Pituitary Leptin-A Paracrine Regulator of Gonadotropes: A Review

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Abstract: Leptin, a potent anorexigenic hormone secreted primarily by adipocytes, is known to be expressed in the anterior pituitary. Studies in our laboratory found leptin proteins and mRNA predominantly in somatotropes in normal male and cycling female rats. In contrast, leptin expression predominated in gonadotropes during pregnancy and lactation. Leptin expression varied with the cycle and was enhanced, in vitro by gonadotropin releasing hormone and Neuropeptide Y. In contrast, ghrelin inhibited pituitary leptin expression. Pituitary leptin in somatotropes or gonadotropes was reduced by nutritional deprivation for 24 h. However, growth hormone (GH), luteinizing hormone (LH) and pituitary leptin recovered if fasted animals were given glucose water. The glucose-mediated recovery suggests that the system is sensitive to changes in serum glucose. Somatotropes and gonadotropes also recovered if pituitary cells from fasted rats were stimulated in vitro with 1-100 pg/ml leptin. This in vitro leptin-mediated recovery suggests that leptin is important in the maintenance of somatotropes and gonadotropes. Collectively, the data suggest that pituitary leptin might serve as a “glucostat”, sensing levels of serum glucose and reporting this nutritional state to somatotropes and gonadotropes in a paracrine manner. A decrease in pituitary leptin might signal nutritional deprivation to somatotropes and gonadotropes, lowering LH and GH production and allowing for conservation of resources. Lower GH would help conserve fat stores and lower LH would promote survival over reproduction.

Keywords: Leptin, somatotropes, gonadotropes, growth hormone, gonadotropins, pituitary, paracrine.

DISCOVERY OF LEPTIN AND ITS ROLE IN REPRODUCTION

Leptin is a recently discovered cytokine produced by white fat cells encoded by the Lep gene [1]. It regulates satiety as well as energy expenditure [2, 3]. This cytokine suppresses appetite by stimulating specific anorexigenic neurons in the arcuate nucleus [2, 4-6] that produce CART/POMC (cocaine and amphetamine RNA transcript / pro-opiomelanocortin) and α-MSH (alpha-melanocyte-stimulating hormone). High leptin levels also inhibit functions of orexigenic neurons producing neuropeptide Y/agouti related peptide (NPY/AgRP) [7, 8], which stimulate appetite [7-12].

Leptin is also an “adipostat,” because it informs the body of the levels of fat stores. However, the widespread distribution of leptin receptors throughout the body has suggested that this cytokine performs additional functions [13]. Schneider et al. [14-18] have reviewed leptin’s effects on fuel utilization, showing that leptin works with insulin to facilitate the use of glucose and free fatty acids.

Leptin may also regulate neuroendocrine systems, particularly in the reproductive system [19-21]. Neuroendocrinologists have suggested that circulating leptin plays a critical role in reproduction because a threshold level of fat is vital for normal puberty and fertility [22-28]. Rats without functional leptin receptors (Zucker fa/fa) and mice without functional leptin proteins (ob/ob) both exhibit characteristics of morbid obesity, insulin resistance, delayed or impaired pubertal development, and pituitaries with low numbers of somatotropes or gonadotropes [29, 30]. Puberty is delayed or absent, and fertility is severely impaired in the absence of leptin receptors or in animals with low leptin levels [28, 31-33].

Infertility is also seen in obese humans bearing a mutation in the leptin gene (LEP) that causes leptin deficiency [34-36]. Humans lacking the full-signaling leptin receptor (LEPR exon 16) are both infertile and have impaired growth [37]. In adult humans, low gonadotropin levels and functional hypothalamic amenorrhea occur when a relative energy deficit (from weight loss, excessive exercise, or eating disorders) disrupts the pituitary–gonadal axis. Women with hypothalamic amenorrhea have low leptin levels and do not express the normal diurnal leptin rhythm [38-45].

Leptin therapy has normalized reproductive hormone levels in a leptin-deficient prepubertal child [34], as well as in adult men [46] and in women with functional amenorrhea [44, 45]. Giving exogenous leptin to leptin-deficient obese animals will cure their infertility [24, 47]. Leptin has also normalized gonadotropins (in women with functional hypothalamic amenorrhea), even if the behavior that led to it has not changed [43-45].

Although leptin may be important for fertility, it may not be directly responsible for the onset of puberty. Early studies showed that leptin accelerated puberty [22, 48, 49], suggesting that leptin might be a metabolic trigger for its
time of onset. However, later studies did not report a good
correlation between normal prepubertal serum leptin levels
and the timing of puberty in normal rodents [50-52] or
primates [53-56]. Therefore, Cheung et al. [52], Urbanski
[33], and Mann and Plant [32] suggested that leptin was not
the trigger for puberty. It is considered a permissive factor
because infertility develops when leptin is deficient [57].
Mechanisms behind its actions in reproduction are only
beginning to be understood.

Leptin may regulate reproduction by direct or indirect
effects on the hypothalamic-pituitary axis. Although the
exact target cells in the hypothalamic part of the circuit are
uncertain [25, 58-60], leptin’s importance in regulating
secretion from Gonadotropin releasing hormone (GnRH)
neurons in the hypothalamus is well established [25, 50, 58,
61-66]. The uncertainty relates to the fact that GnRH
neurons in rats and monkeys may not have leptin receptors
[61-66]. The uncertainty relates to the fact that GnRH
neurons in rats and monkeys may not have leptin receptors
(LEPR) [25, 58, 59], although LEPR are found on
immortalized GnRH neurons [60]. Thus it is possible that
LEPR are expressed early in developing GnRH neurons.
Collectively, the data indicate that leptin regulates GnRH,
directly or indirectly.

Thus, the literature suggests that leptin stimulates the
secretion of both Luteinizing hormone (LH) and
Gonadotropin releasing hormone (GnRH) either directly or
indirectly [58, 64, 67-69]. Leptin also restores LH secretion
in fasted mice, rats, hamsters and monkeys [14-17, 20, 58,
64, 67, 68]. Leptin also directly stimulates LH secretion from
pituitary cells in vitro [70-74]. In addition, leptin regulates
Growth hormone (GH) [19], although there is lack of
agreement about the exact direction of its regulation [19, 75-
78]. Leptin stimulates GH secretion mediated by GH
releasing peptide (GHRP [77, 79] and restores GH pulses in
fasted rats [76, 78]. Luque et al. [80] reported that leptin
restored GH secretion in ob/ob mice that were infused with
exogenous leptin for 7 days. In addition, leptin caused an
increase in Growth hormone releasing hormone (GHRH)
receptors. No changes were noted in GHRH levels
themselves, which suggested that GHRH did not mediate the
restoration.

Collectively, the evidence supports the hypothesis that
leptin may serve as a mediator or modulator in the
neuroendocrine circuit regulating gonadotropes and
somatotropes. The hypothesis is further supported by
evidence showing a reduction in both gonadotropes and
somatotropes in rats or mice that are leptin deficient [29, 30].
Because somatotropes and gonadotropes are present in
leptin-deficient rodents, these data suggest that leptin may
not be needed for the embryonic birth of these cell types.
Rather, the reduced numbers suggest that leptin may
promote later developmental events necessary for the full
functioning of these cells.

Support for a role later in development in rodents comes
from studies by Ahima et al. [81], who mapped postnatal
changes in serum leptin. They reported a discrete surge in
leptin levels during days 7–10 of postnatal development in
mice coinciding with a 6–10-fold increase in leptin mRNA
in the adipose tissue, suggesting that the source was indeed
adipocytes. Their studies showed that this surge did not
depend on changes in fat mass or food intake, which
suggests that it may be linked to an independent source of
regulation not identified in their study. Similar findings in
mice and rats were reported by Devskar et al. [82], who
showed a rise in leptin levels as early as 2 postnatal days
followed by a decline in leptin after postnatal day 14. Ahima
et al. [81] correlated this rise in leptin with a potential role
for leptin as a neuroendocrine hormone and linked it to
significant changes along the neuroendocrine axes.

How would these changes relate to development in the
human? In fact the timing of events in rodents and humans
 correlate well, because rodents are relatively immature at
birth. The postnatal ages that correspond to the rise in leptin
are equivalent to the midgestational stage of human fetal
development [83, 84]. Reitman et al. [85] described a
comparable surge of leptin in the human fetus, showing a
100–150-fold rise from the first to the second trimesters. The
source of leptin is mostly the placenta, which is so important
that Ong et al. [86], correlating leptin levels in cord blood,
found them to be inversely proportional to weight gain after
 birth.

Collectively, this suggests important homeostatic roles
for leptin in early human development. This second trimester
rise in leptin correlates with a similar rise in GH secretion
[87]. Shimon et al. [88] reported both the presence of leptin
receptors, and leptin stimulation of GH secretion in cultured
fetal human pituitary cells, suggesting that pituitary cells
from this stage are responsive to leptin. The expansion of
gonadotropes also coincides with the rise in leptin during
the second trimester [87]. There an increase in GnRH and
gonadotropin levels, especially in females [84, 88-92].
Castillo et al. [89] reported that GnRH stimulated LH release
from cultured fetal human pituitary cells, with higher levels
coming from female fetuses.

THE CASE FOR LEPTIN AS A PARACRINE REGU-
LATOR IN THE ANTERIOR PITUITARY

Most of the early studies assumed that leptin’s functions
are mediated by circulating leptin from adipocytes. However,
a growing body of evidence suggests that the adipocyte
source of leptin may not be able to regulate rapid
neuroendocrine responses. The rise in serum leptin that is
seen at midcycle is too slow for it to be a regulator of the LH
surge [93]. Furthermore, Schneider et al report that nutrition
alone can restore LH pulses lost by fasting [14, 15]. The
timing of this response did not match that for the restoration
of adipocyte leptin [50, 94, 95]. This has led workers to
consider additional sources of leptin for the regulation
(permissive or facilitatory) of neuroendocrine pathways. The
most obvious sources might be from the neuroendocrine
cells themselves.

Evidence is growing that leptin is produced by the
anterior pituitary and the hypothalamus [31, 93, 96-100]. A
pituitary source would point to a paracrine role for this
cytokine. Studies have identified leptin expression in the
pituitary and the hypothalamus of humans and rodents
although during the early part of the decade, studies did not
agree as to the cell type producing leptin. One report of rats
and mice found leptin only in TSH cells [97]. Another group
suggested that leptin was in gonadotropes or thyrotropes, but
not in somatotropes [100]. In humans, leptin proteins were
found in 70% of corticotropes, 21% of somatotropes and 29-
33% of gonadotropes, prolactin cells or thyrotropes [30].
Vidal and Cohen reported labeling for leptin in secretory granules of all pituitary cell types but prolactin cells [101]. In human pituitary adenomas, leptin mRNA and leptin secretion was detected in subsets of all tumorous pituitaries [102, 103]. Thus, a pituitary source of leptin could mediate its local, paracrine effects. Later sections of this review will present data from this laboratory that show both the cell types that produce leptin and also potential regulators for this source of leptin.

The hypothesis that pituitary leptin might be a paracrine factor is further supported by the fact that subsets of all pituitary cells have leptin receptors (LEPR) [30, 96, 97, 99-101]. LEPR are related to the class 1 cytokine receptor superfamily [104, 105]. The long form of the receptor, LEPRb, has a single-pass transmembrane domain and a 302-amino-acid cytosolic domain that binds and activates the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway [106]. In addition, there are multiple splice-variant short isoforms of this receptor, which may be expressed in a tissue-specific manner [107]. They include LEPRa, LEPRc, and LEPRd, each of which contains a single transmembrane domain and 34-, 32-, and 40-amino-acid cytosolic domains, respectively. Another isoform, LEPRe, is soluble and lacks the transmembrane domain. Of all of the isoforms, only LEPRb contains the complete cytosolic domain that activates JAK/STAT pathways. The exact physiologic significance of the other isoforms is uncertain; however, there is speculation that they are involved in transport in serum and through the blood–brain barrier [107]. In db/db mutant mice, deletion of exon 16 of the Lepr interferes with the JAK/STAT pathway binding and renders the mice hyperphagic and obese [108-111]. A similar, rare deletion of LEPR exon 16 has been reported in a human family by Clement et al. [37].

LEPR mRNA was first detected in rat pituitary cells over a decade ago by Zamerano et al. [57]. Cai et al. [112] reported increased expression of LEPR in the anterior pituitaries and somatotropes of mice expressing a human GHRH transgene [113]. Shimon et al. [88] reported LEPR mRNA expression in adult and fetal human pituitaries, as well as in GH- and prolactin-secreting adenomas. Giusti et al. [114] also reported high LEPR expression levels in human GH-secreting adenomas. Jin et al. [96, 97] used RT-PCR to show that LEPRa and LEPRb mRNAs were expressed in most, if not all, of the 12 types of human pituitary tumors, including those secreting GH. Later studies of porcine pituitaries detected LEPR mRNA [115] and also quantified the expression of LEPR in each of the cell types [116]. LEPR immunoreactivity was shown in 69% of somatotropes, which fits well with what we have reported in rats and mice [93, 98, 117]. Sone et al. [99, 100] detected LEPR in 97% of rat pituitary somatotropes.

LEPR expression by gonadotropes was first reported by Jin et al. [96, 97], who detected LEPRa and LEPRb isoforms in human LH adenomas and two mouse cell lines: L6/T2 cells, which produce LH and FSH; and αT3-1 cells, which produce αGSU. In porcine pituitaries [116], LEPR were expressed in 90% of gonadotropes in the pars tuberalis and in 29% of remaining gonadotropes. Later studies by Kaminiski et al. [118] showed regulation of LEPRb with the estrous cycle, reporting that expression of LEPRb peaked during the luteal phase of the cycle and early in pregnancy in porcine pituitaries. Ighal et al. [116] reported LEPR in 70% of somatotropes in ovine pituitaries. In contrast to the foregoing, Sone et al. [99, 100] showed the leptin receptors in 97% of somatotropes (but not the other cell types). They suggested from this evidence that leptin might be produced by gonadotropes for the paracrine regulation of somatotropes. Sone et al. [99, 100] found LEPR in less than 1% of gonadotropes. However, our recent studies have confirmed Kaminiski et al. [118], showing significant expression of LEPR by most gonadotropes [93].

Our studies of pituitary leptin began early this decade with tests of the hypothesis proposed by Sone et al. [98]. We reasoned that if leptin was produced primarily by gonadotropes, we would see changes in leptin expression with the reproductive state. In addition, we recognized that we needed to prove that leptin was actually produced by pituitary cells by detecting leptin mRNA. Our concern, as we began these studies, was that some of the leptin proteins identified by immunolabeling might reflect circulating leptin bound to leptin receptors. Thus the first set of tests identified cells that expressed leptin mRNA, which provided needed proof for leptin production by pituitary cells [93, 98]. The remaining sections of this report discuss our findings, which prove leptin production by specific cell types and our follow up studies, which show evidence that pituitary leptin may act as a paracrine or autocrine regulator in the pituitary [93, 117]. These latest findings are discussed in light of our current understanding of a role for leptin in the regulation of the pituitary.

CHANGES IN PITUITARY LEPTIN EXPRESSION WITH THE REPRODUCTIVE STATE

To detect changes in leptin-bearing cells with the physiologic state, dual immunolabeling for leptin and GH, FSHβ, or LHβ was done, as previously described [98]. In situ hybridization was carried out as described previously [98, 119] with the use of a 48 bp biotinylated oligonucleotide probe complementary to nucleotides 342-389 located within the coding sequence for rat leptin (accession number NM_013076). qRT-PCR assays for leptin mRNA are also described in previous studies [98, 106]. The statistics and power analyses are also described in these studies.

The percentages of cells with leptin proteins rose from a low of 21.0 ± 4.0% on the AM of estrus to a peak of 55.0 ± 3.0% of AP cells on the afternoon of proestrus [93]. This peak during proestrus is higher than that in all other groups (p<0.001), including male rats, which had 39.6 ± 1.0% of AP cells with leptin proteins. All data reported in this and later sections represent the average ± SEM.

The counts of pituitary cells with leptin mRNA show leptin production by 32-37% of pituitary cells in male or in estrous, metestrous, or diestrous female rats [93]. Early in the cycle (estrous and metestrus), there are significantly more cells with leptin mRNA than leptin proteins. In situ hybridization and QRT-PCR assays both detected an interesting significant decline in leptin mRNA on the morning of proestrus [93]. The QRT-PCR assays show that leptin mRNA is maintained at relatively low levels through estrus. Then, levels rise 2—fold and by diestrus, they are higher than all other groups. The increase in mRNA from
metestrus to diestrous was also detected by densitometric analysis of cytochemical label, which showed that the total area of label for leptin mRNA increased and thus signified a higher level of production of leptin mRNA by individual cells. Finally, the most unexpected and interesting finding in this survey was in pregnant animals, which exhibited the highest percentages of AP cells with leptin proteins (60.0 ± 2.0%) or mRNA (44.0 ± 2.0%) [93]. Intermediate levels of leptin-bearing cells, in the range of those seen early in the cycle, were seen in females taken on the 3rd day of lactation.

These studies brought out several significant points about the expression of leptin in pituitary cells [93]. First, leptin expression varies with the stage of the cycle or during pregnancy. Leptin proteins reach a peak just before the LH surge and then are reduced by the morning of estrus. Perhaps leptin stores may have been secreted, although we cannot rule out degradation as a cause of the reduction. The leptin-bearing cells could still be detected, however, by their content of mRNA.

Second, the differential expression of leptin mRNA is important evidence that confirms the production of leptin by pituitary cells. This addresses the concern that pituitary might be there only as a result of uptake of circulating leptin. Pituitary leptin mRNA is detected by both in situ hybridization and QRT-PCR.

Third, more specific paracrine role for pituitary leptin is suggested by the collective evidence showing that leptin is known to be a secretagogue for LH, both in vivo and in vitro [67, 70, 73, 74, 120]. There is no rapid periovulatory rise in serum leptin coming from adipocytes to permit or facilitate an LH surge. However, the rapid rise and fall in pituitary leptin from proestrus to estrus may be timed to adequately facilitate LH secretion. The need for adequate leptin to permit gonadotrope function will be brought out and discussed further in a later section focused on the effects of fasting on LH cells.

The dramatic rise in leptin transcripts on diestrous AM and PM coincides with a time when LH protein synthesis is enhanced. If leptin is important in the regulation of the LH surge, the timing of leptin expression would suggest that it could permit or facilitate LH synthesis or secretion during diestrous and proestrus. The rapid decline in leptin mRNA during estrus is intriguing and consistent with the hypothesis that leptin transcripts are tightly regulated during the cycle, perhaps by rapid degradation.

Our data on pregnant animals suggest that pituitary leptin might be regulated to support gonadotropic or other reproductive functions during pregnancy. This could relate to its role in facilitating the utilization of nutrients, like glucose and free fatty acids [14-18, 121]. It may also play a role in maintaining gonadotropes, or it may regulate placental or gonadal functions.

**PITUITARY CELLS PRODUCING LEPTIN**

**Effects of the Reproductive State**

Tests of regulators for pituitary leptin depended upon our results from studies that determined which cell types produced leptin, especially during the reproductive cycle. Our original hypothesis was based on data from Sone et al [99, 100], which indicated that leptin might be produced by gonadotropes to regulate somatotropes. The changes overall in pituitary leptin certainly supported production by gonadotropes. Dual immunolabeling was therefore done on some of these experimental groups to test this hypothesis. The main question was whether or not somatotropes or gonadotropes, or both, contributed to the rise in the percentage of leptin-bearing cells during proestrus and pregnancy.

Cells co-labeled for leptin proteins and GH proteins were significantly increased on the morning of diestrus to 37.0 ± 4.0% of the population on the morning of proestrus. Further more, the 12 percentage point increment matched the overall increment in pituitary cells bearing leptin [93]. Because the overall percentage of pituitary cells with GH proteins did not change significantly, this may reflect the mobilization of somatotropes that are stimulated to express more leptin during diestrus. Thus, these cells appear to be significant contributors to the rising overall leptin during this period.

The comparative percentages of gonadotropes in the population suggest that these cells probably do not contribute to the rise in pituitary leptin. Gonadotropes with leptin proteins represented 6-8% of pituitary cells in cycling females. Similarly, in male rats, cells co-expressing leptin and GH were most abundant (26% of pituitary cells) and only 4.0% of pituitary cells co-expressed leptin and gonadotropins.

Significant plasticity in the cell types expressing leptin was seen in populations from pregnant rats, as leptin-bearing cells shifted from being predominantly somatotropes to a population that is predominantly gonadotropes [93]. Whereas there were no significant changes in the overall percentages of cells with GH proteins in pregnant or lactating rats, fewer GH cells co-expressed leptin proteins. At the same time, there was a significant rise in percentage of pituitary cells that co-expressed LH or FSH and leptin to 42.0 ± 1.0% or 36.0 ± 4.0% of pituitary cells, respectively. Cells from lactating females also had significantly more cells with LH and leptin (18.7 ± 0.4%) or FSH and leptin (17.0 ± 1.0%) than normal cycling rats [93].

Thus the proportion of leptin-bearing cells with GH declined from 60-70% in normal males or cycling females to less than 20% in pregnant or lactating females [93].

In contrast, the proportion of leptin-bearing cells with gonadotropin rose from 17% in cycling females to 60-70% in pregnant rats [93]. The analysis of dual labeling shows overlap in the percentages of leptin bearing cells with LH or FSH, which supports the hypothesis that leptin is expressed in part by bihormonal gonadotropes.

When, in situ hybridization was done to detect leptin mRNA in cells with GH or LH proteins [93], the data confirmed that from the immunolabeling. Most leptin-mRNA bearing cells in diestrous animals expressed GH and less than 10% expressed LH. In males, 73% of leptin-mRNA bearing cells express GH and 10% express LH. [93]

Thus, our data detecting leptin proteins or mRNA did not support the original hypothesis [99, 100], that leptin production was predominantly in gonadotropes in normal animals. On the other hand, the hypothesis was supported in pregnant and lactating females. There was a significant
decline in cells co-expressing GH and leptin mRNA. Thus, there is plasticity in the expression of leptin across cell types. This association between gonadotropins and leptin in pregnancy and lactation may correlate with the enhanced needs of the animal during these reproductive states. One might speculate that, if it can be secreted, gonadotrope leptin might provide an important continuing source of this hormone, as stores of fat are used to support the pregnancy or lactation. Selective knockout animals in which leptin is ablated from gonadotropes, would be needed to test this hypothesis.

**Effects of the Nutritional State**

The predominance of leptin production by normal somatotropes alerted us to the possibility that leptin may be regulated by different nutritional states, which would be consistent with the one of the somatotropes’ functions, to break down fat and thus optimize body composition. Therefore, the studies of leptin production by the pituitary were continued in male rats following a 24 h fast [117]. It was already known that significant changes in the adipocyte leptin mRNA expression can be seen as early as 8 hours [1, 122, 123], with losses in leptin mRNA of 50% [124] or 85-90% [12, 125] in 24 h. These studies led to tests of potential paracrine actions by pituitary leptin, so they will be described in more detail.

Food deprivation brought about changes in the expression of pituitary leptin. As illustrated by (Fig. 3) in reference [117], there was a significant 64% reduction in the overall percentages of pituitary cells with leptin mRNA from 33 ± 2 to 12 ± 1% of anterior pituitary cells (p<0.001) along with a 40% reduction in the integrated optical density of label (p=0.03). There was also a 22% reduction in the percentages of cells with leptin proteins, from 36 ± 2% to 29 ± 2% of AP cells (±SEM; p<0.029) [117].

The next objective of these studies was to learn which pituitary cell types were most affected by the food deprivation, specifically in their production of leptin. Fig. (4) in ref. [117] illustrates dual labeling for all pituitary hormones, which accounted for over 90% of cells with leptin proteins, with 61% of leptin-bearing cells co-expressing GH, 8-10% co-expressing LH, TSH, or ACTH and only 3% co-expressing prolactin. The remaining 10% could be folliculostellate cells, or monohormonal FSHβ cells, which were not detected. Food deprivation caused a significant loss in leptin protein expression by somatotropes or gonadotropes. In contrast, corticotropes maintained their expression of leptin at 30% of ACTH cells.

When we focused on the pituitary hormone bearing cells themselves to learn about the overall impact on their expression, we found that fasting caused declines in percentages of pituitary cells with GH, prolactin and LH [117]. The percentages of thyrotropes appeared unaffected by fasting. In contrast however, fasting increased percentages of corticotropes over 2X from 10 to 21% of AP cells. The overall increase in corticotropes resulted in a 1.94-fold increase in pituitary cells with leptin and ACTH, from 3.6% to 7% of AP cells (p=0.03). The analysis of leptin in fasted rat pituitary populations accounted for 86% of the leptin cell population, with 45% of leptin cells storing GH (reduced from 61%) and 24% storing ACTH (increased from 8%). Illustrations of these changes are found in photographs in Fig. (4), [117].

We focused then on gonadotropes and somatotropes for continued studies of leptin mRNA expression, because they were most severely affected by the food deprivation. Our analysis showed an 80% decline in cells that co-expressed leptin mRNA and GH proteins from 25 ± 3% to 5 ± 2% of AP cells (p<0.014) [117]. Similarly, fasting caused a significant decline in the co-expression of leptin mRNA in gonadotropes from 3 ± 0.5% of AP cells to 0.7 ± 0.02% of AP cells (p<0.029). The sum of the losses in gonadotropes and somatotropes accounted for the overall loss in leptin mRNA. Fig. (5) in reference [117] illustrates the dual labeling for leptin mRNA and GH in fed and fasted rat populations.

These studies have once more shown leptin plasticity in the anterior pituitary, only this time it was in response to metabolic changes as a result of fasting. The finding highlights the importance of leptin expression by somatotropes and brings out another set of leptin producers, the corticotropes. They will ultimately provide the basis for follow-up studies of the significance of each site. However, this report will focus on our ongoing studies of somatotropes and gonadotropes.

**REGULATION OF PITUITARY LEPTIN**

**Growth Hormone Releasing Hormone (GHRH)**

The first set of studies focused on GHRH as a regulator for pituitary leptin, primarily because most leptin producing cells are somatotropes [98]. Biotinylated GHRH was used to detect target cells with cytochemical (avidin peroxidase) techniques [126]. In diestrous animals, 24 ± 3% (values ± sd) of pituitary cells bound Bio-GHRH and also stored leptin. This co-expression involved 84 ± 10% (values ± sd) of Biotinylated GHRH target cells. Analysis of populations from estrous rats (in which leptin was found in only 20% of pituitary cells), showed that only 11 ± 1.7% of pituitary cells co-expressed Bio-GHRH binding sites and leptin. The patches of peripheral labeling could be on the surface, or in vesicles and early endosomes.

The next set of studies tested responses to GHRH. Estrous rats were used for these experiments to determine if GHRH would stimulate more leptin-bearing cells to levels like those seen in proestrus. Neither estradiol (100 pM) overnight nor 2 nM GHRH alone for 3 hours stimulated expression of more leptin-bearing cells. However, stimulation with both estrogen and GHRH increased expression of leptin to levels similar to those found in diestrous or proestrous animals (41 ± 9%; p=0.02). The changes in the leptin-bearing cells are illustrated in ref [98]. No GHRH stimulated secretion of leptin was noted in these experiments, however.

These studies thus showed an increase in leptin expression by somatotropes to levels like those from diestrous animals. This suggests that rising estrogen may increase GHRH receptivity and hence increase leptin expression. The lack of evidence for secretion of leptin could reflect an autocrine role for this cytokine.
Ghrelin

Ghrelin is a relatively new secretagogue for GH. It was discovered as a product of the stomach in 1999 [127], found in the secretory granules of X/A-enteroendocrine cells [128]. It was named ghrelin on the basis of the Proto-Indo-European word root ghre, which means “to grow.” Ghrelin was reported to be a potent secretagogue for growth hormone (GH) in humans [129] and rats [130]. In both species, ghrelin also stimulated the release of adrenocorticotropin (ACTH) and thyroid-stimulating hormone [129, 130]. Studies and reviews of ghrelin’s actions have proliferated, including those showing its orexigenic functions [128, 131-135]. Ghrelin is released 30 min after the stomach is empty, and signals hunger, stimulating orexigenic neuropeptides [131, 133, 136-140].

Because ghrelin is stimulatory for somatotropes, recent studies were initiated to determine if it also affected pituitary leptin. Fig. (1A) shows that 3 h in 1 or 10 nM ghrelin had no significant effects on the expression of pituitary leptin proteins, as measured by integrated optical density (IOD) of immunolabeling in dispersed pituitary cells. In contrast, ghrelin caused a significant reduction in the expression of pituitary leptin mRNA, as quantified by IOD of fields labeled by in situ hybridization. Counts of leptin mRNA-bearing cells (Fig. 1B) also showed a significant decline with 1 nM (p=0.018) or 10 nM (p=0.013) ghrelin (from 32.3 ± 0.3% to 18.3 ± 0.6% or 17 ± 16%, respectively). The response to ghrelin is illustrated by photographs in Fig. (1C) and (1D).

Dual labeling was then performed to determine if the loss in leptin mRNA was in GH cells. Fig. (2) is a graph showing the significant decline in overall percentages of anterior pituitary (AP) cells dual labeled for leptin and GH proteins (p<0.001). When the counts are expressed as a percentage of leptin bearing cells, 1 nM ghrelin resulted in a decline in the percentage of leptin mRNA-bearing cells that contained GH from 59 ± 6% to less than 4.7 ± 3% of leptin cells (p=0.001). Similarly, 1 nM ghrelin produced a decline in percentages of GH cells with leptin mRNA from 70 ± 3.5% to 18.6 ± 6% of GH cells (p<0.03). The apparent increase with 10 nM was not significantly different from values seen with 1 nM. The decline is illustrated in Fig. (3A) and (3B). In the vehicle control (Fig 3A), there are at least 4-5 dual labeled cells, however the field treated with ghrelin has only one (Fig. 3B). Also, note that the labeling for GH proteins has diminished with the ghrelin to pale amber (note circled cells).

These studies suggest that ghrelin may be a potent regulator of pituitary leptin mRNA, specifically in somatotropes. Ghrelin is an appetite stimulator and it is...
possible that the inhibition of leptin mRNA in somatotropes may reflect a feedback circuit that would prevent somatotrope leptin from contributing to the circulating pool and thus inhibiting appetite. This feedback loop could also stimulate GH, which is lipolytic and reduces leptin expression by adipocytes, thereby lowering leptin.

The role of ghrelin as a mediator of paracrine actions by pituitary leptin is less certain, however. One clue might...
come from reported actions of ghrelin on gonadotropes and luteinizing hormone secretion. Investigators have shown that ghrelin had inhibitory effects on LH and mixed effects on reproductive functions, depending on sex and physiological state [141-143]. Other workers showed reductions in LH pulse frequency in rats [144] or humans [145]. Recently, Iqbal et al. [146] reported that an i.c.v. infusion of ghrelin lowered plasma LH in sheep. Fig. (4) shows serum ghrelin results from the fasting experiments reported in the first section of this review. As expected, serum ghrelin is elevated during the fast, even if glucose water is provided. This ghrelin response to fasting would explain the lower LH and leptin reported by our study [117] and insure that the animal’s survival needs are met before reproductive needs. The higher ghrelin might thus be responsible for the mediation of the lower pituitary leptin. Furthermore, if pituitary (somatotrope) leptin is important for gonadotrope function through paracrine mechanisms, lowering somatotrope leptin might contribute to the lower LH or reduced pulses of LH [145].

Gonadotropin Releasing Hormone (GnRH)

Gonadotropes are regulated by gonadotropin releasing hormone (GnRH) neurons, which are scattered in the preoptic and anterior regions of the hypothalamus and extending to the arcuate nucleus [147-149]. GnRH is secreted in pulses and stimulates both LH and FSH synthesis and release [150-156]. Estrogen from the ovary exerts positive feedback to gonadotropes from diestrus to proestrus and increases the production of GnRH receptors [157] on

Fig. (4). Serum from the fasted groups of animals studied as reported in reference [117] was collected and assayed for ghrelin by EIA. A third group of animals were fasted and given water containing 10% glucose. In both groups of fasted animals, serum ghrelin was elevated significantly (star; p<0.001).

Fig. (5). Freshly dispersed pituitary cells from rats taken on the AM of proestrus were treated for 1 h with neuropeptide Y (NPY) and significant increases in labeling were detected following exposure to 100 pM NPY (p<0.001). The comparable values for 100 pM GnRH are shown in numbers in the lower left of the Figure.
gonadotropes. GnRH pulses are slower during diestrus, thereby favoring FSH synthesis, but faster during proestrus, favoring LH secretion [157-159].

Strong evidence for a stimulatory effect of leptin directly on gonadotropes comes from studies in which the fertility of leptin-deficient ob/ob mice was completely restored by exogenous leptin [24]. Leptin also restored LH secretion in fasted mice, rats, and monkeys [14-17, 20, 58, 64, 67, 68]. Leptin increased LH levels in ovariectomized, estrogen-primed rats [72, 73]. Antiserum to leptin suppressed LH secretion and disrupted the estrous cycles [160]. All of this information assumes that the source of leptin comes from adipocytes.

We reasoned that, if pituitary leptin plays a role in regulating gonadotropes, it might be regulated by GnRH. We realized that pituitary leptin expression by gonadotropes is relatively low except in lactating or pregnant animals. However, the increased expression of leptin seen during proestrus along with that in lactating and pregnant animals suggested that reproductive regulatory factors like GnRH and estrogen may be important in regulating leptin expression.

The first set of studies [93] detected binding sites for GnRH on pituitary cells that contain leptin with 1 nM biotinylated GnRH [161-164] and dual labeling. In diestrous rats, cells with GnRH receptors and leptin proteins are 11.5 ± 2.0% of all pituitary cells. This represented 30.0 ± 3.0% of leptin-bearing cells and 73.0 ± 3.0% of GnRH target cells. The GnRH labeling actually identifies more leptin in cells that are gonadotropes than does labeling for gonadotropins. This is explained further at the end of this section.

Estrogen is a well established modulator of GnRH receptors and our previous studies have shown that 100 pM increases the percentage of GnRH-target cells, when given overnight to diestrous rats [157]. Our studies showed that, whereas estrogen does increase the overall percentage of GnRH-target cells as in our previous studies [157], it does not significantly increase the number of leptin-bearing cells that bind GnRH, which remain at 13.25 ± 2.0% of AP cells [93].

The next phase of our study was designed to learn if estrogen and GnRH could increase leptin mRNA expression by gonadotropes labeled for LH or FSH proteins [93]. Three groups of cells from diestrous rats were treated with and without 100 pM estradiol overnight and then given vehicle or 1 nM GnRH for 1 h the next morning. They were then fixed and labeled for leptin mRNA followed by immunolabeling for LHβ or FSHβ. Neither estrogen nor GnRH alone stimulated more gonadotropes to express leptin mRNA. However, estrogen and GnRH together stimulated a
significant increase in percentage of pituitary cells that co-express LHβ and leptin mRNA from 7.0 ± 2.0% to 11.0 ± 3.0% (p<0.03) of AP cells and an even greater increase in the percentages of cells with leptin mRNA and FSHβ from 8.0 ± 3.0% to 15.0 ± 6.0% (p<0.02)[93]. These findings support the hypothesis that the increase in leptin expression by gonadotropes in pregnant and lactating animals may be brought about by higher estrogen and GnRH stimulation.

Because GnRH receptors are at a peak late in diestrus, extending to the morning of proestrus [157, 164] cells from proestrus AM female rats were studied to learn more about the specific effects of GnRH on leptin mRNA and protein expression [93]. There is a significant increase in the average density of labeling for leptin mRNA or proteins (IOD) after 1 h in 100 pM GnRH (p<0.001), which plateaus at 500 pM. There is a significant decrease in IOD of labeling for leptin proteins after 1 nM GnRH (p<0.03), when compared with that following 500 pM. The value for 1 nM GnRH is still higher than the IOD for the vehicle treated group (p=0.009)[93].

The average IOD for leptin mRNA in the vehicle control is about 5—fold lower than that for the proteins, which reflects the lower expression of mRNA on the AM of proestrus [93]. GnRH increased the density of label for leptin mRNA (the increase with 100 pM is significant by Student’s T test (p<0.001) and there is a further increase to reach a peak with 500 pM (p<0.008). The IOD following 1 nM is not different from that with 500 pM).

GnRH also stimulated secretion of leptin from cultures of pituitary cells taken from diestrous, proestrus or pregnant females. In Fig. 8 in reference [93], we compared basal and GnRH stimulated leptin secretion in these groups. Basal secretion is significantly higher when one compares media from pregnant rat AP cells with that from diestrous rats (p=0.012). GnRH- stimulated secretion is increased over basal in each of the groups (diestrous p=0.016; proestrus p<0.015 and pregnant p=0.01). The cells from pregnant rats show the highest responses to GnRH, when compared with all others. All of these data correlated well with the counts of leptin bearing cells and the findings that leptin is most abundant in gonadotropes in pregnant rats.

This series of studies brings forth more clues about the significance of pituitary leptin. Our studies of GHRH and estrogen indicate that together they increase leptin expression by somatotropes, however they do not stimulate leptin secretion. In contrast, GnRH is a potent secretagogue for leptin as it stimulates an increase in leptin mRNA and proteins. This suggests that GnRH may be one driving force in the plasticity of leptin expression by the pituitary cells.

As stated earlier in this section, it is noteworthy that counts of cells with leptin and GnRH-receptors detected twice as many cells with the potential for gonadotropic activity (defined by their GnRH binding) than are detected by labeling for leptin and gonadotropins. This apparent discrepancy is explained by the appearance, in diestrous and proestrus, of somatotropes, which express LH and FSH mRNAs and GnRH receptors [164, 165]. These “somatogonadotropes” represent 11-16% of the AP population during proestrus and may play a co-gonadotropic role in the support of the reproductive system [166]. The predominance of somatotropes in the leptin-bearing cell population suggests that the GnRH might be affecting leptin from the somatogonadotrope subtype.

Thus, our studies also show that estrogen can work with GnRH to increase the population of gonadotropes that express leptin [93]. In addition GnRH has stimulatory effects on expression of cellular leptin mRNA and proteins in proestrus rats, which express maximal numbers of GnRH receptors [157]. Furthermore, as we will see in a later section in this review, Neuropeptide Y also stimulates increased expression of leptin by gonadotropes.

Whereas there is a significant reduction in expression of leptin mRNA seen on the AM of proestrus, it is worthwhile to note that cells from proestrus females were still able to respond to GnRH by the production of more leptin transcripts and secretion [93]. Thus, the emerging view is of a subset of somatogonadotropes that increase leptin production during the periovulatory period, under the influence of GnRH. Estrogen’s role may be to enhance expression of the GnRH (and the GHRH) receptors. It also stimulates expression of NPY receptors in the proestrus animal. Estrogen may be acting on this converging population of somatogonadotropes. This hypothesis will be tested in future studies that knock out leptin selectively in somatotropes or gonadotropes with Cre-LoxP technology.

Neuropeptide Y

NPY was also chosen as a candidate regulator of pituitary leptin because of the high expression of leptin proteins in proestrus [93]. Neuropeptide Y (NPY) regulates gonadotropes just before the LH surge [167-172]. It is produced by neurons in the arcuate nucleus from which it stimulates appetite and feeding, in a complex circuitry that is regulated by circulating leptin [173-175]. Circulating leptin rises after feeding to inhibit NPY production and thus reduce appetite.

However, NPY secretion is also required for a full GnRH-mediated LH surge in rodents [167-172]. NPY knockout mice have blunted LH surges, which can be enhanced by exogenous NPY [171]. NPY Y1 receptors, stimulated by estrogens, are believed to mediate its actions in the pituitary [176]. To regulate gonadotropes, NPY requires high amplitude GnRH pulses [66, 167, 169, 170], estrogen [176] or progesterone [177]. All of these conditions are seen on the afternoon of proestrus, just before the LH surge. Therefore, our studies of NPY stimulation focused on proestrus rats. Cells from these rats were stimulated for 1 h with 0–1000 pM NPY. Some of the pituitary cells were used for immunocytochemistry and in situ hybridization for leptin (followed by dual labeling for leptin and gonadotropins). Analysis showed that 0.1 nM NPY stimulated a 1.9-fold increase in the density of label for leptin mRNA/cell (Fig. 5). The effects of GnRH and NPY were not additive in any of these experiments (data not shown).

When we expanded the studies to include male pituitary cells, Fig. 6A shows that 3 h in 1 nM NPY also stimulated secretion of leptin. Fig. 6B also shows that NPY stimulated a significant increase in pituitary leptin proteins detected by EIA. The data are expressed as pg leptin/ml in the graph,
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however we also include values expressed as per milligram protein near the graph bars.

Gonadotropes are believed to be the major expression site for NPY receptors. Therefore, single and dual labeling for leptin mRNA and LH proteins was done in cells from male rat pituitaries after NPY stimulation to learn more about the effects of NPY on expression of leptin. Counts of cells bearing leptin mRNA or proteins and LH proteins showed NPY stimulation of all three products (Fig. 7). There was a 25% increase in the percentages of leptin-bearing cells along with impressive increases in percentages of cells with LH proteins or in percentages of pituitary cells dual labeled for leptin mRNA and LH after stimulation by NPY. Collectively these data suggest that NPY may be a potent driver in the regulation of pituitary leptin, particularly that in gonadotropes.

Thus, to summarize, this section of the review identified three hormones that increased expression of pituitary leptin: GHRH, GnRH and NPY. The actions of GHRH had to be potentiated by estrogen, however. Ghrelin, which normally stimulates somatotropes, decreased expression of leptin from this group of cells. Two of the hormones, GnRH and NPY, stimulated secretion of pituitary leptin and increased expression by gonadotropes. Collectively, these data show that pituitary leptin can be regulated, mainly by hormones that regulate somatotropes and the reproductive system. Furthermore, in the studies of leptin secretion, there was an excellent correlation between the abundance of leptin protein-bearining cells in a pituitary cell population and their secretory activity [93].

With respect to secretory pathways inside the cells, it is worthwhile noting that Vidal et al. [101] detected leptin co-expression with pituitary hormones at the electron microscopic level and their photographs depict labeling for leptin in the same granules that store LH or GH. This suggests that the leptin secretory cycle could be similar to that of gonadotropins or GH. Collectively, our experiments with GnRH and the morphological data from Vidal et al [101] support regulatory pathways for leptin that are similar to those that regulate pituitary hormones.

PITUITARY LEPTIN AND PARACRINE INTERACTIONS

Having established regulators for leptin secretion, the final section of this review will describe studies that tested a role for pituitary leptin as a paracrine signaling hormone. However, first, we will present studies that provide evidence for direct interactions of leptin on pituitary gonadotropes and somatotropes.

In earlier sections of this review, the importance of leptin to pituitary gonadotropes and somatotropes was brought out by the reduced numbers of these cell types in animals or humans that are leptin deficient. However, the exact cause of this reduction is controversial. Are the low numbers of somatotropes or gonadotropes caused by the lack of direct leptin stimulation? Alternatively, one may also argue indirect causes from the metabolic disease and/or the hypogonadal condition of these mice. Steroid stimulation of GH cells was needed for their maintenance [106, 119, 178] and GnRH stimulation was needed to promote gonadotrope development. Thus, there is no question that leptin works indirectly through the hypothalamic circuits to affect gonadotropes and somatotropes [25, 50, 58, 61-66]. Indeed, as stated earlier in this review, strong evidence for a stimulatory effect of leptin on gonadotropes comes from studies in which the fertility of leptin-deficient ob/ob mice was completely restored by exogenous leptin [24]. Leptin also restored LH secretion in fasted mice, rats, and monkeys [14-17, 20, 58, 64, 67, 68], GH pulses in fasted rats [68, 76, 77].

Fig. (7). Data from cells from male rats, which were dual labeled for leptin mRNA and LH proteins to detect any changes in co-expression after 1 h in 10 nM NPY. There was an overall increase in the percentages of cells with leptin mRNA (p<0.001) and an increase in percentages of cells with LH proteins (P=0.019). Dual labeling showed a significant increase in the percentages of anterior pituitary cells dual-labeled for leptin mRNA and LH (P=0.006). Star=significant difference.
78], and GH and GHRH receptor expression in leptin-deficient ob/ob mice [80]. Leptin increased LH levels in ovariectomized, estrogen-primed rats [72, 73]. Antiserum to leptin suppressed LH secretion and disrupted the estrous cycles [160].

One of the above in vivo studies highlights the pituitary as an important site of LEPR for the regulation of somatotropes. Luque et al. [80] reported that leptin restored GH secretion in ob/ob mice that were infected with exogenous leptin for 7 days. In addition, leptin caused an increase in GHRH receptors. Interestingly, no changes were noted in hypothalamic GHRH levels themselves, which suggested that GHRH did not mediate the restoration. In addition, several in vitro studies showed that leptin directly stimulated LH secretion [70-74] and may stimulate or inhibit GH [23, 68, 76, 77, 79, 80, 88, 114, 179-184], depending on experimental conditions.

In light of these findings, we hypothesized that, if it was a paracrine regulator, pituitary leptin might itself be regulated by the nutrient status. We thus developed an animal model of food deprivation to test this hypothesis. The model was developed based on information about changes in adipocyte leptin mRNA expression [1, 122, 123] after food deprivation in which losses in leptin mRNA of 50% [124] or 85-90% [12, 125] were seen in 24 h. As shown by (Fig. 4) in reference [117], food deprivation reduced leptin expression by somatotropes and gonadotropes significantly. However, that by corticotropes was increased, largely due to the overall increase in number of corticotropes caused by the stress of fasting [117].

We then modified this model, to determine if pituitary leptin was responsive to an elevation of serum glucose [117]. We added a third group of animals, which were fasted, but had been given 10% glucose water during the period of the fast. This concentration promoted recovery in serum glucose to levels seen in the control animals [117]. We tested serum leptin as well as leptin expression by pituitary cells. Fig. (6) in reference [117] shows the loss in serum leptin in the fasted animals along with a partial recovery in serum leptin from animals that were fasted, but given glucose water during the food deprivation period. The QRT-PCR assays of pituitary leptin mRNA also showed a similar pattern[117]. only the animals given glucose water completely recovered leptin mRNA expression. This recovery in expression of pituitary leptin mRNA was confirmed by in situ hybridization; the percentages of leptin-bearing cells were reduced in the fasted animals, but recovered in animals given glucose water[117]. Similarly, parallel changes in expression of LH mRNA-bearing cells were seen, with a loss in fasted animals and a recovery in fasted animals given glucose water.

Recovery was seen in LH protein expression as well. Dual immunolabeling detected GH, LH or Leptin in fed and the two groups of fasted animals and the graph is shown in Fig. (7) in reference [117]. There was a complete recovery in the percentages of GH and LH cells and a partial recovery in the percentages of leptin protein bearing cells in the fasted animals given glucose water.

At this point, the evidence for leptin as a driving force behind the recovery in somatotropes and gonadotropes was circumstantial. All we had shown was a parallel recovery in pituitary leptin (partial) and LH and GH. This might have occurred simply because of the partial recovery in serum leptin seen in the glucose treated animals [117].

Therefore, the next set of experiments tested exogenous leptin, to learn if it, alone could effect a recovery in gonadotropes and somatotropes in freshly dispersed cells from fasted animals, which had reduced expression of LH, GH and leptin. After treatment for 1 hour with vehicle, the cell cultures showed low percentages of LH, GH and leptin-bearing cells. However, as little as 10 pg/ml leptin for 1 h stimulated a recovery in numbers of somatotropes and gonadotropes. There was also a partial recovery in numbers of leptin-bearing cells. Photographs of the cell populations are illustrated in Fig. (7), [117].

The overall objective of this last set of studies was to determine the physiological significance of pituitary leptin. This has been challenging to prove in vivo because of the abundance of circulating leptin from adipocytes, and the absence of both leptin and leptin receptors in all pituitary cell types. Animal models with leptin or leptin receptor deficiencies have other confounding variables, like hyperglycemia and diabetes, with inherent independent effects on somatotropes and gonadotropes. These models are deficient in gonadotropes and somatotropes and do not respond normally to tests like fasting [185]. We recognize that, ultimately, selective knockouts in the pituitary would be needed to fully test pituitary leptin’s significance.

The parallel restoration of leptin and LH mRNA, or leptin, LH and GH proteins in the glucose treated rats provided circumstantial evidence that further supports an association between these gene products in the pituitary. Food deprivation (signaled by a drop in nutrients, like glucose) is known to result in the attenuation of LH or GH pulses and it is thought that a drop in serum leptin signals some of this. However, in vivo studies have shown that the LH pulses can be restored within hours of giving nutrition, before adipocytes have recovered. Thus, the adipocyte-derived leptin can be too sluggish as a neuroendocrine modulator. LH pulses can also be restored, in vivo, in fasted animals by exogenous leptin as long as glucose can be utilized [14, 15, 17]. Thus, workers have theorized that leptin reports nutritional information, like glucose levels, to the hypothalamic and pituitary cells, permitting reproduction and normal GH or LH cell pulses if nutrition is adequate. Because of the slow nature of that from adipocytes, we suggest that pituitary leptin might serve as a rapid relay for nutritional information.

Our dual labeling evidence showed that the reduction in pituitary leptin following food deprivation is mostly in somatotropes. It is important to recall that ghrelin was stimulated by fasting (Fig. 4) in these studies and ghrelin inhibits pituitary leptin specifically in somatotropes (Fig. 2 and 3). Thus, a rise in serum ghrelin could be an important regulatory mechanism behind the lowering of somatotrope leptin.

It is interesting to note that leptin was not reduced in the expanding population of corticotropes. This suggests that the
regulation of the leptin gene is not uniform across all pituitary cell types. Furthermore, assuming the new corticotropes could secrete their leptin in this environment, it was clearly not sufficient to correct for the losses in somatotropes and gonadotropes. Perhaps the actions of ghrelin on somatotropes were critical in reducing this source of leptin as they were selective for somatotropes.

One of the clues to paracrine regulation is the sensitivity of the response to a candidate factor. In other words, target cells may respond to very low concentrations of a candidate factor in physiologic tests. This sensitivity develops because the candidate factor is not diluted by secretion and transport through the entire blood stream. A local vascular, or extracellular route from cell to cell might allow for effective regulation by very small amounts of a paracrine factor.

Thus, it is significant that, in our study of fasted rats [117], the losses in somatotropes or gonadotropes could be corrected rapidly by a 1 h incubation in as little as 1-10 pg/ml exogenous leptin. Also, these pg/ml levels match those corrected by normal rat pituitary cultures, so the responses match the availability of secreted pituitary leptin.

The recovery in gonadotropes and somatotropes confirms in vivo studies in which exogenous leptin restored LH or GH pulses [20, 58, 67, 68, 76, 78]. The assumption has been made, however that leptin acts mainly on the hypothalamus. In agreement with Luque et al. [80], our current studies suggest that leptin may also act directly on pituitary target cells and that restoration is not limited to actions on the hypothalamus.

Exogenous leptin was not able to fully restore the expression of pituitary leptin. This points to independent regulators for pituitary leptin and our studies show that they include estrogen and GHRH [98] and GnRH [93], all of which would be reduced in a fasted state [20, 58, 67, 68, 76, 78, 186, 187]. NPY would not be reduced, however receptors for this neuropeptide would likely be reduced because of the requirement for estrogen, at least in female rats.

To summarize, we suggest from this evidence that pituitary leptin is needed to maintain LH and GH cell functions. Most of the leptin comes from somatotropes, especially in the male. The findings support the hypothesis that a decline in somatotrope leptin below certain threshold levels might signal nutritional distress and cause parallel reductions in LH and GH cell functions. Endogenous pituitary leptin could thus serve as either a paracrine, or an autocrine regulator.

In our fasted rat model, once serum glucose became normal, there were parallel increases in pituitary leptin, GH and LH. This suggests that pituitary leptin may be sensitive to serum glucose. Thus, pituitary leptin might serve as a glucostat, which would provide one mechanism by which it senses the nutritional state. Recent landmark studies [188, 189] reported glucokinease expression in a subset of pituitary cells including thyrotropes and gonadotropes, which would allow them to monitor the changes in serum glucose. Our studies suggest that pituitary leptin may also be affected by this sensor. A drop in glucose might signal nutritional distress and cause a drop in pituitary leptin. This reduction would, in turn, report nutritional distress to somatotropes and gonadotropes, slowing down their functions and promoting survival activities (hunting for food, and fat conservation) rather than lipolysis (induced by GH) and reproduction (mediated by gonadotropins).

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