6-2010

Motilin and Cecretion of Gonadotropin-Releasing Hormone (GnRH) in Hypothalamic Explants from Male and Female Rats

Maria Del Carmen De Miguel Garijo

MOTILIN AND SECRETION OF GONADOTROPIN-RELASING HORMONE (GnRH) IN HYPOTHALAMIC EXPLANTS FROM MALE AND FEMALE RATS

by

Carmen De Miguel Garijo

B.S., Universidad Autónoma de Madrid, Spain, 2002

A Thesis

Submitted to the Graduate Faculty

Of

St. Cloud State University

in Partial Fulfillment of the Requirements

for the Degree

Master of Science

St. Cloud, Minnesota

June, 2010

This thesis submitted by Carmen De Miguel Garijo in partial fulfillment of the requirements for the Degree of Master of Science at St Cloud State University, is hereby approved by the final evaluation committee.

Chairperson

Dean
School of Graduate Studies

MOTILIN AND SECRETION OF GONADOTROPIN-RELASING HORMONE (GnRH) IN HYPOTHALAMIC EXPLANTS FROM MALE AND FEMALE RATS

Carmen De Miguel Garijo

Undernutrition markedly inhibits reproduction in mammals; however, the mechanisms by which the metabolic status is linked to the reproductive axis remains unclear. Gut-derived factors have been implicated in the central regulation of several physiological processes. Motilin, a peptide produced in the gastrointestinal tract, is secreted during the interdigestive period, and has been implicated in several processes such as inducing the migrating myoelectric complex or inhibiting luteinizing hormone (LH) secretion by mechanisms not presently determined. The objective of the studies presented in this thesis was to determine the effects of administration of exogenous motilin on the secretion of gonadotropin-releasing hormone (GnRH) by hypothalamic explants obtained from male and female Sprague-Dawley rats. To that purpose, hypothalami were obtained post-euthanasia from male, ovariectomized (OVEX) or OVEX implanted with an estradiol mini-pumps (OVEX+E_2) rats, and incubated in artificial cerebrospinal fluid (aCSF) using a shaking water bath. After one hour of equilibration, 600 μl of spent medium were harvested every 7.5 minutes and replaced with the same volume of fresh aCSF for a total of 60 minutes. After that time, 1.4 ng motilin/600 μl or 2.5 ng motilin/600 μl were added to the bath, and sampling continued at the same frequency for 120 minutes. During the last 60 minutes of the experiment 75 mM N-methyl-D-aspartate (NMDA) was added to the bath in order to confirm the viability of the hypothalamic extracts. Results indicated that *in vitro* secretion of GnRH remained pulsatile along the experiment. Our results indicate that administration of motilin did not inhibit hypothalamic GnRH secretion in male, OVEX and OVEX+E_2 rats. As expected, plasma concentrations of LH were significantly elevated in OVEX rats when compared to the rest of experimental animals, and plasma levels of estradiol were significantly greater in OVEX+E_2 rats than in the other animal groups. Based

on our *in vitro* studies, the effects of exogenous motilin on inhibiting LH secretion may be hypothalamus-independent, through direct effects on the pituitary gland or through other cerebral neurotransmitter systems.

_____

Month Year

Approved by Research Committee:

Oladele S. Gazal Chairperson

ACKNOWLEDGEMENTS

I would like to express my appreciation to my advisor, Dr. Oladele Gazal, for his mentorship and guidance. I was fortunate to work in his laboratory for 2 years, and he always provided a wonderful learning atmosphere, in where it was easy to exchange scientific ideas. I look forward to our interactions and collaborations in the future.

I would also like to thank the rest of the members of my thesis committee, Dr. Gordon Shrank, Dr. Maureen Tubbiola, and Dr. David Bacharach, for their guidance and contributions to this project.

I would like to thank the Department of Biological Sciences, and in general, the staff at the Graduate School Office, for all the help lent to me during these years.

Finally, I would also like to thank my husband, Dipendra Karmacharya, for all his love, support and patience during all my graduate years. This thesis work is as much his as it is mine. I also thank my parents, Francisco and Maria del Carmen, for always encouraging me and my sisters to follow our dreams, even if it meant living more than 4,000 miles away from them. My sisters, Arancha and Isabel, are also an important part of my support system, and I

would like to thank them for always being there for me despite the distance. I would also like to thank my family-in-law, for their love and support for the last 15 years.

TABLE OF CONTENTS

Section	Page
LIST OF FIGURES	ix
LIST OF ABBREVIATIONS	xi
INTRODUCTION	1
LITERATURE REVIEW	6
ENDOCRINOLOGICAL BASIS OF REPRODUCTION	6
EFFECT OF UNDERNUTRITION ON REPRODUCTION	9
GUT-DERIVED PEPTIDES AND REPRODUCTION	15
Peptide YY (PYY)	16
Glucagon-like Peptide-1	18
Cholecystokinin (CCK)	20
Ghrelin	22
Motilin	26
HYPOTHESIS	33
MATERIALS AND METHODS	34
EXPERIMENTAL ANIMALS	34
EXPERIMENTAL DESIGN	35

Section Page

RADIOIMMUNOASSAY FOR GnRH 36

RADIOIMMUNOASSAY FOR LH 37

DETERMINATION OF ESTRADIOL IN PLASMA 38

STATISTICAL ANALYSIS 38

RESULTS 39

DISCUSSION 59

REFERENCES 66

LIST OF FIGURES

Figure		Page
1.	Diagram of the experimental design	36
2.	Representative *in vitro* GnRH secretion patterns from hypothalamic explants obtained from male Sprague-Dawley rates treated with: A: aCSF; B: low motilin concentration (1.4 ng/ 600 µl), and C: high motilin concentration (2.5 ng/ 600 µl)	48
3.	Comparison of mean GnRH concentration secreted by hypothalamic explants obtained from male Sprague-Dawley rats during the different experimental periods	49
4.	Representative in vitro GnRH secretion patterns from hypothalamic explants obtained from OVEX Sprague-Dawley rats treated with: A: aCSF; B: low motilin concentration (1.4 ng/ 600 µl), and C: high motilin concentration (2.5 ng/ 600 µl)	50
5.	Comparison of mean GnRH concentration secreted by hypothalamic explants obtained from OVEX Sprague-Dawley rats during the different experimental periods	51
6.	Representative in vitro GnRH secretion patterns from hypothalamic explants obtained from OVEX+E_2 Sprague-Dawley rats treated with: A: aCSF; B: low motilin concentration (2.4 ng/ 600 µl), and C: high motilin concentration (2.5 ng/ 600 µl)	52
7.	Comparison of mean GnRH concentration secreted by hypothalamic explants obtained from OVES+E_2 Sprague-Dawley rats during the different	

experimental periods 53

Figure Page

8. Comparison of mean GnRH concentration secreted during each experimental period by hypothalamic explants obtained from male, OVEX and OVEX+E_2 Sprague-Dawley rats perfused with regular aCSF for the length of the experiment 54

9. Comparison of mean GnRH concentration secreted during each experimental period by hypothalamic explants obtained from male, OVEX and OVEX+E_2 Sprague-Dawley rats 55

10. Comparison of mean GnRH concentration secreted during each experimental period by hypothalamic explants obtained from male, OVEX and OVEX+E_2 Sprague-Dawley rats 56

11. Average concentration of LH in plasma of males, OVEX and Ovex+E_2 Sprague-Dawley rats 57

12. Average concentration of extradiol in plasma of males, OVEX and OVEX+E_2 Sprague-Dawley rats 58

LIST OF ABBREVIATIONS

aCSF	Artificial cerebrospinal fluid
AgRP	Agouti-related protein
BBB	Blood-brain barrier
BMI	Body mass index
CCK	Cholecystokinin
CCK_A receptor	Cholecystokinin receptor isotype A
CCK_B receptor	Cholecystokinin receptor isotype B
CNS	Central nervous system
FSH	Follicle stimulating hormone
GH	Growth hormone
GHSR	Growth hormone secretagogue receptor
GI	Gastrointestinal
GLP-1	Glucagon-like peptide 1
GnRH	Gonadotropin-releasing hormone
HPG axis	Hypothalamic-pituitary-gonadal axis
5-HT_3	5-hydroxytryptamine-3
^{125}I	Iodine 125
i.c.v.	Intracerebroventricular

I.D.	Inner diameter
IMC	Interdigestive migrating contractions
i.p.	Intraperitoneal
LH	Luteinizing hormone
MAP	Motilin associated peptide
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NMDA	N-methyl-D-aspartate
NPY neurons	Neuropeptide Y neurons
O.D.	Outer diameter
OVEX rats	Ovariectomized rats
POMC neurons	Proopiomelancortin neurons
PYY	Peptide YY
RIA	Radioimmunoassay
TBE	Tribromoethanol
Y5 receptors	Neuropeptide Y receptor subtype 5

INTRODUCTION

For many years, nutrition has been known as the most limiting factor for reproductive success (Chagas et al., 2007; Foster et al., 1989); however, the precise mechanisms by which reproduction and nutrition are connected are still unclear. During energetic challenges, several factors such as deficit in oxidizable fuels (Schneider et al., 1998; Wade and Jones, 2004), changes in circulating hormone concentrations (Chowers et al., 1969; Foster et al., 1989; Glass and Swerdloff, 1977) or changes in the secretion of neuropeptides (Badger et al., 1985; Bronson, 1988; Clarker et al., 1990; Dean and Dyer, 1984; Doerr et al., 1980; Dubey et al., 1986; Gruenewald and Matsumoto, 1993), initiate a series of processes that save energy by inhibiting reproductive behavior, and ensure adequate energy availability by motivating food consumption (Roche, 2006; Schneider, 2004). On the other hand, when food is plentiful, sensory detectors send signals to the brain to inform it of the fulfilled energy storage, and, as a result, the animal is more likely to become engaged in reproductive behaviors such as courtship or mating, in order to perpetuate the species (Bronson, 1984; Schneider, 2004).

Reproductive success is closely controlled by hormones, especially by those secreted in the hypothalamic-pituitary-gonadal axis (Blache et al., 2006;

Blache et al., 2007). Gonadotropin-releasing hormone (GnRH) is a peptide hormone that is secreted in an episodic manner from hypothalamic nerve terminals into the hypophyseal portal circulation (Bergendahl and Veldhuis, 1995; Carmel et al., 1976; Clarke and Cummins, 1982; Clayton, 1989; Levine and Ramirez, 1980). After arrival at the anterior pituitary, GnRH induces the biosynthesis and secretion of gonadotropins (luteinizing hormone (LH) and follicular-stimulating hormone (FSH) (Beien et al., 1998), which then travel to the gonads to stimulate critical reproductive processes such as steroid hormone production and gametogenesis. The pulsatility of GnRH secretion is the factor that ultimately regulates those processes (Bergendahl and Veldhuis, 1995; Bucholtz et al., 1996; Nagatani et al., 1996). In this way, any factor that alters the reproductive function has to do so by affecting the synthesis and/or release of GnRH from the hypothalamus.

The ability to monitor internal and external energy availability is essential to the link between reproduction and energy balance (Schneider, 2004; Temple et al., 2002). The list of chemicals and metabolic events that control food intake and reproduction has grown greatly since the discovery in 1994 of the gene coding for leptin (Zhang et al., 1994). Treatment with leptin stimulates reproduction (Burcelin et al., 2003; Clarke et al., 1990; Henry et al., 2001; Nagatani et al., 1998) by increasing GnRH release in the hypothalamus and stimulating LH release in pituitary explants from rats and pigs (Zieba et al., 2005). Besides the above mentioned leptin, other linking factors have been

proposed as mediators between energy balance and reproduction, like glucose availability (Bucholtz et al., 1996; Huang et al., 2008; Nagatani et al., 1996), insulin (Bruning et al., 2000 ; Burcelin et al., 2003 ; DiVall et al., 2007; Griffin et al., 1994 ; Kovacs et al., 2003 ; Szymanski et al., 2007), ghrelin (Barreiro and Tena-Sempere, 2004; Furuta et al., 2001; Nakazato et al., 2001; Seoane et al., 2003; Tschop et al., 2000; Zigman and Elmquist, 2003), cholecystokinin (CCK) (Li et al., 1999; Perera et al., 1993), motilin (Tsukamura et al., 2000), catecholamines (Maeda et al., 1994), neuropeptide Y (Gazal et al., 1998; Li et al., 1999; Raposinho et al., 1999; Shabah et al, 2003; Turi et al., 2003), glucagon-like peptide (Asmar and Holst, 2010; Beak et al., 1998; Mazumdar et al., 2005), corticotrophin-releasing hormone (Jones et al., 2002; Kerdelhue et al., 1997 ; Petraglia et al., 1987 ; Tsukamura et al., 2000), β-endorphin (Chandrashekar et al., 1992; Faletii et al., 2003; Kerdelhue et al., 1997 ; Seifer and Collins, 1990) and orexins (Furuta et al., 2002 ; Ghatei and Bloom, 2003 ; Hagan et al, 1999; Kohsaka et al., 2001).

Several peptides that are secreted in the gastrointestinal tract have also been found expressed in the brain, and, therefore, they are known as gut-brain peptides (Dockray, 1988). Their dual locations suggest the possibility of those peptides being linking agents between the gastrointestinal tract and the central nervous system (CNS) (Dockray, 1988), especially the component that affects reproduction.

Among those peptides is motilin, a 22-amino acid hormone that is secreted in the duodenum during periods of fasting (Itoh, 1997; Peeters et al., 1980). The primary physiological function of this peptide is to induce rhythmic contractions of gastric smooth muscle during interdigestive periods (Gartwaite, 1985; Itoh, 1980, 1977; Peeters et al., 1980; Poitras and Peeters, 2008). The presence of motilin in neural locations such as the pituitary (Beinfeld and Korchak, 1985; Korchak et al., 1984; Nilaver et al., 2004), hypothalamus (Chey et al., 1980; Huang et al., 1998; Jia et al., 2007) and pineal gland (Beinfeld and Korchak, 1985), suggests a role of this peptide in the control of hormones produced in the anterior pituitary (Hill et al., 2002). Samson et al. demonstrated in 1982 that motilin is able to stimulate growth hormone (GH) release *in vitro* (Samson et al., 1984). The relationship of motilin and LH release was also demonstrated in 2002 by Tsukamura et al., who showed that peripheral and central injection of motilin in Wistar rats produces a decrease in circulating LH in those animals (Tsukamura et al., 2002). Since LH secretion is dependent on the pulsatile release of GnRH, it is easy to infer that motilin could be affecting the synthesis or release of GnRH in the hypothalamus, although the cited authors did not investigate that possibility. Therefore, the objective of this study was to determine the effect that motilin application has on the synthesis and secretion of GnRH in hypothalamic explants obtained from male and female Sprague-Dawley rats.

An understanding of the mechanisms that link energy balance and reproduction is important because of their clinical and agricultural implications. In the agricultural field, that understanding is central for improving efforts directed to bring more efficient breeding and lactational performance in dairy and meat animals. In the clinical field, it will help us to understand the rising obesity epidemic that affects our society. Moreover, research in this area has relevance for understanding nutritional infertility, amenorrhea, anovulation and diminished libido associated with eating disorders such as anorexia nervosa or dieting.

LITERATURE REVIEW

ENDOCRINOLOGICAL BASIS OF REPRODUCTION

Reproduction in mammals is under the control of what is known as the hypothalamic-pituitary-gonadal (HPG) axis (Bergendahl and Veldhuis, 1995; Charlton, 2008). The master control of this axis lies in the central nervous system, specifically in the gonadotropin-releasing hormone (GnRH)-releasing neurons, which are located in the area that spans from the preoptic area to the arcuate nucleus in the hypothalamus (Schneider, 2004). GnRH, a decapeptide hormone, was first isolated in mammals, specifically in pig and sheep brains (Guillemin and Burgus, 1972; Schally et al., 1971), and subsequently isolated from all major vertebrate species (reviewed in Kauffman, 2004). This first isolated form of GnRH received the name of GnRH-I, in order to distinguish it from other more recently isolated variants that are also described later in this text. When GnRH-I is released from the hypothalamus into the hypophyseal portal circulation, it is transported to the anterior pituitary lobe, where it stimulates the secretion of luteinizing hormone (LH) and follicular-stimulating hormone (FSH) into the general circulation (Bergendahl and Veldhuis, 1995). After arrival of LH and FSH at the gonads, gonadal steroid secretion and gametogenesis take place. The HPG axis possesses autocontrol mechanisms

that prevent the futile production of either steroids, GnRH or LH, if the needed plasma concentrations of these hormones have been already reached (Schneider, 2004). This is achieved through negative feedback loops, where estrogen produced in the gonads is able to act at the level of the hypothalamus or pituitary to shut down or decrease the secretion of GnRH or LH, respectively (Herbison, 1998).

In the case of the female mammal, estrogen can have a positive or negative effect on the secretion of GnRH, depending on the phase of the ovarian cycle in which the female is at. In that way, there are two modes of secretion of GnRH. A pulsatile secretion occurs during the follicular phase of the ovarian cycle, where low concentrations of estrogen have negative feedback effects on GnRH and LH secretion, limiting their secretion to low concentrations (Clarke, 2005). In a classic experiment, Ernst Knobil determined in 1980 that the pulsatile pattern of GnRH release in ovariectomized monkeys yields about 1 pulse in 60 minutes (Knobil, 1980). The second mode of GnRH secretion is the surge mode, which occurs at the end of the follicular phase, when high concentrations of estrogen exert a positive effect in the secretion of GnRH (Schneider, 2004), and consequently, LH and FSH.

Moreover, it has been repeatedly proven that the pulsatile release of GnRH is essential for the maintenance of a normal reproductive function, as only this mode of delivery can stimulate the secretion of LH and FSH Barkan

et al., 1997; Koch et al., 1973). Experiments with continuous infusion of GnRH into pituitary cells induced pituitary down-regulation (Belchetz et al., 1978) by desensitization of the gonadotrophs, which ultimately resulted in the suppression of gonadal activity.

In addition to the stimulation of gonadotropin secretion, GnRH-I also plays an important role in mammalian reproductive behavior, as demonstrated by the increased sexual receptivity that ovariectomized (OVEX) rats present after administration of this hormone (Kauffman, 2004; Moss and McCann, 1973; Pfaff, 1972), as well as the facilitation of mating behavior that its administration causes in sexually naïve male hamsters whose vomeronasal organ has been removed (Fernandez-Fewel et al., 1995).

In spite of the importance of the pulsatility of GnRH secretion in reproduction, the specific location of the GnRH pulse generator is not known yet. The pulse generator may be a single center located in the mediobasal hypothalamus (Maeda et al., 1995), although other authors maintain that there are two pulse generators: one located in the mediobasal hypothalamus, associated to opioid neurons, and a second one located in the preoptic area and associated with GABA neurons (Kimura and Funabashi, 1998). It is known that endogenous glutamate is involved in the mechanism of pulsatile GnRH secretion, as demonstrated by Bourgignon et al, with the use of glutamate antagonists. This group also suggested the existence of a glutamatergic neuronal system distinct from the GnRH neurons, which would be able to

produce glutamate from glutamine and release it onto the GnRH neurons, controlling in that way the pulsatile secretion of GnRH.

Besides the estrogenic control of GnRH secretion, there has also been demonstrated a relationship between circadian gene expression and regulation of the GnRH secretory patterns (Chappell et al., 2003). Chapell et al. demonstrated the existence of circadian clocks in GnRH neurons, and that perturbation of those clocks' functions disrupts the normal pulse patterns of secretion of the decapeptide (Chappell et al., 2003).

EFFECT OF UNDERNUTRITION ON REPRODUCTION

The most limiting factor for reproductive success in wild and domesticated animals is nutrition. Nutritional status regulates age at puberty, physiological events that control estrous cycle, efficiency of fertilization, implantation of the embryo or even pregnancy (Foster et al., 1989; Schneider, 2004). For instance, an increase in food intake can induce multiple ovulations in females and increase sperm production in male mammals (Martin and Walkden-Brown, 1995; Wade and Schneider, 1992). Adequate body weight is linked to the onset of puberty (Gueorguiev et al., 2001) and large amounts of nutrients and energy are expended by performance athletes and high-producing dairy cattle, which compromises their capacity to reproduce (Judd, 1998). Therefore, the ability of the reproductive system to respond to changes

in nutrients availability is a critical adaptation for survival in a changing environment.

Metabolic status depends on the amount of food consumed, the amount of body reserves, and the rate of expenditure of energy; changes in any of these three components can influence reproductive capacity (Zhang et al., 2005). Metabolic challenges such as food deprivation inhibit the hypothalamic-pituitary-gonadal axis at many levels, the principal locus being the GnRH pulse generator (Bronson, 2000). It has been demonstrated that the activity of the GnRH neurons in the hypothalamus is regulated by signals reflecting metabolic balance (Burcelin et al., 2003; Sullivan et al., 2003), which is an important consideration given that GnRH neuronal activity is a primary controller of gonadal function. As a consequence of food deprivation, a reduced concentration of circulating GnRH results (Bergendahl and Veldhuis, 1995), and a decreased frequency of LH pulses is also observed in female (Bronson, 1986) and male (Bergendahl and Veldhuis, 1995) rats. Curiously, the decreased frequency of LH release is accompanied by increased amplitude of those pulses, what seems to result from an enhanced pituitary sensitivity to GnRH during fasting (Bronson, 1986). All these changes in the reproductive axis are associated with impaired gonadal function, and, subsequent atrophy of sex organs (Bergendahl and Veldhuis, 1995; Cameron et al., 1993; Grewal et al., 1971; Howland and Skinner, 1973). This atrophy

ultimately leads to poor reproduction, even infertility, in male and female animals (Bergendahl and Veldhuis, 1995).

Pulsatile LH secretion, follicle development and ovulation can be reinstated by pulsatile treatment with exogenous GnRH in food-deprived or food-restricted mammals. This effect has been demonstrated in rats (Bronson, 1986; Manning and Bronson, 1991), sheep (Armstrong and Britt, 1987; Foster and Bucholtz, 1995; Kile et al., 1991), pigs (Armstrong and Britt, 1987), cows (Day et al., 1986), monkeys (Cameron, 1996; Cameron and Nosbich, 1991) and women (Nillius, 1975). Pulsatile LH secretion and ovulation can also be simply recovered by refeeding of the animal (Parfitt et al., 1991; Ponzo et al., 2000). Malnutrition inhibits the reproductive axis, in part, through increasing the sensitivity to the negative estrogenic feedback, and, also, through estrogen-independent pathways, such as by the secretion of antigonadotropic peptide (Benson et al., 1976; Benson and Machena, 1994; Bronson, 1988) or melatonin (Chik et al., 1987; Roy and Belsham, 2002) in the pineal gland.

Although the first isolated form of GnRH (GnRH-I) was obtained from hypothalamus (Kauffman, 2004), more than 22 structural variants of the peptide have been isolated from vertebrates since then (Millar et al., 2004) most of them sharing between 10% and 50% sequence homology (Cheng and Leung, 2005). Most vertebrate species possess at least two GnRH forms in the brain: GnRH-I and a variant that was originally isolated from chickens (GnRH-II). The amino acid sequence of this second form of GnRH is 70%

homologous to GnRH-I (Kauffman and Rissman, 2004). GnRH-II has been identified in a wide-range of mammalian species, including human. The omnipresence of GnRH-II in all these species suggests that this isoform of GnRH has been conserved through evolution, and therefore, must have an important function (Kauffman, 2004). Among the physiological functions that have been described for GnRH-II in mammals are stimulation of gonadotropin release (although with only 2% of GnRH-I effectiveness) (Hasegawa et al., 1984; Kaiffman and Rissman, 2004; Millar and King, 1983) and stimulation of ovulation (although, once again, with a decreased potency when compared to GnRH-I–only 10% as effective) (Rissman et al., 1995). In amphibians, GnRH-II has been shown to act as a neuromodulator, producing a long-term increase in the excitability of postsynaptic neurons through inhibition of voltage-dependent K^+ channels (M current) (Jones, 1998; Kauffman, 2004).

Recent evidence suggests that GnRH-II could also be involved in the food-restriction-induced inhibition of reproduction. Female, food-deprived musk shrews and mice exhibit significant decrease in mating behavior, a situation that is significantly reversed by i.c.v. infusion of GnRH-II (Gill and Rissman, 1997; Kauffman, 2004). However, GnRH-II infusion into *ad libitum* fed animals does not increase their mating behavior, what suggests that GnRH-II exerts permissive actions to reproduction, rather than stimulatory (Kauffman and Rissman, 2004). These authors suggested that when food is readily available, GnRH-II is released at a basal rate that permits mating, while

in a situation of food deprivation, where the energy status is compromised, the peptide release decreases, inhibiting mating behavior. In this way, it is suggested that GnRH-II could act as a neurochemical signal of the energetic state in female, acting as a permissive factor that allows reproduction only if the level of energy is the appropriate to support pregnancy and lactation (Kauffman and Rissman, 2004).

The ability of the brain to detect subtle changes in energy availability is important for the maintenance of the normal reproductive function. The nature of the metabolic signals to the GnRH neurons is not completely understood, but a variety of metabolic cues have been suggested to ensure that the brain has an update of the body energetic status, doing so with a certain degree of redundancy (Schneider, 2004). Information about glucose availability is provided to the brain not only through glucodetectors (Nagatani et al., 1996) as the metabolic hypothesis suggests, but also through other signals, such as hormones, growth factors or excitatory amino acids (Schneider, 2004).

Among the hormones that have been suggested to be metabolic messengers to the brain are leptin (Barb and Kraeling, 2004; Blache et al., 2000a and 2000b; Spicer 2001), whose action in the central nervous system appears to be mediated by decreasing the expression of neuropeptide Y (NPY) in the arcuate nucleus, and therefore, stimulating GnRH secretion by decreasing the inhibitory action of NPY on this peptide (Gazal et al., 1998; Li et al., 1999; Plant and Shahab, 2002; Raposinho et al., 1999; Turi et al.,

2003); insulin (Blache et al., 2003; Burcelin, et al, 2003; Miller et al, 2002) and growth hormone (Howland, 1975).

Several authors have demonstrated that circulating concentrations of insulin and leptin are sensitive to changes in food intake and body energy reserves, and that alterations of their secretion is accompanied by changes in the pulsatile secretion of LH, and thus, GnRH (Archer et al., 2002; Blache et al., 2000a; Marie et al., 2001). Furthermore, intracerebral infusion of insulin or leptin increases the frequency of LH pulses (Henry et al., 2001; Miller et al., 1995, 2002; Tanaka et al., 2000). Leptin has been demonstrated to cross the blood-brain barrier and leptin receptors have been shown to be abundantly expressed in the hypothalamic arcuate and paraventricular nuclei (Sone and Osamura, 2001). Besides increasing the release of GnRH, leptin has also been shown to activate the secretion of LH and FSH from the pituitary via activation of nitric oxide synthase in this gland (Yu et al., 1997).

Other adipokines have also been implicated in the control of the hypothalamic-pituitary-gonadal (HPG) axis (Martos-Moreno et al., 2009). These include adiponectin, almost exclusively produced by mature adipocytes, and resistin, mainly produced by the mononuclear cells in the stromal-vascular matrix of the adipose tissue (Budak et al., 2006; Korner et al., 2005). The role of adiponectin in the control of energy homeostasis is uncertain, as unaltered feeding behavior has been shown in mice lacking (Maeda et al., 2002) or over-expressing adiponectin (Combs et al., 2004). However, this peptide has

recently been shown to directly depolarize parvocellular neurons in the hypothalamic paraventricular nucleus, thus controlling neuroendocrine and autonomic functions (Hoyda et al., 2009). Regarding gonadal function, several studies in adiponectin ablated models failed to show any impairment of fertility (Kubota et al., 2002; Ma et al., 2002; Maeda et al., 2002). In contrast, female mice over-expressing adiponectin are infertile, although the precise etiology and extent of this infertility has not been elucidated (Combs et al., 2004). Consequently, an eventual role for adiponectin in linking energy homeostasis and control of the HPG axis cannot be excluded. Indeed, adiponectin has been shown to be produced by the gonads, with both the ovary and testis expressing adiponectin receptors (Caminos et al., 2008; Chabrolle et al., 2007). On the other hand, testicular expression of secretin has been shown to be modulated by gonadotropins and fasting, with this adipokine stimulating testosterone secretion in a dose dependent manner (Nogueiras et al., 2004).

GUT-DERIVED PEPTIDES AND REPRODUCTION

The so called "gut-brain axis" reflects the interactions between the gastrointestinal system and the brain. The gastrointestinal tract communicates with the rest of the body through absorbed nutrients, secreted peptides or by activation of afferent sympathetic nerves (Romjin et al., 2008). The gut is the largest endocrine organ in the body, and it secretes more than 20 different hormones that serve different purposes such as local regulators or regulators

of appetite, satiety, and reproduction (Hameed et al., 2009). In that way, the brain receives both neural and endocrine inputs from the gastrointestinal tract in response to food intake, which are then integrated with signals from other organs to create physiological responses (Hameed et al., 2009). Among this family of gut-derived peptides are peptide YY, glucagon-like peptide 1 (GLP-1), cholecystokinin, ghrelin and motilin. Motilin and ghrelin show striking similar chemical structures, placing them under the same hormonal family, the motilin family (Asakawa et al., 2001; Depoortere, 2001; Tomasetto et al., 2000). Each of these hormones are described in the following sections.

Peptide YY (PYY)

Peptide YY is a member of the PP family of proteins to which NPY also belongs to. Its name is due to the tyrosine residues that this peptide has at both N and C termini. It consists of 36 amino acids (Tatemoto and Mutt, 1980), and it is produced and released from the L-cells of the gastrointestinal tract, particularly from the colon and rectum (Adrian et al., 1985). Most of the circulating PYY is the N-terminal truncated form of the full length peptide, YY_{3-36}. Levels of PYY are low during fasting and peak in the second hour after a meal, remaining elevated for about 6 hours. The strength of the peak is related to the number of consumed calories and the composition of the food (Adrian et al., 1985).

Acute peripheral administration of exogenous PYY_{3-36} in rodents and humans leads to reduction of food intake (Batterham et al., 2002). PYY_{3-36}

exerts its actions through the Y_2 receptor (Keire et al., 2000), which is a member of the Y family of G protein-coupled receptors and is expressed in on the NPY neurons of the arcuate nucleus in the brain (Broberger et al., 1997). One of the models for the mechanism of action of PYY proposes its action as directly inhibiting these neurons, which consequently disinhibits the POMC neurons in the arcuate nucleus (Batterham et al., 2002).

The anorectic effects of PYY have been observed in both lean and obese human subjects (Batterham et al., 2003), suggesting that, unlike leptin, obesity is not a PYY-resistant state and opening the possibility of using this peptide as anti-obesity therapy.

The effects of PYY on reproductive hormones is somehow controversial, given the antagonistic results obtain in different experiments. Administration of increasing doses of PYY_{3-36} to whole pituitary samples obtained from rats elicited LH and FSH secretion in male and female animals (Fernandez-Fernandez, 2004). Interestingly, administration of PYY_{3-36} also increased LH and FSH responsiveness to GnRH *in vitro*. However, systemic i.p. administration of this peptide failed to modify serum LH levels in male and female rats, whereas intracerebroventricular injection inhibits LH secretion *in vivo* and decrease GnRH secretion by hypothalamic fragments in male but not female rats (Fernandez-Fernandez, 2005). All of these data taken together highlights the complex mode of action of this peptide and suggests a role on the neuroendocrine modulation of the reproductive axis.

Glucagon-like Peptide-1

Glucagon-like peptide-1 (GLP-1) is a 30 amino acid peptide member of the glucagon/secretin family of peptides. It is released by the L-cells of the small intestine and colon (Hermann et al., 1995), the alpha cells in the pancreatic islets (Eissele et al., 1992) and by neurons within the brain stem (Jin et al., 1988) as response to food intake. Pre-pro-glucagon is the precursor peptide that is processed by a convertase enzyme to produce glucagon, GLP-1, glucagon-like peptide-2 and oxyntomodulin depending on the site of synthesis (Bell et al., 1983). Immunocytochemical studies have demonstrated the presence of GLP-1 immunoreactive cell bodies in the nucleus of the solitary tract and the medullary reticular nucleus in the rat brain (Jin et al., 1988). This peptide has also been identified in nerve fibers in hypothalamic structures including the paraventricular nucleus and the periventricular strata (Jin et al., 1988; Kreymann et al., 1989; Shimizu et al., 1987). The presence of GLP-1 in the synaptosome fraction of the hypothalamus, its calcium-dependent release from hypothalamic tissue slices by potassium stimulation (Kreymann et al., 1989), and the existence of specific receptors for GLP-1 in the CNS (Kanse et al., 1988) support a putative neurotransmitter or neuromodulatory role for this peptide. Presence of receptors for GLP-1 has been described in the hypothalamus, thalamus and pituitary of rats. Within the hypothalamus, there is a great density of these receptors within the supraoptic, paraventricular, and arcuate nuclei (Kanse et al., 1988).

Levels of circulating GLP-1 rise postpandreally, and fasting reduces proglucagon expression in the intestine (Hoyt et al., 1986). Injections of GLP-1 into the paraventricular nucleus were proven to reduce food and water intake in rats (Tang-Christensen et al., 1996; Turton et al., 1996; Schick et al., 1992), and injections of the specific GLP-1 receptor antagonist exendin to satiated rats increases food intake (Turton et al., 1996). Peripheral administration of GLP-1 to humans has an anorectic effect (Gutzwiller et al., 1999; Verdich et al., 2001) and reduces the rate of gastric emptying, which could also influence food intake (Naslund et al., 1998; Verdich et al., 2001). Verdich et al. also demonstrated that obese individuals have reduced circulating levels of GLP-1 and reduced release of this peptide after food intake.

The role of GLP-1 as a metabolic signal to the brain was demonstrated by Beak et al. They demonstrated that intracerebroventricular injection of GLP-1 produced a quick increase in the plasma LH concentration in male rats. Moreover, they also showed that GLP-1 levels in the hypothalami of fasted male rats were decreased, indicating a relationship between the low LH levels and the negative energy balance (Beak et al., 1998). They also demonstrated *in vitro* that administration of GLP-1 to cultured hypothalamic neurons (GT_1-7 cells) causes a concentration-dependent release of GnRH, further indicating the role of GLP-1 in controlling reproduction.

Cholecystokinin (CCK)

Cholecystokinin was the first gut hormone reported to affect appetite (Gibbs et al., 1973). Levels of CCK in the plasma rise within 15 min of meal initiation (Liddle et al., 1985) and CCK has been shown to reduce food intake in a dose-dependent manner following its administration to rats (Gibbs et al., 1973) and to human subjects (Kissileff et al., 1981).

CCK is widely distributed throughout the GI tract, but the majority of the hormone is synthesized in the L-cells of the duodenum and jejunum (Buffa et al., 1976). In the gastrointestinal tract, CCK exerts local regulatory effects including the inhibition of gastric emptying (Moran and McHugh, 1982) and the stimulation of gallbladder contraction and pancreatic enzyme secretion (Liddle et al., 1985). CCK is also widely distributed in the central nervous system, including the hypothalamus, especially in the ventromedial nucleus and the median eminence (Beinfeld et al., 1981). Two receptor subtypes have been characterized for CCK: CCK_A and CCK_B (Moran et al., 1986; Wank et al., 1992). CCK_A receptors are expressed in vagal afferent and efferent neurons, pancreas and several areas of the brain, such as the nucleus of the tractus solitarius, area postrema and dorsomedial hypothalamus, which are areas known to be linked to control of food intake and reproduction (Moran et al., 1986).

Peripheral administration of CCK to rodents and humans leads to a reduction in food intake by reducing meal size and duration (Kissileff et al.,

1981). Moreover, central administration of the hormone has also been shown to reduce food intake in rodents and this effect is increased by co-administration of leptin, leading to the possibility that CCK may play a role in the long-term regulation of body weight (Matson et al., 2000). The therapeutic potential of this hormone in treatment of obesity has been minimized after some studies showed that constant i.p. infusion for 2 weeks led to tolerance, and therefore no change in food intake or body weight in rats (Crawley and Beinfeld, 1983). In addition, CCK has a very short half life (1-2 minutes), making its use for therapy more difficult (Gibbs et al., 1973).

CCK has been linked to modulation of the migration of GnRH neurons from the nasal placode, where they originate, to the brain via olfactory–vomeronasal axons. If these axons do not penetrate the brain and are directed to no CNS sites, GnRH neurons are found in aberrant locations. CCK guides these axons to the right location (Giacobini et al., 2004), and therefore, influences the placement of these neurons within the hypothalamus, eventually affecting the secretion of this reproductive hormone.

CCK has been shown to be a modulator of neuroendocrine pathways, activating female reproductive behaviors and release of anterior pituitary hormones (Hashimoto et al, 1986; Vijayan et al., 1973). Hashimoto et al. demonstrated that CCK implants in the medial preoptic area of rats inhibited gonadotropin secretion via the dopamine receptor blocker pimozide, and Vijayan et al. demonstrated that intraventricular injections of CCK in the brains

of ovariectomized rats produced significant suppression of plasma LH and elevations in prolactin.

Ghrelin

Ghrelin is mainly produced by the oxyntic cells in the gastric mucosa, although it has also been shown to be produced in the proximal intestine, pituitary, hypothalamus and other organs. It undergoes acylation at its serine amino acid at position 3 by a molecule of n-octanoyl acid (Kojima et al., 1999, 2000). This chemical modification confers ghrelin the ability to cross the blood-brain barrier (BBB) and, more importantly, to bind the 1a subtype of the growth hormone secretagogue receptor (GHSR) (Kojima et al., 1999; Fernández-Fernández et al., 2006; Van der Lely et al., 2004), thus mediating most of the activities of ghrelin, although GHS-R1a independent actions have also been demonstrated (Van der Lely et al., 2004). However, the unacylated isoform of ghrelin is the most abundant form in plasma, and its specific receptor remains unknown. Initially, unacylated ghrelin was considered to be biologically inactive, but it is now accepted that it has a wide range of actions that can overlap or antagonize those of the acylated isoform of the protein (Tena-Sempere, 2008).

Circulating ghrelin concentrations in normal lean subjects are pulsatile, with diurnal and ultradian rhythms, and levels rising at night (Yildiz et al., 2004). Meal intake inhibits both ghrelin and des-acyl ghrelin, but long-term fasting appears to inhibit acylation, although not total ghrelin secretion (Liu et

al., 2008). Serum ghrelin levels are also influenced by growth and pubertal development, with serum levels increasing during the first 2 years of extrauterine life, followed by a later decrease until the end of puberty (Soriano-Guillén et al., 2004b). A notable exception to both of these trends is displayed by patients affected with Prader–Willi syndrome, whose ghrelin levels do not decrease with age. These patients show sustained high levels of ghrelin in blood despite the existence of marked obesity (Haqq et al., 2008). The sex difference in circulating ghrelin levels is controversial. A recent study reports that ghrelin levels are significantly higher in women than in men, and demonstrates a correlation between testosterone levels and ghrelin in men and postmenopausal women (Greenman et al., 2009).

Ghrelin modulates energy homeostasis by stimulating the expression of the genes encoding NPY and AgRP in the arcuate nucleus of the hypothalamus, and by binding to presynaptic terminals of arcuate NPY and POMC neurons, respectively stimulating and inhibiting their activity and peptide release (Lorenzi et al., 2009). This results in a net orexigenic effect, functionally opposite to that produced by leptin (Wynne et al., 2005). In addition, i.c.v. but not peripheral administration of unacylated ghrelin has been shown to increase appetite through the GHS-R independent activation of hypothalamic orexin producing neurons (Toshinai et al., 2006). Levels of circulating ghrelin increase during the fasting state, under the influence of sympathetic innervation, and decrease in the postprandial period, apparently

regulated by nonvagal neurologic control influenced by the increase in insulin levels (Murphy et al., 2006; Wynne et al., 2005). Additionally, a long term decrease or increase in the body's energy stores, for instance during anorexia or obesity, leads to high and low levels of ghrelin, respectively, with a negative correlation observed between body mass index (BMI) and ghrelin levels (Soriano-Guillén et al., 2004a). Based on all of these characteristics, ghrelin can be considered a signal of starvation or energy insufficiency, and a candidate to convey the energy homeostasis status to the central nervous system (Martos-Moreno et al., 2009; Tena-Sempere, 2008).

The effects of ghrelin on gonadotropin secretion have been studied in several species of mammals. Central administration of ghrelin reduces LH pulse frequency in ovariectomized rats and monkeys, and baseline LH levels in intact rats and sheep (Furuta et al., 2001; Lorenzi et al., 2009; Ogata et al., 2009; Tena- Sempere, 2008). This inhibitory effect of ghrelin on LH secretion appears to be more evident in male rats, either intact or gonadectomized and both in the prepubertal and in the adult stages, than in females. Its effects on FSH secretion are poorly characterized or only observed after the infusion of high doses of ghrelin (Tena-Sempere, 2008). This predominant inhibitory effect of ghrelin on LH secretion seems to be exerted mainly through inhibition of hypothalamic GnRH release, as shown by its effects on LH pulsatility and by studies employing hypothalamic explants (Fernández-Fernández et al., 2005b). Additionally, studies of the effects of ghrelin on pituitary explants show

significant decreases in GnRH-stimulated LH secretion, but also stimulatory responses after high doses, thus suggesting a possible direct effect of ghrelin on pituitary LH secretion (Fernández-Fernández et al., 2005b; Tena-Sempere, 2008). Administration of ghrelin to healthy human subjects has also shown to reduce pulse amplitude and frequency of baseline LH secretion, without affecting FSH levels (Lanfranco et al., 2008). Ghrelin acts at different levels of the HPG axis, and those actions can be both induced by ghrelin released from the stomach into the bloodstream or from local producing sites in gonads and CNS (Tena-Sempere, 2008). Expression of ghrelin has been described in human testicle and ovary, as well as in the gonads of other species (Gaytan et al., 2005; Tena-Sempere, 2008). Interestingly, ghrelin has been shown to inhibit the production of testosterone by Leydig cells, and to inhibit estradiol and progesterone secretion in human granulosa-luteal cells (Viani et al., 2008).

A putative role of ghrelin in determining the onset and progression of puberty is suggested by studies performed by several authors. Several authors (Fernandez-Fernandez et al., 2005a; Martini et al., 2006) showed that exogenous administration of ghrelin to male rats during the peri-pubertal period resulted in lower levels of LH and testosterone levels and delayed signs of pubertal maturation, while these markers were not affected in their female siblings. However, the group led by Tena-Sempere reported a deleterious effect of ghrelin on the pubertal development in pubertal female rats (Tena-

Sempere, 2008). These results seem to indicate a more predominant effect of ghrelin on male than female animals, and a different effect depending on the pubertal stage (Martos-Moreno et al., 2009).

Motilin

Motilin is a 22-amino acid hormone (Itoh, 1977) that is produced most abundantly in the upper part of the gastrointestinal tract, specifically in the enterochromaffin cells located in the duodenum (Pearse et al., 1974), although it has also been found in the brain of man (Depoortere, 1997), rabbit (Huang et al., 1998; Depoortere, 1997) and monkey (Huang et al., 1998). Preliminary data from our laboratory also indicate that motilin is present in cerebrospinal fluid obtained from cow. Motilin precursor is also present in medulla oblongata, nucleus of the solitary tract, pituitary, hypothalamus, spinal cord, cerebellum, thyroid (Xu et al., 2001) and even in a human liver metastasis (De Clercq et al., 1995).

Among the species in which motilin has been found are horse (Kitamura, 1984), cow (Huang et al., 1999), dog (Itoh, 1978), human (Daikh et al., 1989), pig (Brown and Dryburgh, 1971), rabbit (Banfield et al., 1992), chicken (De Clercq, 1995), guinea pig (Xu et al., 2001), cat (Depoortere et al., 1993), sheep (De Clercq et al., 1997) and rhesus monkey (Huang et al., 1999). In the rat, motilin immunopositive cells have been described in rat intestine (Sakai et al., 1994), and in parts of the rat central nervous system such as Purkinje cells and dendritic cells of the cerebellum, pyramidal cells

and dendrites of the cerebral cortex, and neurons of the CA3 area of the hippocampus (Lange et al., 1986; O'Donohue et al., 1981).

The human motilin gene has been cloned (Daikh et al., 1989; Yano et al., 1989) and mapped to the p21.3 region of chromosome 6 (Gasparini et al., 1994). It is a single copy gene of 9 Kb, composed of 5 exons separated by 4 introns (Daihk et al., 1989). The structure of this gene is unusual in that the sequence encoding for the 22 amino acid motilin is split near its center by an introns (Daihk et al., 1989). However, to date, the genes encoding motilin and its receptor have never been found in the rat (He et al., 2010; Genome Rat Database, 2010) or the mouse (Mouse Genome Database, 2010). Motilin and ghrelin share almost 50% of similarity in their amino acid sequences, and the receptors of both peptides are part of the same family of G protein-coupled receptors, sharing also more than 53% of their overall amino-acid sequence (Poitras and Peeters, 2008). Given the big similarities between both peptides, they are now considered to be members of the new motilin-ghrelin family (Ohno, et al., 2010). Since the isolation of motilin in rodents remains elusive, it has been proposed that ghrelin may have substituted motilin in these small rodents (Peeters et al., 2003).

Endogenous motilin release occurs in a cyclic fashion in dogs (Itoh, 1978; Lee et al., 1986; Mochiki et al., 1996) and humans (Peeters et al., 1980; Vantrappen et al., 1979), at approximately 100-minute intervals during the interdigestive or fasting period, with its concentration and episodic secretory

pattern reduced by feeding, as demonstrated by Chey et al., 1980. Moreover, glucose was found to suppress motilin secretion in humans (Imura et al., 1980; Saito et la., 1980) and dogs (Barnett et al., 1981). Peak plasma motilin levels correlate with the onset of a characteristic motor pattern in the intestine, known as the migrating myoelectric complex (Peeters et al., 1990).

The motilin peptide is synthesized as a part of a preprohormone precursor consisting of a 25-amino acid signal peptide preceding the 22-amino acid motilin moiety and a 66-amino acid carboxy-terminal peptide (Motilin-associated peptide or MAP) separated from the carboxyl terminus of motilin by a dibasic Lys-Lys dipeptide (Bond et al., 1988). The MAP peptide is released from the protein by proteolytic cleavage in the dibasic site (Huang et al., 1999). Posttranslational processing generates promotilin first, and ultimately, the hormone fragment (Depoortere et al., 1997).

Analysis of the motilin precursor from different species reveals several highly conserved sequences, like the first part of the signal peptide (-25 to -19 amino acids), the N-terminus (1-6 positions), the C-terminus of the motilin itself (15-18 amino acids), as well as the N-terminus (23-26) and middle portion (77-97) of the MAP (Huang et al., 1999). This high level of conservation suggests that these regions are functionally important. The average percentage of homology in the amino acid sequence of motilin among different species is 86% in the motilin itself, 84% in the signal peptide sequence, and 65% in the MAP (Huang et al., 1999). Apparently, the MAP sequence has a higher

evolutionary rate than the rest of the regions in the molecule, what indicates that motilin is an example of mosaic evolution, where each part of the molecule has a different rate of changing (Huang et al., 1999).

The role of the signal peptide is to transfer the mature peptide into the rough endoplasmic reticulum. Although the role of the MAP sequence is not clear yet, it could be important for the posttranslational events that lead to the secretion of motilin (Huang et al., 1999). In 1992, Poitras et al. identified the N-terminus of motilin as the responsible site for the biological action of the molecule. Fragments of motilin of different lengths were injected into rabbit duodenal muscle and it was determined that fragments lacking the two first amino acids in the N-terminus were more than 1,000 times less potent in producing intestinal contractility than the native 22 amino acid molecule (Poitras et al., 1992).

There have been several functions attributed to motilin. The first of them is the regulation of the interdigestive migrating myoelectric complex (Itoh, 1997). Motilin plasma levels increase cyclically every 90-120 minutes during the interdigestive fasting period (Itoh, 1997), and that cyclic secretion of motilin drastically disappears after ingestion of a meal. These cyclical peaks of plasma motilin are associated to strong peristaltic contractions starting in the stomach and propagating to the duodenum and small intestine. This pattern of migrating waves is known as phase III contraction of the interdigestive migrating contractions (IMC) (Szurszewski, 1969). These contractions are

assumed to have an important housekeeping role, as they forcefully push the content of the intestine forward while cleaning the bowels of debris and bacteria that otherwise would accumulate in the lead to bacterial overgrowth, compromising nutrient absorption in the small intestine (Ohno et al., 2010). Exogenous administration of motilin has been shown to initiate premature phase III contractions in the stomach (Itoh et al., 1976). Other studies also demonstrated that these peristaltic contractions concur with plasma peaks of motilin (Peeters et al., 1980), and that suppression of circulating motilin with antibodies suppresses phase III contractions in dogs (Lee et al., 1983). The effects of motilin on phase III contractions in dogs are blocked by a 5-hydroxytryptamine-3 (5-HT_3) antagonist (Itoh et al., 1991), suggesting that the motilin-induced signal may be mediated by 5-HT_3 receptors on the vagal afferents and transmitted to the stomach by the vagal efferents, inducing the release of acetylcholine. Other studies propose that motilin acts directly on the smooth muscle cells (Adachi et al., 1981; Lüdtke et al., 1989; Strunz et al., 1975)

Another function of this peptidic hormone is the control of feeding intake. Central and peripheral administration of motilin has been proven to stimulate feeding in non-fasted and fasted rats, respectively (Rosenfeld and Garthwaite, 1987; Garthwaite, 1985), suggesting that this hormone could be acting as a hunger hormone during periods of fasting. Asakawa et al. demonstrated that the same was also true in mice when they administered

motilin i.c.v. and observed a significant increase in food intake (Asakawa et al., 1998). The mechanism proposed for this action of motilin is that this hormone promotes the evacuation of the intestine contents. The emptying of the intestine produces a stimulation of the hunger, and therefore, it also stimulates food intake.

As explained earlier in this literature review, it has also been reported that fasting affects reproduction. In times of food scarcity some physiological processes cannot be compromised, and animals adjust their metabolic necessities. There are some activities that are not essential for the individual survival, such as reproduction, and therefore processes like ovulatory cycles are suspended during periods of undernutrition. There have been a series of studies indicating that fasting significantly suppresses pulsatile LH secretion in normal and estrogen-primed ovariectomized rats (Bronson, 1986; Bergendahl and Veldhuis, 1995; Cagampang, et al., 1991). Since plasma levels of motilin increase under the same circumstances, it has been proposed that motilin could act as one of the peripheral substances that convey information about fasting or glucose deprivation to the brain, therefore leading to the suppression of GnRH or LH secretion. Other studies have also described that fasting-induced suppression of LH secretion is reversed by gastric vagotomy, indicating that the vagus nerve may be implicated in LH suppression (Cagampang et al., 1992). The coincidental participation of the vagus nerve in both stimulation of motilin release (Diamant et al., 1994) and the suppression

of LH release during fasting further suggests the participation of motilin in the fasting-induced suppression of LH release.

A study performed by Tsukamura et al. demonstrated that peripheral or central administration of motilin suppresses the release of LH in female rats (Tsukamura et al., 2000). In that study, motilin was administered intravenously and intracerebrally into ovariectomized (OVEX) or ovariectomized and estradiol treated (OVEX+E_2) rats. They observed that intravenous administration of motilin resulted in a significantly reduced average concentration of LH in plasma in both OVEX and OVEX+E_2 rats, and in a significant reduction of the pulsatile secretion pattern of this hormone in the OVEX+E_2 rats. Similar results were also obtained when motilin was administered intracerebrally. These results suggest that motilin has an inhibitory role in the control of LH secretion. Since LH secretion was recovered when the animals received exogenous administration of GnRH, the authors assumed that the effect of motilin on this secretion was through inhibiting the secretion of GnRH, instead of by direct effects on the pituitary; however, they did not test this hypothesis directly in their studies.

HYPOTHESIS

Based on the evidence presented in the literature review section of this thesis, we hypothesized that the *in vitro* administration of exogenous motilin to whole hypothalamic extracts obtained from male and female ovariectomized Sprague-Dawley rats inhibits GnRH secretion and, therefore, reproduction.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Fifty-four post-pubertal Sprague-Dawley rats were used in this study. Eighteen of them were intact males (average weight of 550 g), and the rest 36 rats were female (average weight of 280 g). All the female animals were ovariectomized (OVEX) at least 2-weeks before starting the experiments; 18 of them receiving also an estradiol implant immediately after ovariectomy (Tygon flexible plastic tubing with I.D. of 1/16", O.D. 1/8" and wall 1/32"; VWR Scientific Products, GA, USA).

The animals were kept in the Animal Room of the Wick Science Building at St. Cloud State University, MN, under *ad libitum* conditions of food and water. The light cycle was 14-hour light, 10-hour darkness, with the lights turned on at 5:00 a.m. and turned off at 7:00 p.m.

The research protocol used during this study was approved by the Institutional Animal Care and Usage Committee (IACUC) of St. Cloud State University on October 14, 2004.

EXPERIMENTAL DESIGN

The animals were divided in three experimental groups: intact males (n=18), OVEX females (n=18), and OVEX, estradiol-treated females (n = 18). Within each of those three groups, the animals were subdivided in three subgroups: control animals (n = 6), animals receiving low concentration of motilin (1.4 ng/600 μl; n = 6), and animals receiving high concentration of motilin (2.5 ng/600μl; n = 6).

On the day of the experiment, the animals were euthanized with an overdose of 2.5% Tribromoethanol (TBE), and rapidly decapitated. Trunk blood was collected immediately after decapitation for determination of estradiol and LH concentrations in plasma. The brain was quickly removed from the skull and the hypothalamus isolated. Hypothalami were then incubated in a shaking water bath (Baxter Shaking Water bath, model YB521) in the presence of artificial cerebrospinal fluid (aCSF) bubbled with 95% O_2 and 5% CO_2. The hypothalami were allowed to equilibrate for a period of 60 minutes before onset of the experiment. aCSF contained 0.13 M NaCl, 0.025 M $NaHCO_3$, 1.25 mM NaH_2PO_4, 2.5 mM KCl, 1mM $MgCl_2$, 0.025 M glucose and 1M $CaCl_2$.

After equilibration, 600 μl of spent media were harvested every 7.5 minutes and replaced with the same volume of fresh aCSF for 60 minutes (Period 1). After that time, the treatment was applied in Period 2. The treatment consisted in the application of either 600 μl of aCSF (control group),

or low (1.4 ng/600 μl) or high (2.5 ng/600 μl) porcine motilin, during the following 120 minutes of perfusion (Period 2), with the sampling continuing at the same frequency as before. During the last hour of perfusion (Period 3), the hypothalami were challenged with 75 mM NMDA in order to test their ability to synthesize GnRH along the experiment. During this period of time, the samples were collected every 15 minutes. Porcine motilin and NMDA were purchased from Sigma, St Louis, MO.

The collected samples were stored at -80°C until the determination of GnRH by radioimmunoassay.

Figure 1. Diagram of the experimental design.

RADIOIMMUNOASSAY FOR GnRH

GnRH concentration in the spent media was determined by radioimmunoassay (RIA) techniques as described before (Ellinwood et al., 1985), using duplicate 150 μl aliquots of the perfusates. The GnRH standards used in the assay were 1, 5, 10, 25, 50, 100, 250, 500, and 1000 pg/ml. The GnRH used for standards and iodination was purchased from Sigma. Iodinated GnRH (Iodine-125 purchased from Perkin Elmer Laboratories) was used as tracer. Antiserum R-1245 was obtained from Dr. Terry Nett (Colorado State University) and used in 1:12k dilution, and 95% ethanol as precipitating

agent. The minimum GnRH concentration detected by the assay was 1pg/ml, and average intra- and inter-assay coefficients of variation were 3% and 15%, respectively.

RADIOIMMUNOASSAY FOR LH

Plasma LH concentration was also determined in duplicate 50 μl aliquots of the blood samples collected after decapitation, by the means of RIA techniques following previously described methods (Bernard et al., 1983), using the NIDDK kit obtained from Dr. A. F. Parlow. Iodinated LH (Iodine-125 purchased from Perkin Elmer Laboratories) was used as tracer. NIDDK-rLH-I-10, AFP 1156B was used for iodination. rLH-RP3 was used as the standard and NIDDK anti-rLH-S-11 was used as the antiserum at 1:50,000 dilution. Sheep anti-rabbit Gamma Globulin (SARGG) was used as the second antibody for precipitation of the bound antibody. The standards used in the assay were 0.8, 1.6, 3.1, 6.2, 12.5, 25, and 50 ng/ml. Briefly, on day 1, standards and antibody were added. Twenty-four hours later, tracer was added and tubes were incubated for 48 hours, after which SARGG and 6% polyethylene glycol was used to separate the bound and unbound fractions. The minimum LH concentration detected by this assay was 0.8 ng/ml, and the average intra- and inter-assay coefficients of variation were 9% and 12%, respectively.

DETERMINATION OF ESTRADIOL IN PLASMA

The concentration of estradiol in plasma in the moment of decapitation was determined with a 17-β- Estradiol-^{125}I RIA commercial kit (ICN Biomedicals Inc., Costa Mesa, CA). Fifty μl aliquots of the plasma samples were used in duplicate. The standards that were used during the assay were the following: 0, 10, 30, 100, 300, 1000, and 3000 pg/ml. The minimum estradiol concentration detected by the assay was 8.65 pg/ml. Average intra- and inter-assay coefficients of variation were 4.7% and 9.1%, respectively.

STATISTICAL ANALYSIS

Two-way repeated measures ANOVA was used to test significant differences in GnRH concentrations between group and treatment. One-way ANOVA analyses were performed to test differences in LH and estradiol plasma concentrations among experimental groups. In all the cases, a p value of less than 0.05 was considered significant.

RESULTS

In Vitro hypothalamic explants secrete GnRH in a pulsatile manner. Figures 2, 4 and 6 illustrate GnRH secretion patterns by representative male, OVEX and OVEX+E_2 hypothalamic explants. These figures indicate that the hypothalamic explants maintain their capacity to secrete GnRH in a pulsatile manner during the perfusion time. Interestingly, the baseline levels of each of the explants studied were different from one another.

In all the cases, the capability of the explants to secrete GnRH was tested in the last hour of the experiment by adding NMDA 75 mM to the bath. NMDA is a glutamate receptor agonist and therefore, emulates the action of this excitatory neurotransmitter on the GnRH-producing neurons in the hypothalamus, producing a massive release of GnRH. All hypothalamic explants responded to NMDA administration by significantly increasing their GnRH secretion ($p<0.05$). This effect can be clearly seen in the last part of the graphs depicted in Figures 2, 3 and 4 (from 240 min on).

Effects of motilin on GnRH secretion in male hypothalamic explants. Administration of motilin at a concentration of 1.4 ng/ 600 μl or 2.5 ng/ 600 μl to male hypothalamic explants did not have any significant effect on the pulsatile secretion of GnRH when compared to the pulses presented during

the first 120 minutes (Period 1), when the explants were perfused with regular aCSF (Figure 2).

Interestingly, some of the male explants demonstrated an acute and modest increase in GnRH secretion right after administration of the low dose of motilin, as indicated in Figure 2-B; however, that increase was short-lived and secretion of GnRH quickly returned to normal values.

Administration of 75 mM NMDA during the last hour of the experiment significantly increased the amplitude of the GnRH pulses in all the male explants studied (Figure 2).

There were no significant differences between the GnRH concentration secreted by male hypothalamic explants treated with regular aCSF (control), low motilin or high motilin, as indicated in Figure 3. In fact, administration of low or high doses of motilin was shown to be ineffective in causing a significant reduction on GnRH secretion.

During period 1, the explants that were later treated with high concentration of motilin showed significantly elevated baseline levels of GnRH secretion when compared to the levels presented during the same period by the groups later treated with regular aCSF or low concentration of motilin (high motilin group: 29.5 ± 7.3 pg/ml vs. control group: 7 ± 0.6 pg/ml and low motilin group: 15.2 ± 7.67.3 pg/m; n = 4-5 / group) (Figure 3). However, administration of high concentration of motilin to this group did not increase GnRH concentration from baseline levels (period 1: 29.5 ± 7.3 pg/ml vs. period 2:

26.1 ± 6.5 pg/ml; n = 5), indicating that the concentration of motilin used was not high enough to inhibit secretion of GnRH.

NMDA administration produced a massive release of GnRH from the explants at the end of the experiment, averaging 2,185.1 ± 999.3 pg/ml; this indicates that the capability of GnRH secretion had remained intact during the *in vitro* experiment.

Effects of motilin on GnRH secretion in hypothalamic explants Obtained from OVEX rats. Administration of motilin at a concentration of 1.4 ng / 600 μl or 2.5 ng / 600 μl to hypothalamic explants obtained from OVEX rats did not have any significant effect on pulsatile GnRH secretion when compared to the values observed in these explants during the first 120 minutes (period 1), when the explants were perfused with regular aCSF (Figure 4).

As observed in the male explants, administration of 75 mM NMDA during the last hour of the experiment significantly increased the amplitude of the GnRH pulses in all OVEX explants studied, as indicated in the last part of tracings represented on Figure 4 (from 240 min on).

Figure 5 illustrates a comparison of the average concentration of GnRH secreted by hypothalamic explants obtained from OVEX Sprague-Dawley rats during the different periods of the experiment.

There was no significant difference detected in the concentration of secreted GnRH between the experimental groups during period 1 (control

group: 54.8 ± 7.6 pg/ml, low motilin group: 64.6 ± 30.3 pg/ml, and high motilin group: 27.9 ± 7.6 pg/ml; n = 4-5/group).

However, during period 2, administration of a low concentration of motilin significantly elevated the mean concentration of GnRH secreted by OVEX hypothalamic explants (from 64.6 ± 30.3 pg/ml to 190.9 ± 20 pg/ml; n = 4) when compared to the levels measured during period 1. In fact, the mean concentration of GnRH secreted by hypothalamic explants treated with low dose of motilin was significantly greater than the mean concentration secreted by control or high motilin groups during the same period (low motilin: 190.9 ± 20 pg/ml, control: 17.7 ± 8.1 pg/ml, and high motilin: 25.5 ± 6.1 pg/m; n = 4-5/group; Figure 5).

After administration of NMDA in the last hour of the experiment, the GnRH concentration significantly increased in all the experimental groups, with an average concentration of GnRH of 1,494 ± 726.6 pg/ml.

Effects of motilin on GnRH secretion in hypothalamic explants Obtained from OVEX+E_2 rats. Administration of motilin at a concentration of 1.4 ng / 600 μl or 2.5 ng / 600 μl to hypothalamic explants obtained from OVEX+E_2 rats did not have any significant effect on pulsatile GnRH secretion when compared to the values observed in these explants during the first 120 minutes (Period 1), when the explants were perfused with regular aCSF (Figure 6).

Administration of 75 mM NMDA during the last hour of the experiment significantly increased the amplitude of the GnRH pulses in all the OVEX+E_2 explants studied, as indicated in the last part of the tracings represented on Figure 6.

Figure 7 illustrates a comparison of the concentrations of GnRH secreted by OVEX+E_2 rats administered aCSF, low or high motilin during each experimental period. There were no significant differences between experimental treatments in periods 1 and 2. Average GnRH concentrations during these periods were 27.6 ± 6.5 pg/ml and 31 ± 6.1 pg/ml, respectively (n = 5/group).

As expected, concentrations of secreted GnRH were significantly elevated during the third period after administration of NMDA. Interestingly, the concentration secreted from control explants was significantly higher (30,802 ± 12,615 pg/ml; n = 5) than from the low or high motilin groups (903 ± 379.1 pg/ml and 396 ± 141.9 pg/ml, respectively).

Comparison of GnRH secretion among control groups. Figure 8 compares the GnRH concentration measured during each of the experimental periods in the control groups for males, OVEX and OVEX+E_2 rats.

This figure indicates that the concentrations of GnRH secretion in OVEX and OVEX+E_2 are significantly greater than in male explants in each of the experimental periods, indicating that the baseline secretion in these females is significantly higher than in males (During period 1: OVEX rats: 54.8

± 7.6 pg/ml, OVEX+E_2 rats: 40.6 ± 10.5 pg/ml and males: 7.0 ± 0.6 pg/ml; n = 4-5/group).

Although OVEX+E_2 rats tended to secrete a smaller concentration of GnRH than OVEX rats, the difference between both groups did not reach significance (OVEX rats: 54.8 ± 7.6 pg/ml vs. OVEX+E_2 rats: 40.6 ± 10.5 pg/ml; Period 1).

Similarly to the other periods, average concentration of GnRH after challenge with NMDA was significantly greater in OVEX and OVEX+E_2 explants when compared to the response obtained in male explants (OVEX: 1777.9 ± 896.1 pg/ml, OVEX+E_2: 30802.6 ± 12615 pg/ml, and males: 2438.7 ± 915 pg/ml, respectively; n = 4-5/group). These results seem to indicate that male hypothalamic explants have a smaller capability for producing GnRH when compared to OVEX or OVEX+E_2.

Comparison of GnRH secretion among low motilin groups. Figure 9 illustrates a comparison of secreted GnRH concentration between males, OVEX and OVEX+E_2 rats during the different experimental periods.

During period 1, OVEX rats secreted significantly greater concentrations of GnRH than males (OVEX: 64.6 ± 30.3 pg/ml vs. males: 7.7 ± 2.3 pg/ml; n= 4-5/group), but those concentrations were not significantly different from the concentrations of GnRH secreted by the OVEX+E_2 group during the same period (OVEX: 64.6 ± 30.3 pg/ml vs. OVEX+E_2: 22.2 ± 6 pg/ml; n = 4-5/group).

Administration of motilin at a concentration of 2.3 x 10^{-3} μg/μl significantly increased GnRH secretion in OVEX hypothalamic explants from 64.6 ± 30.3 pg/ml to 190.854 ± 20 pg/ml (n = 5).

After administration of motilin (period 2), OVEX rats secreted significantly greater concentrations of GnRH than both males and OVEX+E_2 rats (OVEX: 110 ± 20 pg/ml; males: 11.4 ± 5.7 pg/ml; OVEX+E_2: 28.4 ± 7.3 pg/ml; n = 4-5/group).

As expected, secretion of GnRH in response to administration of NMDA was greatly increased in the three experimental groups, but this time the concentration of GnRH secreted by male hypothalamic explants was significantly greater than the levels secreted by OVEX and OVEX+E_2 explants (males: 2939.5 ± 1684 pg/ml, OVEX: 639.7 ± 416.8 pg/ml, and OVEX+E_2: 903.6 ± 379.1 pg/ml; n = 4-5/group).

Comparison of GnRH secretion among high motilin groups. Figure 10 represents a comparison between secreted GnRH concentration among males, OVEX and OVEX+E_2 rats along the different experimental periods within the groups of animals receiving high concentration of motilin.

There was no significant difference among the secreted concentrations of GnRH among the groups during periods 1 and 2. Average concentration of secreted GnRH during period 1 was 25.8 ± 2.9 pg/ml.

Administration of high concentration of motilin to the explants did not change the secretion of GnRH in any of the groups respect to their baseline

levels, and those concentrations were not different among the experimental groups (males: 27.7 ± 7.4 pg/ml; OVEX: 34.8 ± 15 pg/ml; OVEX+E_2: 25.2 ± 8.1 pg/ml; n = 4-5/group).

Administration of NMDA greatly increased the secretion of GnRH in all the hypothalamic explants, and there was no difference between the levels secreted by the different experimental groups, averaging 1,004.2 ± 313.1 pg/ml during that period.

Plasma LH concentrations in male, OVEX and OVEX+E_2 rats. Plasma levels of LH were measured in blood samples collected in the moment of decapitation. Figure 11 illustrates the average concentration of plasma LH found in males, OVEX and OVEX+E_2 rats.

As expected, plasma concentrations of LH were significantly greater in the OVEX group when compared to the levels presented by males or OVEX+E_2 rats (OVEX: 3.2 ± 0.5 ng/ml; males: 1 ± 0.1 ng/ml, and OVEX+E_2: 1.9 ± 0.2 ng/ml; n = 4-5/group). Plasma concentrations of LH were also significantly higher in the OVEX+E_2 group than in the males.

Plasma estradiol concentrations in male, OVEX and OVEX+E_2 rats. Figure 12 illustrates the average concentration of plasma estradiol measured in males, OVEX and OVEX+E_2 rats at the moment of decapitation. As expected, plasma concentrations of estradiol were significantly elevated in the rats that had received the estradiol mini-pump (OVEX+E_2), when compared to

the levels found in males and OVEX rats (OVEX+E_2: 762 ± 341 pg/ml; males: 39.7 ± 15.2 pg/ml, and OVEX: 69.1 ± 9.1 ng/ml; n= 7-18/group). There was no significant difference in plasma estradiol concentration between male and OVEX rats.

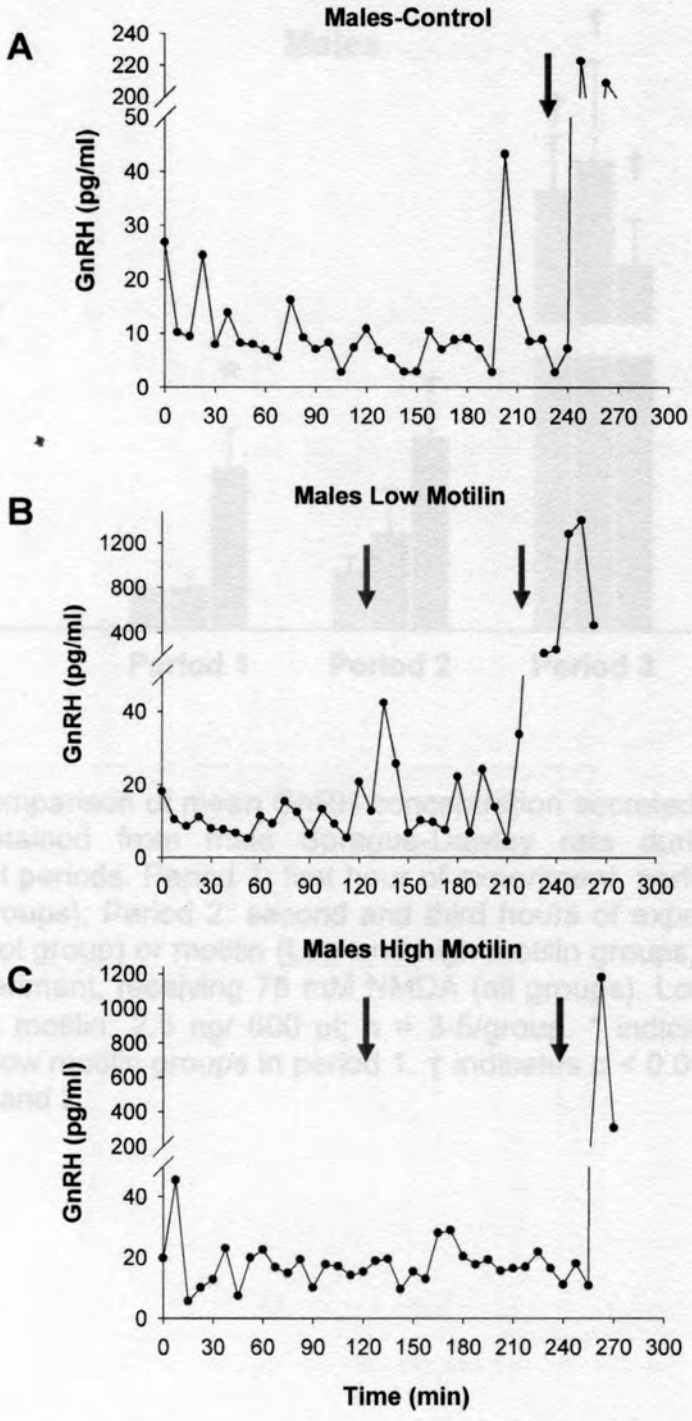

Figure 2. Representative *in vitro* GnRH secretion patterns from hypothalamic explants obtained from male Sprague-Dawley rats treated with: A: aCSF; B: low motilin concentration (1.4 ng/ 600 μl), and C: high motilin concentration (2.5 ng/ 600 μl). Administration of motilin or NMDA is indicated by a green or red arrow, respectively, in each of the graphs.

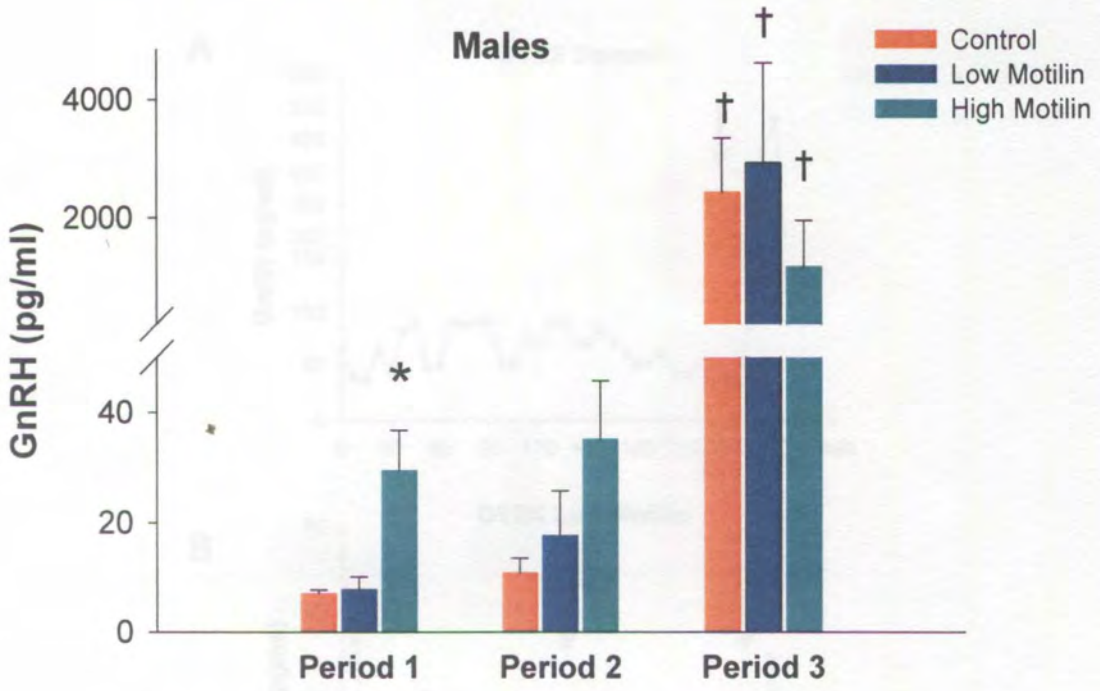

Figure 3. Comparison of mean GnRH concentration secreted by hypothalamic explants obtained from male Sprague-Dawley rats during the different experimental periods. Period 1: first hour of experiment, perfused with regular aCSF (all groups); Period 2: second and third hours of experiment, receiving aCSF (control group) or motilin (Low and high motilin groups); Period 3: fourth hour of experiment, receiving 75 mM NMDA (all groups). Low motilin: 1.4 ng/ 600 μl; high motilin: 2.5 ng/ 600 μl; n = 3-5/group. * indicates $p < 0.05$ vs. control and low motilin groups in period 1. † indicates $p < 0.05$ vs. same group in periods 1 and 2.

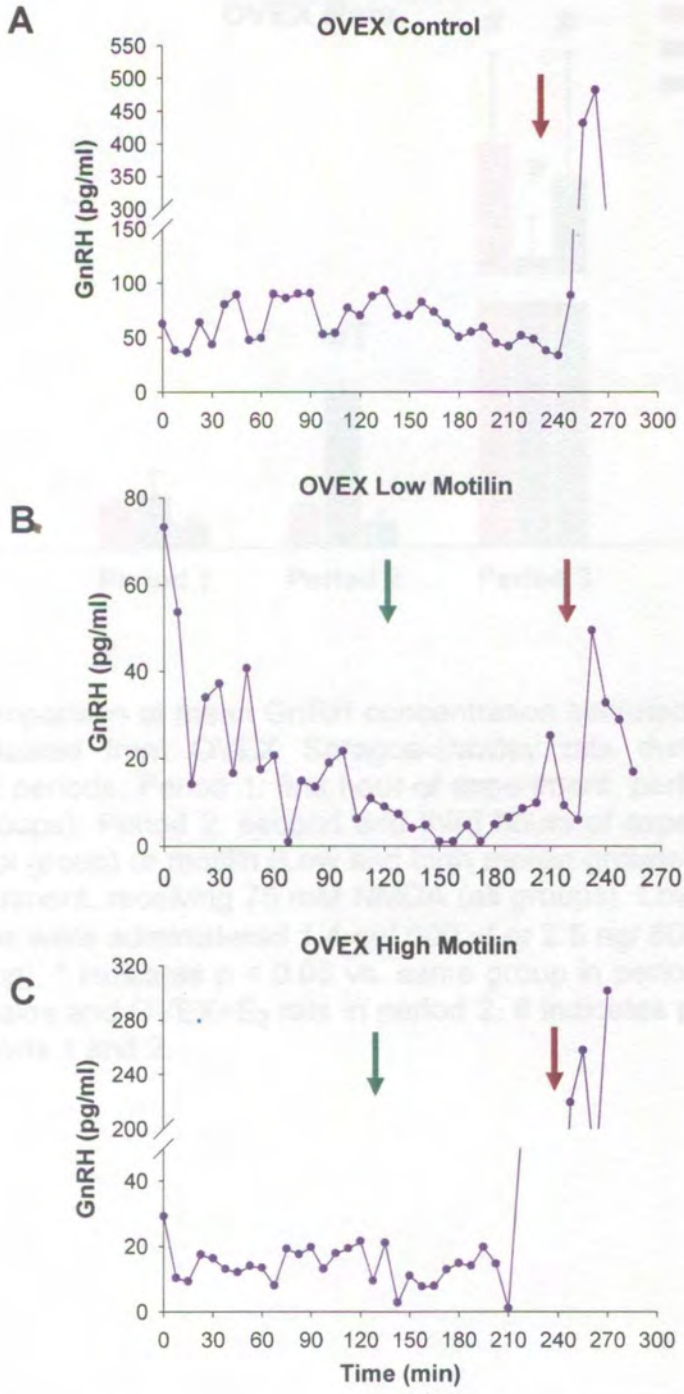

Figure 4. Representative *in vitro* GnRH secretion patterns from hypothalamic explants obtained from OVEX Sprague-Dawley rats treated with: A: aCSF; B: low motilin concentration (1.4 ng/ 600 μl), and C: high motilin concentration (2.5 ng/ 600 μl). Administration of motilin or NMDA is indicated by a green or red arrow, respectively, in each of the graphs.

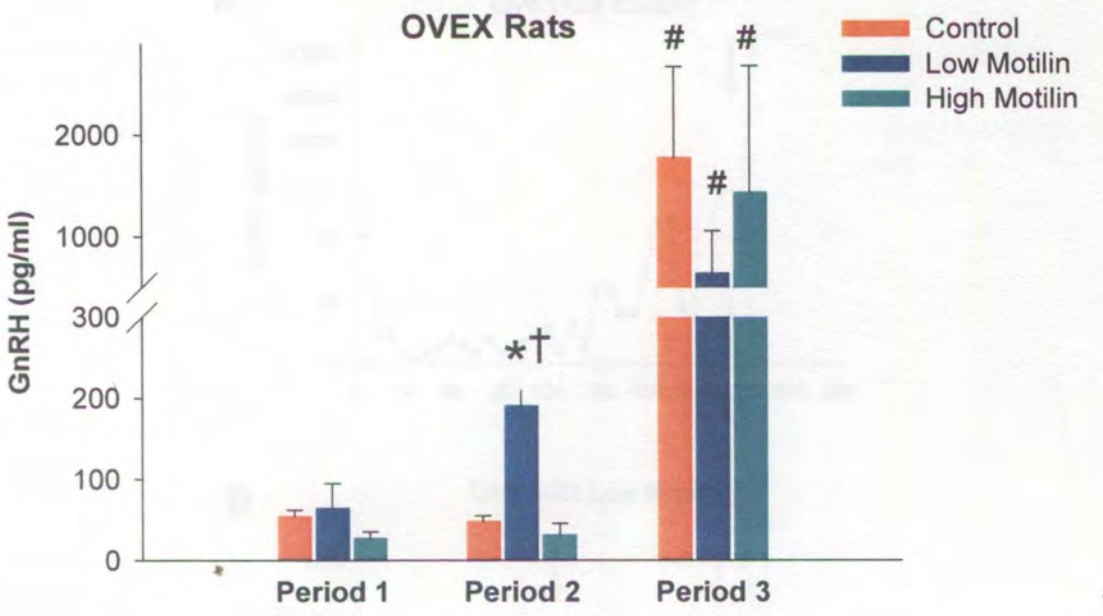

Figure 5. Comparison of mean GnRH concentration secreted by hypothalamic explants obtained from OVEX Sprague-Dawley rats during the different experimental periods. Period 1: first hour of experiment, perfused with regular aCSF (all groups); Period 2: second and third hours of experiment, receiving aCSF (control group) or motilin (Low and high motilin groups); Period 3: fourth hour of experiment, receiving 75 mM NMDA (all groups). Low motilin and high motilin groups were administered 1.4 ng/ 600 μl or 2.5 ng/ 600 μl, respectively. (n = 4-5/group). * indicates $p < 0.05$ vs. same group in period 1. † indicates $p < 0.05$ vs. males and OVEX+E_2 rats in period 2. # indicates $p < 0.05$ vs. same group in periods 1 and 2.

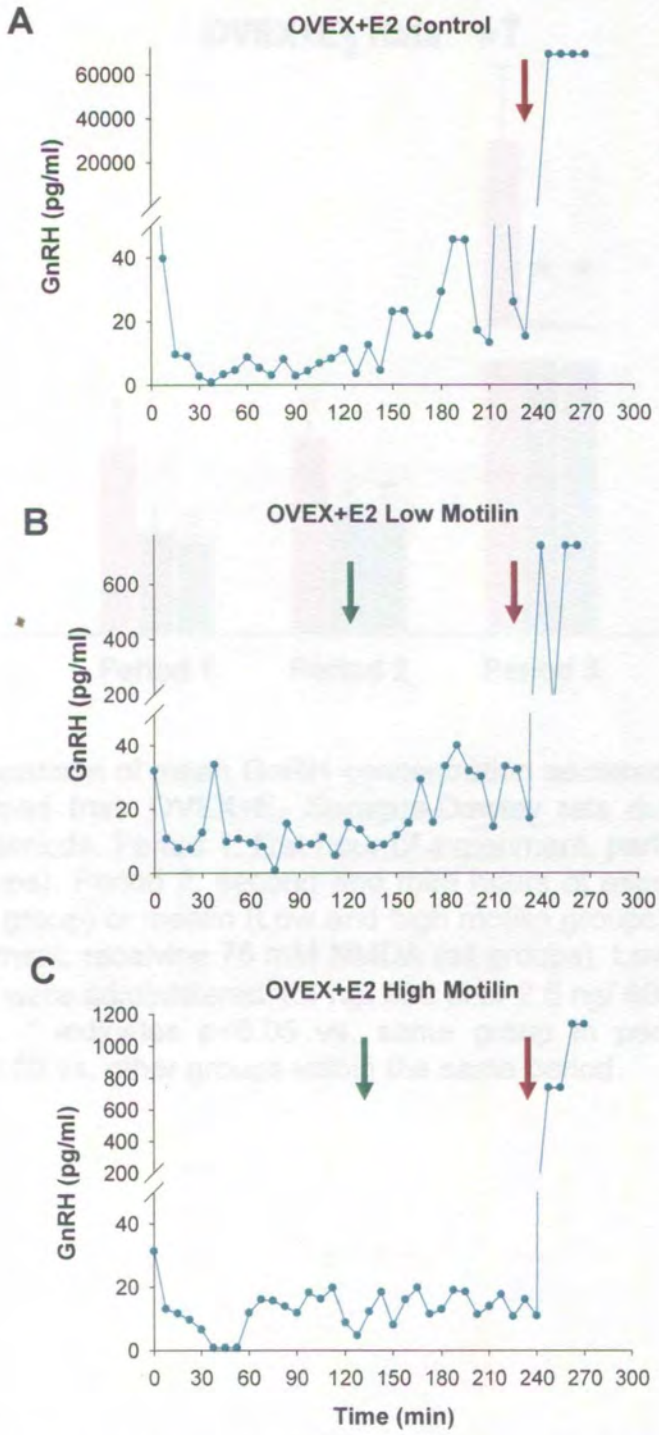

Figure 6. Representative *in vitro* GnRH secretion patterns from hypothalamic explants obtained from OVEX+E_2 Sprague-Dawley rats treated with: A: aCSF; B: low motilin concentration (1.4 ng/ 600 μl), and C: high motilin concentration (2.5 ng/ 600 μl). Administration of motilin or NMDA is indicated by a green or red arrow, respectively, in each of the graphs.

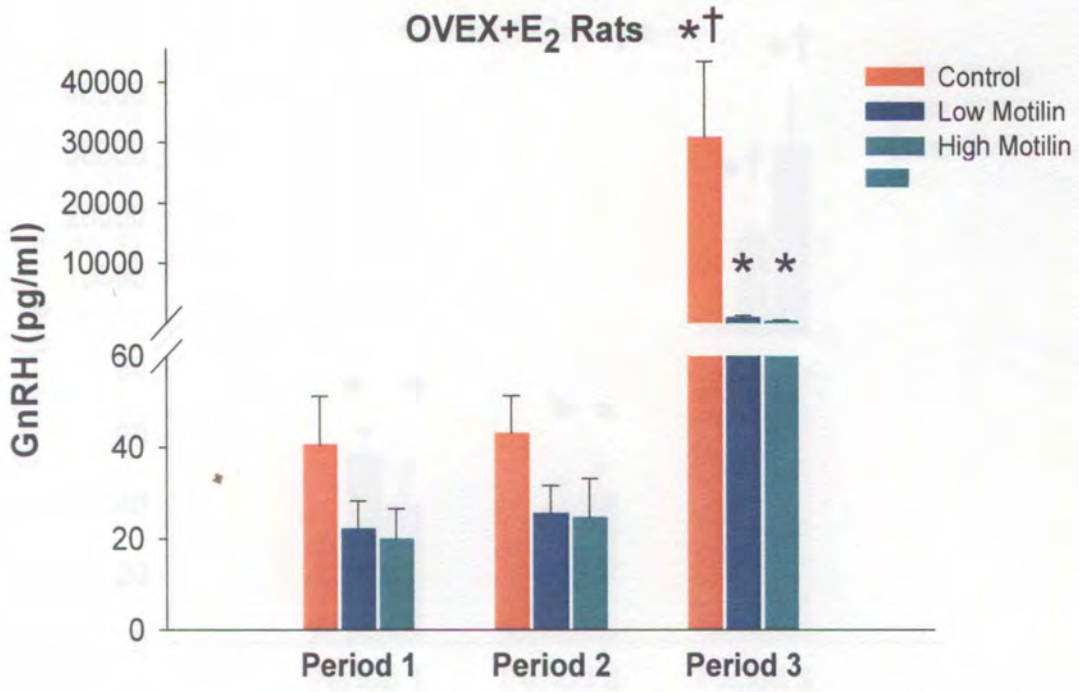

Figure 7. Comparison of mean GnRH concentration secreted by hypothalamic explants obtained from OVEX+E_2 Sprague-Dawley rats during the different experimental periods. Period 1: first hour of experiment, perfused with regular aCSF (all groups); Period 2: second and third hours of experiment, receiving aCSF (control group) or motilin (Low and high motilin groups); Period 3: fourth hour of experiment, receiving 75 mM NMDA (all groups). Low motilin and high motilin groups were administered 1.4 ng/ 600 μl or 2.5 ng/ 600 μl, respectively. (n = 5/group). * indicates $p<0.05$ vs. same group in periods 1 and 2; † indicates $p < 0.05$ vs. other groups within the same period.

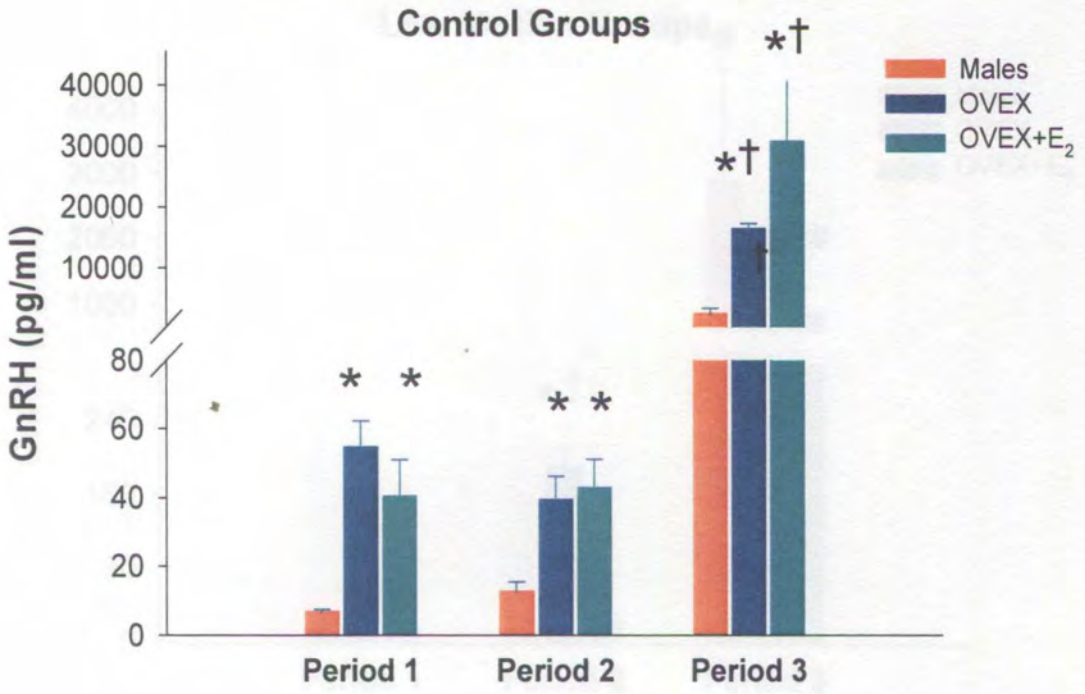

Figure 8. Comparison of mean GnRH concentration secreted during each experimental period by hypothalamic explants obtained from male, OVEX and OVEX+E_2 Sprague-Dawley rats perfused with regular aCSF for the length of the experiment. Period 1: first hour of experiment; Period 2: second and third hours of experiment; Period 3: fourth hour of experiment, receiving 75 mM NMDA (n = 4-5/group). * indicates $p < 0.05$ vs. male rats within the same period. † indicates $p < 0.05$ vs. same group in periods 1 and 2.

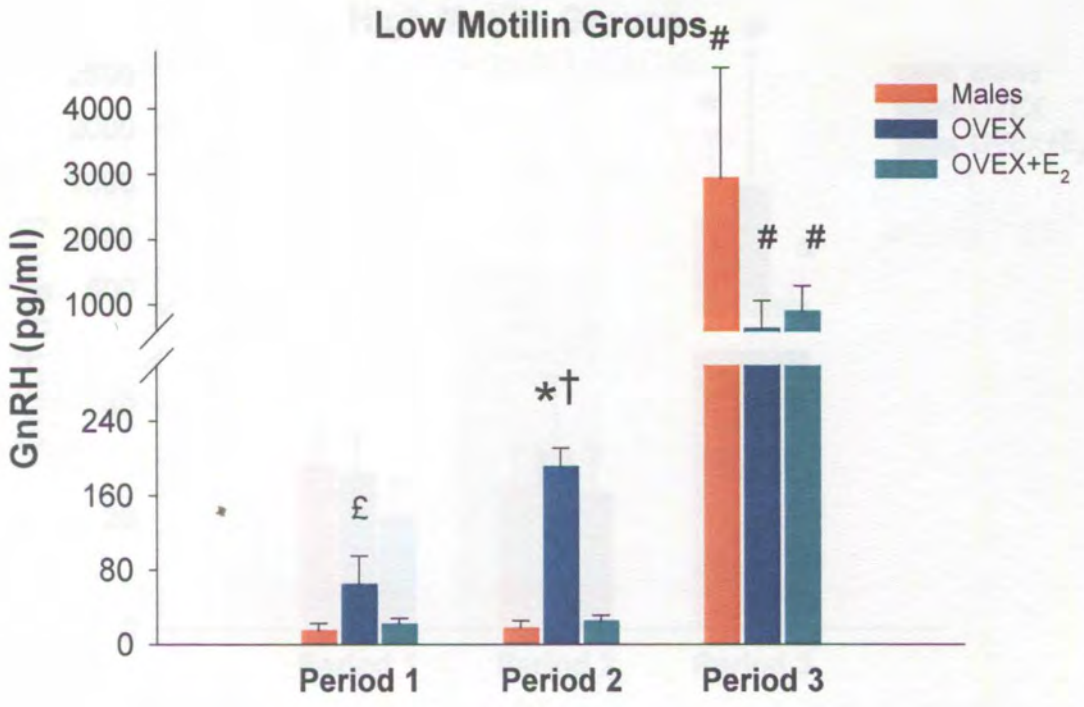

Figure 9. Comparison of mean GnRH concentration secreted during each experimental period by hypothalamic explants obtained from male, OVEX and OVEX+E_2 Sprague-Dawley rats. Period 1: first hour of experiment, perfused with regular aCSF; Period 2: second and third hours of experiment, receiving low concentration of motilin (1.4 ng/ 600 μl); Period 3: fourth hour of experiment, receiving 75 mM NMDA. (n = 4-5/group). * indicates $p < 0.05$ vs. same group in period 1. † indicates $p < 0.05$ vs. male and OVEX+E_2 within the same period. # indicates $p < 0.05$ vs. same group in periods 1 and 2. £ indicates $p < 0.05$ vs. males within the same period.

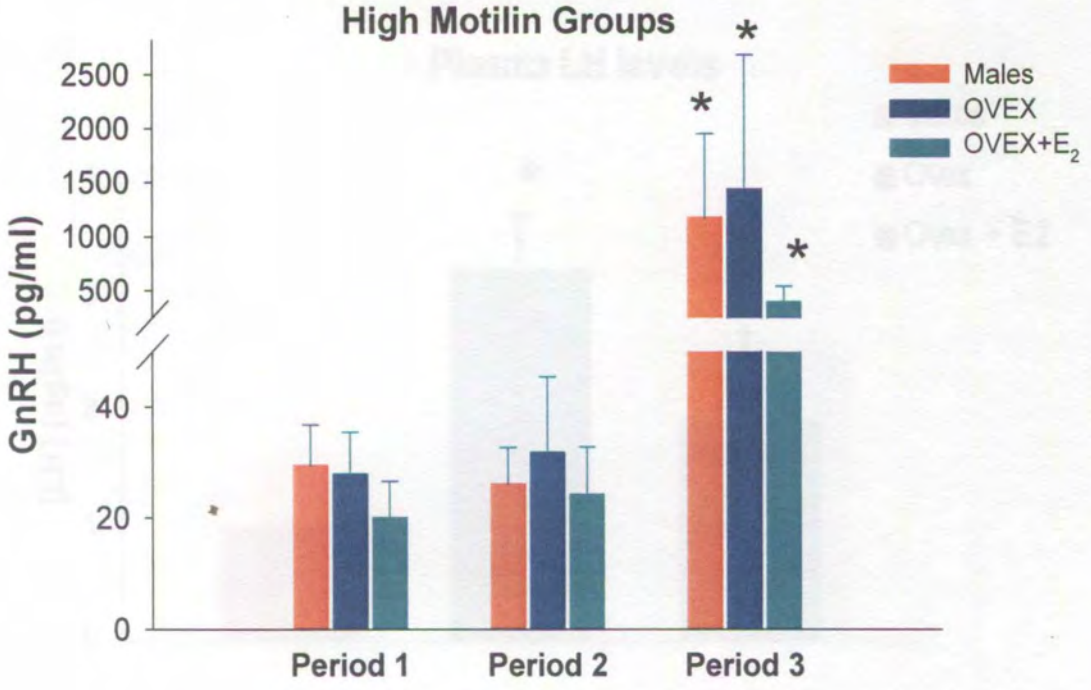

Figure 10. Comparison of mean GnRH concentration secreted during each experimental period by hypothalamic explants obtained from male, OVEX and OVEX+E_2 Sprague-Dawley rats. Period 1: first hour of experiment, perfused with regular aCSF; Period 2: second and third hours of experiment, receiving high concentration of motilin (2.5 ng/ 600 μl); Period 3: fourth hour of experiment, receiving 75 mM NMDA. (n = 4-5/group). * indicates $p < 0.05$ vs. same group in periods 1 and 2.

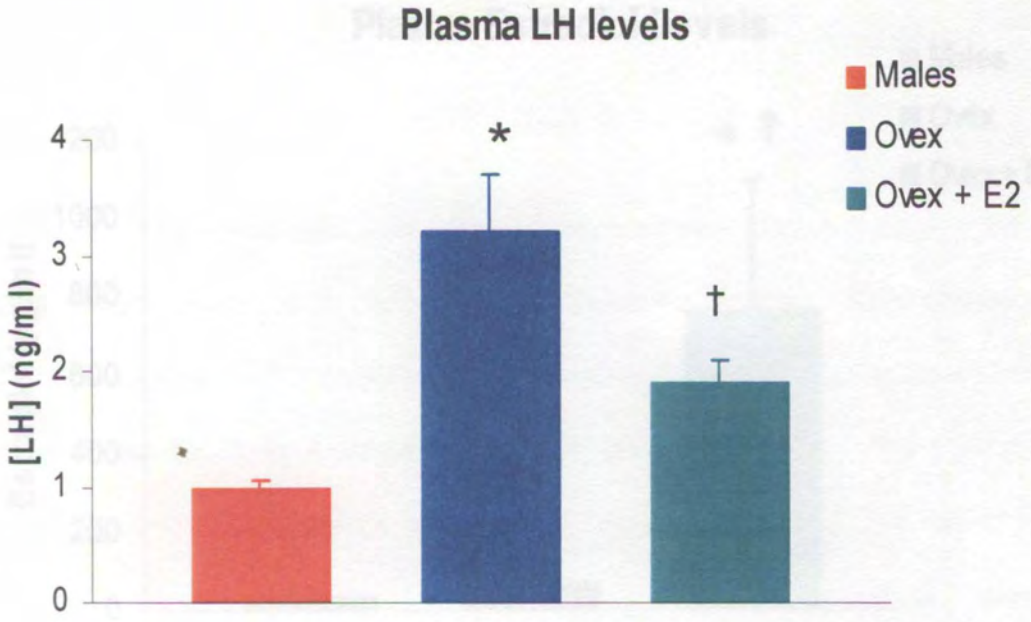

Figure 11. Average concentration of LH in plasma of males, OVEX and OVEX+E_2 Sprague-Dawley rats (n = 4-5/group). * and † indicate < 0.05 vs. males.

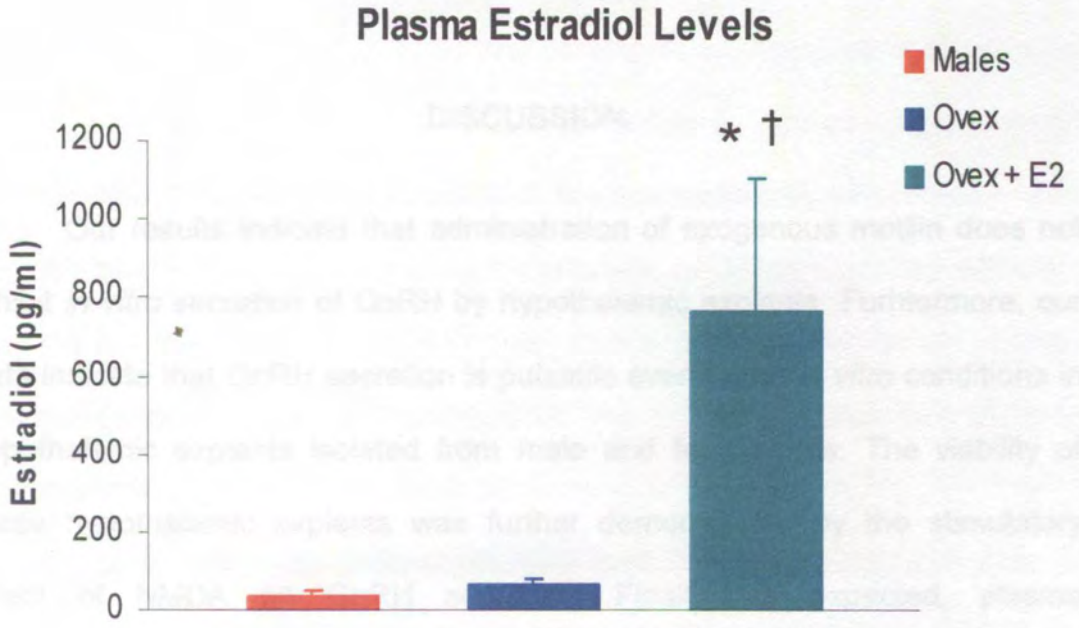

Figure 12. Average concentration of estradiol in plasma of males, OVEX and OVEX+E_2 Sprague-Dawley rats (n = 7-18/group). * indicates $p < 0.05$ vs. males and † indicates $p < 0.05$ vs. OVEX rats.

DISCUSSION

Our results indicate that administration of exogenous motilin does not inhibit *in vitro* secretion of GnRH by hypothalamic explants. Furhtermore, our data indicate that GnRH secretion is pulsatile even under *in vitro* conditions in hypothalamic explants isolated from male and female rats. The viability of these hypothalamic explants was further demonstrated by the stimulatory effect of NMDA on GnRH secretion. Finally, as expected, plasma concentrations of LH were significantly elevated in the OVEX rats when compared to either male or ovariectomized rats, and levels of plasma estradiol were significantly greater in OVEX+E_2 rats than in the other animal groups.

GnRH is produced in the hypothalamus and secreted into the hypophyseal portal blood, from where it reaches the anterior pituitary in order to control LH and FSH secretion (Beien et al., 1998). Both LH and FSH regulate gamete and hormone production in the gonads. Therefore, secretion of GnRH is essential for reproduction, and all factors, internal or external, that affect reproduction must do so through the regulation of either GnRH synthesis, secretion or both processes.

One of the most important factors known to suppress reproduction is undernutrition (Chagas et al., 2007; Foster et al., 1989); however, the identity

of the link between metabolic state and reproductive axis remains unclear. Tsukamura et al. reported in 2000 that peripheral or intracerebral injection of motilin, an intestinal peptide, into OVEX or OVEX+E_2 rats inhibited secretion of LH *in vivo*. The authors assumed that the mechanism by which motilin was exerting its effects was through the suppression of GnRH secretion, although they did not test this hypothesis in their studies. The present results indicate that exogenous motilin application does not inhibit secretion of GnRH in hypothalamic explants.

Results from the present studies indicate that GnRH secretion is pulsatile even under *in vitro* conditions in hypothalamic explants isolated from male and female rats. Interpulse intervals occurred approximately every 37 minutes, which is the characteristic pulsatile secretion followed by rats *in vivo* (Purnelle et al., 1997). These observations indicate that the isolation process was successful, and that the GnRH pulse generator was intact in those hypothalamic fragments after isolation. The viability of the hypothalamic explants was further confirmed when they were administered NMDA in the last period of the experiment. Administration of this glutamate mimetic produces the generalized depolarization of the cell membrane, leading to a massive secretion of GnRH in all the tested explants, further demonstrating their capability to produce and secrete GnRH.

Tonic release of GnRH from the hypothalamus occurs in a pulsatile manner in both male and female rats (Guillemin and Burgus, 1972;

Krsmanovic et al., 1992; Schally et al., 1972). However, in males the pituitary secretion of LH is under a tonic regulatory control, while in females the control depends on the phase of the ovarian cycle in which the animal is at.

Our results indicate that baseline levels of secreted GnRH are between 3 and 4 times greater in OVEX rats than intact males. This is due to the removal of the gonads in the OVEX rats. When ovariectomies are performed, the major source of estradiol in the body is removed, and therefore, the inhibitory feedback signal that this hormone exerts on the secretion of GnRH and LH is eliminated, increasing the secretion of these reproductive hormones. Intact males produce testosterone in their gonads, which signals the hypothalamus and pituitary to stop secreting GnRH and LH, respectively.

The hypothesis tested in these studies was that motilin would inhibit the secretion of GnRH; however the opposite was observed. Contrary to our expectations, administration of low concentration of motilin resulted in a significant increase in the secretion of GnRH by the OVEX explants, while it did not appear to have any effect on GnRH secretion by explants obtained from male and OVEX+E_2 rats. There are several possible reasons for this unexpected increase in GnRH secretion.

A possible explanation is that motilin could act through other neurotransmitters that, due to the isolation process, may not be present during our experiments. During the isolation, the hypothalamus is separated from

other areas of the brain that could be the source of neurotransmitters that could be mediating motilin actions on reproduction.

A second possibility is that motilin could be inducing GnRH secretion instead of inhibiting it. There have been reports in the literature indicating that GnRH secretion is dependent upon an increased intracellular concentration of Ca^{2+} (Krasmanoic et al., 1992), and motilin has been associated with the release of this ion from intracellular storage (Depoortere and Peeters, 1995). This evidence suggests that motilin could stimulate GnRH secretion by increasing the intracellular release of Ca^{2+}.

Motilin could also be acting as an antagonist of NPY, which is a neuropeptide produced in the paraventricular nucleus of the hypothalamus. NPY actions have been described to be very dependent on the steroid environment, increasing GnRH secretion in intact rodents and rabbits, but decreasing this secretion in the absence of gonadal steroids (Khorram et al., 1987; McDonald et al., 1989; Urban et al., 1996). In our OVEX rats, motilin could be antagonizing the action of NPY, and thus the increase in GnRH secretion observed in these animals. Y5 receptors for NPY have been implicated in the action of this neuropeptide on GnRH neurons (Lebrethon et al., 2000). It could be that motilin may be binding to those receptors, blocking the action of NPY, and, therefore, producing an increase of GnRH secretion as a consequence.

The putative inhibitory action of motilin could also be mediated by GABA neurons. GABA is the chief inhibitory neurotransmitter in the mammalian nervous system. In fact, GABA or inhibitory neurons have been described in different areas of the brain, including the hypothalamus (Vincent et al., 1982). As described before, it is possible that during the isolation of the hypothalamic explants there could be a disruption of the connections existing between different neurons and the hypothalamus. Motilin may mediate inhibition of GnRH secretion by stimulating GABA neurons to secrete this inhibitory neurotransmitter that could then act on GnRH neurons. If disruption of the cytoarchitecture occurred during isolation of the hypothalamic explants, GABA secretion could have been altered, and therefore, inhibition of GnRH secretion by motilin would not take place.

Other intermediates of motilin action could be endogenous opioid peptides, which are also known to decrease GnRH secretion by acting on GnRH neurons.

Interestingly, ghrelin, a member of the same family as motilin, has been shown to effectively reduce *in vitro* GnRH secretion in rat hypothalamic explants (Fernandez-Fernandez et al., 2005). The genes encoding for motilin and motilin receptor in rats and mice have not yet been found, in spite of these two genomes being completely sequenced. It has been proposed that ghrelin may have substituted motilin in rodents; therefore, it could also be possible that motilin receptors in GnRH neurons could have been substituted by ghrelin

receptors. The absence of motilin receptors in the hypothalamus would explain the lack of response to motilin in our experiments, and the divergent results obtained when using these two closely related peptides on hypothalamic explants.

Finally, it is also possible that the effects of exogenous motilin on *in vivo* LH secretion described by Tsukamura et al. could be hypothalamus-independent, and purely due to the direct action of this hormone on the pituitary or on other neurotransmitter systems acting exclusively at the level of the pituitary.

In order to determine if the action of motilin on GnRH secretion is dependent on the presence of estradiol, some of the OVEX rats used in these studies were implanted with an estradiol minipump. Implantation of such a minipump attempts to maintain a constant level of estradiol in the body of that animal, without the fluctuations that occur during a normal estrous cycle. The exogenous estradiol blunts the secretion of the reproductive hormones from the brain. Estradiol is a hormone that can cross cell membranes, and, therefore, it enters the target cells to bind to its intracellular receptors. Once estradiol binds to its receptor, both molecules form a complex that binds to the DNA, inhibiting the transcription of the target protein, in this case, GnRH. Our results indicate that the presence of estradiol did not facilitate the inhibitory effect of motilin on the secretion of GnRH.

In conclusion, the results presented in this thesis demonstrate that hypothalamic explants are capable of secreting GnRH in a pulsatile manner, and that those explants are responsive to NMDA stimulation, as demonstrated by the massive secretion of GnRH observed after administration of this glutamate mimetic. Our data also indicate that exogenous motilin administration did not inhibit hypothalamic GnRH secretion in male, OVEX and OVEX+E_2 Sprague-Dawley rats. These results may indicate that the effects of exogenous motilin on LH secretion may be hypothalamus-independent. Conversely, the effect of motilin may be hypothalamus-dependent whereby some other neurotransmitter systems that might have been disrupted in the hypothalamic explants are crucial for motilin action. Finally, it is possible that motilin does not affect the synthesis or secretion of GnRH but acts through the post-GnRH receptor binding at the level of the anterior pituitary.

REFERENCES

REFERENCES

Adachi H, Toda N, and Hayashi S. 1981. Mechanism of the excitatory action of motilin on isolated rabbit intestine. *Gastroenterology* **80**(4) 783-788.

Adrian T, Ferri GL, Bacarese-Hamilton AJ, Fuessl HS, Polak JM, and Bloom SR. 1985. Human distribution and release of a putative new gut hormone, peptide YY. *Gastroenterology* **89** 1070-1077.

Archer Z, Rhind SM, Findlay PA, Kyle CE, Thomas L, Marie M, and Adam CL. 2002. Contrasting effects of different levels of food intake and adiposity on LH secretion and hypothalamic gene expression in sheep. *J Endocrinol* **175** 383-393.

Armstrong J and Britt JH. 1987. Nutritionally-induced anestrus in gilts: metabolic and endocrine changes associated with cessation and resumption of estrous cycles. *J Anim Sci* **65** 508–523.

Asakawa A, Inui A, Momose K, Ueno N, Fujino MA, and Kasuga M. 1998. Motilin increases food intake in mice. *Peptides* **19**(6) 987-990.

Asakawa A, Inui A, Kaga T, Yuzuriha H, Nagata T, Ueno N, Makino S, Fujimiya M, Niijima A, Fujino MA, and Kasuga M. 2001. Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. *Gastroenterology* **120** 337–345.

Asmar M, and Holst JJ. 2010. Glucagon-like peptide 1 and glucose-dependent insulinotropic polypeptide: new advances. *Curr Opin Endocr Diab Obes* **17** 57–62.

Badger T, Lynch EA, and Fox PH. 1985. Effects of fasting on luteinizing hormone dynamics in the male rat. *J Nutr* **115** 788–797.

Banfield D, MacGillivray RT, Brown JC, and McIntosh CH. 1992. The isolation and characterization of rabbit motilin precursor cDNA. *Biochim Biophys Acta* **1131** 341-344.

Barash I, Cheung CC, Weigle DS, Ren H, Kabigting EB, Kuijper JL, Clifton DK, and Steiner RA. 1996. Leptin is a metabolic signal to the reproductive system. *Endocrinology* **137** 3144-3147.

Barb C and Kraeling RR. 2004. Role of leptin in the regulation of gonadotropin secretion in farm animals. *Anim Reprod Sci* **82-83** 155-167.

Barkan A, Jaffe CA, and Padmanabhan V. 1997. In vivo Investigation of Hypothalamic Secretory Activity. *TEM* **8** 105-111.

Barnett J and Owyang C. 1981. Serum glucose concentration as a modulator of interdigestive gastric motility. *Gastroenterology* **94** 739-744.

Barreiro M, and M Tena-Sempere.2004. Ghrelin and reproduction: a novel signal linking energy status and fertility? *Molecular and Cellular Endocrinology* **226** 1-9.

Batterham R, Cowley MA, Small CJ, Herzog H, Cohen MA, Dakin CL, Wren AM, Brynes AE, Low MJ, Ghatei MA, Cone RD, and Bloom SR. 2002. Gut hormone PYY (3-36) physiologically inhibits food intake. *Nature* **418** 650-654.

Batterham R, Cohen MA, Ellis SM, Le Roux CW, Withers DJ, Frost GS, Ghatei MA, and Bloom SR. 2003. Inhibition of food intake in obese subjects by peptide YY3-36. *N Engl J Med* **349** 941-948.

Beak S, Heath MM, Small CJ, Morgan DGA, Ghatei MA, Taylor AD, Buckingham JC, Bloom SR, and Smith DM. 1998. Glucagon-like Peptide-1 Stimulates Luteinizing Hormone–releasing Hormone Secretion in a Rodent Hypothalamic Neuronal Cell Line. *J Clin Invest* **101** 1334-1341.

Beien S, Virendra BM, Ganapathy KB, Lin P, and Darrell W. 1998. Evidence of a role of bradikinin neurons in the control of gonadotropin-releasing hormone secretion. *Neuroendocrinology* **67** 209-218.

Beinfeld M, Meyer DK, Eskay RL, Jensen RT, and Brownstein MJ. 1981. The distribution of cholecystokinin immunoreactivity in the central nervous system of the rat as determined by radioimmunoassay. *Brain Res* **212** 51–57.

Beinfeld M and Korchak DM. 1985. The regional distribution and the chemical, chromatographic, and immunologic characterization of motilin brain peptides: the evidence for a difference between brain and intestinal motilin-immunoreactive peptides. *J Neurosci* **5** 2502-2509.

Belchetz P, Plant TM, Nakai Y, Keogh EJ, and Knobil E. 1983. Hypophysial responses to continuous and intermittent delivery of hypopthalamic gonadotropin-releasing hormone. *Science* **202** 631-633.

Bell G, Santerre RF, and Mullenbach GT. 1976. Hamster preproglucagon contains the sequence of glucagon and two related peptides. *Nature* **302** 716–718.

Benson B, Matthews MJ, and Hruby VJ. 1976. Characterization and effects of a bovine pineal antigonadotropic peptide. *Amer Zool* **16** 17-24.

Benson B and Machena N. 1994. Infusion of a pineal gland-derived antigonadotropic decapeptide into the lateral ventricle depresses prolactin levels in male rats. *Life Sciences* **55** 263-268.

Bergendahl M, and Veldhuis JD. 1995. Altered pulsatile gonadotropin signaling in nutritional deficiency in the male. *TEM* **6** 145-159.

Bernard C, Valet JP, Beland R, and Lambert RD. 1983. Prediction of bovine ovulation by a rapid radioimmunoassay for plasma LH. *J Reprod Fert* **68** 425-430.

Blache D, Chagas LM, Blackberry MA, Vercoe PE, and Martin GB. 2000. Metabolic factors affecting the reproductive axis in male sheep. *J Reprod Fertil* **120** 1-11.

Blache D, Tellam RL, Chagas LM, Blackberry MA, Vercoe PE, and Martin GB. 2000. Level of nutrition affects leptin concentrations in plasma and cerebrospinal fluid in sheep. *J Endocrinol* **165** 625-637.

Blache D, Zhang S, and Martin GB. 2003. Fertility in male sheep: modulators of the acute effects of nutrition on the reproductive axis of male sheep. *Reprod* **61** 387-402.

Blache D, Zhang S, and Martin GB. 2006. Dynamic and integrative aspects of the regulation of reproduction by metabolic status in male sheep. *Reprod Nutr Dev* **46** 379–390.

Blache D, Chagas LM, and Martin GB. 2007. Nutritional inputs into the reproductive neuroendocrine control system—A multidimensional perspective. *Reprod Suppl* **64** 124–139.

Bond C, Nilaver G, Godfrey B, Zimmerman EA, Adelman JP. 1988. Characterization of complementary deoxyribonucleic acid for precursor of porcine motilin. *Mol Endocrinol* **2** 175-180.

Bourguignon J, Gerard A, Alvarez Gonzalez ML, Purnelle G, and Franchimont P. 1995. Endogenous glutamate involvement in pulsatile secretion of gonadotropin-releasing hormone: evidence from effect of glutamine and developmental changes. *Endocrinology* **136** 911-916.

Broberger C, Landry M, Wong H, Walsh JN, and Hökfelt T. 1997. Subtypes Y1 and Y2 of the neuropeptide Y receptor are respectively expressed in pro-opiomelanocortin- and neuropeptide-Y-containing neurons of the rat hypothalamic arcuate nucleus. *Neuroendocrinology* **66** 393-408.

Bronson F. 1986. Food-restricted, prepubertal, female rats: rapid recovery of luteinizing hormone pulsing with excess food, and full recovery of pubertal development with gonadotropin-releasing hormone. *Endocrinology* **118** 2483-2487.

Bronson F. 1988. Effect of food manipulation on the GnRH-LH-estradiol axis of young female rats. *Am J Phys* **254** R616-621.

Bronson F. 2000. Puberty and energy reserves: a walk on the wild side. *In:* Wallen K, Schneider JE, editors Reproduction in context: social and environmental influences on reproduction MIT Press; Cambridge 15-34.

Brown J and Dryburgh JR. 1971. A gastric inhibitory polypeptide. II. The complete amino acid sequence. *Can J Biochem* **49** 867-872.

Bruning J, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC, Klein R, Krone W, Muller-Wieland D, and Kahn CR. 2000. Role of brain insulin receptor in control of body weight and reproduction. *Science* **289** 2122-2125.

Bucholtz D, Vidwans NM, Herbosa CG, Schillo KK, and Foster DL. 1996. Metabolic interfaces between growth and reproduction. V. Pulsatile luteinizing hormone secretion is dependent on glucose availability. *Endocrinology* **137** 601-607.

Budak E, Fernández Sánchez M, Bellver J, Cerveró A, Simón C, and Pellicer A. 2006. Interactions of the hormones leptin, ghrelin, adiponectin, resistin, and PYY3-36 with the reproductive system. *Fertil Steril* **85** 1563-1581.

Buffa R, Solcia E, and Go VL. Immunohistochemical identification of the cholecystokinin cell in the intestinal mucosa. *Gastroenterology* **70** 528–532.

Burcelin R, Thorens B, Glauser M, Gaillard RC, and Pralong FP. 2003. Gonadotropin-releasing hormone secretion from hypothalamic neurons: stimulation by insulin and potentiation by leptin. *Endocrinology* **144** 4484-4491.

Cagampang FRA, Maeda K-I, Tsukamura H, Ohkura S, and Ota K. 1991. Involvement of ovarian steroids and endogenous opioids in the fasting-induced suppression of pulsatile LH release in ovariectomized rats. *J Endocrin* **129** 321-328.

Cagampang FRA, Maeda K-I and Ota K. 1992. Involvement of the gastric vagal nerve in the suppression of pulsatile LH release during acute fasting in rats. *Endocrinology* **130** 3003-3006.

Cameron J and Nosbisch C. 1991. Suppression of pulsatile luteinizing hormone and testosterone secretion during short term food restriction in the adult male rhesus monkey (Macaca mulatta). *Endocrinology* **128** 1532–1540.

Cameron J, Helmreich DL, and Schreihofer DA. 1993. Modulation of reproductive hormone secretion by nutritional intake: stress signals versus metabolic signals. *Human Reproduction* **8** 162-167.

Cameron J. 1996. Regulation of reproductive function in primates by short-term changes in nutrition. *Rev Reprod* **1** 117–126.

Caminos J, Nogueiras R, Gaytán F, Pineda R, González CR, Barreiro ML, Castaño JP, Malagón MM, Pinilla L, Toppari J, Diéguez C, and Tena-Sempere M. 2008. Novel expression and direct effects of adiponectin in the rat testis. *Endocrinology* **149** 3390-3402.

Carmel P, Araki S, and Ferin M. 1976. Pituitary stalk portal blood collection in rhesus monkeys: evidence for pulsatile release of gonadotropin-releasing hormone (GnRH). *Endocrinology* **99** 243–248.

Chabrolle C, Tosca L, and Dupont J. 2007. Regulation of adiponectin and its receptors in rat ovary by human chorionic gonadotrophin treatment and potential involvement of adiponectin in granulosa cell steroidogenesis. *Reproduction* **133** 719-731.

Chagas L, Bass JJ, Blache D, Burke CR, Kay JK, Lindsay DR, Lucy MC, Martin FB, Meier S, Rhodes FM, Roche JR, Thatcher WW, and Webb

R. 2007. Invited Review: New Perspectives on the Roles of Nutrition and Metabolic Priorities in the Subfertility of High-Producing Dairy Cows. *J Dairy Sci* **90** 4022-4032.

Chandrashekar V. and Bartke A. 1992. The influence of beta-endorphin on testicular endocrine function in adult rats. *Biology of Reproduction* **47** 1-5.

Chappell P, White RS, and Mellon PL. 2003. Circadian gene expression regulates pulsatile gonadotropin-releasing hormone (GnRH) secretory patterns in the hypothalamic GnRH-secreting GT1-7 cell line. *J Neurosci* **23** 11202-11213.

Charlton H. 2008. Hypothalamic control of anterior pituitary function: A history. *J Neuroendocrinology* **20** 641-646.

Cheng C and Leung PC. 2005. Molecular biology of gonadotropin-releasing hormone (GnRH)-I, GnRH-II, and their receptors in humans. *Endocr Rev* **26** 283-306.

Chey W, Escoffery FT, Roth F, Chang TM, and Yajrma H. 1980. Motilin-like immunoreactivity (MLI) in the gut and neurons of peripheral and central nervous system. *Regul Pept* Suppl **7** 519.

Chey W, and Lee KY. 1980. Actions of motilin on gastrointestinal motility and plasma immunoreactive motilin concentration in interdigestive and postprandial states. *Endocrinol Jpn* **27** Suppl 1.

Chik C, Ho AK, and Brown GM. 1987. Effect of food restriction on 24-h serum and pineal melatonin content in male rats. *Acta Endocrinologica* **115** 507–513.

Chowers I, Einat R, and Feldman S. 1969. Effects of starvation on levels of corticotropin releasing factor, corticotropin and plasma corticosterone in rats. *Acta Endocrinol (Copenh)* **61** 687–694.

Clarke I. and Cummins JT. 1982. The temporal relationship between gonadotropin releasing hormone (GnRH) and luteinizing hormone (LH) secretion in ovariectomized ewes. *Endocrinology* **111** 1737–1739.

Clarke I, Horton BB, and Doughton JB. 1990. Investigation of the mechanism by which insulin-induced hypoglycemia decreases luteinizing hormone secretion in ovariectomized ewes. *Endocrinology* **127** 1470–1476.

Clarke IJ, and Pompolo S. 2005. Synthesis and secretion of GnRH. *Anim Repr Sci* **88** 29-55.

Clayton R. 1989. Gonadotrophin-releasing hormone: its actions and receptors. *J Endocrinology* **120** 11–19.

Combs T, Pajvani UB, Berg AH, Lin Y, Jelicks LA, Laplante M, Nawrocki AR, Rajala MW, Parlow AF, Cheeseboro L, Ding YY, Russell RG, Lindemann D, Hartley A, Baker GR, Obici S, Deshaies Y, Ludgate M, Rossetti L, and Scherer PE. 2004. A transgenic mouse with a deletion in the collagenousdomain of adiponectin displays elevated circulating adiponectin and improved insulin sensitivity. *Endocrinology* **145** 367–383.

Crawley J, and Beinfeld MC. 1983. Rapid development of tolerance to the behavioral actions of cholecystokinin. *Nature* **302** 703–706.

Daikh D, Douglass JO, and Adelman JP. 1989. Structure and expression of the human motilin gene. *DNA* **8** 615-621.

Date Y, Kojima M, Hosoda H, Sawaguchi A, Mondal MS, Suganuma T, Matsukura S, Kangawa K, and Nakazato M. 2000. Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. *Endocrinology* **141** 4255-4261.

Day M, Imakawa K, Zalesky DD, Kittok RJ, and Kinder JE. 1986. Effects of restriction of dietary energy intake during the prepubertal period on secretion of luteinizing hormone and responsiveness of the pituitary to luteinizing hormone-releasing hormone in heifers. *J Anim Sci* **62** 1641–1647.

Dean A, and RG Dyer. 1984. Fasting reverses the response of neurons in the mediobasal hypothalamus (MBH) of female rats to electrical stimulation of the ventral noradrenergic tract (VNAT). *J Physiol* **353** 101.

De Clercq P, Depoortere I, and Peeters T. 1997. Isolation and sequencing of the cDNA encoding the motilin precursor from sheep intestine. *Gene* **202** 187-191(185).

De Clercq P, Vandermeers A, Van Assche G, Fiasse R, Depoortere I, Vandermeers-Piret MC, Deprez P, and Peeters T. 1995. Purification and amino acid sequence of human motilin isolated from a motilin containing liver metastasis. *Regul Pept* **55** 79–84.

Depoortere I, Peeters TL, Vandermeers A, Vandermeers-Piret MC, Christophe J, and Vantrappen G. 1993. Purification and amino acid sequence of motilin from cat small intestine. *Regul Pept* **49** 25–32.

Depoortere I, De Clercq P, Svoboda M, Bare L, and Peeters TL. 1997. Identification of motilin mRNA in the brain of man and rabbit. Conservation of polymorphism of the motilin gene across species. *Peptides* **18** 1497–1503.

Depoortere I. 2001. Motilin and motilin receptors: characterization and functional significance. *Verh K Acad Geneeskd Belg* **63** 511–529.

Diamant NE, Chung SA, and Hall KE. 1994. The vagus, motilin and the migrating motor complex. *Gastroenterology* **107**(6) 1912-3.

DiVall S, Radovick S, and Wolfe A. 2007. Egr-1 binds the GnRH promoter to mediate the increase in gene expression by insulin. *Mol Cell Endocrinol* **270** 64–72.

Dockray G. 1988. Regulatory peptides and the neuroendocrinology of gut-brain relations. *Quart J Exp Phys* **73** 703-727.

Doerr P, Fichter M, Pirke K-M, and Lund R. 1980. Relationship between weight gain and hypothalamic pituitary adrenal function in patients with anorexia nervosa. *J Steroid Biochem* **13** 529–537.

Dubey A, Cameron JL, Steiner RA, and T.M. 1986. Plant. Inhibition of gonadotropin secretion in castrated male rhesus monkeys (Macaca mulatta) induced by dietary restriction: analogy with the prepubertal hiatus of gonadotropin release. *Endocrinology* **118** 518–525.

Eissele R, Göke R, and Willemer S. 1992. Glucagon-like peptide-1 cells in the gastrointestinal tract and pancreas of rat, pig and man. *Eur J Clin Invest* **22** 283–291.

Faletti A, Mohn C, Farina M, Lomniczi A, and Rettori V. 2003. Interaction among beta-endorphin, nitric oxide and prostaglandins during ovulation in rats. *Reproduction* **125** 469–477.

Fernández-Fernández R, Aguilar E, Tena-Sempere M, and Pinilla L. 2004. Effects of polypeptide YY3-36 upon luteinizing hormone-releasing hormone and gonadotropin secretion in prepubertal rats: in vivo and in vitro studies. *Endocrinology* **146** 1403–1410.

Fernández-Fernández R, Tena-Sempere M, Navarro VM, Barreiro ML, Castellano JM, Aguilar E, and Pinilla L. 2005. Effects of ghrelin upon gonadotropin-releasing hormone and gonadotropin secretion in adult female rats: in vivo and in vitro studies. *Neuroendocrinology* **82** 245–255.

Fernández-Fernández R, Navarro VM, Barreiro ML, Vigo EM, Tovar S, Sirotkin AV, Casanueva FF, Aguilar E, Dieguez C, Pinilla L, and Tena-Sempere M. 2006. Effects of chronic hyperghrelinemia on puberty onset and pregnancy outcome in the rat. *Endocrinology* **146** 3018–3025.

Fernández-Fernández R, Martini AC, Navarro VM, Castellano JM, Dieguez C, Aguilar E, Pinilla L, and Tena-Sempere M. 2006. Novel signals for the integration of energy balance and reproduction. *Mol Cell Endocrinol* **254-255** 127–132.

Fernández-Fewell G, and Meredith M. 1995. Facilitation of mating behavior in male hamsters by LHRH and AcLHRH5-10: interaction with the vomeronasal system. *Physiol Behav* **57** 213-221.

Foster D, Ebling FJ, and Micka AF. 1989. Metabolic interfaces between growth and reproduction. I. Nutritional modulation of gonadotropin, prolactin, and growth hormone secretion in the growth-limited female lamb. *Endocrinology* **125** 342–350.

Foster D and Bucholtz DC. 1995. Glucose as a possible metabolic cue timing puberty. *In: Bergada C, Moguilevsky JA, editors Serono International Symposium on Puberty: basic and clinical aspects Rome, Italy: Ares-Serono Symposia.*

Furuta M, Funabashi T, and Kimura F. 2001. Intracerebroventricular administration of ghrelin rapidly suppresses pulsatile luteinizing hormone secretion in ovariectomized rats. *Biochem Biophys Res Commun* **288** 780–785.

Furuta M, T Funabashi, and F Kimura. 2002. Suppressive action of orexin A on pulsatile luteinizing hormone secretion is potentiated by a low dose of oestrogen in ovariectomised rats. *Neuroendocrinology* **75** 151-157.

Garthwaite T. 1985. Peripheral motilin administration stimulates feeding in fasted rats. *Peptides* **6** 41–44.

Gasparini P, Grifa A, Savasta S, Merlo I, Bisceglia L, Totaro A, and Zelante L. 1994. The motilin gene: subregional localisation, tissue expression, DNA polymorphisms and exclusion as a candidate gene for the HLA-associated immotile cilia syndrome. *Hum Genet* **94** 671–674.

Gaytan F, Morales C, Barreiro ML, Jeffery P, Chopin LK, Herington AC, Casanueva FF, Aguilar E, Dieguez C, Tena-Sempere M. 2005. Expression of growth hormone secretagogue receptor type 1a, the functional ghrelin receptor, in human ovarian surface epithelium, mullerian duct derivatives, and ovarian tumors. *J Clin Endocrinol Metab* **90** 1798–1804.

Gazal O, Leshin LS, Stanko RL, Thomas MG, Keisler DH, Anderson LL, and Williams GL. 1998. Gonadotropin-Releasing Hormone Secretion into Third-Ventricle Cerebrospinal Fluid of Cattle: Correspondence with the Tonic and Surge Release of Luteinizing Hormone and Its Tonic Inhibition by Suckling and Neuropeptide Y. *Biol Reprod* **59** 676–683.

Ghatei M, and Bloom SR. 2003. Orexin A interactions in the hypothalamo-pituitary gonadal axis. *Endocrinology* **142** 5294-5302.

Giacobini P, AS Kopin, PM Beart, LD Mercer, A Fasolo, and S Wray. 2004. Cholecystokinin Modulates Migration of Gonadotropin-Releasing Hormone-1 Neurons. *The Journal of Neuroscience* **24** 4737–4748.

Gibbs J, Young RC, and Smith GP. 1973. Cholecystokinin elicits satiety in rats with open gastric fistulas. *Nature* **245** 323–325.

Gill C, and Rissman EF. 1997. Female sexual behavior is inhibited by short- and long-term food restriction. *Physiol Behav* **61** 387–394.

Glass AR, and Swerdloff RS. 1977. Serum gonadotropins in rats fed a low-valine diet. *Endocrinology* **101** 702–707.

Greenman Y, Rouach V, Limor R, Gilad S, and Stern N. 2009. Testosterone is a strong correlate of ghrelin levels in men and postmenopausal women. *Neuroendocrinology* **89** 79-85.

Grewal T, Mickelson O, and Hafs HD. 1971. Androgen secretion and spermatogenesis in rats following semistarvation. *Proc Soc Exp Biol Med* **138** 723-727.

Griffin M, South SA, and Yankov VI. 1994. Insulin-dependent diabetes mellitus and menstrual dysfunction. *Ann Med* **26** 331–340.

Gruenewald D, and Matsumoto AM. 1993. Reduced gonadotropin-releasing hormone gene expression with fasting in the male rat brain. *Endocrinology* **132** 480–482.

Gueorguiev M, Goth ML, and Korbonits M. 1972. Leptin and puberty: a review. *Pituitary* 4: 79-86, 2001.Guillemin R, and Burgus, R. The hormones of the hypothalamus. *Sci Amer* **227** 24–33.

Gutzwiller J, Göke B, and Drewe J. 1999. Glucagon-like peptide-1: a potent regulator of food intake in humans. *Gut* **44** 81–86.

Hagan J, Leslie RA, and Patel S. 1999. Orexin A activates locus coeruleus cell firing and increases arousal in the rat. *Proc Natl Acad Sci* **96** 10911–10916.

Hameed S, Dhillo WS, and Bloom SR. 2009. Gut hormones and appetite control. *Oral Diseases* **15** 18–26.

Haqq A, Grambow SC, Muehlbauer M, Newgard CB, Svetkey LP, Carrel AL, Yanovski JA, Purnell JQ, and Freemark M. 2008. Ghrelin concentrations in Prader-Willi syndrome (PWS) infants and children: changes during development. *Clin Endocrinol* **69** 911–920.

Hasegawa Y, Miyamoto K, Igarashi M, Chino N, and Sakakibara S. 1984. Biological properties of chicken luteinizing hormone-releasing hormone: gonadotropin release from rat and chicken cultured anterior pituitary cells and radioligand analysis. *Endocrinology* **114** 1441–1447.

Hashimoto R and Kimura F. 1986. Inhibition of gonadotropin secretion induced by cholecystokinin implants in the medial preoptic area by the dopamine receptor blocker, pimozide, in the rat. *Neuroendocrinology* **42** 32–37.

He J, Irwin DM, Chen R, and Z Y-P. 2010. Stepwose loss of motilin and its specific receptor genes in rodents. *J Mol Endo* **44** 37–44.

Henry B, Goding JW, Tilbrook AJ, Dunshea FR, and Clarke IJ. 2001. Intracerebroventricular infusion of leptin elevates the secretion of luteinizing hormone without affecting food intake in long-term food-restricted sheep, but increases growth hormone irrespective of bodyweight. *J Endocrinol* **168** 67–77.

Herbison A. 1998. Multimodal influence of estrogen upon gonadotropin-releasing hormone neurons. *Endocrin Reviews* **19** 302–330.

Herrmann C, Göke R, Richter G, Fehmann HC, Arnold R, and Göke B. 1995. Glucagon-like peptide-1 and glucose-dependent insulin-releasing polypeptide plasma levels in response to nutrients. *Digestion* **56** 117–126.

Hill J, Szekeres P, Muir A, and Sanger GJ. 2002. Molecular, functional and cross-species comparisons between the receptors for the prokinetic neuropeptides, motilin and ghrelin. *Gastroenterology* **122** Supp 1: A54.

Howland B and Skinner KR. 1973. Effect of starvation on LH levels in male and female hamsters. *J Reprod Fert* **32** 505–507.

Howland B. 1975. The influence of feed restriction and subsequent refeeding on gonadotropin secretion and serum testosterone levels in male rats. *J Reprod Fert* **44** 429-436.

Hoyda T, Samson WK, and Ferguson AV. 2009. Adiponectin depolarizes parvocellular paraventricular nucleus neurons controlling neuroendocrine and autonomic function. *Endocrinology* **150** 832–840.

Hoyt E, Lund PK, and Winesett DE. 1996. Effects of fasting, refeeding, and intraluminal triglyceride on proglucagon expression in jejunum and ileum. *Diabetes* **45** 434–439.

Huang W, M Acosta-Martínez, TH Horton, and JE Levine. 2008. Fasting-Induced Suppression of LH Secretion Does Not Require Activation of ATP-Sensitive Potassium (KATP) Channels. *Am J Physiol Endocrinol Metab* **295** E1439–E1446.

Huang Z, De Clercq P, Depoortere I, and Peeters TL. 1998. Isolation and sequence of cDNA encoding the motilin precursor from monkey intestine. Demonstration of the motilin precursor in the monkey brain. *FEBS Lett* **435** 149–152.

Huang Z, Depoortere I, De Clercq P, and Peeters T. 1999. Sequence and characterization of cDNA encoding the motilin precursor from chicken, dog, cow and horse. Evidence of mosaic evolution in prepromotilin. *Gene* **240** 217–226.

Imura H, Seino Y, Mori K, Itoh Z, and Yanaihara N. 1980. Plasma motilin levels in normal subjects and patients with diabetes mellitus and certain other diseases. Fasting levels and responses to food and glucose. *Endocrinol Jpn* **27**(Suppl 1) 151–155.

Itoh Z. 1977. Motilin-a hormone to control interdigestive gut motility. *Seikagaku Apr* **49** 197–212.

Itoh Z. 1978. Interdigestive gastrointestinal motor activity controlled by motilin in conscious dogs. *Nippon Heikatsukin Gakkai Zasshi* **14** Suppl 43–44.

Itoh Z. 1980. Humoral control of gastrointestinal motility. *Horumon To Rinsho* **28** 1161–1168.

Itoh Z, Mizumoto A, Iwanaga Y, Yoshida N, Torii K, and Wakabayashi K. 1991. Involvement of 5-hydroxytryptamine 3 receptors in regulation of interdigestive gastric contractions by motilin in the dog. *Gastroenterology* **100**(4) 901–908.

Itoh Z. 1997. Motilin and clinical application. *Peptides* **18** 593–608.

Jia Y, Liu CQ, Tang M, and Jiang ZY. 2007. Expression of motilin in the hypothalamus and the effect of central erythromycin on gastric motility in diabetic rats. *Neurosci Bull* **23** 75–82.

Jin S, Han VK, Simmons JG, Towle AC, Lauder JM, and Lund PK. 1988. Distribution of glucagon-like peptide I (GLP-I), glucagon, and glicentin in the rat brain: an immunocytochemical study. *J Comp Neurol* **271** 519–532.

Jones J, Pick RR, and Davenport MD. 2002. Disinhibition of female sexual behavior by a CRH receptor antagonist in Syrian hamsters. *Am J Physiol Regul Integr Comp Physiol* **283** R591–R597.

Jones S. 1987. A muscarine-resistant M-current in C cells of bullfrog sympathetic ganglia. *Neurosci Lett* **74** 309–314.

Judd S. 1998. Disturbance of the reproductive axis induced by negative energy balance. *Reprod Nutr Dev* **10**.

Kanse S, Kreymann B, Ghatei MA, and Bloom SR. 1988. Identification and characterization of glucagon-like peptide-1 7-36 amide-binding sites in the rat brain and lung. *FEBS Lett* **241** 209–212.

Kauffman A. 2004. Emerging Functions of Gonadotropin-Releasing Hormone II in Mammalian Physiology and Behaviour. *Journal of Neuroendocrinology* **16** 794–806.

Kauffman A and Rissman EF. 2004. A critical role for the evolutionarily conserved gonadotropin-releasing hormone II: mediation of energy status and female sexual behavior. *Endocrinology* **145** 3639–3646.

Keire D, Mannon P, Kobayashi M, Walsh JH, Solomon TE, and Reeve JR Jr. 2000. Primary structures of PYY, [Pro(34)]PYY, and PYY-(3-36) confer different conformations and receptor selectivity. *Am J Physiol Gastrointest Liver Physiol* **279** G126-131.

Kerdelhue B, Lenoir V, Kolm P, Seltman HJ, Jones HW Jr, and Jones GS. 1997. ACTH, beta-endorphin, substance P, and corticotrophin releasing hormone in plasma and follicular fluid in hormonally stimulated menstrual cycles for in-vitro fertilization in the human. *Hum Reprod* **12** 231–235.

Khorram O, Pau KYF, Spies HG. 1987. Bimodal effects of neuropeptide Y on hypothalamic release of gonadotrophin-releasing hormone in conscious rabbits. *Neuroendocrinology* **45** 290–297.

Kile J, Alexander BM, Moss GE, Hallford DM, and Nett TM. 1991. Gonadotropin-releasing hormone overrides the negative effect of reduced dietary energy on gonadotropin synthesis and secretion in ewes. *Endocrinology* **128** 843–849.

Kimura F, and Funabashi T. 1998. Two Subgroups of gonadotropin releasing hormone neurons control gonadotropin secretion in rats. *News Physiol Sci* **13** 225–231.

Kissileff H, Pi-Sunyer FX, Thornton J, and Smith GP. 1981. C-terminal octapeptide of cholecystokinin decreases food intake in man. *Am J Clin Nutr* **34** 154–160.

Kitamura N, Yamada J, Calingasan NY, and Yamashita T. 1984. Immunocytochemical distribution of endocrine cells in the gastrointestinal tract of the horse. *Equine Vet J* **16** 103–107.

Knobil E. 1980. The neuroendocrine control of the menstrual cycle. *Recent Prog Horm Res* **36** 53–88.

Koch Y, Wilchek M, Fridkin M, Chobsieng P, Zor U, and Lindner HR. 1973. Production and characterization of an antiserum to synthetic gonadotropin-releasing hormone. *Biochem Biophys Res Commun* **55** 616–622.

Kohsaka A, Watanobe H, Kakizaki Y, Suda T, and Schio HB. 2001. A significant participation of orexin-A, a potent orexigenic peptide, in the preovulatory luteinizing hormone and prolactin surges in the rat. *Brain Res* **898** 166–170.

Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, and Kangawa K. 1999. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* **402** 656–660.

Korchak D, Nilaver G, and Beinfeld MC. 1984. The development of motilin-like immunoreactivity in the rat cerebellum and pituitary as determined by radioimmunoassay. *Neuroscience Letters Volume* **48** 267–272.

Korner A, Kratzsch J, and Kiess W. 2005. Adipocytokines, leptin–the classical, resistin – the controversical, adiponectin – the promising, and more to come. *Best Pract Res Clin Endocrinol Metab* **19** 525–546.

Kovacs P, Morales JC, and Karkanias GB. 2003. Central insulin administration maintains reproductive behavior in diabetic female rats. *Neuroendocrinology* **78** 90–95.

Kreymann B, Ghatei MA, Burnet P, Williams G, Kanse S, Diani AR, and Bloom SR. 1989. Characterization of glucagon-like peptide-1-(7-36) amide in the hypothalamus. *Brain Res* **502** 325–331.

Krsmanovic LZ, Stojilkovic SS, Merelli F, Dufour SM, Virmani MA, and Catt KJ. Calcium signaling and episodic secretion of gonadotropin-releasing hormone in hypothalamic neurons. *Proc Natl Acad Sci USA* **89** 8462–8466.

Kubota N, Terauchi Y, Yamauchi T, Kubota T, Moroi M, Matsui J, Eto K, Yamashita T, Kamon J, Satoh H, Yano W, Froguel P, Nagai R, Kimura S, Kadowaki T, and Noda T. 2002. Disruption of adiponectin causes insulin resistance and neointimal formation. *J Biol Chem* **277** 25863–25866.

Lanfranco F, Bonelli L, Baldi M, Me E, Broglio F, and Ghigo E. 2008. Acylated ghrelin inhibits spontaneous luteinizing hormone pulsatility and responsiveness to naloxone but not that to gonadotropin-releasing hormone in young men: evidence for a central inhibitory action of ghrelin on the gonadal axis. *J Clin Endocrinol Metab* **93** 3633–3639.

Lange W, Unger J, Pitzl H, and Weindl A. 1986. Is motilin a cerebellar peptide in the rat? *Anat Embryol* **173** 371–376.

Lebrethon MC, Vandermissen E, Gérard A, Parent S, Junien JL and Bourguignon. 2000. *In vitro* stimulation of the prepubertal rat gonadotropin-releasing hormone pulse generator by leptin and neuropeptide y through distinct mechanisms. *Endocrinology* **141**(4) 1464–1469.

Lee K, Shiratori K, Chen YF, Chang TM, and Chey WY. 1986. A hormonal mechanism for the interdigestive pancreatic secretion in dogs. *Am J Physiol* **251** G759–764.

Lee KY, Chang M, and Chey WY. 1983. Effects of rabbit antimotilin serum on myoelectric activity and plasma motilin concentration in fasting dog. *Am J Physiol* **245**(4) G547–553.

Levine J, and Ramirez VD. 1980. In vivo release of luteinizing hormone-releasing hormone estimated with push-pull cannulae from the mediobasal hypothalami of ovariectomized, steroid-primed rats. *Endocrinology* **107** 1782–1790.

Li C, Kaba H, Saito H, and Seto K. 1992. Cholecystokinin: critical role in mediating olfactory influences on reproduction. *Neuroscience* **48** 707–713.

Li C, Chen P, and Smith MS. 1999. Morphological evidence for direct interaction between arcuate nucleus neuropeptide Y (NPY) neurons and gonadotropin releasing hormone neurons and the possible involvement of NPY Y1 receptors. *Endocrinology* **140** 5382–5390.

Liddle R, Goldfine ID, Rosen MS, Taplitz RA, and Williams JA. 1985. Cholecystokinin bioactivity in human plasma. Molecular forms, responses to feeding, and relationship to gallbladder contraction. *J Clin Invest* **75** 1144–1152.

Liu J, Prudom CE, Nass R, Pezzoli SS, Oliveri MC, Johnson ML, Veldhuis P, Gordon DA, Howard AD, Witcher DR, Geysen HM, Gaylinn BD, and Thorner MO. 2008. Novel ghrelin assays provide evidence for independent regulation of ghrelin acylation and secretion in healthy young men. *J Clin Endocrinol Metab* **93** 1980–1987.

Lorenzi T, Meli R, Marzioni D, Morroni M, Baragli A, Castellucci M, Gualillo O, and Muccioli G. 2009. Ghrelin: a metabolic signal affecting the reproductive system. *Cytokine Growth Factor Rev* **20** 137–152.

Lüdtke FE, Müller H, and Golenhofen K. 1989. Direct effects of motilin on isolated smooth muscle from various regions of the human stomach. *Pflugers Archiv European Journal of Physiology* **414**(5) 558–563.

Ma K, Cabrero A, Saha PK, Kojima H, Li L, Chang BH, Paul A, and Chan L. 2002. Increased beta -oxidation but no insulin resistance or glucose intolerance in mice lacking adiponectin. *J Biol Chem* **277** 34658–34661.

Maeda K, Cagampang FR, Coen CW, and Tsukamura H. 1994. Involvement of the catecholaminergic input to the paraventricular nucleus and of corticotropin-releasing hormone in the fasting-induced suppression of luteinizing hormone release in female rats. *Endocrinology* **134** 1718–1722.

Maeda K, Tsukamura H, Ohkura S, Kawakami S, Nagabukuro H, and Yokoyama A. 1995. The LHRH pulse generator: a mediobasal hypothalamic location. *Neurosci Biobehav Rev* **19** 427–437.

Maeda N, Shimomura I, Kishida K, Nishizawa H, Matsuda M, Nagaretani H, Furuyama N, Kondo H, Takahashi M, Arita Y, Komuro R, Ouchi N, Kihara S, Tochino Y, Okutomi K, Horie M, Takeda S, Aoyama T,

Funahashi T, and Matsuzawa Y. 2002. Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. *Nat Med* **8** 731–737.

Manning J and Bronson FH. 1991. Suppression of puberty in rats by exercise: effects on hormone levels and reversal with GnRH infusion. *Am J Physiol Endocrinol Metab* **260** R717–723.

Marie M, Findlay PA, Thomas L, and Adam CL. 2001. Daily patterns of plasma leptin in sheep: effects of photoperiod and food intake. *J Endocrinol* **170** 277–286.

Martin G, and Walkden-Brown, SW. 1995. Nutritional influences on reproduction in mature male sheep and goats. *J Reprod Fert* **49** 437–449.

Martini A, Fernández-Fernández R, Tovar S, Navarro VM, Vigo E, Vazquez MJ, Davies JS, Thompson NM, Aguilar E, Pinilla L, Wells T, Dieguez C, and Tena-Sempere M. 2006. Comparative analysis of the effects of ghrelin and unacylated ghrelin on luteinizing hormone secretion in male rats. *Endocrinology* **147** 2374–2382.

Martos-Moreno G, Chowen JA, and Argente J. 2009. Metabolic signals in human puberty: Effects of over and undernutrition. *Mol Cell Endocrinol* [Epub ahead of print].

Matson C, Reid DF, Cannon TA, and Ritter RC. 2000. Cholecystokinin and leptin act synergistically to reduce body weight. *Am J Physiol Regul Integr Comp Physiol* **278** R882–R890.

Mazumdar M, Singru P, Sakharkar A, Deshmukh M, and Subhedar N. Glucagon-like peptide-1 (GLP-1) immunoreactivity in the forebrain and pituitary of the teleost, Clarias batrachus (Linn.). *Fish Physiology and Biochemistry* **31** 173–176.

McDonald JK, Lumpkin MD, DePaolo LV. 1989. Neuropeptide Y suppresses pulsatile secretion of luteinizing hormone in ovariectomized rats: possible site of action. *Endocrinology* **125** 186–191.

Millar R and King JA. 1983. Synthesis, luteinizing hormone-releasing activity, and receptor binding of chicken hypothalamic luteinizing hormone-releasing hormone. *Endocrinology* **113** 1364–1369.

Millar R, Lu Z-L, Pawson AJ, Flanagan CA, Morgan K, and Maudsley SR. 2004. Gonadotropin-Releasing Hormone Receptors. *Endocrine Reviews* **25** 235–275.

Miller D, Blache D, and Martin GB. 1995. The role of intracerebral insulin in the effect of nutrition on gonadotrophin secretion in mature male sheep. *J Endocrinol* **147** 321–329.

Miller D, Findlay PA, Morrison MA, Raver N, and Adam CL. 2002. Seasonal and dose-dependent effects of intracerebroventricular leptin on lh secretion and appetite in sheep. *J Endocrinol* **175** 395–404.

Mochiki E, Satoh M, Tamura T, Haga N, Suzuki H, Mizumoto A, Sakai T, and Itoh Z. Exogenous motilin stimulates endogenous release of motilin

through cholinergic muscarinic pathways in the dog. *Gastroenterology* 111: 1456-1464, 1996.

Moran T and McHugh PR. 1982. Cholecystokinin suppresses food intake by inhibiting gastric emptying. *Am J Physiol* **242** R491–R497.

Moran T, Robinson PH, Goldrich MS, and McHugh PR. 1986. Two brain cholecystokinin receptors: implications for behavioral actions. *Brain Res* **362** 175–179.

Moss R, and McCann SM. 1973. Induction of mating behavior in rats by luteinizing hormone-releasing factor. *Science* **181** 177–179.

Mouse Genome Database, Jackson Laboratories, Bar Harbor, Maine. World Wide Web (URL: www.informatics.jax.org). Data retrieved on May 14, 2010.

Murahashi K, Bucholtz DC, Nagatani S, Tsukahara S, Tsukamura H, Foster DL, and Maeda K-I. 1996. Suppression of LH pulses by restriction of glucose availability is mediated by sensors in the brain stem. *Endocrinology* **137**1171–1176.

Murphy K, Dhillo WS, and Bloom SR. Gut peptides in the regulation of food intake and energy homeostasis. *Endocr Rev* **27** 719–727.

Nagatani S, Bucholtz DC, Murahashi K, Estacio MA, Tsukamura H, Foster DL, and Maeda KI. 1996. Reduction of glucose availability suppresses pulsatile luteinizing hormone release in female and male rats. *Endocrinology* **137** 1166–1170.

Nagatani S, Guthikonda P, Thompson RC, Tsukamura H, Maeda KI, and Foster DL. 1998. Evidence for GnRH regulation by leptin: leptin administration prevents reduce pulsatile LH secretion during fasting. *Neuroendocrinology* **67** 370–376.

Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, and Matsukura S. 2001. A role for ghrelin in the central regulation of feeding. *Nature* **409** 194–198.

Näslund E, Gutniak M, Skogar S, Rössner S, and Hellström PM. 1998. Glucagon-like peptide 1 increases the period of postprandial satiety and slows gastric emptying in obese men. *Am J Clin Nutr* **68** 525–530.

Nilaver G, MC Beinfeld, C T Bond, D Daikh, B Godfrey, and JP Adelman. 2004. Heterogeneity of motilin immunoreactivity in mammalian tissue. *Synapse* **2** 266–275.

Nillius S. 1975. Proceedings: promotion of fertility in women: induction of ovulation. *J Endocrinol* **66** 14P–15P.

Nogueiras R, Barreiro ML, Caminos JE, Gaytán F, Suominen JS, Navarro VM, Casanueva FF, Aguilar E, Toppari J, Diéguez C, and Tena-Sempere M. 2004. Novel expression of resistin in rat testis: functional role and regulation by nutritional status and hormonal factors. *J Cell Sci* **117** 3247–3257.

O'Donohue TL, Beinfeld MC, Chey WY, Chang T-M, Nilaver G, Zimmerman EA, Yajima H, Adachi H, Poth M, McDevitt RP, and Jacobowitz DM.

1981. Identification, characterization and distribution of motilin immunoreactivity in the rat central nervous system. *Peptides* **2** 467–477.

Ogata R, Matsuzaki T, Iwasa T, Kiyokawa M, Tanaka N, Kuwahara A, Yasui T, and Irahara M. 2009. Hypothalamic Ghrelin suppresses pulsatile secretion of luteinizing hormone via beta-endorphin in ovariectomized rats. *Neuroendocrinology* **90** 364–370.

Ohno T, Mochiki E, and Kuwano H. 2010. The roles of motilin and ghrelin in gastrointestinal motility. *Int J Peptides* Article ID 820794.

Parfitt D, Church KR, and Cameron JL. 1991. Restoration of pulsatile luteinizing hormone secretion after fasting in rhesus monkeys (Macaca mulatta): dependence on size of the refeed meal. *Endocrinology* **129** 749–756.

Pfaff D. 1972. Steroid sex hormones in the rat brain: specificity of uptake and physiological effects. *UCLA Forum Med Sci* **15** 103–112.

Pearse A, Polak JM, Bloom SR, Adams C, Dryburgh JR, and Brown JC. 1974. Enterochromaffin cells of the mammalian small intestine as the source of motilin. *Virchows Arch B Cell Pathol* **16** 111–120.

Peeters T, Vantrappen G, and Janssens J. 1980. Fasting plasma motilin levels are related to the interdigestive motility complex. *Gastroenterology* **79** 716–719.

Peeters T, Romanski K, Bormans V, and Vantrappen G. 1990. Interrelations between the presence or lack of bile and bile acids in the small-intestinal lumen, release of motilin and pancreatic polypeptide and migrating myoelectric complex in dogs. *Pol Arch Weter* **30** 57–70.

Peeters TL. 2003. Central and peripheral mechanisms by which ghrelin regulates gut motility. *J Physiol Pharmacol* **54**(Suppl 4) 95–103.

Perera A, JG Verbalis, N Mikuma, SS Majumdar, and TM Plant. 1993. Cholecystokinin stimulates gonadotropin-releasing hormone release in the monkey (Macaca mulatta). *Endocrinology* **132** 1723–1728.

Petraglia F, Sutton S, Vale W, and Plotsky P. 1987. Corticotropin-releasing factor decreases plasma luteinizing hormone levels in female rats by inhibiting gonadotropin-releasing hormone release into hypophysial-portal circulation. *Endocrinology* **120** 1083–1088.

Plant T, and Shahab M. 2002. Neuroendocrine mechanisms that delay and initiate puberty in higher primates. *Physiol Behav* **77** 717–722.

Poitras P, Gagnon D, and St-Pierre S. 1992. N-terminal portion of motilin determines its biological activity. *Biochem Biophys Res Commun* **183** 36–40.

Poitras P, and TL Peeters. 2008. Motilin. *Curr Opin Endocr Diab Obes* **15** 54–57.

Ponzo O, Cresta MA, Rondina D, Szwarcfarb B, Basabe JC, and Scacchi P. 2000. Effect of aproteic diet and fasting on insulin, pancreatic

noradrenaline and luteinizing hormone. Changes after 24-hour refeeding. *Neuro Endocrinol Lett* **21** 441–446.

Purnelle G, Gerard A, Czajkowski V, and Bourguignon J-P. 1997. Pulsatile secretion of gonadotropin-releasing hormone by rat hypothalami explants of GnRH neurons with cell bodies. *Neuroendocrinology* **66** 305–312.

Raposinho P, Broqua P, and Pierroz DD. 1999. Evidence that the inhibition of luteinizing hormone secretion exerted by central administration of neuropeptide Y (NPY) in the rat is predominantly mediated by the NPY-Y5 receptor subtype. *Endocrinology* **140** 4046–4055.

Rat Genome Database Web Site, Medical College of Wisconsin, Milwaukee, Wisconsin. World Wide Web (URL: http://rgd.mcw.edu/). Data retrieved on May 14, 2010.

Rissman E, Alones VE, Craig-Veit CB, and Millam JR. 1995. Distribution of chicken-II gonadotropin-releasing hormone in mammalian brain. *J Comp Neurol* **357** 524–531.

Roche J. 2006. The effect of nutritional management of the dairy cow on reproductive efficiency. *Anim Reprod Sci* **96** 282–296.

Romjin J, Corssmith EP, Havekes LM, and Pjil H. 2008. Gut-brain axis. *Curr Opin Clin Nutr Met Care* **11** 518–521.

Rosenfeld DJ and Garthwaite TL. 1987. Central administration of motilin stimulates feeding in rats. *Physiol Behav* **39**(6) 753–756.

Roy D and Belsham DD. 2002. Melatonin receptor activation regulates GnRH gene expression and secretion in GT1–7 GnRH neurons. *J Biol Chem* **277** 251–258.

Saito S, Ogawa T, Saito H, Ishimaru K, Oshima I, and Sonaka Y. 1980. Regulation of motilin secretion in the postprandial state in man. *Endocrinol Jpn* **27**(Suppl 1) 157–162.

Sakai T, Satoh M, Koyama H, Iesaki K, Umahara M, Fujikura K, and Itoh Z. 1994. Localization of motilin-immunoprotective cells in the rat intestine by light microscopic immunocytochemistry. *Peptides* **15**(6) 987–991.

Samson W, Lumpkin MD, Nilaver G, and McCannb SM. 1984. Motilin: A novel growth hormone releasing agent. *Brain Research Bulletin* **12** 57–62.

Schally A, Arimura A, Kastin AJ, Matsuo H, Baba Y, Redding TW, Nair RMG, Debeljuk L, and White F. 1971. Gonadotropin-Releasing Hormone: One Polypeptide Regulates Secretion of Luteinizing and Follicle-Stimulating Hormones. *Science* **173** 1036–1038.

Schick R, Zimmerman P, Walde T, Schusdziarra V, and Classen M. 1992. Glucagon-like peptide (GLP)-1-(7–36) amide: a central suppressor of food intake in fasted rats. *Gastroenterology* **102** A756.

Schneider J. 2004. Energy balance and reproduction. *Physiology and Behavior* **81** 289–317.

Schneider J, Goldman MD, Tang S, Bean B, Ji H, and Friedman MI. 1998. Leptin indirectly affects estrous cycles by increasing metabolic fuel oxidation. *Hormones and Behavior* **33** 217–228.

Schneider JE, and Zhu Y. 1994. Caudal brain stem plays a role in metabolic control of estrous cycles in Syrian hamsters. *Brain Res* **661** 70–74.

Seifer D, and Collins RL. 1990. Current concepts of beta-endorphin physiology in female reprodutive dysfunction. *Fertil Steril* **54** 757–771.

Seoane L, Lopez M, Tovar S, Casanueva FF, Señaris RM, and Dieguez C. 2003. Agouti-related peptide, neuropeptide Y, and somatostatin-producing neurons are targets for ghrelin actions in the rat hypothalamus. *Endocrinology* **144** 544–551.

Shahab M, Balasubramaniam A, Sahu A, and Plant TM. 20003. Central nervous system receptors involved in mediating the inhibitory action of neuropeptide Y on luteinizing hormone secretion in the male rhesus monkey (Macaca mulatta). *J Neuroendocrinol* **15** 965–970.

Shimizu I, Hirota M, Ohboshi C, and Shima K. 1987. Identification and localization of glucagon-like peptide-1 and its receptor in rat brain. *Endocrinology* **121** 1076–1082.

Sone M, Nagata H, Takekoshi S, and Osamura RY. 2001. Expression and localization of leptin receptor in the normal rat pituitary gland. *Cell Tissue Res* **305** 351–356.

Soriano-Guillén L, Barrios V, Chowen JA, Sánchez I, Vila S, Quero J, and Argente J. 2004. Ghrelin levels from fetal life through early adulthood: relationship with endocrine and metabolic and anthropometric measures. *J Pediatr* **144** 30–35.

Soriano-Guillén L, Barrios V, Campos-Barros A, and Argente J. 2004. Ghrelin levels in obesity and anorexia nervosa: effect of weight reduction or recuperation. *J Pediatr* **144** 36–42.

Spicer L. 2001. Leptin: a possible metabolic signal affecting reproduction. *Domest Anim Endocrinol* **21** 251–270.

Strunz U, Domschke W, and Mitznegg P. 1975. Analysis of the motor effects of 13 norleucine motilin on the rabbit, guinea pig, rat, and human alimentary tract *in vitro*. *Gastroenterology* **68**(6) 1485–1491.

Sullivan S, DeFazio RA, and Moenter SM. 2003. Metabolic regulation of fertility through presynaptic and postsynaptic signaling to gonadotropin-releasing hormone neurons. *J Neurosci* **23** 8578–8585.

Szurszewski JH. 1969. A migrating electric complex of canine small intestine. *Am J Physiol* **217**(6) 1757–1763.

Szymanski L, Schneider JE, Friedman MI, Ji H, Kurose Y, Blache D, Rao A, Dunshea FR, and Clarke IJ. 2007. Changes in insulin, glucose and ketone bodies, but not leptin or body fat content precede restoration of luteinizing hormone secretion in ewes. *J Neuroendocrinol* **19** 449–460.

Tanaka T, Nagatani S, Bucholtz DC, Ohkura S, Tsukamura H, Maeda K, and Foster DL. 2000. Central action of insulin regulates pulsatile luteinizing

hormone secretion in the diabetic sheep model. *Biol Reprod* **62** 1256–1261.

Tang-Christensen M, Larsen PJ, Goke R, Fink-Jensen A, Jessop DS, Moller M, and Sheikh SP. 1996. Central administration of GLP-1(7– 36) amide inhibits food and water intake in rats. *Am J Physiol* **271** R848–R856.

Tatemoto K, Mutt V. 1980. Isolation of two novel candidate hormones using a chemical method for finding naturally occurring polypeptides. *Nature* **285** 417–418.

Temple J, Schneider JE, and Scott DK.2002. Mating behavior is controlled by acute changes in metabolic fuels. *Am J Physiol Regul Integr Comp Physiol* **282** R782–R790.

Tena-Sempere M. 2008. Ghrelin and reproduction, ghrelin as novel regulator of the gonadotropic axis. *Vitam Horm* **77** 285–300.

Tomasetto C, Karam SM, Ribieras S, Masson R, Lefebvre O, Staub A, Alexander G, Chenard MP, and Rio MC. 2000. Identification and characterization of a novel gastric peptide hormone: the motilin-related peptide. *Gastroenterology* **119** 395–405.

Toshinai K, Yamaguchi H, Sun Y, Smith RG, Yamanaka A, Sakurai T, Date Y, Mondal MS, Shimbara T, Kawagoe T, Murakami N, Miyazato M, Kangawa K, and Nakazato M. 2006. Des-acyl ghrelin induces food intake by a mechanism independent of the growth hormone secretagogue receptor. *Endocrinology* **147** 2306–2314.

Tschop M, DL Smiley, and ML Heiman. 2000. Ghrelin induces adiposity in rodents. *Nature* **407** 908–913.

Tsukamura H, Nagatani S, and Cagampang FR. 1994. Corticotropin releasing hormone mediates suppression of pulsatile luteinizing hormone secretion induced by activation of alpha-adrenergic receptors in the paraventricular nucleus in female rats. *Endocrinology* **134** 1460–1466.

Tsukamura T, Maekawa, Moriyama, Reyes, Sakai, Niwa, and Foster. 2000. Peripheral or central administration of motilin suppresses LH release in female rats: a novel role for motilin. *Journal of Neuroendocrinology* **12** 403–408.

Turi G, Liposits Z, Moenter SM, Fekete C, and Hrabovszky E. 2003. Origin of neuropeptide Y-containing afferents to gonadotropin-releasing hormone neurons in male mice. *Endocrinology* **144** 4967–4974.

Turton M, O'Shea D, and Gunn I. 1996. A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature* **379** 69–72.

Ugrumov M, IP Ivanova, MS Mitskevich, Z Liposits, G Setalo, and B Flerko. 1985. Axovascular relationships in developing median eminence of perinatal rats with special reference to luteinizing hormone-releasing hormone projections. *Neuroscience* **16** 897–906.

Urban JH, Das I, Levine JE. 1996. Steroid modulation of neuropeptide Y-induced luteinizing hormone releasing hormone release from median eminence fragments from male rats. *Neuroendocrinology* **63**112–119.

Van der Lely A, Tschöp M, Heiman ML, and Ghigo E. 2004. Biological, physiological, pathophysiological, and pharmacological aspects of ghrelin. *Endocr Rev* **25** 426–457.

Vantrappen G, Janssens J, Peeters TL, Bloom SR, Christofides ND, and Hellemans J. 1979. Motilin and the interdigestive migrating motor complex in man. *Dig Dis Sci* **24** 497–500.

Verdich C, Flint A, and Gutzwiller JP. 2001. A meta-analysis of the effect of glucagon-like peptide-1 (7-36) amide on ad libitum energy intake in humans. *J Clin Endocrinol Metab* **86** 4382–4389.

Viani I, Vottero A, Tassi F, Cremonini G, Sartori C, Bernasconi S, Ferrari B, and Ghizzoni L. 2008. Ghrelin inhibits steroid biosynthesis by cultured granulosa-lutein cells. *J Clin Endocrinol Metab* **93** 1476–1481.

Vijayan E, Samson WK, and McCann SM. 1979. In vivo and in vitro effects of cholecystokinin on gonadotropin, prolactin, growth hormone and thyrotropin release in the rat. *Brain Res* **172** 295–302.

Vincent SR, Hökfelt T and Wu J-Y. 1982. GABA neuron systems in hypothalamus and the pituitary gland. Immunohistochemical demonstration using antibodies against glutamate decarboxylase. *Neuroendocrinology* **34**(2) 117–125.

Wade G, and Schneider JE. 1992. Metabolic fuels and reproduction in female mammals. *Neurosci Biobehav Rev* **16** 235–272.

Wade G, and Jones JE. 2004. Neuroendocrinology of nutritional infertility. *Am J Physiol Regul Integr Comp Physiol* **287** R1277–1296.

Wank S, Pisegna JR, and de Weerth A. 1992. Brain and gastrointestinal cholecystokinin receptor family: structure and funtional expression. *Proc Natl Acad Sci USA* **89** 8691–8695.

Wynne K, Stanley S, McGowan B, and Bloom S. 2005. Appetite control. *J Endocrinol* **184** 291–318.

Xu L, Depoortere I, Tang M, and Peeters TL. 2001. Identification and expression of the motilin precursor in the guinea pig. *FEBS Lett* **490** 7–10.

Yano H, Seino Y, Fujita J, Yamada Y, Inagaki N, Takeda J, Bell GI, Eddy RL, Fan YS, and Byers MG. 1989. Exon-intron organization, expression, and chromosomal localization of the human motilin gene. *FEBS Lett* **249** 248–252.

Yildiz B, Suchard MA, Wong ML, McCann SM, and Licinio J. 2004. Alterations in the dynamics of circulating ghrelin, adiponectin, and leptin in human obesity. *Proc Natl Acad Sci U S A* **101** 10434–10439.

Yu W, Kimura M, Walczewska A, Karanth S, and McCann SM. 1997. Role of leptin in hypothalamic–pituitary function. *Proc Natl Acad Sci USA* **94** 1023–1028.

Zhang S, Blache D, Vercoe PE, Adam CL, Blackberry MA, Findlay PA, Eidne KA, and Martin GB. 2005. Expression of orexin receptors in the brain and peripheral tissues of the male sheep. *Regul Pept* **124** 81–87.

Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, and Friedman JM. 1994. Positional cloning of the mouse obese gene and its human homologue. *Nature* **372** 425–432.

Zieba D, Amstaldena M, and Williams GL. 2005. Regulatory roles of leptin in reproduction and metabolism: A comparative review. *Domestic Animal Endocrinology* **29** 166–185.

Zigman J, and Elmquist JK. 2003. From anorexia to obesity—the Yin and Yang of body weight control. *Endocrinology* **144** 3749–3756.