH pulse frequency. In the model, a total delay of 120 minutes was achieved by adding the 100-minute delay for aromatization to the 20-minute delay for the inhibitory action of estradiol ($t_{OE\text{-}PG}$). The relatively brief delay for estradiol action was chosen on the basis of recent studies with ovariectomized ewes, suggesting the inhibitory effect of estradiol on LH secretion involved a nongenomic (and hence rapid) mechanism (47). In further simulations, the delay due to aromatization was increased 2- or 4-fold, and various combinations of delays in feedback and sensitivities of the PULSAR to estradiol inhibition were tested, with the aim of obtaining combinations, and hence simulations that approximated experimental observations. The three time delays (100, 200, 400 min) were thus combined with high, medium, and low levels of sensitivity to estradiol inhibition (K_{OE} = 700, 1400, or 2800 ng) chosen on the basis of outcomes of simulations in stage 1. The value of K_{OE} = 700 ng was included because, in the initial simulation, the target frequency of five pulses per 24 hours was considered to reflect a high sensitivity to steroid feedback. The values of 1400 and 2800 ng were chosen because, as the model was explored, they produced changes in pulse frequency and the shape of the secretory profiles that reflected experimental observations, when the time delay for aromatization was set at 200 or 400 minutes. In addition, this range of values for K_{OE} gave the model scope for assessing responses to reductions in sensitivity to negative feedback.

The combinations of time delays in aromatization and sensitivities to estradiol, outlined above, were also tested under two other conditions: 1) a reduction of T concentration (and thus estradiol concentration), which simulated castration; and 2) infusion of exogenous T, at the same doses used in earlier model (75 ng/min and 150 ng/min) that simulated the effect of T implants in castrated rams. These simulations were undertaken to describe

the effects of delays in the feedback process on increases and decreases in the frequency of LH pulses.

Plasma volume

The results of simulations for LH and TE are presented as nanograms per milliliter, assuming a plasma volume of 3000 mL.

Sources of data for model validation

As far as possible, data obtained from normal, adult, intact Merino rams under controlled environmental conditions in our laboratory (15, 21, 45), were used for all model testing and validation so we could maintain consistency and reduce variation introduced in environment, genotype and laboratory technique. Additional parameter values were derived from rational prediction by model simulation.

Computer software

The models were simulated with SAAM II software (46), using a Runge-Kutta integrator and a modified Gauss-Newton method for optimization.

Results

Stage 1 model: the GnRH-LH-T-GnRH loop

Fit of the model of GnRH-LH positive feed-forward

Through the fitting process, we obtained estimates for the parameters of LH release and their respective coefficients of variation (CV): LH_{BASAL} = 2300.20 ng/min (CV 6.7%); a_{LH} = 11 427.60 minutes^{-1} (CV 0.85%); k_{LH} = 0.02 minutes^{-1} (CV 1.8%). The model provided an estimate of the half-life of LH as 35.5 ± 0.7 minutes.

Simulation of GnRH-LH-T positive feed-forward

In the model with no delay compartment for T release, the CVs for the parameters in the T compartment were unacceptably high (Figure 2A): LH-T multiplier (a_{TE}) = 0.029 minutes^{-1} (CV 38.5%); fractional rate constants for T (k_{TE}) = 0.022 minutes^{-1} (CV 45.4%). Adding a delay compartment for T release (Figure 2B) resolved the problem of excessively high CVs: a_{TE} = 0.079 minutes^{-1} (CV 23.8%); k_{TE} = 0.066 minutes^{-1} (CV 24.8%); time delay for T release after LH stimulation ($t_{LH–TE}$) = 15.15 minutes (CV 13.0%). The simulations produced an estimate for the delay of 15 ± 2 minutes. The interval from LH peak to T peak was not affected by the addition of the delay (47 min without a delay vs 48 min with a delay).

T negative feedback

The inclusion of T negative feedback ($t_{TE\text{-}PULSAR}$) led to a GnRH pulse interval of 6 hours (Figure 3) when the value for sensitivity to T (S_{TE}) was set at 700 ng/min. Each GnRH pulse evoked an LH pulse that stimulated the production of a T pulse. The interval between pulses was approximately 300 minutes.

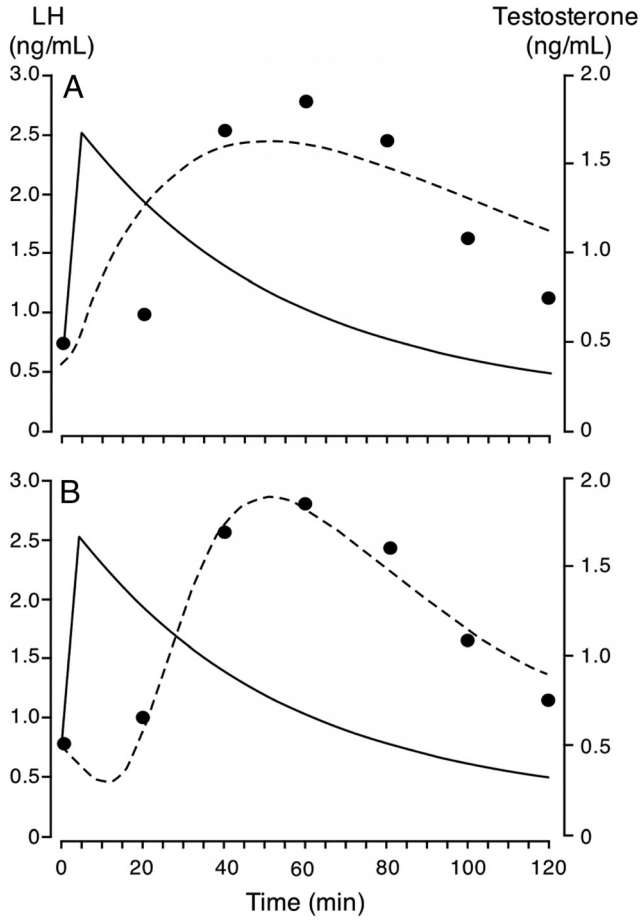

Figure 2. Effect of the absence (A) and presence (B) of a delay in LH-T feed-forward on the fit with experimental data for LH and T. Stimulation by an LH pulse is represented by the solid line. The model solutions for T concentration (dashed lines), representing the best fit of the model to the experimental data (closed circles), show the advantage of including the delay.

Simulation of responses to castration and T infusion

Switching the system to a castration state by decreasing the T concentration to zero after 24 hours of simulation (Figure 3) caused a decrease in GnRH pulse interval, after a delay of approximately 60 minutes, from intervals of 5–6 hours to a minimum of 40 minutes. This response was followed immediately by a decrease in LH pulse interval and by a rise in basal LH concentration. T infusion into the castration state increased the GnRH/LH pulse interval (Figure 3). At the lower dose (75 ng/min), the increase in pulse interval was small (from about 40 to about 44 min), but when the dose was doubled to 150 ng/min, the pulse interval increased to approximately 16 hours. In both cases, basal LH concentrations declined.

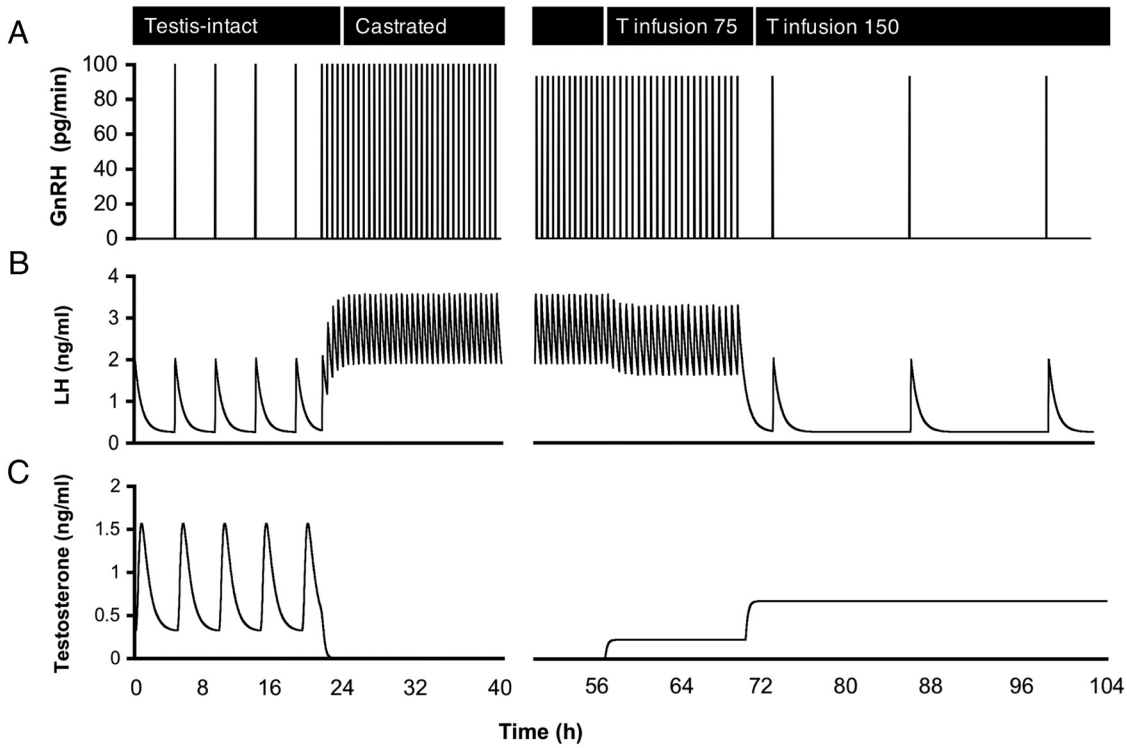

Figure 3. Simulation of the effects of castration and T replacement on the pulsatile secretion of GnRH (A), LH (B), and T (C) in sexually mature male sheep. Model outputs are presented for simulations of three conditions: 1) testis intact (0–24 h); 2) immediately after castration (24–56 h); and 3) immediately after the infusion of exogenous T in the castration state, initially at 75 ng/min (56–72 h) and then at 150 ng/min (72–108 h).

Stage 2: addition of an aromatization-based time delay in feedback

Obtaining the initial values of k_{AO} (S_{OE} = 700 ng; n_{oe} = 4; no time delays for aromatization or estradiol action)

In these simulations, the expected frequency of five GnRH/LH pulses per 24 hours (constant pulse interval of about 300 min) was obtained when the rate of conversion of T to estradiol (k_{AO}) was set at 3.2×10^{-5}/min.

The effects of changes in a time delay in aromatization

The following simulations were run with the time delay in estradiol negative feedback fixed at 20 minutes but with different delays in T aromatization and sensitivities of the GnRH pulse generator to estradiol (S_{OE}). The aim was to reveal the effect of changes in a time delay in the conversion of T to estradiol on the GnRH/LH pulse profile in the testis-intact state, after castration, and after T replacement at the following two difference doses.

S_{OE} = 700 ng

When S_{OE} was set at a high value, a delay in T aromatization of 100 minutes produced the highest peak estradiol concentrations (Table 1) and led to the release of double pulses of LH, separated by a silent period of about 480 minutes (Figure 4A). When the time delay for aromatization was increased to 200 minutes, the peak estradiol concentration decreased a little (Table 1), but triplets of LH pulses were produced with a silent period of about 600 minutes between triplets (Figure 4C). When the time delay for aromatization was increased to 400 minutes, the peak estradiol concentration fell to the lowest values (Table 1), but, notably, the LH output returned to single pulses at a frequency of four pulses per 24 hours (Figure 4E).

In the simulation of castration, with T concentration reduced to its lowest value, the LH pulse frequency increased and reached equilibrium earlier with a 100-minute delay for aromatization (Figure 4A and Table 1) than with 200- or 400-minute delays (Figure 4, C and E, and Table 1). The longest delays to the increase in pulse frequency and to achievement of equilibrium were observed when the delay for aromatization was increased to 400 minutes (Table 1).

When the delay for aromatization was set at 100 minutes, infusion of T at 75 ng/min reduced pulse frequency after 5 hours, and equilibrium was reached after about 12.5 hours (Figure 4B and Table 1). When the aromatization delay was increased from 100 to 200 or 400 minutes, both the reduction in LH pulse frequency and the attainment of equilibrium were achieved (Figure 4, D and F, and Table 1). When the rate of infusion

Table 1. Model Outputs Showing the Effects of Various Delays Caused by Aromatization on the Peak Estradiol Concentration (Nanograms per Milliliter) in the Testis-Intact State and on the Changes in GnRH Secretion After Castration and After Infusion of T (75 nanograms per Minute and Then 150 Nanograms per Minute) in the Castration State

	Delay in Aromatization, min		
	100	**200**	**400**
Peak estradiol concentration, ng/mL)	0.0017	0.0015	0.0009
After loss of T in the castration state	Delay, min, until GnRH pulse frequency increase		
	340	375	413
	Delay, min, to decrease in GnRH pulse frequency		
After T infusion at 75 ng/min in the castration state	295	370	587
After T infusion at 150 ng/min in the castration state	46	126	219
	Delay, min, to equilibrium in GnRH pulse frequency		
After loss of T in the castration state	542	620	835
After T infusion at 75 ng/min in the castration state	745	994	1515
After T infusion at 150 ng/min in the castration state	568	754	1385

The delay in the inhibitory effect of estradiol was constant ($t_{OE\text{-}PULSAR}$ = 20 min) and sensitivity of the pulsar to steroid inhibition (S_{OE}) was set at 700 ng.

of T was increased from 75 to 150 ng/min, there was a change in both the reduction in pulse frequency and the time needed to reach secretory equilibrium for all three delays in aromatization (Figure 4, B, D and F, and Table 1).

S_{OE} = 1400 ng

Simulations with medium sensitivity and a delay of 100 minutes in aromatization produced triplet pulses separated by a silent period of approximately 265 minutes (Figure 5A) and, again, a high peak of estradiol concen-

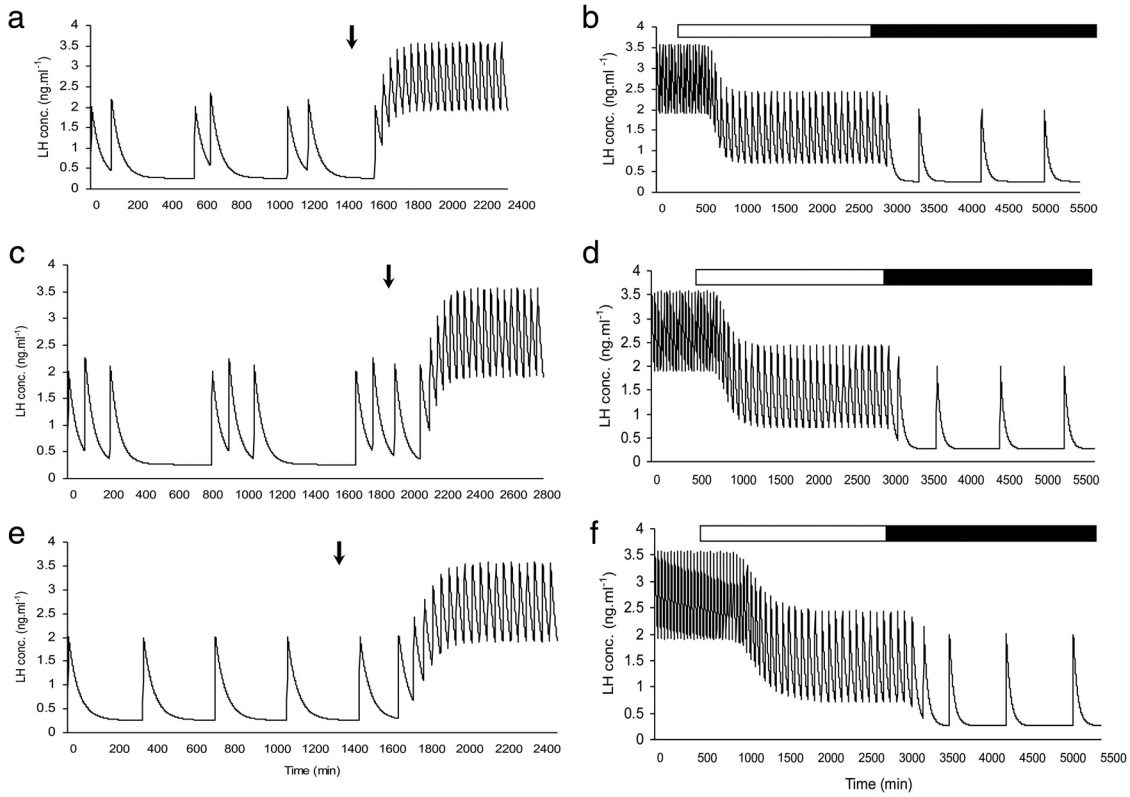

Figure 4. Simulated patterns of pulsatile LH secretion with the sensitivity of the GnRH pulse generator to estradiol (S_{OE}) set at 700 ng. Simulations tested three different delays in T aromatization in the testis-intact state (panels A, C, and E: first 1440 min), after castration (arrows), then after infusion of exogenous T (panels B, D, and F) at 75 ng/min (open bars) and then 150 ng/min (solid bars). The delay for estradiol inhibition was set at 20 minutes, and the delay for T aromatization was set at 100 minutes (panels A and B), 200 minutes (panels C and D), or 400 minutes (panels E and F). Time = 0 is the state of initial equilibrium.

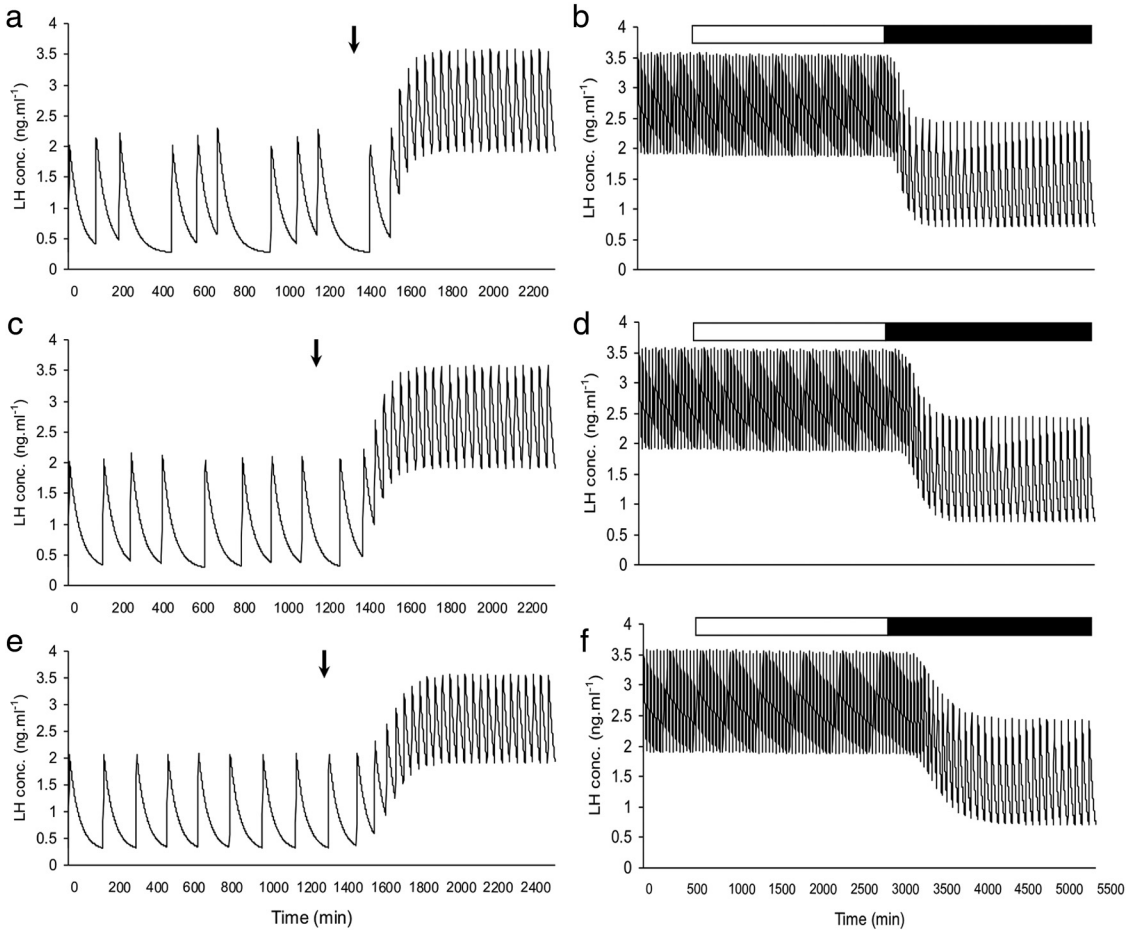

Figure 5. LH pulse profiles with S_{OE} set at 1400 ng after the changes in delay for T aromatization in the intact state (panels A, C, and E: first 1440 min), after castration (panels A, C, and E: arrows), and then infusion of exogenous T (panels B, D, and F) at 75 ng/min (open bars) and 150 ng/min (solid bars). The delay for estradiol inhibition was set at 20 minutes, and the delay for T aromatization set at 100 minutes (panels A and B), 200 minutes (panels C and D), or 400 minutes (panels E and F). Time = 0 is the state of equilibrium.

tration (Table 2). A delay in aromatization of 200 minutes produced a peak estradiol concentration 20% lower than for a delay of 100 minutes (Table 2), and LH pulses were released in groups (Figure 5C). A long aromatization delay of 400 minutes led to a slightly lower peak of estradiol concentration than with a delay of 200 minutes (Table 2),

Table 2. Effects of Medium (1400 ng) and High (2800 ng) Levels of Sensitivity of the Pulsar to Steroid Inhibition (S_{OE}) and Three Delays in Aromatization of T, and Thus Peak Estradiol Concentrations (Nanograms per Milliliter), on the Time Required for GnRH Pulse Frequency to Change After Castration, and After an Infusion of Exogenous Testosterone at Two Different Rates (75 and Then 150 ng/min) in the Castration State

S_{OE}, ng	1400			2800		
Delay, min, in T aromatization	**100**	**200**	**400**	**100**	**200**	**400**
Peak estradiol concentrations, ng/mL	0.0020	0.0060	0.0015	0.0032	0.0026	0.0024
Delay, min, Until GnRH Pulse Frequency Increase						
After loss of T in the castration state	224	268	260	85	223	243
Delay, min, Until GnRH Pulse Frequency Decrease						
After T infusion at 75 ng/min in the castration state	>30 h	>30 h	>30 h	>30 h	>30 h	>30 h
After T infusion at 150 ng/min in the castration state	159	256	364	>30 h	>30 h	>30 h
Delay, min, Until GnRH Pulse Frequency Equilibrium						
After loss of T in the castration state	428	527	714	341	442	686
After T infusion at 75 ng/min in the castration state	>30 h	>30 h	>30 h	>30 h	>30 h	>30 h
After T infusion at 150 ng/min in the castration state	712	965	1432	>30 h	>30 h	>30 h

The delay in the inhibitory effect of estradiol of the pulsar was constant ($t_{OE\text{-}PULSAR}$ = 20 min). The simulations were set to end at 30 hours.

but, again, in contrast to the earlier two simulations, single pulses were produced and released at the frequency of nine pulses per 24 hours (Figure 5E).

After changing to the castration state, with a 100-minute delay for aromatization, the pulse frequency started to increase and reached equilibrium earlier than with the longer aromatization delays (Figure 5A and Table 2). In contrast, with the aromatization delay set to 200 or 400 minutes, the increase in pulse frequency was retarded (Figure 5, C and E, and Table 2), although equilibrium was reached 3 hours earlier with a delay of 200 minutes than with a delay of 400 minutes (Table 2).

A reduction in pulse frequency was not observed after the infusion of exogenous testosterone at 75 ng/min for more than 30 hours, with any of the three values for aromatization delay (Figure 5, B, D, and F, and Table 2). On the other hand, pulse frequency decreased markedly after T infusion at 150 ng/min. Pulse frequency fell earlier with the aromatization delay set at 100 minutes than with delays of 200 or 400 minutes (Table 1). When the aromatization delay was increased to 200 or 400 minutes, pulse frequency decreased after 4.3 hours or 6 hours, and equilibrium was reached after 16 hours or 23.9 hours (Figure 5, D and F, and Table 2).

S_{OE} = 2800 ng

With $S_{OE} = 2800$ ng (low sensitivity), the peak estradiol values were higher or medium with 100-minute or 200-minute delays for aromatization (Table 2), both of which led to pulses released in groups, similar to those illustrated in Figures 4 and 5. In contrast, single pulses were released at a frequency of 16 pulses per 24 hours when the delay for aromatization was increased to 400 minutes, and the resulting estradiol concentration was lower than in the simulations with 100- or 200-minute delays for aromatization (Table 2).

After castration, the pulse frequency rose and reached equilibrium earlier with a delay of 100 minutes for aromatization than with longer delays (Table 2). The results were strikingly different when the aromatization delay was increased to 200 or 400 minutes: pulse frequency increased after 3.7 or 4 hours and the time needed to reach equilibrium was about 30%–100% longer than with a delay of 100 minutes (Table 2). Simulation of an infusion of exogenous T at 75 ng/min and 150 ng/min in the castration state did not affect pulse frequency (Table 2).

Discussion

Our compartmental model of the reproductive endocrine system of the male sheep produced outputs that correlate strongly with available experimental data for every component of the feedback loop. For GnRH-LH positive feed-forward, the simulation of LH secretion showed the typical pulsatile pattern with a rapid increase in concentration to a sharp peak within minutes, followed by an exponential decrease (37), clearly reflecting the changes in secretion measured by frequent sampling of cephalic arteriovenous blood every 20 seconds and every 5 minutes (49). This justifies the assumption in previous modeling studies that an LH pulse comprises a very short period of secretion, approximated as an instantaneous event (maximum concentration reached at $t = 0$), followed by a decline that reflects clearance alone (50). The present model improves on that by showing peak values being reached after about 5 minutes of secretion because it incorporates a simulation of square-wave GnRH pulses of a 4-minute duration. We used a constant duration of GnRH pulses in all of our simulations, as did Heinze et al (41), although this is not an absolutely accurate representation of the real situation in sheep: for example, Moenter et al (37) observed GnRH pulses ranging from 4 to 7 minutes in duration in female sheep. For positive feed-forward from LH to T, the simulations suggested that the best fit to experimental data required a delay of 15 minutes before T was released after stimulation by LH. The presence or absence of this delay had no effect on the time needed to reach the peak concentration of T, which, at 48 minutes, was similar to that observed in Merino rams in our hands (40–100 min: 16, 30) as well as other sheep models (35–160 min: 5, 17, 43, 44).

The good fit of these initial components of the model justified the next step in development, namely the addition of negative feedback, and outputs were again in excellent agreement with experimental observations. A long interval between pulses, accurately reflecting situations in which the inhibitory effect of steroid feedback is strong (7, 12), was obtained when the pulsar was made highly sensitive to inhibition by T. The response to castration was successfully simulated, with outputs similar to experimental observations in mature male sheep (13, 14, 51), and the model was also able to simulate a dose-responsive increase in pulse interval after T administration (13, 14).

In the second phase of model development, the aim was to test the effects of hypothetical changes in time delays associated with aromatization of T to estradiol in brain tissue, and thus changes in local concentration of estradiol, on the frequency of GnRH pulses. This hypothesis was tested after the initial settings achieved the base frequency of five pulses per 24 hours and provided an estimate of the potential rate of the conversion of T to estradiol. Having produced normal pulse profiles, the model was run with variations in the other parameters. The out-

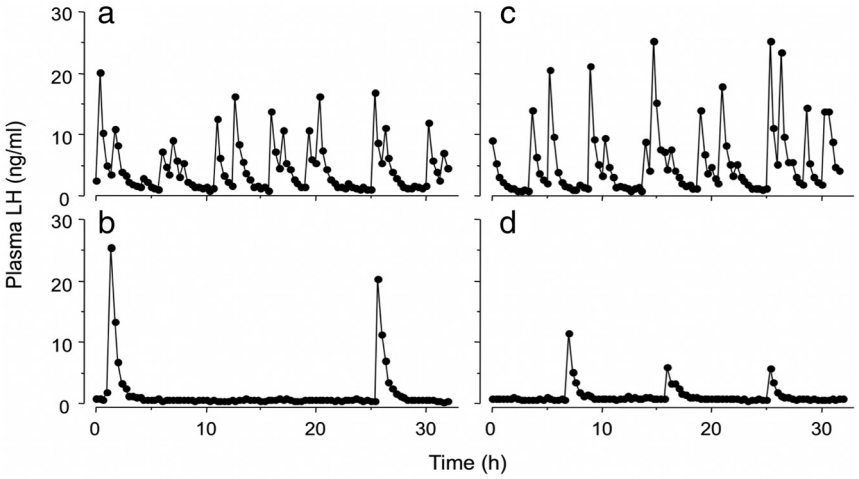

Figure 6. Examples of multiple-pulse events in LH profiles observed in four young males that were well fed (panels A and C) or underfed (panels B and D) during the period leading to the onset of puberty (reproduced with permission from reference 16). Similar phenomena are also seen in mature males (15, 45). Our modeling suggests that this could be caused by a long delay in negative feedback, effectively inducing a brief castration response with the pulsar triggering high-frequency pulses of GnRH before it responds to the inhibitory steroid signal. By contrast, the lower profiles (panels B and D) are consistent with outputs in which the delay in inhibitory feedback is short. In other words, change in a delay in inhibitory feedback could underpin changes in responsiveness to inhibitory feedback, a foundation concept in many aspects of reproductive homeostasis, including puberty, seasonal breeding, postpartum anestrus, and response to nutrition in the sheep (14, 52, 53, 54).

puts showed how changes in the process of aromatization could affect negative feedback on the GnRH pulse generator, but the result also depended on the level of sensitivity of the pulse generator to the inhibitory effects of estradiol. A reduction in the rate of aromatization, as observed in the simulations with longer delays, led to lower estradiol concentrations and to changes in the frequency of GnRH pulses. When the sensitivity of the pulse generator to estradiol was reduced, it allowed the pulse frequency to increase to 16 pulses per 24 hours, mimicking the observation in male Merino sheep receiving an acute dietary supplement (14, 21).

Overall, the coherence of the model outputs with our extensive bank of experimental data, including steroid feedback, suggests that our model is functional at this stage of its development and can be used for testing hypotheses about the regulation of GnRH pulse frequency. However, we need to remember that the coherence between model outputs and experimental data may simply be a self-serving consequence of the assumptions and rules of regulation that were used in developing the models in the first instance. Moreover, these assumptions and rules were based on experimental observations that are themselves limited by experimental methods: in other words, limitations in the strength of the experimental data may affect the validity of assumptions used in the models. In the development of any model, there will always be a risk that the major assumption(s) are invalid, so the whole model may be invalid. In this context, one of the outputs of the present model requires particular attention: the appearance of multiple-pulse events in the LH profile with the variation in S_{OE} (was set at 700 ng and 1400 ng) and combination with different time delays of T aromatization (100, 200, and 400 min; Figures 4 and 5). This is a remarkable reflection of real-world experimental data in male sheep (Figure 6), but the phenomenon of multiple pulses was not used to generate the model.

Our simulations also suggest that GnRH pulse frequency responds to changes in the sensitivity of the GnRH pulse generator to the inhibitory effects of estradiol. In the literature, there is a wealth of experimental data supporting this concept, although nearly all are limited to changes in responsiveness to a given dose of steroid, rather than estimation of sensitivity (the minimum dose that effects a response). Most interesting is the observation, in mature Merino rams, that a change in the level of nutrition causes a change in the responsiveness of GnRH/LH pulse frequency to exogenous steroid (14). The processes through which the GnRH pulse generator is controlled by steroid feedback in our model seems to be similar to those modeled by Keenan and Veldhuis (28). However, we implemented inhibitory feedback as a combination of three factors: 1) steroid concentration, 2) time delay, and 3) sensitivity of PULSAR to steroid inhibition. Obviously we still need to determine the mechanisms behind the time delay and the sensitivity of the PULSAR, for which one speculation might be changes in the steroid receptors (32, 55).

We compared the inputs and outputs of our model with experimental data from male Merino sheep, in which brain aromatase plays a role in steroid-negative feedback (31, 32, 33). In our in vivo experiments, we were unable to show that nutrition affects central aromatase activity, but this was almost certainly because the tissue samples assayed for the enzyme were too large and thus too heterogeneous for detecting changes that probably involve only small numbers of highly localized cells (32). It has been suggested that brain aromatase activity is controlled passively by substrate availability (31, 35, 36), but other factors besides T concentration could be involved: for example, in the hamster, central aromatase activity is af-

fected by photoperiod in both intact and castrated males (36). For male sheep, changes in aromatase activity could explain two observations: 1) differences between the breeding and nonbreeding seasons in LH pulse frequency in castrated rams (42); and, 2) the fact that estradiol as well as androgens can reduce GnRH pulse frequency (8, 14, 29, 57). Indeed, intracerebral infusion of an aromatase inhibitor increases the frequency of GnRH pulses in testis-intact Merino rams (33). Clearly the predictions of the present model support the need for further study of the roles of brain aromatase activity in feedback in animal experiments.

We simulated a classical experiment in endocrinology by observing the increase in GnRH pulse frequency after castration and the reversal of the outcome by T replacement, mimicking quantitative observations in male sheep (8, 14, 29, 51). Interestingly, the model was able to show variation in the time required to respond to castration with an increase in pulse frequency and also variation in the time required to reach equilibrium. Similarly, the model controlled the time required for exogenous T to reduce pulse frequency, with a clear demonstration of a dose response. In fact, acute responses to surgical castration are very difficult to observe because anesthesia interferes with GnRH secretion, unless pharmacological castration is used with T secretion acutely blocked by treatment with hormone antagonists. In such experiments with rams (30), the time required for GnRH pulse frequency to increase after the fall in T concentrations, and the time required for pulse frequency to fall after the recovery of steroid feedback, agree well with the output of the present model. However, the model also indicated that these response times could vary with, for example, changes in the delay by aromatization and the sensitivity of the pulse generator to estradiol. This situation demonstrates the advantage of mathematical models.

In conclusion, our model successfully simulates the processes that control the output of the GnRH pulse generator, and the feed-forward and feedback processes that control LH and T secretion in male sheep. The second phase model appears to be particularly robust, as indicated by the spontaneous output of multiple-pulse events, and clearly supports the view that inhibitory feedback is a dynamic process rather than the static process assumed in steady state in vivo models. If experimentation were to show that negative feedback involves a changeable delay caused by, for example, aromatization, we would need to add an extra dimension, arguably essential, to our understanding of the reproductive endocrine axis that, at present relies only on changes in the responsiveness of the GnRH pulse generator to steroid inhibition. Changes in delays and sensitivity might even be part of the sensor-integrator concept proposed by Blache et al (22, 58). Our model is sufficiently robust to be used to test hypotheses concerning the effects of internal or external factors that affect reproductive activity, such as nutrition, photoperiod, and sociosexual signals.

Several limitations of the model need to be mentioned: 1) for LH, T, and estradiol, the model of synthesis is linear, yet many biological interactions are nonlinear because of saturation or limitations in supply of substrates and enzymes, or ligand and receptor; 2) we used linear kinetics for clearance that might not reflect reality; and 3) the model for the Pulsar comprises only a small number of parameters, without inputs from circadian rhythms, for example, thus limiting its flexibility. While acknowledging these limitations, we point out that any modeling exercise must begin with the principle of parsimony and then add complexity when the data justify it. Indeed, the whole reason for modeling is to indicate where experimental data are needed to further our understanding.

Finally, further development of this model should help us to combine mechanistic and empirical processes in ways that will support research aimed at improvement of reproductive function for farm animals (59, 60). An obvious direction is to develop the compartmental model so that it can respond to the homeorhetic regulatory processes that have been used to describe a long-term change in small ruminant production systems (18, 61, 62).

Appendix 1

Glossary

Variables

PULSAR generator of the pulsatile signal that controls GnRH secretion (dimensionless)

GnRH mass (picograms)

LH concentration (nanograms per milliliter)

TE T concentration (nanograms per milliliter)

OE mass of estradiol (nanograms per milliliter)

Parameters

$P_{\text{INT-FB}}$ is the interval between successive pulses (minutes)

$P_{\text{INT-MIN}}$ is the limit factor for minimum pulse interval (dimensionless)

a_{LH} is the GnRH-LH multiplier (minutes^{-1})

a_{TE} is the LH-T multiplier (minutes^{-1})

k_{GnRH}, k_{LH}, k_{TE}, and k_{OE} are fractional rate constants for GnRH, LH, T, and estradiol (minutes^{-1})

k_{AO} is the conversion rate of T to estradiol (minutes^{-1})

LH_{BASAL} is the basal secretion rate for LH (nanograms per minute)

$t_{LH–TE}$ is the time delay for T release after LH stimulation (minutes)

$t_{TE\text{-}PULSAR}$ is the time delay for T negative feedback on the PULSAR (minutes)

S_{TE} is the sensitivity of the pulsar to inhibition by T (nanograms per minute)

R_{TE} is the threshold at which the pulsar responds to T (dimensionless)

S_{OE} is the sensitivity of the pulsar to inhibition by estradiol (nanograms per minute)

R_{OE} is the threshold at which the pulsar responds to estradiol (dimensionless)

$t_{AA\text{-}OE}$ is the time delay in T aromatization (minutes)

$t_{OE\text{-}PULSAR}$ is the time delay for T negative feedback on PULSAR (minutes)

Acknowledgments

We thank the Animal Science Group in the School of Animal Biology for their support.

Address all correspondence and requests for reprints to: Professor Graeme Martin, School of Animal Biology M092, The University of Western Australia, Crawley 6009, Australia. E-mail: graeme.martin@uwa.edu.au.

Current address for T.R.F.: Faculty of Veterinary Medicine, Syiah Kuala University, Banda Aceh 23111, Indonesia.

P.H.R. Barrett is a research fellow of the National Health and Medical Research Council of Australia.

This work was supported by The School of Animal Biology at the University of Western Australia . T.R.F. was supported by an International Postgraduate Research Scholarship and a University Postgraduate Award from the University of Western Australia.

Disclosure Summary: The authors have nothing to disclose.

References

1. **Caldani M, Batailler M, Thiery JC, Dubois MP.** LHRH-immunoreactive structures in the sheep brain. *Histochemistry*. 1988;89:129–139.
2. **Lehman MN, Robinson JE, Karsch FJ, Silverman AJ.** Immunocytochemical localization of luteinizing hormone-releasing hormone (LHRH) pathways in the sheep brain during anestrus and the midluteal phase of the estrous cycle. *J Comp Neurol*. 1986;244:19–35.
3. **Martin GB.** Factors affecting the secretion of luteinizing hormone in the ewe. *Biol Rev Camb Philos Soc*. 1984;59:1–87.
4. **Smith JT, Hawken PAR, Lehman MN, Martin GB.** The role of kisspeptin in reproductive function in the ewe. In: Juengel JI, Miyamoto A, Price C, Smith MF, Webb R, eds. *Reproduction in Domestic Ruminants VIII*. UK: Context; 2014:105–116.
5. **Sanford LM, Winter JSD, Palmer WM, Howland BE.** The profile of LH and testosterone secretion in the ram. *Endocrinology*. 1974;95:627–631.
6. **Jackson GL, Kuehl D, Rhim TJ.** Testosterone inhibits gonadotropin-releasing hormone pulse frequency in the male sheep. *Biol Reprod*. 1991;45:188–194.
7. **Lincoln GA.** Central effects of photoperiod on reproduction in the ram revealed by the use of a testosterone clamp. *J Endocrinol*. 1984;103:233–241.
8. **Tilbrook AJ, de Kretser DM, Cummins JT, Clarke IJ.** The negative feedback effects of testicular steroids are predominantly at the hypothalamus in the ram. *Endocrinology*. 1991;129:3080–3092.
9. **Clarke IJ, Cummins JT.** The temporal relationship between gonadotropin releasing hormone (GnRH) and luteinizing hormone (LH) secretion in ovariectomized ewes. *Endocrinology*. 1982;111:1737–1739.
10. **Moenter SM.** Leap of faith: does serum luteinizing hormone always accurately reflect central reproductive neuroendocrine activity? *Neuroendocrinology*. 2015;102:256–266.
11. **Blanc F, Martin GB, Bocquier F.** Modelling reproduction in farm animals: a review. *Reprod Fertil Dev*. 2001;13:337–353.
12. **Hötzel MJ, Markey CM, Walkden-Brown SW, Blackberry MA, Martin GB.** Morphometric and endocrine analyses of the effects of nutrition on the testis of the mature Merino ram. *J Reprod Fertil*. 1998;113:217–230.
13. **D'Occhio MJ, Schanbacher BD, Kinder JE.** Relationship between serum testosterone concentration and patterns of luteinizing hormone secretion in male sheep. *Endocrinology*. 1982;110:1547–1554.
14. **Tjondronegoro S, Martin GB, Sutherland SRD, Boukhliq R.** Interactions between nutrition, testosterone and inhibin in the control of gonadotrophin secretion in mature rams. *Reprod Fertil Dev*. 1996;8:855–862.
15. **Martin GB, Sutherland SRD, Lindsay DR.** Effects of nutritional supplements on testicular size and the secretion of LH and testosterone in Merino and Booroola rams. *Anim Reprod Sci*. 1987;12:267–281.
16. **Martin GB, White CF.** Effects of dietary zinc deficiency on gonadotrophin secretion and testicular growth in young male sheep. *J Reprod Fertil*. 1992;96:497–507.
17. **Lincoln GA.** The temporal relationship between plasma levels of FSH and LH in the ram. *J Reprod Fertil*. 1978;53:31–37.
18. **Adam CL, Findlay PA, Miller DW.** Blood-brain leptin transport and appetite and reproductive neuroendocrine responses to intracerebroventricular leptin injection in sheep: influence of photoperiod. *Endocrinology*. 2006;147:4589–4598.
19. **Lincoln GA.** Changes in pituitary responsiveness to luteinizing hormone releasing hormone in rams exposed to artificial photoperiods. *J Endocrinol*. 1977;73:519–527.
20. **Rhim TJ, Kuehl D, Jackson GL.** Seasonal changes in the relationship between secretion of gonadotropin-releasing hormone, luteinizing hormone, and testosterone in the ram. *Biol Reprod*. 1993;48:197–204.
21. **Zhang S, Blache D, Blackberry MA, Martin GB.** Dynamics of the responses in the secretion of luteinising hormone, leptin and insulin following an acute increase in nutrition in mature male sheep. *Reprod Fertil Dev*. 2004;16:823–829.
22. **Blache D, Zhang S, Martin GB.** Fertility in male sheep: modulators of the acute effects of nutrition on the reproductive axis of male sheep. *Reprod Suppl*. 2003;61:387–402.
23. **Adams NR, Atkinson S, Martin GB, Briegel JR, Boukhliq R, Sanders MR.** Frequent blood sampling changes the plasma concentration of LH and FSH and the ovulation rate in Merino ewes. *J Reprod Fertil*. 1993;99:689–694.
24. **Jacquez JA.** Compartmental analysis in biology and medicine. 2nd ed. Ann Arbor, MI: University of Michigan Press; 1985.
25. **Smith WR.** Hypothalamic regulation of pituitary secretion of luteinizing hormone. II. Feedback control of gonadotropin secretion. *Bull Math Biol*. 1980;42:57–78.
26. **Soboleva TK, Peterson AJ, Pleasants AB, McNatty KP, Rhodes FM.**

A model of follicular development and ovulation in sheep and cattle. *Anim Reprod Sci*. 2000;58:45–57.
27. **Cartwright M, Husain M.** A model for the control of testosterone secretion. *J Theor Biol*. 1986;123:239–250.
28. **Keenan DM, Veldhuis JD.** A biomathematical model of time delayed in the human male hypothalamic-pituitary-Leydig cell axis. *Am J Physiol Endocrinol Metab*. 1998;275:E157–E176.
29. **Blache D, Tjondronegoro S, Blackberry MA, Anderson ST, Curlewis JD, Martin GB.** Gonadotrophin and prolactin secretion in castrated male sheep following subcutaneous or intracranial treatment with testicular hormones. *Endocrine*. 1997;7:235–243.
30. **Blache D, Chagas LM, Caraty A, Deghenghi R, Delaleu B, Blackberry MA, Martin GB.** GnRH secretion into CSF in rams treated with a GnRH antagonist. *J Neuroendocrinol*. 1997;9:887–892.
31. **Roselli CE, Stormshak F, Resko JA.** Distribution and regulation of aromatase activity in the ram hypothalamus and amygdala. *Brain Res*. 1998;811:105–110.
32. **Sharma TP, Blache D, Roselli CE, Martin GB.** Distribution of aromatase activity in brain and peripheral tissues of male sheep; effect of nutrition. *Reprod Fertil Dev*. 2004;16:709–715.
33. **Sharma TP, Blache D, Blackberry MA, Martin GB.** Role of peripheral and central aromatization in the control of gonadotrophin secretion in the male sheep. *Reprod Fertil Dev*. 1999;11:293–302.
34. **Roselli CE, Resko JA.** Androgens regulate brain aromatase activity in adult male rats through a receptor mechanism. *Endocrinology*. 1984;114:2183–2189.
35. **Roselli CE, Ellinwood WE, Resko JA.** Regulation of brain aromatase activity in rats. *Endocrinology*. 1984;114:192–200.
36. **Callard GV, Mak P, Solomon DJ.** Effects of short days on aromatization and accumulation of nuclear estrogen receptors in the hamster brain. *Biol Reprod*. 1986;35:282–291.
37. **Moenter SM, Brand RM, Midgley AR, Karsch FJ.** Dynamics of gonadotropin-releasing hormone release during a pulse. *Endocrinology*. 1992;130:503–510.
38. **Blache D, Caraty A, Miller DW, Blackberry MA, Martin GB.** Effect of intracerebroventricular injection of GnRH, GnRH agonist or antagonist on LH secretion in male sheep. Paper presented at: 13th International Congress on Animal Reproduction; 1996; Sydney, Australia; p 3–13.
39. **Naylor AM, Porter DWF, Lincoln DW.** Inhibitory effect of central LHRH on LH secretion in the ovariectomized ewe. *Neuroendocrinology*. 1989; 49:531–536.
40. **Padmanabhan V, Evans NP, Dahl GE, McFadden KL, Mauger DT, Karsch FJ.** Evidence for short or ultrashort loop negative feedback of gonadotropin-releasing hormone secretion. *Neuroendocrinology*. 1995;62:248–258.
41. **Heinze K, Keener RW, Midgley JAR.** A mathematical model of luteinizing hormone release from ovine pituitary cells in perifusion. *Am J Physiol Endocrinol Metab* 1998;275:E1061–E1071.
42. **Lincoln GA.** Seasonal variation in the episodic secretion of luteinizing hormone and testosterone in the ram. *J Endocrinol*. 1976;69: 213–226.
43. **Schanbacher BD, Ford JJ.** Seasonal profiles of plasma luteinizing hormone, testosterone and estradiol in the ram. *Endocrinology*. 1976;99:752–757.
44. **Terqui M, Garnier DH, De Reviers MM, Huet S, Pelletier J.** La structure chronologique du dialogue entre l'hypophyse et les gonades chez les mammifères domestiques. In: Ortavant R, Reinberg A, eds. *Rythes et Reproduction*. Paris: Masson; 1980:59–72.
45. **Hötzel MJ, Walkden-Brown SW, Fisher JS, Martin GB.** Determinants of the annual pattern of reproduction in mature male Merino and Suffolk sheep: responses to a nutritional stimulus in the breeding and non-breeding seasons. *Reprod Fertil Dev*. 2003;15:1–9.
46. **Barrett PHR, Bell BM, Cobelli C, et al.** SAAM II: simulation, analysis, and modeling software for tracer and pharmacokinetic studies. *Metabolism*. 1998;47:484–492.
47. **Adams NR, Abordi JA, Briegel JR, Sanders MR.** Effect of diet on the clearance of estradiol-17β in the ewe. *Biol Reprod*. 1994;51:668–674.
48. **Arreguin-Arevalo JA, Nett TM.** A non-genomic action of estradiol as the mechanism underlying the acute suppression of secretion of luteinizing hormone in ovariectomized ewes. *Biol Reprod*. 2006; 74:202–208.
49. **Rasmussen DD, Malven PV.** Characterization of cephalic arteriovenous LH differences by continuous sampling in ovariectomized ewes. *Neuroendocrinology*. 1982;34:415–420.
50. **Martin GB, Thomas GB, Terqui M, Warner P.** Pulsatile LH secretion during the preovulatory surge in the ewe: experimental observations and theoretical considerations. *Reprod Nutr Dev*. 1987;27: 1023–1040.
51. **Caraty A, Locatelli A.** Effect of time after castration on secretion of LHRH and LH in the ram. *J Reprod Fertil*. 1988;82:263–269.
52. **Foster DL, Ryan KD.** Endocrine mechanisms governing transition into adulthood: a marked decrease in inhibitory feedback action of estradiol on tonic secretion of luteinizing hormone in the lamb during puberty. *Endocrinology*. 1979;105:896–904.
53. **Legan SJ, Karsch FJ.** Photoperiodic control of seasonal breeding in ewes: modulation of the negative feedback action of estradiol. *Biol Reprod*. 1980;23:1061–1068.
54. **Wright PJ, Geytenbeek PE, Clarke IJ, Findlay JK.** Evidence for a change in oestradiol negative feedback and LH pulse frequency in post-partum ewes. *J Reprod Fertil*. 1981;61:97–102.
55. **Sharma SC, Jeffrey WC, Margareta DP, JoAnne SR.** Expression and function of estrogen receptor subtypes in granulosa cells: regulation by estradiol and forskolin. *Endocrinology*. 1999;140:4320–4334.
56. **Romeo RD, Wade J, Venier JE, Sisk CL.** Androgenic regulation of hypothalamic aromatase activity in prepubertal and postpubertal male golden hamsters. *Endocrinology*. 1999;140:112–117.
57. **Schanbacher BD.** Regulation of luteinizing hormone secretion in male sheep by endogenous estrogen. *Endocrinology*. 1984;115: 944–950.
58. **Blache D, Chagas LM, Blackberry MA, Vercoe PE, Martin GB.** Metabolic factors affecting the reproductive axis in male sheep. *J Reprod Fertil*. 2000;120:1–11.
59. **Maas JA, Garnsworthy PC, Flint APF.** Modelling responses to nutritional, endocrine and genetic strategies to increase fertility in the UK dairy herd. *Vet J*. 2009;180:356–3623.
60. **Vetharaniama I, Peterson AJ, McNatty KP, Soboleva TK.** Modelling female reproductive function in farmed animals. *Anim Reprod Sci*. 2010;122:164–173.
61. **Puillet L, Martin O, Sauvant D, Tichit M.** An individual-based model simulating goat response variability and long-term herd performance. *Animal*. 2010;4:2084–2098.
62. **Puillet L, Martin O, Tichit M, Sauvant D.** Simple representation of physiological regulations in a model of lactating female: application to the dairy goat. *Animal*. 2008;2:235–246.