Ghrelin and reproduction: a novel signal linking energy status and fertility?

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Abstract

Ghrelin was originally identified in 1999 as the endogenous ligand of the growth hormone (GH) secretagogue receptor (GHS-R). Since then, an ever growing number of publications have reported the potential involvement of this molecule in the regulation of a large array of endocrine and non-endocrine functions, including the control of GH secretion and several other neuroendocrine axes as well as food intake and energy balance. On the basis of its proposed role as indicator of energy insufficiency and the proven reproductive effects of other regulators of energy homeostasis and growth (such as the adipocyte-derived hormone leptin), it is tempting to hypothesize that ghrelin might play a role in the control of reproductive function and fertility. Indeed, although evidences in this area are still fragmentary, we review herein data from different research groups, which have recently substantiated the reproductive facets of this newly identified hormone. Thus, expression of ghrelin has been demonstrated in human and rodent placenta, and ghrelin has been reported to inhibit early embryo development. In addition, ghrelin was shown to suppress luteinizing hormone (LH) secretion in vivo, and to decrease LH responsiveness to LH-releasing hormone (LHRH) in vitro. Moreover, ghrelin was able to inhibit stimulated testicular testosterone secretion, whereas androgens have been proven independent modulators of circulating ghrelin levels. In this context, our group has recently provided extensive evidence for the expression of ghrelin and its putative receptor, the type 1a GHS-R, in rat and human gonads. Testicular expression of ghrelin was highly selective for mature Leydig cells and under the hormonal control of pituitary LH, while in the ovary, expression of ghrelin was demonstrated in steroidogenically active luteal and interstitial hilus cells. Likewise, expression of GHS-R type 1a was demonstrated in Sertoli and Leydig cells of the testis and follicular, luteal and interstitial hilus cells in the ovary. In summary, the data so far available indicate that ghrelin may operate at different levels of the reproductive system, including the testis and the ovary, which are potential targets for systemic ghrelin actions. In addition, ghrelin is produced locally within the human and rodent gonads, where the presence of both components (ligand and receptor) of ghrelin signaling system is highly suggestive of a conserved regulatory role for this newly discovered molecule in the regulation of mammalian gonadal function. Overall, it is proposed that ghrelin may cooperate with other regulatory signals, such as leptin, in the integrated control of energy balance and reproduction.

Keywords: Ghrelin, GH-secretagogue receptor (GHS-R); Leydig cell; Seminiferous; Tubules; Testis; Ovary; Rat; Human

1. Introduction

Identification of ghrelin, in late 1999, as the natural ligand of the growth hormone (GH) secretagogue receptor (GHS-R) was the end-point of a long search for the endogenous counterpart of a large family of peptidyl and non-peptidyl synthetic compounds, globally termed GH-secretagogues (GHS), with ability to elicit GH release in vivo and in vitro in a wide spectrum of species, including humans (Casanueva and Dieguez, 1999a; Kojima et al., 1999, 2001). The functional ghrelin peptide results from the cleavage of a precursor form, the prepro-ghrelin, which is composed of 117 amino acids. In the human and rat, the mature ghrelin peptide consists of 28 amino acids, with divergence in two residues only (Kojima et
tions of ghrelin as well as of synthetic GHSs have been pre-
ligand binding and signal transduction capacity (Howard et
domains 6 and 7, and it is apparently devoid of high-affinity
is the functionally active, signal transducing form of the re-
1b (Howard et al., 1996; McKee et al., 1997). The GHS-R1a
alternative splicing of a single gene, have been described: the
full-length type 1a receptor and the truncated GHS type
neuroendocrine tissues such as the pituitary and hypothala-
nic cell system expressing the GHS-R. Thus, ghrelin, as
endogenous counterpart of synthetic GHSs, was first demon-
strated to potently elicit GH secretion both in vivo and in vitro.
This GH secretagogue action is conducted both at the pitu-
tory and the hypothalamus, where ghrelin has been proven
to regulate GH-releasing hormone and somatostatin systems
(Sesane et al., 2003). Moreover, ghrelin may serve additional
central neuroendocrine functions, such as modulation of lac
totropic, corticotropic and gonadotrophic axes (Arvat et al.,
2001; Furuta et al., 2001). Shortly after its cloning, ghrelin
was also identified as orexigenic signal acting at the hypotha-
lamus, through regulation of several food-intake controlling
neuropeptides such as neuropeptide Y (NPY), Agouti-related
protein (AgRP) and orexin (Tschop et al., 2000; Nakazato et
al., 2001; Wren et al., 2000; Gualillo et al., 2003; Toshinai
et al., 2003; Chen et al., 2004). To note, gastric expression
of ghrelin is enhanced after food deprivation, and ghrelin has
been proposed as molecular signal for energy insufficiency
(Zigman and Elmquist, 2003). Indeed, the involvement of
ghrelin in the long-term control of body weight in humans
has been recently proposed (Cummings et al., 2002).
In addition to the central actions listed above, growing evi-
dence indicates that ghrelin might be involved also in the reg-
ulation of quite diverse peripheral functions. These include
regulation of gastric motility and acid secretion, different ef-
effects upon the cardiovascular system, modulation of glucose
metabolism and pancreatic insulin secretion as well as con-
trol of cell proliferation in several cancer cell lines. These
have been recently revised in detail elsewhere (De Ambrogi
et al., 2003; Gualillo et al., 2003; Korbonits et al., 2004; van
der Lely et al., 2004).

2. Ghrelin: central and peripheral actions

In recent years, a large body of evidence has demonstrated
that the biological actions of ghrelin are much wider than
those originally anticipated. Indeed, a striking feature of ghe-lin is its widespread pattern of expression (Gualillo et al.,
2003; Korbonits et al., 2004; van der Lely et al., 2004). No-\tably, ghrelin was originally identified in the stomach, which
is by far the major source of circulating ghrelin, account-
ing for at least two-thirds of its plasma levels (Kojima et al.,
2001; Gualillo et al., 2003). In addition, ghrelin expression
has been also demonstrated in an array of tissues and cell
types including small intestine, pancreas, lymphocytes, plas-
centa, kidney, lung, pituitary and brain (Gualillo et al., 2003;
Caminos et al., 2003a; Korbonits et al., 2004). In this context,
our group has recently provided pioneering evidence for the
expression of ghrelin, and its functional receptor, in rat and
human gonads (as reviewed in detail herein). Overall, such
a ubiquitous pattern of expression strongly suggests that in
addition to systemic actions of the gut-derived peptide, lo-

cally produced ghrelin is provided with paracrine/autocrine
regulatory effects in different tissues (Korbonits et al., 2004;
vander Lely et al., 2004). This seems to be the case in the
gonads.

As indicated above, identification of ghrelin was achieved
by means of an orphan receptor strategy using a heterolo-
gous cell system expressing the GHS-R. Thus, ghrelin, as
endogenous counterpart of synthetic GHSs, was first demon-
strated to potently elicit GH secretion both in vivo and in vitro.
This GH secretagogue action is conducted both at the pitu-
tory and the hypothalamus, where ghrelin has been proven
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der Lely et al., 2004).

3. Neuroendocrine integrators: the case of leptin

Recent advances in our knowledge on the neuroendocrine
networks controlling different pivotal body functions have
help to identify close connections between the systems gov-
erning somatic growth, energy balance and reproduction. In-
deed, such a link had been long anticipated on the basis of
the well-known need of sufficient energy stores for proper
pubertal development, growth and fertility (Casanueva and
Dieguez, 1999b). However, only recently, identification of
the molecular signals responsible for this integrated control
has been initiated.
In this context, leptin is probably the most illustrative paradigm of neuroendocrine integrator. Leptin was originally identified in 1994 as the satiety factor, primarily produced in the white adipose tissue, which signals the amount of body energy stores to the hypothalamic centers controlling food intake, thus serving an essential role in body weight homeostasis (Casanueva and Dieguez, 1999b; Ahima et al., 2000). However, soon after its identification, it became evident that in addition to its involvement in energy balance, leptin functions also as a pleotropic mediator in a wide range of neuroendocrine functions, including growth and reproductive axes (Wauters and Considine, 2000). It is likely that other neuroendocrine integrators cooperate with leptin in such a coordinated regulatory function.

Evidence from experimental and epidemiological studies clearly indicates that leptin is a pivotal regulator of reproductive function, especially in females where threshold leptin levels are absolutely essential for normal pubertal development and fertility. Likewise, disturbance of reproductive function is observed in male models of leptin insufficiency (Tena-Sempere and Barreiro, 2002). Interestingly, the mechanisms whereby leptin regulates fertility are multifaceted and likely involve actions at different levels of the hypothalamic–pituitary–gonadal axis (Tena-Sempere and Barreiro, 2002). Undoubtedly, the hypothalamus, the key site for the central control of food intake and neuroendocrine function, is the primary target for the reproductive actions of leptin, and stimulatory effects of this peptide on hypothalamic LHRH release have been reported. These likely account for the net stimulatory action of leptin upon the reproductive axis. However, direct effects of leptin at the pituitary and gonads have also been demonstrated. On the latter, expression for the net stimulatory action of leptin upon the reproductive axis is observed in male models of leptin insufficiency (Tena-Sempere and Barreiro, 2002). Nonetheless, ghrelin has been also involved in the control of gonadal function observed in the presence of highly elevated leptin, and stimulatory effects of ghrelin upon the reproductive system, analysis of the potential reproductive actions of ghrelin has received little attention. Yet, although fragmentary, several lines of evidence suggest that, indeed, ghrelin might participate in the control of gonadal axis. This phenomenon likely includes both systemic effects at different levels of the reproductive system, as well as direct gonadal actions of locally produced ghrelin. These are revised in detail in the following sections.

4. Ghrelin and reproduction

As stated above, it is highly probable that additional neuroendocrine integrators may cooperate with leptin in the joint control of energy balance and reproduction. The data so far available make it tempting to speculate that ghrelin might be a candidate for such a physiological function. Indeed, leptin and ghrelin have been implicated, with opposite roles, in body weight homeostasis: leptin, as satiety factor that signals for energy abundance, and ghrelin, as orexigenic factor that signals for energy insufficiency (Zigman and Elmquist, 2003). However, in contrast to the well-documented effects of leptin upon the reproductive system, analysis of the potential reproductive actions of ghrelin has received little attention. Yet, although fragmentary, several lines of evidence suggest that, indeed, ghrelin might participate in the control of gonadal axis. This phenomenon likely includes both systemic effects at different levels of the reproductive system, as well as direct gonadal actions of locally produced ghrelin. These are revised in detail in the following sections.

4.1. Systemic actions of ghrelin upon the reproductive axis

Growing evidence indicates that ghrelin is expressed and/or operates at different levels of the gonadotropic axis as well as in other reproductive tissues. However, the data so far available are still limited, and the characterization of the mechanisms of action of ghrelin and its potential interplay with other known regulators of the reproductive system remains largely unexplored. Concerning gonadotropin secretion, initial data evidenced that central administration of ghrelin suppressed pulsatile LH secretion in ovariectomized female rats (Furuta et al., 2001). In addition, we have recently demonstrated that ghrelin is able to inhibit LH secretion in vivo in prepubertal male rats as well as in gonadectomized males and females, whereas FSH secretion was not affected (Fernandez-Fernandez et al., 2004). Interestingly, ghrelin directly stimulated the secretion of both gonadotropins at the pituitary and differentially affected the response to LHRH; the LH response was inhibited while the FSH response was enhanced. The latter might contribute to the dissociation of both gonadotropins that is observed in different physiological and experimental situations.

In addition, ghrelin has been also involved in the control of prolactin secretion, and stimulatory effects of ghrelin upon serum prolactin levels have been demonstrated in adult humans (Arvat et al., 2001). In the rat, however, ghrelin has been recently reported to inhibit prolactin secretion in prepubertal male and female rats, arguing mainly at the hypothalamus (Tena-Sempere et al., 2004). The reasons for such an apparent discrepancy between rat and human species remain to be solved, but one tempting possibility is that the inhibitory actions of ghrelin upon prolactin secretion are restricted to the period of (pre)pubertal maturation, as chronic administration of ghrelin to adult cyclic rats enhanced serum prolactin levels (Pinilla and Tena-Sempere, unpublished observation). The functional relevance of ghrelin-induced inhibition of prolactin secretion around puberty in the rat, and whether a similar phenomenon operates also in peripubertal humans, is yet to be established.

Expression of ghrelin and GHS-R genes has been described in non-pregnant and decidualized endometrium, and ghrelin has been involved as paracrine/autocrine regulator of decidualization of human endometrial stromal cells, and tentatively, in the cross-talk between endometrium and embryo during implantation (Tanaka et al., 2003). Notably, ghrelin
levels in uterine fluid dramatically increased during fasting in mice, and ghrelin has been recently reported to inhibit the development of mouse preimplantation embryos in vitro (Kawamura et al., 2003). In good agreement, we have recently observed that chronic ghrelin treatment during the first half of pregnancy in the rat induced a significant reduction in the litter size (our unpublished results). Overall, it is tempting to propose that ghrelin may operate as signal for energy insufficiency during early stages of gestation, acting as inhibitory factor in early embryo development in order to avoid the excessive metabolic drain linked to pregnancy and lactation in situations of malnutrition (Kawamura et al., 2003). In addition, ghrelin has been detected in human and rat placenta (Gualillo et al., 2001), and ghrelin has been demonstrated in human fetal circulation (Cortelazzi et al., 2003). The role of placental and fetal ghrelin in the regulation of gestational growth and metabolism remains to be fully elucidated.

4.2. Ghrelin system in the testis

Given the documented actions of leptin upon the male and female gonads (Tena-Sempere and Barreiro, 2002), direct gonadal effects of ghrelin appeared feasible. Indeed, besides data demonstrating the inhibitory effect of ghrelin on pulsatile LH secretion (Furuta et al., 2001), initial analyses identified a testis-specific ghrelin gene derived transcript (GGDT) in the mouse (Tanaka et al., 2001), and expression of ghrelin gene in human testis was preliminarily reported (Gnanapavan et al., 2002).

In parallel, our research group undertook the characterization of the pattern of expression and potential biological actions of ghrelin in the male gonad. Thus, expression of ghrelin in rat and human testis was demonstrated through molecular and immunological approaches (Tena-Sempere et al., 2002; Gaytan et al., 2004). In the rat testis, ghrelin expression was selectively detected in Leydig cells at advanced stages of maturation, regardless of their fetal or adult origin, as demonstrated during post-natal development and after selective elimination of mature Leydig cells by administration of the cytotoxic compound ethylene dimethane sulfonate (Tena-Sempere et al., 2002; Barreiro et al., 2002a). Similarly, immunohistochemical analyses evidenced that ghrelin is strongly expressed in interstitial mature Leydig cells of the human testis. However, a specific feature of testicular expression of ghrelin in the human is the presence of this peptide, albeit at low levels, in Sertoli cells (Gaytan et al., 2004). Analyses of its regulation by hormonal signals revealed that ghrelin expression in the testis is, at least partially, under the control of pituitary LH. This is in good agreement with the fact that testicular LH/LH receptors are expressed in Leydig cells (Tena-Sempere and Huhtaniemi, 2003).

Concerning the putative ghrelin receptor, testicular expression of GHS-R1a has been demonstrated in the rat and human (Tena-Sempere et al., 2002; Barreiro et al., 2003; Gaytan et al., 2004). Intriguingly, in the rat testis, expression of GHS-R gene was detected at rather constant relative levels throughout post-natal development. In contrast, isoform-specific GHS-R1a mRNA was undetectable at prepubertal stages of post-natal development and it sharply increased thereafter, suggesting that during pubertal development a shift in the pattern of splicing of the GHS-R gene takes place in rat testis that favors expression of the biologically active type 1a form of the receptor. In good agreement, GHS-R1a mRNA was also detected in adult human testis. Location analyses in the rat and human by means of immunohistochemistry and/or in situ hybridization revealed a scattered pattern of distribution of GHS-R1a, with specific expression in somatic Sertoli and Leydig cells, as well as in germ cells, mainly in pachytenic spermatocytes, in the human (Barreiro et al., 2003; Gaytan et al., 2004). As was the case for the ligand, testicular expression of GHS-R gene appeared under hormonal regulation, as GHS-R1a mRNA levels were stimulated by ghrelin and pituitary FSH. Thus, testsis sensitivity to ghrelin is likely regulated by homologous and heterologous signals, which is highly suggestive of a finely tuned, direct action of ghrelin in the control of testicular function.

Assessment of the biological actions of ghrelin in the testis was undertaken in our laboratory using an in vitro setting. In this model, ghrelin was able to significantly inhibit, in a dose-dependent manner, stimulated testosterone secretion. The fact that ghrelin equally decreased human chorionic gonadotropin (hCG) and cAMP-induced testosterone secretion indicates that this inhibitory action must take place in a step beyond cAMP formation. The inhibitory effect of ghrelin upon testosterone secretion was associated with a significant decrease in hCG-stimulated levels of the mRNAs encoding 17β-hydroxysteroid dehydrogenase (HSD) and testis-specific 17β-HSD type III (Tena-Sempere et al., 2002). Of interest, the ability of ghrelin to inhibit human CG-stimulated testosterone secretion in vitro might be in apparent contrast with the stimulatory role of pituitary LH upon testicular ghrelin expression, as LH is the major elictor of testosterone production (Tena-Sempere and Huhtaniemi, 2003). A tempting possibility, however, is that ghrelin might operate as a local regulator in the fine-tuning of the steroidogenic actions of LH, as ghrelin would participate in the auto-limitation of testicular testosterone response to gonadotropic stimulation (Barreiro et al., 2002a).

In addition to its steroidogenic effects, ghrelin might also directly regulate seminiferous tubule functions, as expression of GHS-R1a was demonstrated in the tubular compartment of the tests. In this sense, our initial analyses indicate that ghrelin might inhibit expression of the gene encoding stem cell factor (SCF) (Barreiro and Tena-Sempere, submitted). Notably, SCF is a Sertoli cell product that has been pointed out as the major paracrine stimulator of germ cell development, acting as a survival factor for spermatogonia, spermatocytes and spermatids in the adult rat seminiferous epithelium (Hakovirta et al., 1999; Yan et al., 2000b). In addition, testic-
ular SCF has been involved in Leydig cell development and survival (Yan et al., 2000a). Thus, the action of ghrelin upon tubular SCF mRNA expression may have implications both in Leydig cell proliferation and the control of spermatogenesis. Moreover, besides implications in testicular function, our current data open up the possibility that ghrelin may participate in additional biological systems through modulation of SCF gene expression.

4.3. Ghrelin system in the ovary

As was the case for the testis, we have recently provided evidence for the expression of ghrelin and its functional receptor in the ovary. In the rat, expression of ghrelin gene was demonstrated in the ovary throughout the estrous cycle, with the lowest levels in proestrus and peak expression values in diestrous 1; i.e. during the luteal phase of the cycle. In good agreement, ghrelin immunoreactivity was predominantly located in the luteal compartment of the ovary; with intense immunostaining being detected in steroidogenic cells from corpus luteum of the current cycle as well as in regressing corpora lutea (Caminos et al., 2003b). Likewise, strong ghrelin immunostaining was observed in young and mature corpora lutea of the human ovary, whereas it was not detected in ovarian follicles at any developmental stage (Gaytan et al., 2003). Worthy to note, the profile of ghrelin expression in the human corpus luteum is roughly coincident with its peak in functional activity within the ovarian cycle, suggesting a potential regulatory role of locally produced ghrelin in the control of corpus luteum function. In addition, ghrelin immunoreactivity was also demonstrated in interstitial hilus cells of the human ovary. This cell type is steroidogenically active, with ability to secrete testosterone in response to LH stimulation, and shows distinctive morphological characteristics (e.g. presence of crystals of Reinke) identical to those of differentiated testicular Leydig, i.e. the source of ghrelin expression within the testis (Gaytan et al., 2003).

Concerning the functional receptor, expression of GHS-R1a protein in the human ovary showed a wide pattern of tissue distribution, with detectable expression in oocytes as well as somatic follicular cells, luteal cells from young, mature, old and regressing corpora lutea, and to a lower extent, in interstitial hilus cells (Gaytan et al., 2003). To note, expression of GHS-R1a peptide in somatic cells from ovarian follicles roughly paralleled follicular development. This suggests a potential relationship between GHS-R expression and follicle growth, which remains to be proven. Overall, the simultaneous expression of ghrelin and its cognate receptor in several ovarian compartments is compatible with a potential action of locally produced ghrelin in the auto/paracrine regulation of human ovarian function. In addition, the wide pattern of GHS-R1a expression makes it possible that circulating ghrelin may operate upon specific cell targets within the cyclic ovary, as has been demonstrated for other factors with key roles in body weight homeostasis, such as leptin.

5. Ghrelin and reproduction: future perspectives and conclusions

Identification of ghrelin has been a major breakthrough in the field of neuroendocrinology. From a methodological point of view, its discovery illustrates a clear example of the usefulness of ‘reverse’ pharmacology and orphan receptor strategies in the search for potentially relevant endogenous ligands. From a physiological standpoint, cloning of ghrelin has forced us to revisit the accepted models of central control of GH secretion. In addition, ghrelin has turned out to be a rather ubiquitous molecule involved in a wide range of endocrine and non-endocrine actions. Notably, these include regulation of food intake and energy balance. The latter has drawn considerable attention, given that obesity (as disturbance of energy balance) is a highly prevalent disease worldwide with elevated rates of co-morbidity and mortality. In fact, it is now established that ghrelin operates as an important indicator of energy insufficiency (Zigman and Elmquist, 2003), which likely interplay with other peripheral signals (such as leptin) in the control of common central circuits primarily involved in the long-term control of body weight homeostasis.

Compelling evidence indicates that common regulatory signals are implicated in the integrated control of energy balance and reproduction. Leptin, as peripheral signal for energy abundance, serves an important role in reproduction, and threshold leptin levels (as indicator of sufficient energy stores) are needed for proper activation and function of the reproductive axis (Casanueva and Dieguez, 1999b). Conversely, ghrelin, as peripheral signal for energy insufficiency, might play an opposite role. However, the analysis of the reproductive actions of ghrelin remains largely incomplete. Nevertheless, data from different groups, including ours, strongly suggest that systemic ghrelin may indeed participate in the control of reproduction, as ghrelin inhibits LH secretion in different experimental models and reduces circulating prolactin levels in pubertal rats (Furuta et al., 2001; Fernandez-Fernandez et al., 2004; Tena-Sempere et al., 2004). In addition, enhanced ghrelin levels, as those observed in malnutrition states, could carry out a direct inhibitory effect upon testicular steroidogenesis, as inferred from our in vitro data (Tena-Sempere et al., 2002), and might hamper early embryo development and reduce the litter size in order to minimize the energetic drain associated to pregnancy and lactation (Kawamura et al., 2003).

It is obvious, however, that the ‘reproductive’ facet of ghrelin is not restricted to the potential actions of the gut-derived systemic hormone. As demonstrated for other tissues and cell systems, ghrelin is locally produced in the male and female gonads of rat and human species, with conserved patterns of cellular distribution. Similarly, the functional ghrelin receptor, the GHS-R1a, is also expressed in the gonads. Moreover, gonadal expression of both components (ligand and receptor) of ghrelin system is under the precise control of hormonal signals, such as pituitary gonadotropins and ghrelin. It is likely that locally produced ghrelin might be serv-
ing additional functions to those of systemic ghrelin. Thus, ghrelin, which is expressed in the testis under the stimulus of pituitary LH, may operate as local mediator of some of the testicular actions of this gonadotropin (Barreiro et al., 2002a). Moreover, expression of ghrelin in testicular Leydig cells is dependent on the degree of cell differentiation, and ghrelin peptide is detected at the highest levels in Leydig cells at advanced stages of maturation regardless of their fetal- or adult-type origin (Barreiro et al., 2003). Notably, rat and human mature Leydig cells, which do express ghrelin and its functional receptor, are devoid of significant proliferative activity (Tena-Sempere and Huhtaniemi, 2003). In contrast, proliferating rat Leydig cell progenitors and poorly differentiated human Leydig tumor cells do not show ghrelin immunoreactivity (Barreiro et al., 2002b; Gaytan et al., 2004). Considering that ghrelin and its synthetic counterparts (GHSs) have been shown to carry out anti-proliferative actions in different tumor cell lines (Broglio et al., 2002), we are presently investigating whether ghrelin may function as an autocrine regulator of Leydig cell proliferation, both in normal and tumor conditions. Finally, expression of GHS-R1a in the seminiferous tubules strongly suggests that the seminiferous epithelium might be a target for ghrelin actions. In fact, our initial unpublished evidence indicates that ghrelin is able to inhibit the tubular expression of SCF gene, whose relevance as survival factor in the control of spermatogenesis is well demonstrated (Yan et al., 2000a,b).

To be noted, recent reports indicate that both ghrelin and GHS-R knockout mouse models are apparently devoid of an overt metabolic phenotype, neither they show major reproductive defects (Sun et al., 2003, 2004). In contrast, leptin insufficiency (as that observed in ob/ob and db/db mice as well as in obese Zucker rats) is clearly associated not only with obesity and metabolic alterations, but also with impaired reproductive function (Rivier and Olster, 1997; Casanueva and Dieguez, 1999b; Tena-Sempere and Barreiro, 2002). Although the reproductive phenotype of the available models of ghrelin deficiency remains to be studied in detail, such data would argue against an essential role of this molecule in the control of fertility and reproduction. The above observations, however, do not exclude the possibility that ghrelin may cooperate in the integrated control of energy balance and reproduction, as compensatory mechanisms might have been activated to overcome the lack of endogenous ghrelin. Moreover, an interesting possibility is that whereas the lack of leptin as signal for energy abundance is clearly detrimental for fertility, the absence of ghrelin may not be as deleterious in terms of reproductive function as its overexpression, given its proposed role as molecular signal for energy deficit.

There are still many open questions concerning the potential reproductive actions of ghrelin. Concerning its steroidogenic effects, the data so far available are limited to the rat testis, and we are presently engaged in the elucidation of the role, if any, of ghrelin in rat and human ovarian steroidogenesis. To note, recent studies evidenced that androgens are independent modulators of ghrelin levels in male and female humans, thus confirming an interaction between ghrelin and sex steroid synthesis (Pagotto et al., 2002, 2003; Gambineri...
et al., 2003). Ghrelin, however, does not appear to have a universal effect upon steroidogenic tissues, as it was unable to modify adrenal corticosterone secretion in rats (Barreiro et al., 2002b; Andreis et al., 2003). Moreover, gonadal actions of ghrelin other than the control of the steroidogenic function, which are likely conducted by the locally produced peptide, remain largely unexplored. Regarding the effects of ghrelin upon the gonadotropin axis, we are currently analyzing the primary sites and mechanisms of action whereby ghrelin regulates LH and prolactin secretion. Similarly, studies on the effects of chronic administration of ghrelin upon several reproductive parameters of the rat are in progress.

In summary, ghrelin, the endogenous ligand of the GHS-R, has recently emerged as a pleotropic neuroendocrine modulator involved in the control of a wide spectrum of biological functions, including GH secretion and energy balance. In addition, growing evidence indicates that ghrelin may participate in the regulation of different aspects of the reproductive function. These actions likely involve two partially overlapping ghrelin systems, which are tentatively depicted in Fig. 1: the systemic gut-derived hormone, with potential actions at different levels of the hypothalamic–pituitary–gonadal axis, and the locally produced ghrelin, which may serve additional autocrine/paracrine roles in the control of gonadal function. Overall, it is proposed that ghrelin may cooperate with other regulatory signals, such as the adipocyte-derived hormone leptin, in the integrated control of energy balance and reproduction.

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