Many LH peaks are needed to physiologically stimulate testosterone secretion: modulation by fasting and NPY

Pierroz, DD; Aebi, Anne Catherine; Huhtaniemi, IT; Aubert, Michel

How to cite

PIERROZ, DD et al. Many LH peaks are needed to physiologically stimulate testosterone secretion: modulation by fasting and NPY. In: The American journal of physiology, 1999, vol. 276, n° 4 Pt 1, p. E603–610. doi: 10.1152/ajpendo.1999.276.4.E603

This publication URL: https://archive-ouverte.unige.ch/unige:74360
Publication DOI: 10.1152/ajpendo.1999.276.4.E603

Many LH peaks are needed to physiologically stimulate testosterone secretion: modulation by fasting and NPY

DOMINIQUE D. PIERROZ,[1] AUDREY C. AEBI,[1]
ILPO T. HUHTANIEMI,[2] AND MICHEL L. AUBERT[1]
[1]*Division of Biology of Growth and Reproduction, Department of Pediatrics, University of Geneva School of Medicine, 1211 Geneva 14, Switzerland; and* [2]*Department of Physiology, Institute of Biomedicine, University of Turku, 20520 Turku, Finland*

Pierroz, Dominique D., Audrey C. Aebi, Ilpo T. Huhtaniemi, and Michel L. Aubert. Many LH peaks are needed to physiologically stimulate testosterone secretion: modulation by fasting and NPY. *Am. J. Physiol.* 276 (*Endocrinol. Metab.* 39): E603–E610, 1999.—The pulsatile luteinizing hormone (LH) and testosterone secretions were studied during serial blood collections performed at 7-min time intervals in the male rat. In fed rats, a discontinuous pattern of LH secretion was observed. Periods without secretion alternated with active secretory episodes consisting in trains of three to four LH peaks that triggered testosterone secretion usually 1–2 h later. The magnitude of the testosterone response was not correlated with the amplitude of the LH peaks. Isolated, single peaks of LH did not evoke clear testosterone responses. Forty-eight hours after initiation of fasting, testosterone secretion was markedly decreased, but integrated LH secretion was only partly reduced. Chronic infusion of neuropeptide Y (NPY; 18 μg/day, icv) reduced testosterone secretion to very low levels and abolished pulsatile LH secretion or testosterone response to isolated LH peaks. In conclusion, the stimulation of testosterone secretion by LH necessitates several LH peaks organized in a proper sequence, and the testosterone response is not immediate. Low testosterone secretion in fasting rats appears to result from disappearance of coordinated, multiple LH peaks of sufficient size. Inhibition of the gonadotropic axis achieved by central NPY administration is due to either absence of LH peak "clusters" or occurrence of nonfunctional single LH peaks.

luteinizing hormone; pulsatile secretion

THE CONCEPT THAT testosterone secretion by Leydig cells is mainly under the influence of the pituitary gonadotrophin luteinizing hormone (LH) has been recognized for several decades (7, 11, 18). It is also now well recognized that LH is secreted in pulses and that the frequency and amplitude of these pulses is essential for appropriate stimulation of gonadal function (2, 28). In the rat, previous studies have indicated that there is no direct relationship between the trains of LH pulses and the induction of testosterone secretory episodes, with often an active LH secretory period being dissociated from the testicular response (13, 26). The assessment of the pulsatile release of LH in rats has been difficult; the frequency of pulsing (20–30 min) necessitates frequent blood sampling ideally at 5-min time intervals (14, 32), and the relative lack of sensitivity of the available RIAs for rat LH make the sample volumes requested too high. The recent availability of a supersensitive immunofluorometric assay (17), allowing accurate determinations of LH in small blood specimens (30 μl of plasma), has made the study of pulsatile LH release in rats much easier. Also, the development of an automated serial blood collecting machine (10), allowing the simultaneous collection of small blood volumes at set time intervals in 12 rats for up to 16 h without human intervention, has made studies of pulsatile patterns of rat hormone secretion easier. LH secretion is reduced or abolished in several adverse metabolic conditions such as fasting (3, 4, 6) or dietary restriction (12), resulting usually in hypogonadism. Recently, our laboratory has demonstrated that chronic, central infusion of neuropeptide Y (NPY) resulted in hypogonadism and in reduction in growth hormone (GH) and insulin-like growth factor I (IGF-I) secretion, both in male (23) and female (8) adult rats. Because this hypogonadism is produced by a central inhibition, it has been assumed, but not demonstrated, that LH secretion would be reduced or abolished.

The availability of improved techniques to study the pulsatile LH release prompted us to compare pulsatile LH release and testosterone secretion in control, fed adult male rats and in two situations of presumably reduced or abolished LH secretion, i.e., fasting and NPY-induced hypogonadism.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats were purchased from Iffa-Credo (L'Arbresle, France) and were received at the age of 40 days. They were provided with standard laboratory chow ad libitum unless indicated otherwise. Room temperature was maintained between 21 and 23°C, and a 12:12-h light-dark cycle was used. All experiments were reviewed by the University of Geneva School of Medicine Ethical Committee for Animal Experimentation and were approved by the State of Geneva Veterinary Office.

Peptide. Synthetic porcine NPY (lot 138–45–15) was synthesized as previously described (5) and was donated by Dr. Jean Rivier (The Salk Institute, La Jolla, CA). Lyophilized NPY was dissolved in 0.04 M phosphate buffer containing 0.15 M NaCl, 0.01% ascorbic acid, and 0.2% BSA, adjusted to pH 7.4. The phosphate buffer used as solvent was sterilized and filtered using 0.2-μm filters (Nalgene, Rochester, New York).

Experimental procedures. Four series of blood collections were performed using exactly the same protocol. Each series included 12 rats corresponding to the capacity of the blood collecting machine. At 50 days of life, male rats were cannulated in the right jugular vein under pentobarbital sodium anesthesia (5 mg/100 g body wt, ip) as described previously (8)

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

and then each rat was placed in one of the cages of the blood collecting machine. Three days later, a 5.5-h serial blood collection at 7-min time intervals was initiated at 1100 or 1400, as indicated. The following three types of rats were studied: *1*) normal rats without treatment (controls), *2*) fasting rats (fast initiated 48 h before start of blood collection), and *3*) rats receiving a chronic intracerebroventricular NPY infusion (18 μg/day) beginning 3 days before blood collection.

Series 1 included four control, four fasted, and three NPY-treated rats. For this initial series, only the pulsatile LH secretion was analyzed, and plasma testosterone was determined only at the end of blood collection. For the remaining series, plasma LH and testosterone were measured in each plasma sample, allowing the parallel analysis of the secretory pattern of both hormones. *Series 2* included only control animals. *Series 3* included equal numbers of control and NPY-treated animals, and *series 4* included fasting rats. The maximum sample number for a 5.5-h blood collection was 48. A collection was considered meaningful only if 43 or more blood fractions were obtained. At the end of blood collection, the rats were killed by decapitation, and weight of testes and seminal vesicles was measured.

Collection in NPY-treated rats. A guide cannula was placed in the lateral ventricle at the age of 43 days, 1 wk before the jugular vein cannulation, as described in detail previously (8). During the cannulation of the jugular vein, an Alzet pump (model 2001; flow rate 1.0 μl/h, 200 μl capacity) filled with an NPY solution calculated to deliver 18 μg NPY/day was implanted under the skin dorsally and was connected to the intracerebroventricular cannula as described (8).

Automated blood collection. An automated repetitive blood microsampling apparatus was built according to the machine originally described by Clark et al. (10) and was adapted in our laboratory as described (15). In the present study, 60 μl of blood were automatically collected from freely moving rats during 5.5 h at 7-min time intervals. The obtained blood samples were then pushed in the tubings to the fraction collector, diluted by the Ringer-heparin solution used as solvent in all tubings, and sampled as 300-μl aliquots in the refrigerated collector tray. The diluted blood samples were then centrifuged, and the supernatants representing 1:10 dilution of plasma (assuming 50% hematocrit for rat blood) were stored at −20°C until required for hormone measurements.

LH and testosterone measurements. Rat LH was measured using the time-resolved immunofluorometric assay (Delphia; Wallac OY, Turku, Finland), as described previously (17), using National Institute of Diabetes and Digestive and Kidney Diseases rat LH RP-2 preparation as standard. Testosterone was assayed according to a previously described RIA technique (19) with a 500 fg/tube assay sensitivity. A 2.5-μl aliquot of plasma (i.e., 25 μl of the dilution) was assayed in duplicate for testosterone; hence, the assay sensitivity was 0.2 ng/ml. Minimum detectable concentration for LH was 0.05 ng/ml when assaying 10 μl of undiluted plasma. The intra-assay coefficient of variation (CV) was 19% at 0.05 ng/ml and <5% at levels above 1 ng/ml. The interassay CV was 12.5% at an LH concentration of 0.24 ng/ml and 7.8% at 7.8 ng/ml.

Statistical analysis. The patterns of pulsatile LH release were analyzed using a PC version of the Pulsar program (22) prepared by Dr. Sten Rosberg. This program allows determination of significant peaks of secretion and integrated secretion (area under the curve). The following aftervalues were used in the program: G1 = 6.4, G2 = 4.6, G3 = 3.9, G4 = 3.4, and G5 = 3.1. Similarly, the pattern of testosterone secretion was analyzed using the same program, with the following G values: G1 = 4.4, G2 = 2.6, G3 = 1.9, G4 = 1.4, and G5 = 1.1. G values were established empirically based on the recommendations made by Merriam and Wachter (22). Analysis of variance was first performed to evaluate the overall variation due to NPY or fasting, then individual variations were analyzed by the Duncan's range test or unpaired *t*-test when appropriate.

RESULTS

General aspects. Seventeen fed controls, 8 fasting rats, and 7 NPY-treated rats were analyzed. Comprehensive analysis of all patterns of LH and testosterone secretion is presented in Table 1. The additional data, obtained in the first series of rats (4 fed controls, 4 fasted rats, and 3 NPY-infused rats) are presented in Table 2. As expected, fasting and central infusion of NPY clearly resulted in hypogonadism with low LH and testosterone secretion and a marked reduction in weight of testes and seminal vesicles, indicating that LH and testosterone secretion, respectively, were consistently reduced during the experimental days.

Secretory profiles in control rats. Careful examination of LH and testosterone secretory patterns, analyzed in parallel in 14 control rats, clearly indicated that there is no immediate relationship between LH peaks and testosterone response. Whereas pulsatility of LH secretion is obvious, a more integrated pattern for testosterone secretion was observed with the occurrence of sporadic sharp peaks. Another general finding of this study was that the induction of the testosterone response always required multiple peaks (trains of usually 3 peaks), but the amplitude of these peaks varied widely. Indeed, the maximal testosterone response could be obtained by trains of either large or small LH pulses. Figures 1 and 2 illustrate representative examples of the different patterns observed in 14 rats. Three examples of vigorous LH secretion with an

Table 1. *Effects of 48-h fasting or NPY central infusion on LH and testosterone secretion in adult male rats (all series)*

Time Period	Integrated LH Secretion, ng/ml for 5.5 h	Number of LH Peaks, peaks/5.5 h	Maximum Plasma LH, ng/ml	Points in LH Peaks, %	Integrated Testosterone Secretion, ng/ml for 5.5 h
Controls (17)	0.85 ± 0.16	5.6 ± 0.6	1.27 ± 0.20	35.5 ± 4.2	5.5 ± 0.8
Fasted (8)	0.39 ± 0.07	4.1 ± 0.5	0.76 ± 0.19	22.6 ± 4.3	1.4 ± 0.3* (4)
NPY infused (7)	0.22 ± 0.10*	1.4 ± 0.5†	0.65 ± 0.20*	8.2 ± 3.3†	1.7 ± 0.4* (4)
ANOVA (*P* value)	<0.01	<0.01	<0.05	<0.005	<0.01

Values are means ± SE; no. of rats is in parentheses. LH, luteinizing hormone. Integrated testosterone secretion was only measured in 4 rats of the fasted (*series 3*) and neuropeptide Y (NPY; 18 μg/day)-treated groups (*series 4*). Significance of changes versus controls: *$P < 0.05$ and †$P < 0.001$.

Table 2. *Effects of 48-h fasting or NPY central infusion on pulsatile LH release in adult male rats (series 1 only)*

Time Period	Body Weight, g	Seminal Vesicle Weight, mg	Testes Weight, g	Plasma Testosterone Levels, ng/ml
Controls (4)	260 ± 2	673 ± 24	3.19 ± 0.06	3.84 ± 1.34
Fasted (4)	251 ± 10	391 ± 28†	2.82 ± 0.12*	0.30 ± 0.02*
NPY infused (3)	252 ± 11	90 ± 7†	2.51 ± 0.08‡	0.21 ± 0.01*
ANOVA (*P* value)	NS	<0.001	<0.005	<0.025

Values are means ± SE; no. of rats is in parentheses. Plasma testosterone levels were measured at the end of blood collection. Significance of changes with respect to controls: $*P < 0.05$, $\dagger P < 0.001$, and $\ddagger P < 0.01$. NS, not significant.

adequate testosterone response are illustrated in Fig. 1. As can be seen, trains of relatively large LH peaks were observed, often in groups of three, clearly dissociated from the testosterone response, as could be evaluated in this time window of 5.5 h. Pulse frequency for these rats was 30.7 ± 2.7 min. In general, the testosterone response followed a train of LH peaks by 1–2 h. In *rats 2–2* and *2–5* (Fig. 1), generous LH pulsing ended up in a marked increase in testosterone secretion with a 2-h delay. In *rat 2–6* (Fig. 1, *bottom*), a sharp testosterone peak was observed (8 ng/ml) together with the third major LH peak. Four examples of less coordinated patterns of LH and testosterone secretion are illustrated in Fig. 2. For *rat 3–10* (Fig. 2*A*), a first train of three small LH peaks (<0.5 ng/ml) resulted in activation of testosterone secretion; a second train of three LH peaks of even smaller amplitude induced three additional testosterone peaks of very high amplitude (16 ng/ml). A similar pattern was seen for *rat 3–12*, with four even smaller LH peaks progressively activating testosterone secretion. A very different pattern was seen for *rat 2–14* (Fig. 2*C*), for which a decreasing trend of testosterone secretion with small peaks was observed in the absence of LH secretion; the decrease in testosterone secretion finally induced two sharp peaks of LH with the impression that a third peak would have been seen if the collection had lasted longer, thus again representing a train of three peaks. For *rat 2–15* (Fig. 2*D*), the same progressive decrease in testosterone secretion was observed, but at the end of the 5.5-h collection no new LH peak occurred. Mean values for the different parameters analyzed in these control rats are shown in Table 1. The mean number of LH peaks was 5.5 ± 0.6, representing one peak per hour, but the range was considerable, from one to nine peaks for this 5.5-h collecting period.

Secretory profiles in fasting rats. It was expected that a 2-day fast would reduce LH and testosterone secretion. In the first series studied, mean plasma testosterone measured at the end of blood collection was markedly decreased in the four fasting rats studied, and the weight of seminal vesicles was reduced by a factor of two (Table 2). In the four rats studied in *series 4*, integrated testosterone secretion over 5.5 h was also markedly decreased from 5.5 ± 0.8 to 1.4 ± 0.3 ng/ml, clearly indicating that testosterone secretion was inhib-

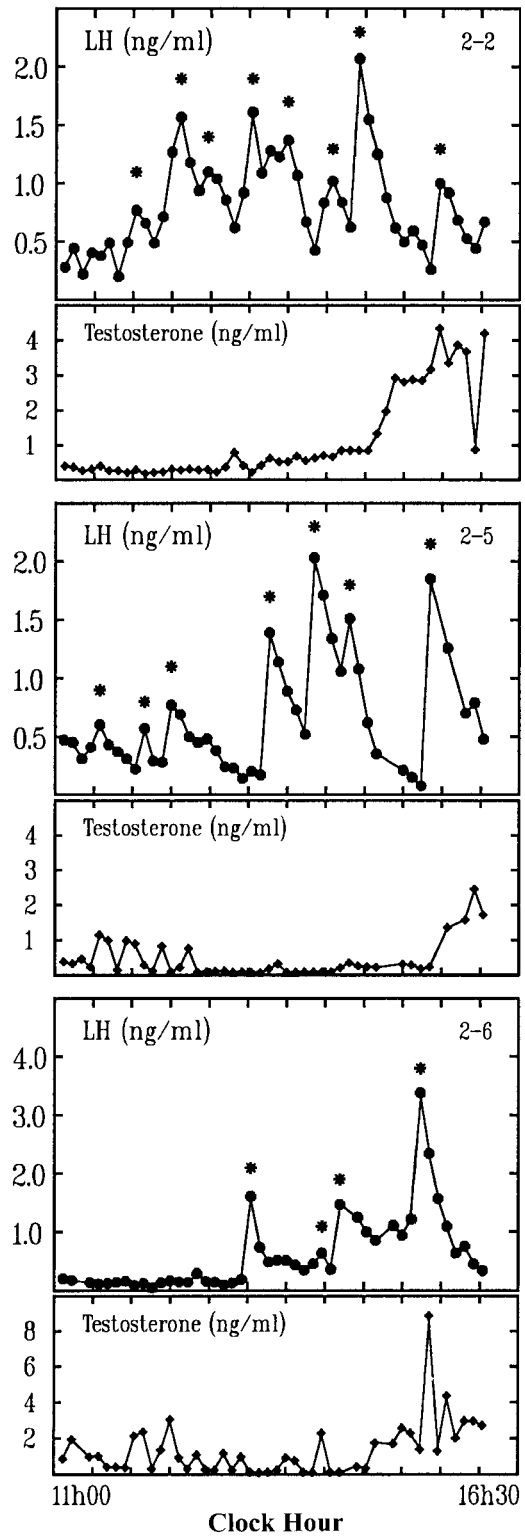

Fig. 1. Relationship between pulsatile luteinizing hormone (LH) release and testosterone secretion in fed male rats. Three examples are shown of rats exhibiting trains of LH peaks of high amplitude resulting in a clear Leydig cell response with an increase in plasma testosterone levels. Significant LH peaks are shown with asterisks. Blood collection was performed between 1100 and 1630 at 7-min time intervals. One mark on the time scale represents 30 min. Note the difference in scale for *rat 2–6* shown at *bottom*.

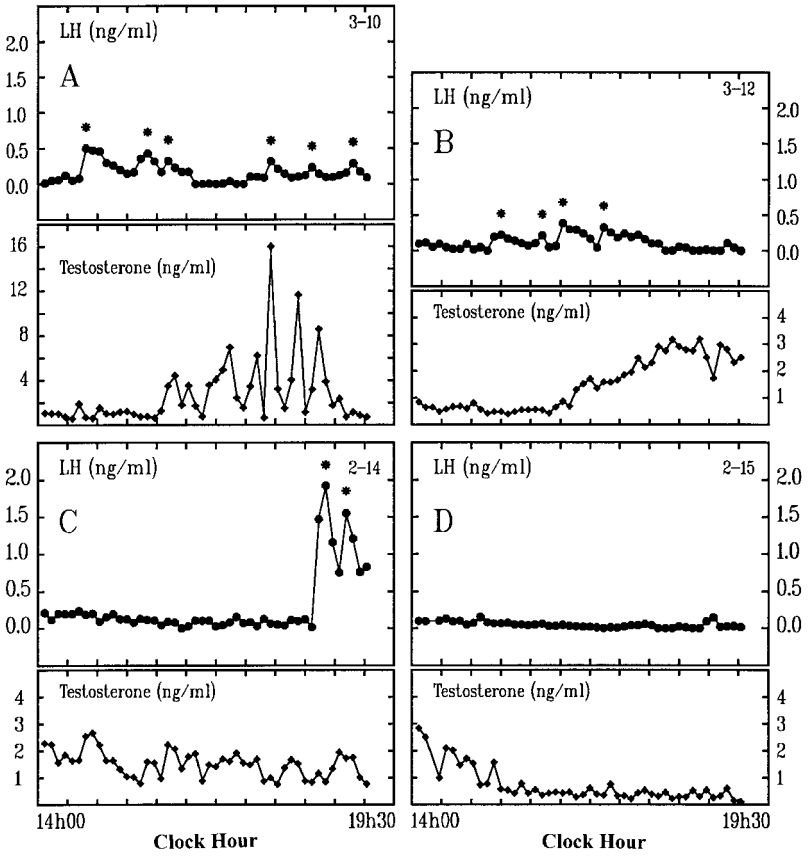

Fig. 2. Relationship between pulsatile LH release and testosterone secretion in fed male rats. Four examples of rats exhibiting no direct relationship between LH peaks and testosterone secretion. *A*: trains of 3 small LH peaks entraining a vigorous testosterone secretion. *B*: very small LH peaks inducing a steady increase in testosterone secretion. *C*: decrease in testosterone secretion resulting in the induction of 2 vigorous LH peaks. *D*: decreasing testosterone secretion to low values without increase in LH. Blood collection was performed between 1400 and 1930 at 7-min time intervals. One mark on the time scale represents 30 min. Note the difference in scale for plasma testosterone for rat in *A*.

ited in fasting rats (Table 1). Consistent with low testosterone secretion, LH secretion was reduced but not fully abolished in these fasting rats. Either no LH secretion was observed, as in *rat 4–12* (Fig. 3), or several small LH peaks were seen as in *rats 4–3* and *4–9*. In these two rats, there was no relationship between these LH peaks and the sporadic testosterone secretion. In the fourth rat shown in Fig. 3 (*rat 4–6*), an

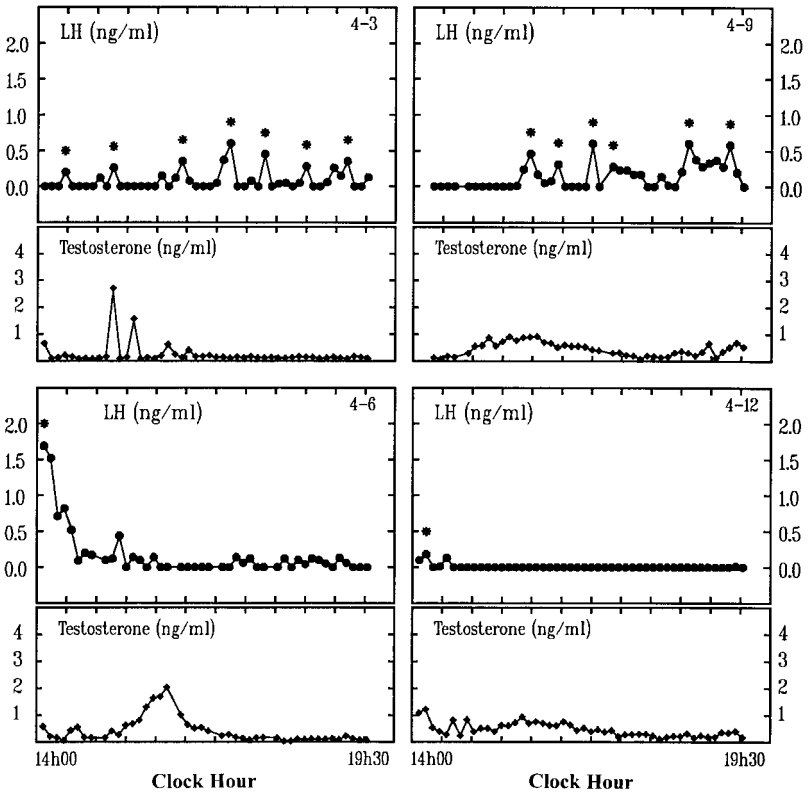

Fig. 3. Relationship between pulsatile LH release and testosterone secretion in fasting rats. Food was removed 48 h before initiation of blood collection. Four examples of rats exhibiting various types of LH secretion. Significant LH peaks are shown with asterisks. Blood collection was performed between 1400 and 1930 at 7-min time intervals. One mark on the time scale represents 30 min.

initial large LH peak resulted in a discrete testosterone peak 2 h later. Comparing all controls and fasting rats, none of the LH parameters were significantly decreased in fasting rats despite the suppressed testosterone levels (Table 1); integrated LH secretion was decreased but not significantly ($P = 0.07$), as was the percentage of points in peaks. The number of LH peaks was not decreased.

Secretory profiles in centrally NPY-infused rats. Previous studies from our laboratory have shown that chronic, central infusion of NPY resulted in hypogonadism (8, 23). In the two series of NPY-treated rats of the present study, testosterone secretion was very low, with a mean value for plasma testosterone measured at the end of blood collection of 0.21 ± 0.01 ng/ml compared with 3.84 ± 1.34 ng/ml in control rats (*series 1*, Table 2) and an integrated testosterone secretion of 1.7 ± 0.4 ng/ml for 5.5 h in treated rats versus 5.5 ± 0.8 ng/ml for 5.5 h in control rats (Table 1). The effects of decreased testosterone secretion were quite obvious from the markedly reduced weight of seminal vesicles (7-fold decrease) and the 20% decrease in testicular weight achieved after 3 days of NPY infusion (Table 2). LH secretion was clearly impaired in NPY-treated rats; both integrated LH secretion and number of LH peaks were reduced by a factor of four, and the percentage of points in LH peaks averaged only 8.2 ± 3.3% in treated rats versus 35.5 ± 4.2 in control rats (Table 1). The pattern of LH secretion was characterized either by an absence of any LH peaks as seen in four out of seven rats (*rat 3–9* as shown in Fig. 4) or by discrete, isolated LH peaks, as seen for the three other rats shown in Fig. 4 (*rats 3–1*, *3–7*, and *3–14*). These LH peaks did not entrain any testosterone secretion, as clearly seen for *rat 3–14*, for which the relatively important LH peak seen at the beginning of blood collection did not induce any increase in testosterone during the remaining 5 h of blood collection. Body weight was not affected by NPY treatment.

DISCUSSION

It is a common finding that the mean values for plasma LH or testosterone in rat studies display large standard errors, reflecting important individual variations. It is less well perceived that these variations are mostly due to the highly pulsatile pattern of both LH and testosterone rather than to poorly controlled sample collection or hormone assay conditions.

In their elegant study, Ellis and Desjardins (13) first demonstrated in 1982 that the LH and testosterone secretion episodes are often dissociated and that, for both hormones, periods without secretion often occur in normally fed, adult male rats. They described as "normal pattern of secretion" a well-organized sequence of multiple LH pulses, usually three to five peaks, that induce a testosterone response only 1–2 h later with no real pulsatility and finally a "resting" period with no LH pulses with decreasing or absent testosterone secretion (13). In addition, this pattern of LH/testosterone secretion was not different between day and night collection periods, and most importantly, individual rats were not programmed for a particular pattern of secretion, since repeated collections in the same rats at 5-day time intervals yielded very different patterns.

With the availability of much more sensitive assays for LH and testosterone (17), and an automated blood collecting machine, we reasoned that it would be useful

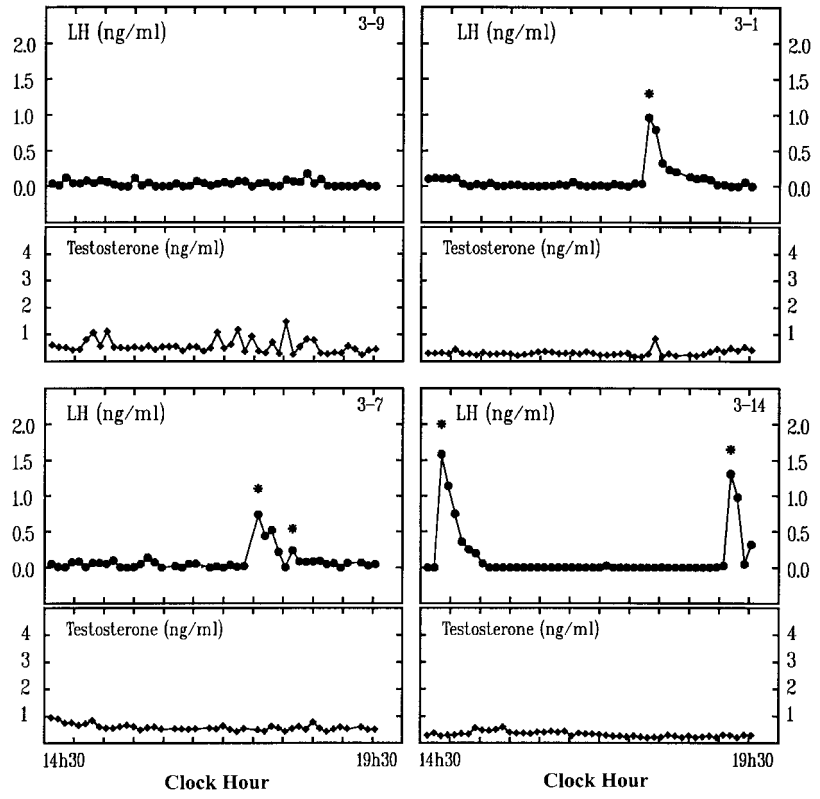

Fig. 4. Relationship between pulsatile LH release and testosterone secretion in rats chronically infused with neuropeptide Y (NPY; 18 μg/day). NPY-containing Alzet pumps were connected to an icv cannula and were implanted dorsally 3 days before initiation of blood collection. Four rats displayed complete absence of LH release such as for *rat 3–9* as in this figure and *rats 9*, *10*, and *12* from *series 1*, not shown. Examples of silent LH peaks in the presence of very low testosterone secretion are seen for *rats 3–7* and *3–14*. Significant LH peaks are shown with asterisks. Blood collection was performed between 1400 and 1930 at 7-min time intervals. One mark on the time scale represents 30 min.

to reevaluate the relationship between LH and testosterone secretion in normal rats and also to investigate the alterations of this peculiar relationship between the two hormones in two situations known for decreased testosterone secretion, fasting and central infusion of NPY.

The present study confirms that for each LH peak detected, an immediate response in testosterone secretion is almost never seen in the rat. Whereas no direct relationship between the LH peaks and testosterone responses is present, there is however a clear requirement of multiple LH peaks allowing a testosterone secretory episode to occur, generally 1–2 h later. The organization of LH peaks in trains of three peaks appears to be an essential feature for proper testosterone stimulation. The amplitude of these LH peaks appears to be less essential, since maximal testosterone responses were seen with trains of LH peaks of very different amplitude. This relationship between LH and testosterone secretion in the rat differs from that seen in humans, since more direct testosterone responses were seen in male subjects after trains of LH peaks (27). However, also in humans, testosterone secretion was found to lag behind LH secretion by ~40 min (27).

The absence of immediate testosterone response to LH peaks in vivo is an uncommon endocrine concept and is surely unexpected, since Leydig cells in culture immediately respond to LH. Thus, in vivo, the Leydig cells are programmed to release testosterone in function not only of LH stimulation but also in response to various local paracrine factors transducing local conditions, most likely related to Sertoli cell function and process of spermatogenesis (18, 21). It is also possible that access of LH to the Leydig cells is quite different in the normal, in vivo situation than in the cell culture conditions. Indeed, Turner and Rhoades (31) demonstrated restricted availability of LH to the testicular interstitial fluid. This would imply that Leydig cells are not exposed through the testicular interstitial fluid to similar LH pulses as occur in circulation and therefore do not "read" these pulses. Testosterone response would thus be more dependent on the overall LH secretion over a period of time rather than to the nature of pulses. Three LH pulses would produce the proper LH presence in the testicular interstitial fluid that would trigger a significant testosterone response. With this type of restricted permeability of LH to the testicular compartment, it is clear that isolated LH peaks have much less chance to stimulate a significant testosterone response.

A clear difference in the LH/testosterone secretory pattern was seen between fasting and NPY-treated rats. In fasting rats, there was an apparent discrepancy between the important reduction in testosterone secretion and the persistence of substantial LH secretion. Two alternatives exist for explaining this discrepancy. Either the scattered small LH peaks seen do not constitute the proper sequence of trains of peaks required for Leydig cell stimulation, or testosterone secretion is inhibited by the metabolic consequences of fasting. Alterations of circulating metabolic components could induce some kind of desensitization of the Leydig cells that would no longer respond to LH. Such a situation is seen for the stimulation of IGF-I by GH at the hepatic level, with hepatocytes of fasting animals becoming refractory to the action of GH (30). Previous studies however have indicated that the testicular target for LH is intact in fasting rats, since human chorionic gonadotropin administration to fasting rats induced a normal testosterone response (4). Thus the most likely cause for reduction in testosterone secretion in the fasting rat is the absence of a proper train of LH peaks that is able to reach the interstitial space and Leydig cells and that is able to produce their optimal activation.

We have previously demonstrated that chronic, central infusion of NPY induces hypogonadism in adult intact female (8) and male (23) rats. Such constant infusion also prevents sexual maturation in female rats (16, 24). Because it is known that NPY synthesis and release in the hypothalamus is markedly increased in several situations of adverse metabolic conditions associated with hypogonadism (20), the working hypothesis was formulated that such an elevation of endogenous NPY release could represent the neuroendocrine vector for inhibition of gonadotropin-releasing hormone (GnRH) release (16). In the previous studies on the effects of central infusion of NPY, obvious hypogonadism was reported as seen by arrested estrous cycles in female rats (8), arrested pubertal development in immature female rats (24), or very low testosterone secretion in adult male rats (23). In these studies, it was implied that gonadotropin secretion was reduced or abolished, but this last point was never formally demonstrated. In the present study, we are confirming that central NPY infusion to male rats produced a rapid inhibition of the testicular function by the spectacular losses of seminal vesicle and testis weights as seen after 3 days of constant NPY infusion (Table 2), an apparent consequence of chronically decreased testosterone secretion (Tables 1 and 2). We are now demonstrating that the NPY-induced inhibition of the reproductive axis is associated with markedly reduced LH secretion. In four NPY-infused rats, no LH secretion was seen; in the three others, isolated, "silent" peaks were seen that did not induce any testosterone response (Fig. 4). Thus, as expected, the NPY-induced central inhibition of reproductive function is associated with altered LH secretion.

The occurrence of isolated, silent LH peaks, which could be best qualified as nonfunctional peaks, is one of the most intriguing findings of this study. Whereas in fasting rats it is possible that some of these isolated LH peaks were possibly functional, with the presence of a small testosterone response (Fig. 3, *rat 4–6*), for the three NPY-treated rats with isolated LH peaks shown in Fig. 4, the LH peaks seen remained without the testosterone response. This is particularly true for *rat 3–14*, for which a superb spontaneous LH peak was seen at initiation of blood collection with an amplitude of 1.6 ng/ml. Plasma LH concentration then decreased following first-order kinetics with a half-life of 10 min

and reached baseline after 40 min. There were no changes in testosterone secretion over the next 5 h, after which time a second smaller isolated LH peak occurred. This provides further evidence that isolated LH peaks alone are unable to stimulate testosterone secretion once the testicular function is arrested.

A key role for leptin as a regulator of reproduction in function of prevailing metabolic conditions has also been evoked. Leptin, the recently described hormone originating from adipose tissue, was shown to control food intake, possibly acting on NPY release for this action (25, 29), and also to control reproductive function (9). In their elegant study, Ahima and colleagues (1) showed that leptin administrated to fasting mice could prevent the inhibitory effects of fasting on LH and testosterone secretion. Of particular interest is the observation that, in these mice, leptin treatment concomitantly prevented the increase in hypothalamic NPY synthesis seen in untreated, fasting mice (1). Thus the action of leptin to rescue gonadotropin secretion in fasting mice was associated with prevention of the increase in NPY activity seen in fasting, thus reinforcing the working hypothesis that NPY may be the main effector of fasting for the inhibition of GnRH/LH release (16). The present study confirms this hypothesis since fasting and NPY treatment produced the same effects on LH and testosterone secretion, but NPY treatment produced these effects much faster. The fast action of NPY results most likely from the fact that NPY action within the hypothalamus is immediate, whereas during the first day of fasting no inhibition is yet effective on GnRH release, with the hypothalamus of the rats becoming more conscious of the fasting situation on the 2nd and then 3rd day of absence of food.

In conclusion, the stimulation of testosterone secretion by LH in the male rat necessitates several LH peaks organized in a proper sequence, and the testosterone response to these peaks is not immediate. Possibly, LH pulsatility is not perceived within the testicular interstitial fluid, and only the repetition of several peaks can produce the necessary increase in LH to trigger a meaningful testosterone secretory episode. Single LH peaks, irrespective of their size, often remain silent. Central infusion of NPY produced a more complete inhibition of the pituitary testicular axis than fasting within this 3-day time frame. Low testosterone secretion in fasting rats appears to result from disappearance of coordinated, multiple LH peaks of sufficient size. These data confirm that the inhibition of the gonadotropic axis achieved by central NPY administration is due to the absence of significant LH peaks. Finally, this study further highlights the fact that proper evaluation of LH and testosterone secretions requires analysis of multiple samples.

We thank Brigitte Delavy in Geneva and Aila Metsävuori and Tarja Laiho in Turku for excellent technical assistance. We also thank Jean Jacques Goy for excellent work in our animal facilities. We are grateful to Dr. Janet Roser (University of California, Davis, CA) for the $518B_7$ antibody used in the rat LH assay.

This study was supported by National Research Science Foundation Grant 31–39729.93 and by a grant from the Sigrid Jusélius Foundation.

Address for correspondence and reprint requests: M. L. Aubert, Hôpital des Enfants, HUGs, 6, rue Willy-Donzé, 1211 Geneva 14, Switzerland (E-mail: aubert@cmu.unige.ch).

Received 8 July 1998; accepted in final form 20 November 1998.

REFERENCES

1. **Ahima, R. S., D. Prabakaran, C. Mantzoros, D. Qu, B. Lowell, E. Maratos-Flier, and J. S. Flier.** Role of leptin in the neuroendocrine response to fasting. *Nature* 382: 250–252, 1996.
2. **Bartke, A., R. E. Steele, N. Musto, and B. V. Caldwell.** Fluctuation in plasma T levels in adult male rats and mice. *Endocrinology* 92: 1223–1228, 1973.
3. **Bergendahl, M., A. Perheentupa, and I. Huhtaniemi.** Effect of short-term starvation on reproductive hormone gene expression, secretion and receptor levels in male rats. *J. Endocrinol.* 121: 409–417, 1989.
4. **Bergendahl, M., A. Perheentupa, and I. Huhtaniemi.** Starvation-induced suppression of pituitary-testicular function in rats is reversed by pulsatile gonadotropin-releasing hormone substitution. *Biol. Reprod*. 44: 413–419, 1991.
5. **Boublik, J. H., N. A. Scott, M. R. Brown, and J. E. Rivier.** Synthesis and hypertensive activity of neuropeptide Y fragments and analogs with modified N- or C-termini or D-substitutions. *J. Med. Chem*. 32: 587–601, 1989.
6. **Bronson, F. H.** Food-restricted, prepubertal female rats: rapid recovery of luteinizing hormone pulsing with excess food, and full recovery of pubertal development with gonadotropin-releasing hormone. *Endocrinology* 118: 2483–2487, 1986.
7. **Catt, K. J., J. P. Harwood, R. N. Clayton, T. F. Davies, V. Chan, M. Katikineni, K. Nozu, and M. L. Dufau.** Regulation of peptide hormone receptors and gonadal steroidogenesis. *Recent Prog. Horm. Res.* 36: 557–622, 1980.
8. **Catzeflis, C., D. D. Pierroz, F. Rohner-Jeanrenaud, J. E. Rivier, P. C. Sizonenko, and M. L. Aubert.** Neuropeptide Y administered chronically into the lateral ventricle profoundly inhibits both the gonadotropic and somatotropic axis in intact adult female rats. *Endocrinology* 132: 224–234, 1993.
9. **Chehab, F., M. Lim, and R. Lu.** Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin. *Nat. Genet.* 12: 318–320, 1996.
10. **Clark, R. G., G. Chambers, J. Lewin, and I. C. A. F. Robinson.** Automated repetitive microsampling of blood: growth hormone profiles in conscious male rats. *J. Endocrinol*. 111: 27–35, 1986.
11. **Desjardins, C.** Endocrine signaling and male reproduction. *Biol. Reprod*. 24: 1–21, 1981.
12. **Dong, Q., M. Bergendahl, I. Huhtaniemi, and D. J. Handelsman.** Effect of undernutrition on pulsatile luteinizing hormone (LH) secretion in castrate and intact male rats using an ultrasensitive immunofluorometric LH assay. *Endocrinology* 135: 745–750, 1994.
13. **Ellis, G. B., and C. Desjardins.** Male rats secrete luteinizing hormone and T episodically. *Endocrinology* 110: 1618–1627, 1982.
14. **Ellis, G. B., and C. Desjardins.** Mapping episodic fluctuations in plasma LH in orchidectomized rats. *Am. J. Physiol*. 247 (*Endocrinol. Metab*. 10): E130–E135, 1984.
15. **Gruaz, N. M., Y. Arsenijevic, W. B. Wehrenberg, P. C. Sizonenko, and M. L. Aubert.** Growth hormone (GH) deprivation induced by passive immunization against rat GH-releasing factor does not disturb the course of sexual maturation and fertility in the female rat. *Endocrinology* 135: 509–519, 1994.
16. **Gruaz, N. M., D. D. Pierroz, F. Rohner-Jeanrenaud, P. C. Sizonenko, and M. L. Aubert.** Evidence that neuropeptide Y could represent a neuroendocrine inhibitor of sexual maturation in unfavorable metabolic conditions in the rat. *Endocrinology* 133: 1891–1895, 1993.
17. **Haavisto, A. M., K. Pettersson, M. Bergendahl, A. Perheentupa, J. F. Roser, and I. Huhtaniemi.** A supersensitive immunofluorometric assay for rat luteinizing hormone. *Endocrinology* 132: 1687–1691, 1993.
18. **Huhtaniemi, I. T., R. N. Clayton, and K. J. Catt.** Gonadotropin binding and Leydig cell activation in the rat testis in vivo. *Endocrinology* 111: 982–987, 1982.

19. **Huhtaniemi, I. T., H. Nikula, and S. Rannikko.** Treatment of prostatic cancer with a gonadotropin-releasing hormone agonist analog: acute and long-term effects on endocrine function of testis tissue. *J. Clin. Endocrinol. Metab*. 61: 698–704, 1985.
20. **Kalra, S. P., M. G. Dube, A. Sahu, C. P. Phelps, and P. S. Kalra.** Neuropeptide Y secretion increases in the paraventricular nucleus in association with increased appetite for food. *Proc. Natl. Acad. Sci. USA* 88: 10931–10935, 1991.
21. **Klinefelter, G., and W. R. Kelce.** Leydig cell responsiveness to hormonal and nonhormonal factors in vivo and in vitro. In: *The Leydig Cell*, edited by A. H. Payne, M. P. Hardy, and L. D. Russell. Cache River Press, 1996, vol. 1, p. 535–553.
22. **Merriam, G. R., and K. W. Wachter.** Algorithms for the study of episodic hormone secretion. *Am. J. Physiol*. 243 (*Endocrinol. Metab*. 6): E310–E318, 1982.
23. **Pierroz, D. D., C. Catzeflis, A. C. Aebi, J. E. Rivier, and M. L. Aubert.** Chronic administration of neuropeptide Y into the lateral ventricle inhibits both the pituitary-testicular axis and growth hormone and insulin-like growth factor-I secretion in intact adult male rats. *Endocrinology* 137: 3–12, 1996.
24. **Pierroz, D. D., N. M. Gruaz, V. D'Allèves, and M. L. Aubert.** Chronic administration of neuropeptide Y into the lateral ventricle starting at 30 days of life delays sexual maturation in the female rat. *Neuroendocrinology* 61: 293–300, 1995.
25. **Schwartz, M. W., R. J. Seeley, L. A. Campfield, P. Burn, and D. G. Baskin.** Identification of targets of leptin action in rat hypothalamus. *J. Clin. Invest*. 98: 1101–1106, 1996.
26. **Södersten, P., P. Eneroch, and A. Q. Pettersson.** Episodic secretion of luteinizing hormone and androgen in male rats. *J. Endocrinol*. 97: 145–153, 1983.
27. **Spratt, D. I., L. S. L. O'Dea, D. Schoenfeld, J. Butler, P. N. Rao, and W. F. Crowley, Jr.** Neuroendocrine-gonadal axis in men: frequent sampling of LH, FSH, and testosterone. *Am. J. Physiol*. 254 (*Endocrinol. Metab*. 17): E658–E666, 1988.
28. **Steiner, R. A., W. J. Bremner, and D. K. Clifton.** Regulation of luteinizing hormone pulse frequency and amplitude by T in the adult male rat. *Endocrinology* 111: 2055–2061, 1982.
29. **Stephens, T. W., M. Basinski, P. K. Bristow, J. M. Bue-Valleskey, S. G. Burgett, L. Craft, J. Hale, J. Hofmann, H. M. Hsiung, A. Kriauciunas, W. MacKellar, P. J. Rsteck, Jr., B. Schoner, D. Smith, F. C. Tinsley, X. Y. Zhang, and M. Heiman.** The role of neuropeptide Y in the antiobesity action of the obese gene product. *Nature* 377: 530–532, 1995.
30. **Thissen, J. P., J. M. Ketelslegers, and L. E. Underwood.** Nutritional regulation of the insulin-like growth factors. *Endocr. Rev.* 15: 80–101, 1994.
31. **Turner, T. T., and C. P. Rhoades.** Testicular capillary permeability: the movement of luteinizing hormone from the vascular to the interstitial compartment. *J. Androl*. 16: 417–423, 1995.
32. **Urban, R. J., W. S. Evans, A. D. Rogol, D. L. Kaiser, M. L. Johnson, and J. D. Veldhuis.** Contemporary aspects of discrete peak-detection algorythm. I. The paradigm of the luteinizing hormone pulse signal in men. *Endocr. Rev*. 9: 3–36, 1987.