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IN VIVO MODELS FOR THE STUDY OF GONADOTROPIN AND LHRH SECRETION

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Summary—The objective of this paper is to describe recent data from my laboratory dealing with the *in vivo* control of LHRH secretion in male rabbits and rats using the technique of push–pull perfusion (PPP) that allows repetitive determinations of this neuropeptide in quasi-normal physiological conditions. In addition, we have applied this method to simultaneously measure LHRH and LH in freely behaving male rats bearing a push–pull cannula (PPC) in the anterior pituitary. A description of the validation of this technique and its potential use will be discussed as well as data indicating that castration in the male rat induces a significant increase in the LHRH and LH signals; however, following testosterone treatment, in spite of a clear return of LH output to intact levels, even higher levels of LHRH reaching the anterior pituitary were detected. Curiously, in the rabbit no changes in LHRH release were noticed with castration, but following testosterone treatment, a transient but robust 5–8-fold increase in LHRH release was noticed.

In short, these studies have demonstrated the existence of apparently opposite rather than similar responses in the testicular control of the hypothalamic–hypophysial axis of the male rat as compared with those of the male rabbit.

INTRODUCTION

The central role of hypothalamic LHRH in the control of gonadotropin secretion is a universally accepted concept that applies across several species [1–7] including humans [8–10].

The objective of this paper is to describe recent data from our laboratory dealing with the *in vivo* control of LHRH secretion using the technique of push–pull perfusion (PPP) which allows repetitive determinations of this neuropeptide under quasi-normal physiological conditions. In addition, we have applied this method to simultaneously measure LHRH (the incoming signal to the gonadotropes) and LH (the outgoing signal) in freely behaving male rats bearing a push–pull cannula (PPC) in the anterior pituitary.

The two most salient features of the PPP method are its capacity to perfuse a specific neuroanatomical locus several times within the same animal and its adaptability, that is it can be used in different species, to perfuse different loci in the brain and to measure synthesis/metabolism as well as release *in vivo* of several neuropeptides. The present data were generated in male rats and male rabbits using this general procedure which has been recently critically reviewed [11]. In this paper three major issues will be discussed:

1. the role of the testis on *in vivo* LHRH and LH secretion in the male rat;
2. the role of the testis on *in vivo* LHRH release in the male rabbit and
3. the description of an *in vivo* model to study the LHRH–LH interactions at the pituitary level of freely behaving rats.

THE RAT LHRH–LH TESTIS AXIS

It is without dispute and practically a universal finding, at least in mammals, that blood levels of gonadotropins raise following castration [3, 12–17]. However, the role of hypothalamic LHRH in this neurohormonal circuit in response to castration is unclear since apparent conflicting results have been reported among laboratories (for review see [18]) and even within our own laboratory [19–21] probably due to shortcomings of the techniques used to estimate the response of the LHRH–LH axis. Also, the site of action of testosterone (T) and the precise role of this

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Table 1. Summary of mean results (±SEM) from Pulsar analysis of LHRH release profiles obtained from push–pull perfusion of the anterior pituitary and serum LH levels of intact and castrate male rats

	Mean LHRH release (pg/10 min)	Amplitude (pg)	Frequency (Pulses/h)	Serum LH (ng/ml)
Intact	1.5 ± 0.12^a	1.3 ± 0.14^b	1.7 ± 0.07	103.8 ± 14.4^c
Castrate	2.4 ± 0.23^a	3.3 ± 0.26^b	1.7 ± 0.05	475.4 ± 44.1^c

These data represent the analysis of LHRH release profiles from 4–5 hour push–pull perfusion of the anterior pituitary gland of intact ($n = 21$) and castrated ($n = 18$) male rats. Additionally, a single blood sample was obtained from 16 of these intact and 17 of these castrated male rats. Serum concentration of LH is presented. Significant differences ($P < 0.001$) between values obtained for each group are indicated by superscripted letters.

steroid molecule as a negative feedback signal and/or as a possible trophic factor in the CNS needs further study.

Therefore, we have decided to re-examine this issue in the male rat using the PPC in the anterior pituitary in order to estimate first, the response of the LHRH–LH axis to castration and second, the response of this system in the same castrated animals but following T treatment. Previously we reported the effect of castration on LHRH release in male rats bearing a PPC in the pituitary [20]. Herein we have used a larger PPC (20 gauge outer cannula vs 24 gauge in the previous report) and measured both LHRH and serum LH levels to overcome some of the pitfalls in the assessment of our previous results. Table 1 shows that position of the PPC with this dimension in the anterior pituitary does not alter the expected elevation in serum LH concentrations after 14 days of castration as compared with serum LH concentrations of intact animals indicating that in these pituitary-PPC bearing rats, the functional response of the gonadotropes to the absence of testicular hormones is unabated. Two representative examples of the LHRH release pattern following castration compared with the pattern detected in intact males is shown in Fig. 1. Two major features are evident: larger LHRH amplitude signals and a two-fold increase in mean LHRH release are seen in 14-day castrated male rats compared with those of intact rats. However, under such conditions the frequency of the LHRH pulse generator was practically identical as depicted in Table 1 which summarizes those results in 21 intact male vs 18 castrated pituitary-PPC bearing

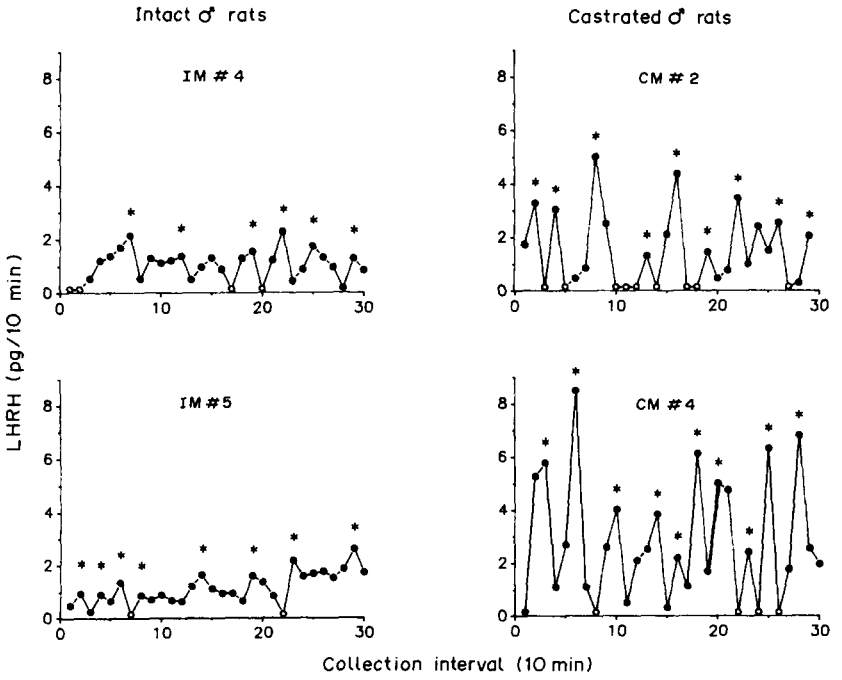

Fig. 1. Representative *in vivo* LHRH release profiles from two intact (IM No. 4, IM No. 5) and two 14-day castrated (CM No. 2, CM No. 4) male rats. LHRH was measured in push–pull perfusion samples collected at 10 min intervals from the anterior pituitary. Release profiles are plotted on the same vertical scale for both groups to facilitate a direct comparison of LHRH release between intact and castrated animals. Asterisks (*) represent peaks identified as significant LHRH pulses using the Pulsar method for pulse detection. Open circles represent undetectable levels of LHRH.

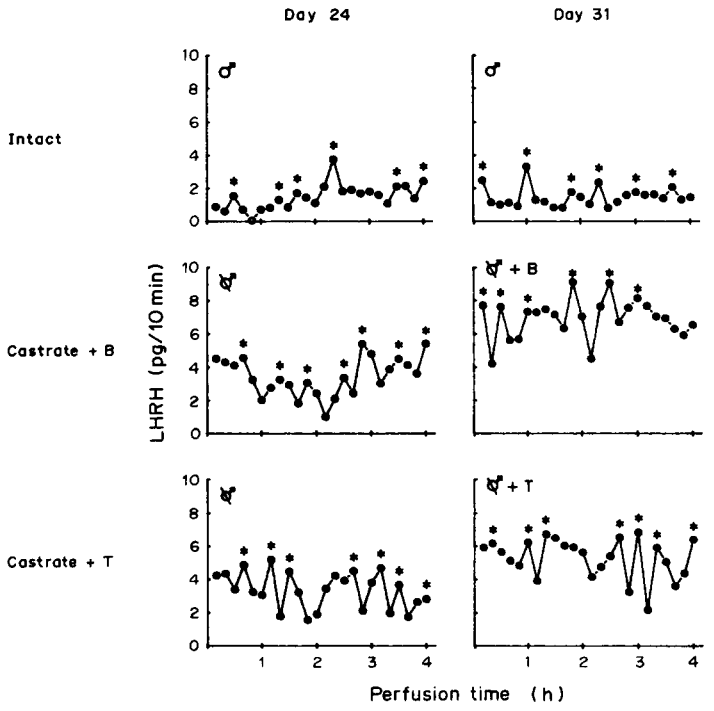

Fig. 2. Representative LHRH release profiles (pg/10 min) obtained from individual animals which were perfused 24 and 31 days subsequent to implantation of a push–pull cannula in the anterior pituitary gland. Animals in the intact group ($n = 5$) were perfused as intact animals during both perfusions. Animals in the castrate + B group ($n = 4$) were perfused on day 24 (corresponding to day 14 of castration) and again on day 31 after 7 days of subcutaneous exposure to an empty Silastic capsule. Animals in the castrate + T group ($n = 6$) were perfused on day 24 (castration day 14) and again on day 31 following 7 days subcutaneous exposure to a Silastic capsule containing crystalline testosterone (T). Mean plasma T levels resulting from this mode of T replacement were indistinguishable from mean intact levels. To facilitate direct comparisons between each treatment or group, the same vertical scale was utilized. Peaks identified as significant pulses by Pulsar analysis are indicated by asterisks (*), and the open circle represents undetectable levels of LHRH. The hormonal condition of the animals are indicated on the top left of each profile.

animals. Thus, it seems firmly established under these new conditions of perfusion of the anterior pituitary that castration does not lead to changes in the rate at which the LHRH pulse generator discharges the LHRH signals toward the anterior pituitary and that the main control of the testis is either exerted at the LHRH neuron by regulating the amount of LHRH discharge in response to an action potential and/or in regulating the number of active LHRH neurons.

If testosterone (T) is the main feedback signal from the testes that regulates the function of the LHRH–LH axis, castration should increase the release of these two tightly coupled LHRH and LH signals as documented above and T treatment should decrease their release. To clarify the role of T, 14-day castrated rats were perfused before receiving a T Silastic capsule (♂ + T) and an intact group (I) to control for repetitive perfusion was also included. In addition, blood samples were obtained through cardiac puncture in lightly ether anesthetized rats to measure LH levels at the end of each perfusion. Examples of the LHRH release profiles in these three groups, intact, castrated + blank Silastic (♂ + B) implant and castrated + T implants (♂ + T) are shown in Fig. 2 and mean concentrations of pituitary LHRH levels and serum LH levels of these three groups are depicted in Fig. 3A and B, respectively. It is self-evident from these data that repetitive perfusions of the pituitary in intact rats does not lead to changes in the normal release profile of LHRH or alter significantly mean concentration levels of LH. Castrated rats who received blank implants behaved as expected [12, 22, 23] since blood LH levels rose over intact levels and higher concentrations of LH were detected in rats castrated for 21 days than in animals castrated for 14 days [24]. Interestingly, a similar pattern of increase in LHRH release was observed in those animals. Surprisingly, when the rats received Silastic capsules containing T, the behavior of the LHRH–LH

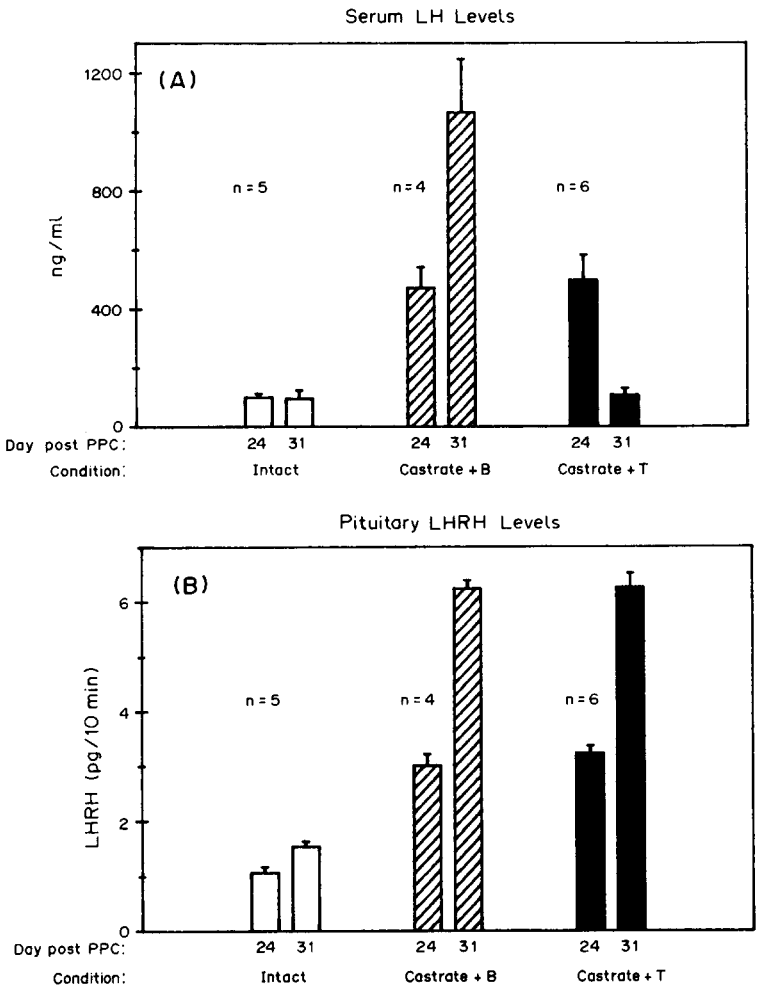

Fig. 3. (A) Mean Serum LH levels (±SEM) from intact, castrate + blank Silastic capsule (B) and castrate + testosterone implant (T) obtained from blood samples taken immediately after 4 h push–pull perfusion (PPP) on day 24 and 31 subsequent to push–pull cannula (PPC) implantation. Hormonal condition of the animals and number of animals comprising the mean are indicated on each of the graphs. (B) Mean LHRH release (±SEM) obtained by PPP of the anterior pituitary for each of the experimental groups on day 24 and 31 post PPC-implant. Hormonal status of the animals and number of animals comprising the mean are indicated on each of the graphs.

axis was affected differentially by this type of treatment since LH levels returned to normal intact range levels, whereas LHRH levels rose even further to reach values similar to those observed in the castrated control animals bearing blank implants. These data, obtained in the same freely behaving male rats who underwent first castration followed by T treatment, lead to the unambiguous conclusion that removal of the testes unleashes a significant increase in the secretion of LHRH and LH. Although T administered for 7 days in a continuous manner had a clear negative feedback action on LH secretion, it did not affect the secretion of LHRH suggesting among several options that either the pituitary is more sensitive than the hypothalamus to this dose and mode of administration of T or the hypothalamic LHRH neurosecretory apparatus is insensitive to this mode of T administration since the normal episodic release of this steroid [25–27] was not reproduced by this treatment. Therefore, it would be argued that to normalize the activity of the LHRH pulse generator in castrated rats an episodic mode of administration of T is essential. Current experiments in our laboratory are addressing such a basic question.

THE LHRH–LH TESTIS AXIS IN THE RABBIT

As in the male rat, castration in the male rabbit leads to high and maintained elevated concentration of both LH and FSH [28–30] as well as in the concentration of LHRH receptors in the anterior pituitary [31]. In addition, these

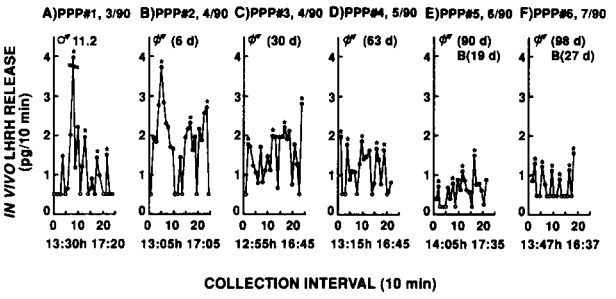

Fig. 4. LHRH release profiles in rabbit No. 161 under intact (panel A), following castration (panels B–D) and after receiving a subcutaneous blank (B) T implant (panels E–F). A total of six push–pull perfusions (PPP) of the hypothalamus were performed in this same animal during a five-month period as indicated. Notice the absence of increase in LHRH release after castration. Asterisks (*) indicate LHRH pulses.

effects of castration can be prevented by androgen replacement [31]. As far as we know the release of LHRH in castrated male rabbits has not been reported. Hence we asked two questions: first, does removal of the testes lead to an increase in LHRH secretion and second, does T treatment affect the LHRH pulse generator in long-term castrated rabbits? The PPP technique is an ideal method to examine these two basic questions, since it allows the use of the same animal under three different endocrine conditions: intact, castrated and castrated plus T implants or blank implants. The implants consisted of Silastic capsules containing a rod of T suspended in a mixture containing the steroid and special Silastic 382 or no hormone, generously provided by D. A. Ladd. A paper reporting levels of T in plasma following the use of these implants was previously reported by this group [31].

To achieve these goals 10 New Zealand white male rabbits were stereotaxically implanted with a PPC, the tip aiming towards the medial basal region of the hypothalamus, during the period of 14 February–18 May with the majority in March and April 1990, using procedures previously reported [6]. Push–pull perfusates were collected every 10 min into tubes on ice, acidified with 1 N HCl to a final concentration of 0.1 N and frozen until LHRH measurements were performed by RIA [32]. A detailed description of these results has been submitted to *J. Fert. Steril.* [33].

Figure 4 shows examples of the *in vivo* LHRH release profile in rabbit No. 161 perfused under the intact condition and several times after castration. The pulsatile release of this decapeptide from the hypothalamus in this freely behaving animal did not change after 6 days of castration with a tendency in the long-term castrated condition (over 90 days) for a reduction rather than an increase in the activity of the LHRH pulse generator. A summary of such results is presented in Table 2, showing that removal of the testis in the rabbit does not lead to significant changes in the release parameters of this neuropeptide, particularly in the mean amplitude and mean LHRH release, at least during the post-castration intervals examined in these experiments that covers 5–10 to 50–64 days post-castration.

The lack of increase in LHRH release following removal of the testes in the male rabbit is at odds with data reported above in castrated male rats [34] as well as with data published in ovariectomized rabbits [3] bearing a PPC in the tuberal region of the hypothalamus in which clear and maintained increases in mean LHRH release were detected. The reasons for such a dichotomy is not readily apparent, though it

Table 2. The mean LHRH release, mean amplitude and mean frequency of LHRH signals from 37 perfusions in 10 rabbits ($x \pm$ SE) are summarized

		Castrated		
	Intact	5–10 day	22–31 day	50–64 day
Mean LHRH release (pg/10 min)	1.00 ± 0.16	1.18 ± 0.14	1.05 ± 0.12	1.00 ± 0.09
Mean amplitude (pg)	1.27 ± 0.23	1.46 ± 0.33	1.03 ± 0.08	1.40 ± 0.28
Mean frequency	0.88 ± 0.10	0.98 ± 0.13	1.20 ± 0.13	1.10 ± 0.15

All 10 animals were perfused as intact rabbits and during different periods following castration. Notice the lack of changes in the activity of the LHRH pulse generator after castration

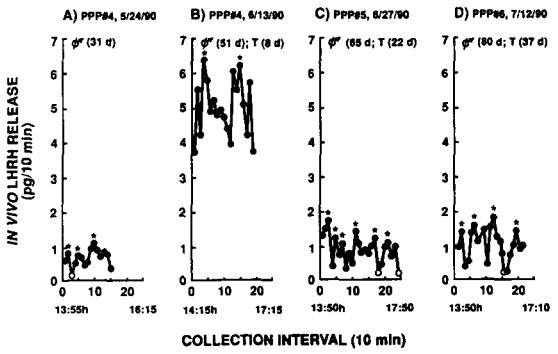

Fig. 5. An example of the LHRH release profiles of castrated rabbit No. 155 before and after receiving a subcutaneous T implant. Notice the low activity of the neural LHRH apparatus before T implant and the remarkable but transient increase in its activity detected on day 8 after T implant with a subsequent dropped to control levels by day 22 and 37 in spite of the continuous administration of T. In parenthesis are indicated the days of castration and bearing-time of the T implants. Asterisks (*) indicate LHRH pulses.

may indicate a different role for T in the control of the LHRH–LH axis in this photoperiodic species. It could further be argued that the main or the only negative feedback site of action of T occurs at the level of the gonadotropes in the pituitary as several reports suggest that this is indeed the case in several species [17, 35–37]. In addition, the steroid may exert a potent trophic action on the hypothalamus, essential for the maintenance of the normal activity of the LHRH neurosecretory apparatus. If this tenet is correct, T treatment in castrated rabbits should induce an increase in LHRH release. Figure 5 is an example that this is indeed true, since a rather dramatic increase in LHRH release of about 8-fold was observed in this rabbit, No. 155, receiving a T implant as indicated above; however, not surprisingly, the effect though robust, was transient since mean levels of LHRH dropped to intact levels or below by 25 days post-implant, as depicted in Fig. 6, showing that maintenance of fixed levels of T in blood within normal range [31] are only transiently effective in stimulating the LHRH neural apparatus, due perhaps to a rapid down regulation of T receptors in the hypothalamus. It is interesting to note that only mean LHRH levels were increased without changes in the amplitude or frequency of the LHRH signals, suggesting that one of the trophic actions of T might be of activating silent LHRH neurons. Further studies will be required to clarify this unexpected lack of effect of castration in the male rabbit and the robust, but transient, increase in LHRH release following T treatment.

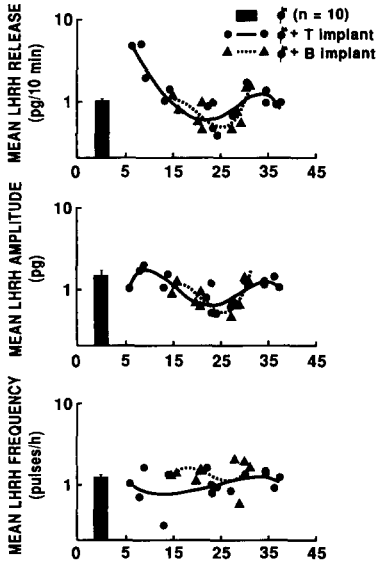

Fig. 6. The effect of testosterone implants on the activity of the LHRH pulse generator in castrated rabbits. Regression plots of mean LHRH release, mean amplitude and mean frequency vs days after T implants (circles) or control B implants (triangles).

A NOVEL *IN VIVO* MODEL TO STUDY THE INPUT–OUTPUT RELATIONSHIP OF LHRH–LH AT THE PITUITARY SITE IN FREELY BEHAVING RATS

In this section we will describe recent data demonstrating the feasibility of using the PPC in the anterior pituitary to examine local *in vivo* interactions among signals arriving to and signals originating from anterior pituitary cells in freely

behaving rats. The possible universal use of this powerful application of the PPC will be illustrated by showing the input–output relationship at this locus between LHRH and LH signals in male rats. The idea is rather simple, since it implies the simultaneous measurement of the moment to moment fluctuations in the pituitary perfusates of LHRH and LH and correlate such measurements in animals undergoing continuous perfusion of the pituitary for protracted periods of time, apparently without disturbing the physiological condition of the animals. Obviously, the likelihood of success of such an approach will depend on the analytical tools available allowing the specific measurements of two or more chemicals simultaneously. Therefore in our first attempt to validate such procedure, we tested if different dilutions of pituitary perfusates will displace iodinated LH from its binding to a monoclonal antibody against the β-subunit of the LH molecule in a parallel fashion to that induced by a rat-LH standard. That this was the case is shown in Fig. 7A, since pituitary perfusates from 40 to 320 μl from castrated male rats bearing a PPC in the pituitary, clearly did so. In addition, an array of dilutions of a male rat pituitary homogenate from 1:128,000 to 1:2000 also displaced the radiolabeled LH bound to the antibody in a parallel fashion to that induced by the rat-LH standard (Fig. 7B). The input–output relationship between LHRH and LH was then examined in 14-day castrated rats bearing a PPC in the anterior pituitary. Figure 8 illustrates the temporal relationship and magnitude of the LH release (bottom) in response to a single pulse of 1 ng of LHRH infused directly into the anterior pituitary (top) through the push-side of the PPC for 6 min at interval 6. In this paradigm samples were collected every 10 min for 1 h pre- and for 1 h post-infusion of LHRH. The results illustrate several important features of this technique. First, one can define the temporal relationship between these two events, that in this case were out of phase by about 20 min, that is, the peak

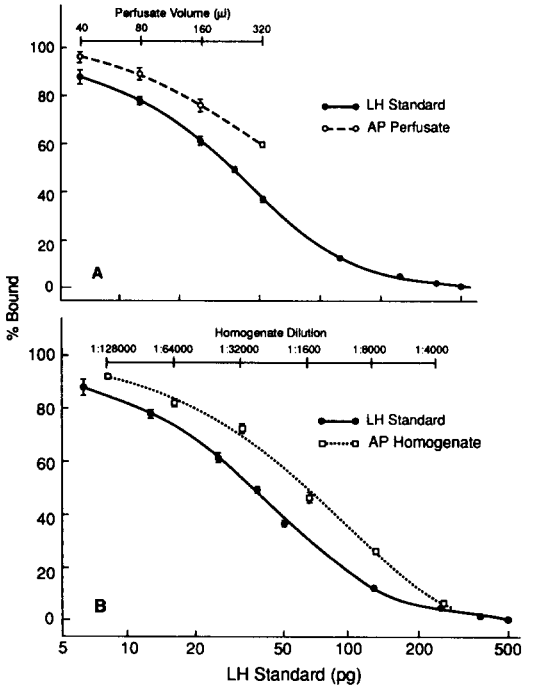

Fig. 7. Increasing amounts of anterior pituitary push–pull perfusate (A) and serial doubling dilutions of anterior pituitary homogenates (B), demonstrate the capacity to decrease the amount of ^{125}I-labeled LH to bind to anti-LH-β monoclonal antibody (provided by J. F. Roser, University of California, Davis) at a dilution of 1:1 million. These manipulations demonstrate specific binding of LH which is parallel to the standard curve generated from NIDDK-rLH-RP-3 standard (provided by the National Hormone and Pituitary Program). Each point represents the mean ± SEM for triplicate samples.

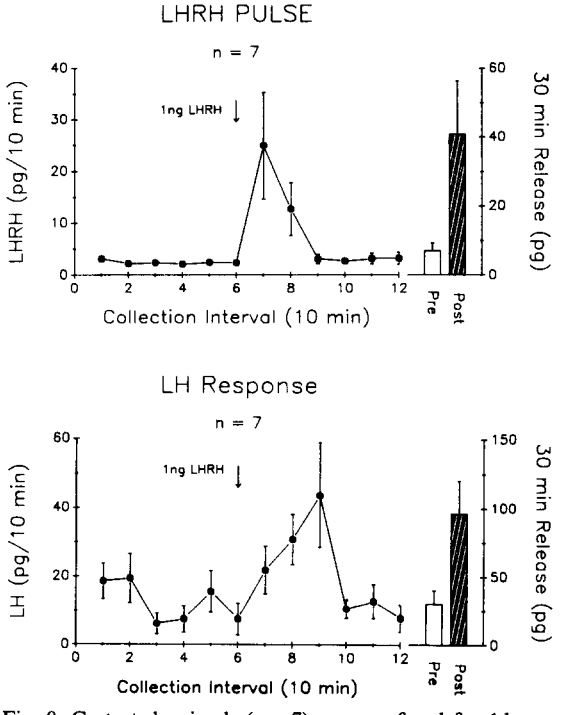

Fig. 8. Castrated animals ($n = 7$) were perfused for 1 h to establish basal secretory profiles for LHRH and LH. A 6 min pulse of LHRH totalling 1 ng was then introduced through the push side of the push–pull cannula and collections were continued for a second hour. The top panel depicts the LHRH profile and the bottom panel, the LH profile obtained from the same push–pull perfusion sample. Arrows indicate the time at which the LHRH pulse was administered. Bar diagrams indicate the amount of hormone measured in the 30 min pre-treatment and 30 min post-treatment intervals. Significant increases are observed in the amount of both LHRH and LH ($P < 0.01$ between pre- and post-treatment intervals.

levels of LHRH were achieved 10 min after the infusion while the mean peak LH response was achieved 20 min afterwards, corresponding to interval 9 in the figure. Second, the amount of LHRH recovered under the condition of this experiments was less than 5% of the total amount infused, indicating a rapid clearance of the neuropeptide from the tip of the cannula. Third, the levels of LH in the perfusate were about 0.1 ng/ml, whereas in the peripheral circulation they were about 450 ng/ml, a sizeable difference which indicates that only a small portion of the LH secreted by the pituitary gonadotropes was being collected. And fourth, under these conditions, a four-fold increase in the total amount of LH release was evoked by the infusion of 1 ng of LHRH. Assuming that the gonadotropes will respond in like fashion to endogenous pulses of LHRH, we are able to propose an operational definition of temporal coincidence between LHRH and successive LH pulses, based on these *in vivo* responses to exogenous LHRH. Pulses of LH and LHRH are then considered to be temporally associated if the peak of the LH pulse occurs within two sampling intervals of the peak of an LHRH pulse. Using this operational definition, an additional study was performed in 14-day castrated male rats to examine the moment to moment relationship between LH and LHRH. Figure 9 illustrates three examples using this method. At a first glance, it is evident that pulses of LH and LHRH as identified by the asterisks can be clearly detected in these animals and under these conditions using Pulsar analysis [38, 39]. Detailed analysis of these release profiles reveal that of the 31 identified LH pulses in these animals, 100% were temporally associated with an LHRH pulse. Furthermore, the frequency of pulses for both LHRH and LH are not significantly different, with one pulse of LHRH occurring every 28.8 ± 1.1 min and one pulse of LH occurring every 32.5 ± 0.9 min. The slight difference between the LH and LHRH pulse frequencies reflects the occurrence of three silent LHRH pulses in these animals. These pulses of LHRH were not temporally associated with an LH pulse and are a consistent observation in studies examining the release of both LH and LHRH [2–4, 7, 13, 16, 17, 40]. Interestingly, in preliminary results, we have shown that such tight coupling between LHRH and LH signals shown in castrated rats does not exist in intact rats as depicted in Fig. 10. The simultaneous measurement of LHRH and LH in intact rats (top and bottom of left panel) is drastically different to that of castrated rats (top and bottom of right panel) as

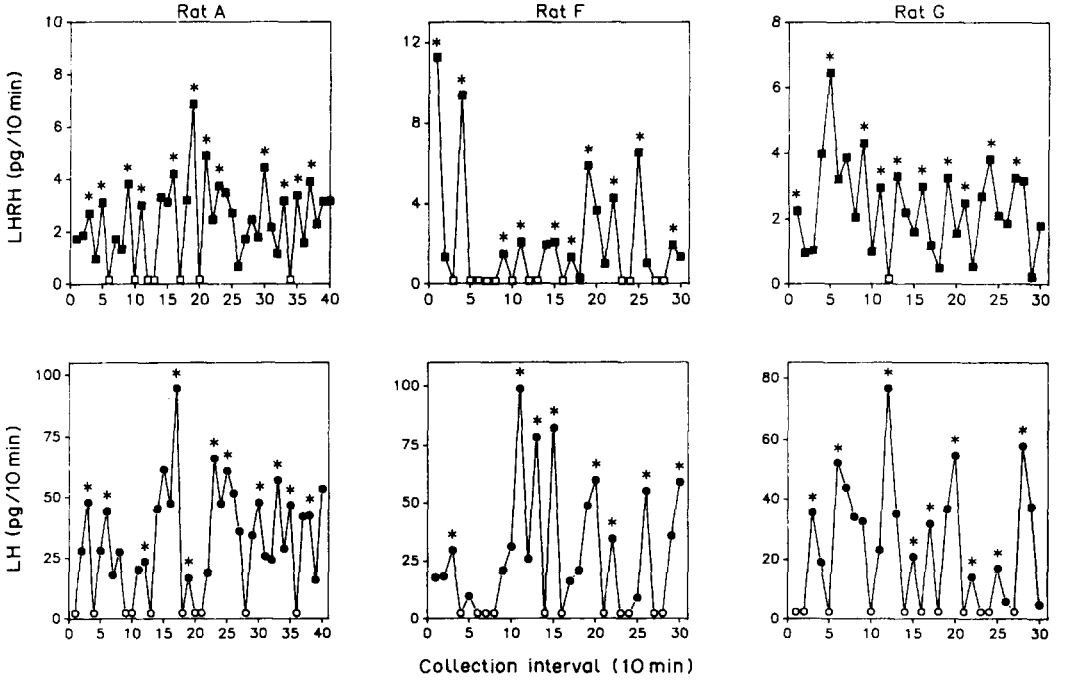

Fig. 9. Individual LHRH (top, ■) and LH (bottom, ●) release profiles obtained from the same anterior pituitary push–pull perfusion sample from three orchidectomized (14-day) rats. Asterisks (*) represent peaks identified as significant LHRH pulses using the PULSAR method for pulse detection. Open symbols represent undetectable levels of LHRH (□) and LH (○).

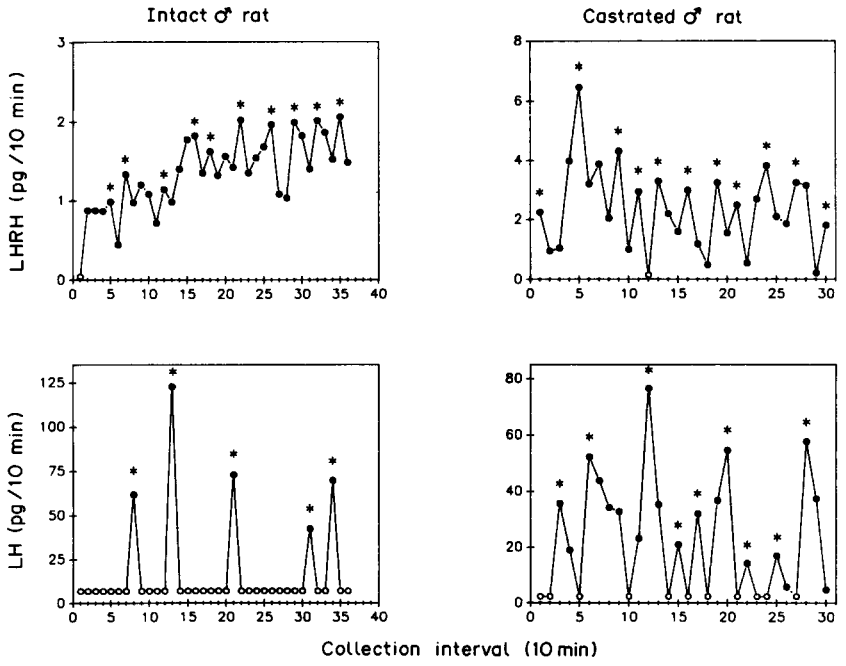

Fig. 10. Preliminary results of the simultaneous measurement of LH and LHRH from the same anterior pituitary push–pull perfusion sample in one representative intact and castrated male rat. Asterisks (*) represent peaks identified as significant LHRH pulses using the Pulsar method for pulse detection. Open circles represent undetectable hormone levels.

shown in these two examples. In the intact rat Pulsar analysis identified 10 pulses; only five pulses of LH were detected, whereas in the castrated rat 11 LHRH pulses and 9 LH pulses were measured. Analysis of the pulse frequency from simultaneous LH and LHRH release profiles from five intact animals and five castrated rats is shown in Table 3. Two important features are evident: one, the frequency of the LHRH signals in intact and castrated animals is invariant, whereas the frequency of the LH signals increased from 1.2 pulse/h in the intact condition to 1.6 pulse/h in the castrated condition. In other words, approximately 40% of the LHRH pulses were silent, that is, were not accompanied by an LH pulse in intact animals, whereas in castrated rats the relationship between these two signals was close to 100%. It is tempting to speculate that this major discrepancy in the ratio of LHRH and LH pulses between castrated and intact male animals most likely is due to the so called cloaking effect of T on the gonadotropes as proposed by Levine and Duffy [40]. The use of this novel technique to prove or deny such a challenging question in animals undergoing perfusion of the pituitary in the presence or absence of T will be the topic of a future communication.

Table 3. Summary of mean pulse frequency from Pulsar analysis of LHRH and LH release profiles obtained from the same anterior pituitary push–pull perfusion sample from intact and castrated male rats

	Pulse frequency LHRH (pulses/h)	LH (pulses/h)
Intact	1.70 ± 0.08	1.17 ± 0.11*
Castrate	1.70 ± 0.06	1.68 ± 0.03

These data represent the analysis of LH and LHRH release profiles obtained from intact ($n = 5$) and 14-day castrated ($n = 5$) male rats. Significant differences (*) were observed only in the pulse frequency of LH between intact and castrated animals ($P < 0.01$).

A description of an *in vivo* model to study these interactions is shown in Fig. 11. Herein, the LHRH pulse generator is considered to fire at a fixed frequency, typical for each species, but close to 1 pulse/h in monkeys [16], rabbits [41] and sheep [2, 13]. In the rat it appears to function at a higher rate of 1 pulse/30–40 min. However, the actual amount of LHRH delivered to the pituitary portal vessels is under a complex set of controls of which the inhibitory neurons, particularly endorphins [42] and GABA [43] could play pivotal roles in permitting more or less amounts of LHRH to be discharged into the portal system following an action potential. T, a main secretory product of the testis, appears to exert a negative feedback action on the control of LH at the level of the anterior pituitary. Depending on its mode of administration, "trophic effects" on the LHRH neurosecretory apparatus

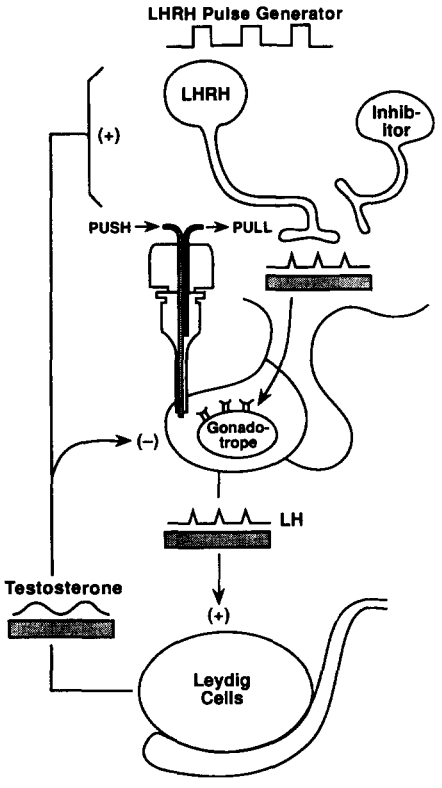

Fig. 11. Testicular control of the rat hypothalamic–hypophysial axis: pulses of LHRH from hypothalamic neurons are conducted to the vicinity of the pituitary gonadotropes where they stimulate the release of LH into the systemic circulation. Pulses of LH elicit an episode of testosterone (T) secretion from the Leydig cells of the testis. T feeds back at the level of the pituitary causing a decrease in the release of LH and by decreasing the number of LHRH receptors on the gonadotropes. Simultaneously, testicular feedback exerts a facilitatory effect at the levels of the hypothalamus on the synthesis of LHRH, and the maintenance of an inhibitory control on the magnitude of its pulsatile release, insuring that when plasma levels of T fall to levels no longer adequate to restrain the gonadotrope, sufficient LHRH will be available to stimulate the release of LH, and thus re-establish the impetus for another volley of episodic T secretion.

leading to either increases on *in vivo* LHRH release, as demonstrated herein in the rabbit, and/or to subtle activations of the synthetic machinery responsible for LHRH production as shown in the rat [44–46] appear to be important effects of T. Further studies will be required to prove the "trophic effects" of T on the LHRH neurosecretory apparatus of *in vivo* animals under physiological conditions.

The PPC placed in the anterior pituitary with the capability of measuring simultaneously several input and output signals is limited only by the sensitivity of the assays and quantity of the signals arriving to and leaving from the pituitary cells, offers an elegant and powerful technique to examine *in vivo* these interactions at a local site in the hypothalamic–pituitary axis of freely behaving animals.

In conclusion, these studies have demonstrated the existence of apparently opposite rather than similar responses in the testicular control of the hypothalamic–hypophyseal axis of the male rat as compared with those of the male rabbit. In the rat, castration unleashes significant increases in mean release of LHRH and LH with the neuropeptide being released at an invariant frequency, whereas the frequency of LH increases to match the fixed frequency of the LHRH pulse generator. In the rabbit, castration does not appear to modify the normal activity of the LHRH neurosecretory apparatus, whereas T treatment leads to a robust but transient stimulatory effect, since several-fold increases in the release of this decapeptide were observed within 5–10 days post T implants. Intriguingly, in the male rat a similar administration of T did not modify the already high levels of LHRH detected in castrated animals in spite of a clear return of blood LH levels to normal intact levels. Lastly, the simultaneous measurements of LHRH and LH from the same pituitary perfusates showed an uncoupling between these two signals in male intact rats, whereas in the castrated animals a tight coupling was detected.

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