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**REVIEW [REVISIÓN]**

# NEUROENDOCRINE EFFECTS OF INSULIN, IGF-I AND LEPTIN ON THE SECRETION OF THE GONADOTROPIN-RELEASING HORMONE (GnRH)

# [EFECTOS NEUROENDOCRINOS DE INSULINA, IGF-I Y LEPTINA SOBRE LA SECRECIÓN DE HORMONA LIBERADORA DE GONADOTROPINAS (GnRH)]

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## SUMMARY

Animal energy balance greatly determines his reproductive success. In the majority of mammals, under a negative energy balance, there is a decrease in the synthesis of the gonadotropin-releasing hormone (GnRH) which reduces the activity of the hypothalamic-pituitary-gonadal axis. When the energy balance is improved, the hypothalamus reacts to this change and reestablishes the secretion of GnRH. Insulin, the insulin growth factor I (IGF-I) and leptin seem to be the main messengers that signal the hypothalamus on the animal energy balance. It has been seen that the peripheral concentrations of these hormones under positive or negative energy states are associated with changes in GnRH secretion. This review shows how IGF-I acts directly on GnRH neurons affecting its synthesis and secretion, whereas that insulin and leptin act on neurons in the arcuate nucleus (ARC), which synapses with GnRH neurons in the medial preoptic zone. Both insulin and leptin decrease the expression of neuropeptide Y (NPY) and therefore the negative effect of NPY on GnRH secretion. On the other hand insulin and leptin stimulating the synthesis of galanin-like peptide (GALP) and proopiomelanocortin (POMC). Both GALP, as well as the POMC metabolites (mainly the melanocyte stimulating hormone) increase the synthesis of GnRH. Finally, leptin increases the expression of kisspeptin in ARC neurons. Kisspeptin has a positive effect on the synthesis and secretion of GnRH.

**Key words:** GnRH secretion, insulin, IGF-I, leptin, neurotransmitters, nutritional stress

## RESUMEN

El balance energético del individuo determina en gran medida su eficiencia reproductiva. Bajo condiciones de balance negativo de energía, en la mayoría de los mamíferos, hay una reducción en la síntesis de hormona liberadora de gonadotropinas (GnRH), lo cual disminuye la actividad del eje hipotálamo-hipófisis-gónadas. Cuando el balance energético es revertido, el hipotálamo puede monitorear este cambio y restablecer la secreción de GnRH. La Insulina, el Factor de Crecimiento similar a la Insulina I (IGF-I) y Leptina parecen ser los principales mensajeros que informan al hipotálamo sobre el estado energético del animal puesto que las concentraciones periféricas de estas hormonas en situaciones energéticas negativas o positivas, se han asociado con los cambios en la secreción de GnRH. En la presente revisión se muestra como IGF-I actúa directamente sobre neuronas secretoras de GnRH, afectando su síntesis, en tanto que insulina y leptina actúan sobre neuronas en el núcleo arcuato, las cuales hacen sinapsis con neuronas GnRH en el área preóptica medial. Sobre neuronas productoras de neuropéptido Y (NPY) insulina y leptina reducen su expresión y por lo tanto el efecto negativo del NPY sobre neuronas GnRH. En cambio insulina y leptina estimulan la síntesis de péptido similar a la galanina (GLAP) y propiomelanocortina (POMC). Tanto GALP como los metabolitos de POMC (hormona estimulante de melanocitos principalmente) incrementan la síntesis de GnRH. Finalmente, la leptina, incrementa la expresión de kispeptina en neuronas del núcleo ARC. Kispeptina por su parte también tiene un efecto positivo sobre la síntesis y secreción de GnRH.

**Palabras clave:** Secreción de GnRH, Insulina, IGF-I, Leptina, Neurotransmisores, Estrés Nutricional

## INTRODUCTION

Energy and/or energy reserves control the reproductive function in several species. In humans, anorexia, cachexia and excessive exercise block the reproductive cycle (De Souza *et al*., 1998). In rodents, overnight fasting, show a decrease in the secretion of the luteinizing hormone (LH; Gamba and Pralong, 2006). In beef and dairy cows, low body condition score (BCS) at calving prolong postpartum anestrus compared to cows in good BCS (Crowe, 2008). Heifers in nutritional anestrus fed a high energy diet (16.2 Mcal EM) resume ovulatory cycles in a shorter time span (57 days) than heifers on a moderate energy diet (10.2 Mcal EM) (80 days) (Bossis *et al*., 2000).

The loss of the reproductive function under negative energy balance is associated to a decrease in the synthesis, concentration and secretion of GnRH, and therefore reduction of LH which blocks follicular maturation, ovulation and the reproductive cycle itself (Gamba and Pralong, 2006; Crowe, 2008; Hill *et al*., 2008). The concentrations of LH during anestrus induced by food restriction in cows and heifers were low in comparison to animals that were fed a maintenance diet during anestrus. In both cases, re-feeding increased animal body weight, BCS and reestablished LH concentrations (Richards *et al*., 1989a; Bossis *et al.*, 2000). It has been reported that feed restrictions, and extreme exercise in rodents, sheep and monkeys causes a shutdown of GnRH pulses within minutes to hours while reverting (usually between an hour or two) when an adequate amount of energy is available (Hill *et al.*, 2008).

Although it is clear that the availability of energy reserves control the reproductive function regulating the secretion of GnRH, the mechanisms by which the hypothalamus monitors the animal energy status and the regulation of the neurotransmitters involved in signaling have not been well established.

The main molecules involved in transmitting the message to the hypothalamus on the animal energy status are insulin, IGF-I and leptin. The objective of this review is to gather information on the regulation of GnRH synthesis and secretion by insulin, IGF-I abd leptin under nutritional stress. In the first part of the review, a brief description is made on the hypothalamic nuclei and the neurotransmitters involved in the secretion of GnRH. Next an analysis of the mechanisms of action of insulin, IGF-I and leptin, as well as the role these molecules on the regulation of the function of GnRH neurons under conditions of nutritional stress.

## Hypothalamic nuclei that control GnRH secretion

The hypothalamus is located at the level of the third ventricle under the thalamus and above the hypophysis. Frontal and limited by the optic chiasma and caudal by the mammillary bodies (Daniel, 1976; Saleem *et al.*, 2007). Anatomically and functionally, the hypothalamus is divided into the anterior, intermediate and posterior lobes, as well as a medial and lateral area where different hypothalamic nuclei are found (Table 1; Loes *et al.*, 1991; Sahar *et al.*, 2007). The best characterized nuclei are: the supraoptic nucleus (SON) and the paraventricular nucleus (PVN) almost totally formed by long neurons that mainly produce oxytocin and vasopressin (Daniel, 1976). The medial preoptic nucleus (MPO) and the ARC are important hypothalamic structures involved in the control of GnRH secretion. The MPO is located in the preoptic area and within are the cellular bodies of GnRH neurons (Schneider, 2004). In the ARC, there are three main neuronal populations, those POMC, NPY (Coll *et al.*, 2007) and GALP (Crown *et al.*, 2007). A fourth population of neurons in this nucleus are those producing kisspeptin (Goodman *et al.*, 2007; Gottsch *et al.*, 2004a), although in the anteroventral periventricular nucleus, there is a large amount of kiss-1 neurons (Gottsch *et al*., 2004a). It has been shown that 50-75% of the axonic projections of the GnRH neurons in the MPO in rodents are directed towards the median eminence (ME; Smith and Jennes, 2001) where GnRH is released into the perivascular space of the fenestrated capillaries that form part of the primary plexus of the hypophysiary portal system (Jennes and Conn, 1994). The axons of the neuronal populations in ARC are directly or indirectly connected to the GnRH neurons. This may serve as a path for regulating the metabolism of this neuropeptide (Crown *et al.*, 2007; Hill *et al*., 2008; Xu *et al.*, 2009a).

## Neurotransmitters involved in the secretion of GnRH

Although there is a large amount of neurotransmitters involved in the control of GnRH secretion only four neurotransmitters relate with the energetic state of the animal and with the concentrations of the metabolic hormones, insulin, IGF-I and leptin.

### Neuropeptide Y (NPY)

The NPY is a 36 amino acid peptide widely distributed in the central nervous system, both in humans and rodents. This peptide is expressed in the hypothalamus (mainly ARC), amygdala, hippocampus, nucleus of the solitary tract and cerebral cortex. The NPY interacts with at least six receptors linked to protein G (Y1, Y2, Y3, Y4, Y5 and Y6). NPY´s functions are very

diverse (Eva *et al.*, 2006). Among other functions NPY acts as the link between animal energy status and its reproductive function (Crown *et al.*, 2007). The axons of NYP neuron are in contact with the cell bodies of GnRH neuron in MPO, as well as with the axons of GnRH neurons in the median eminence (Xu *et al.*, 2009a), for which the NPY neurons could regulate both the synthesis, as well as the release of GnRH. Although NPY seems to stimulate LH in cycling animals (Eva *et al.*, 2006), in general this peptide blocks the gonadotrophic axis in situations of negative energy balance (Gamba and Pralong, 2006; Hill *et al.*, 2008). Fasting for 48 h in ovariectomized rats decreases LH concentrations and increases NPY expression even in the presence of estradiol (Kalamatianos *et al.*, 2008). It has recently been shown that the binding of NPY with its Y5 receptor, hyperpolarizes the membrane potential of GnRH neurons inhibiting the secretion of GnRH (Xu *et al.*, 2009a). In addition, the inhibition of the Y5 receptor by the use of an antagonist depolarizes the membrane of GnRH neurons (Xu *et al.*, 2009a) and eliminates the inhibitory effect that NPY exerted on LH (Raposinho *et al.*, 1999). In ob/ob infertile rats with Y4 receptor knockouts, total male fertility and partial female estral cycles are reestablished, moreover there is an increase of GnRH expression (Sainsbury *et al.*, 2008).

**Propiomelancortin (POMC)**

The POMC gene is actively transcribed in several tissues, including ARC neurons. In the central nervous system, POMC is enzymatically processed and gives rise to at least four small peptides: β-endorphins, and α-, β-, g-melanocyte-stimulating hormone (α-MSH, β-MSH and g-MSH, respectively; Coll *et al.*, 2004). The nerve endings of POMC in ARC project towards MPO where they release β-endorphin and α-MSH, both involved in the control of GnRH (Hill *et al.*, 2008; Ward *et al.*, 2009). In fasting rats, LH concentrations decrease in comparison to that in well-fed rats and this decrease in LH is associated to a decrease in the release of GnRH by the hypothalamus. Under these conditions, a lesser baseline concentration of α-MSH in MPO and in the ARC-ME region in fasting rats has been seen in comparison to those well-fed animals (Watanobe, 2002). *In vitro* studies with immortalized GnRH neurons show that both α-MSH, and g-MSH increase AMPc concentration and the GnRH secretion in dose-dependent way (Stanley *et al.*, 2003). In addition, the infusion of g-MSH directly into MPO increases the plasmatic concentrations of LH in rats (Stanley *et al.*, 2003). Recent reviews by Crown *et al.* (2007) and Hill *et al.* (2008) suggest that β-endorphin inhibits the secretion of GnRH/LH. In sheep, it has been reported that β-endorphin is involved in the inhibition of the pulsatile release or surges of GnRH in an endocrine medium dominated by $P_4$ (Taylor *et al.*, 2007).

**Galanin-like Peptide (GALP)**

The GALP is a 60 amino acid neuropeptide originally isolated from the hypothalamus of pigs that is partially homologous to the galanin orexigenic neurotransmitter. Although GALP is widely distributed in the central nervous system, GALP is mainly found in ARC and the median eminence of rats (Man and Lawrence, 2008). The role of GALP as a link between the status of energy and the animal´s reproductive function has been widely reviewed (Gottsch *et al.*, 2004b; Kageyama *et al.*, 2005; Crown *et al.*, 2007). These reports have shown that GALP neurons in ARC project their axons towards the GnRH neurons in MPO and that GALP stimulates the secretion of GnRH and sexual behavior both in male, and in female rats.

Table 1. Location of hypothalamic nuclei with respect to the medial lateral and rostral caudal axes and nuclei involved in the control of GnRH*

| Region | Medial Area | Lateral Area |
|---|---|---|
| Anterior | Medial preoptic nucleus (MPO)* | Lateral preoptic nucleus (LPN) |
| | Supraoptic nucleus (SON) | Lateral nucleus |
| | Anteroventral periventricular nucleus (AVPV)* | |
| | Paraventricular nucleus (PVN) | |
| | Anterior nucleus (AN) | |
| | Suprachiasmatic nucleus (SC) | |
| Intermediate | Dorsal-medial nucleus (DMN) | Lateral nucleus |
| | Ventromedial nucleus (VMN) | Tuberolateral nucleus (TLN) |
| | Arcuate nucleus (ARC)* | |
| Posterior | Mammillary Nucleus (MN) | Lateral nucleus |
| | Posterior Nucleus (PN) | |

Modified by Saleem *et al.*, 2007, Hill *et al.*, 2008

The intracerebroventricular infusion of GALP in ovariectomized rats increases the mean concentration and pulsing frequency of LH in the presence of estrogens, suggesting that the effect of GALP on LH is estrogen-dependent (Uenoyama *et al.*, 2008). In contrast, knockout male and female rats for the GALP gene did not show any differences in LH concentrations with respect to the wild-type rats, both while fasting as well as well-fed (Dungan-Lemko *et al.*, 2008).

**Kisspeptin**

Kisspeptin is a ligand codified by the kiss-1 gene that acts through receptors coupled to the G protein (GPR54). Kisspeptin is the main regulator of the GnRH neurons (Kadokawa *et al.*, 2008). The kiss-1 gene is expressed by the AVPV nucleus and ARC, while the GPR54 receptor is expressed by GnRH neurons and its mutation causes hypogonadotrophism and hypogonadism in humans and rats (Hill *et al.*, 2008; Catellano *et al.*, 2009; Clarkson and Herbison, 2009). Treatment with kiss-10, in prepubertal heifers increases mean LH blood concentrations (Kadokawa *et al.*, 2008). The effect of kisspeptin is proposed as the stimulator of GnRH secretion (Roseweir and Millar, 2009) or acting directly on the gonadotrope in the hypophysis (Suzuki *et al.*, 2008).

**IGF-I**

**IGF System and Mechanism of Action**

The IGF-I is a basic polypeptide consisting of 70 amino acids (Trojan *et al.*, 2007) that together with IGF-II, two receptors of IGF, IGF binding proteins (IGFBP) and IGFBP proteases form the IGF system which regulates somatic growth, cell proliferation and apoptosis (Trojan *et al.*, 2007).

The IGF-I has important effects on the metabolism of glucose together with insulin (Sandhu *et al.*, 2002). Growth hormone (GH) is the main stimulator for the production of IGF-I in the liver, but this polypeptide can be produced by the majority of tissues in response to GH and other factors such as insulin (Le Roith, 2003; Werner *et al.*, 2008).

The biological effects of IGF-I are mediated by its interaction with its receptor I (IGFR-I), although it may also bind to the insulin receptor and the receptor related to insulin. The IGFR-I is a member of the super family of receptors linked to kinase proteins, is a tetramere formed by two α-subunits located towards the extracellular space where the IGF-I is linked and two β subunits within the cell where the kinase domain is found (Adams *et al.*, 2000). The union of the ligand with the receptor causes a conformational change of the receptor allowing the linking of ATP and the phosphorylation of the tyrosine dominion of the receptor. This phosphorylation increases the receptor kinase activity in order to phosphorylate a series of cytoplasmatic substrates that together are known as downstream signaling transductions mediators (Werner *et al.*, 2008). Among the mediators that are activated in response to the IGF-I are, the mitogen activated protein kinases (MAPK), phosphatidylinositol-3-kinase/protein kinase C (IP3K/PKC) and the blockage of the glycogen synthetase enzyme. The activation of these mediators triggers cell mitosis (via MAPK), inhibits apoptosis (since IP3K inhibits caspases, BAD and increases the expression of bcl-2). Due to the increase of the kinase activity in the cell, IGF-I can activate glucose and amino acids transporters, stimulate protein synthesis by activating transcription factors and inhibit gluconeogenesis (Le Roith, 2003; Trojan *et al.*, 2007; Werner *et al.*, 2008). The bioavailability of IGF-I is regulated by at least 6 IGF binding proteins (IGFBPs). The IGFBP3 is the protein of highest molecular weight and found predominantly in serum. The binding of this protein with IGF-I blocks proteolysis of the factor increasing its half life (Baxter, 2000). In general, the IGFBPs inhibit the metabolic and proliferative effects of IGF-I (Werner *et al.*, 2008).

**IGF-I and GnRH**

The onset of lactation after calving in dairy and beef cows causes negative energy balance in the animal. Schillo (1992) suggests that IGF-I concentrations may be inversely related with the duration of the postpartum anestrus. In cycling beef cows, IGF-I concentrations are greater than in cows in anestrus, while the relative abundance of IGFBP-2 in serum was less in cycling cows (Roberts *et al.*, 1997). In cattle, IGF-I serum concentrations are associated with the amount of energy reserves (Roberts *et al.*, 1997; Guzmán *et al.*, 2008). Similar results are seen in dairy cows (Kawashima *et al.*, 2007). In cows and heifers, food restriction decreases IGF-I concentrations which in turn has been associated to a decrease in LH serum concentrations (Richards *et al.*, 1991). It is known that in heifers, re-feeding increases IGF-I concentrations (León *et al.*, 2004) while shortening the time between the start of re-feeding and the first ovulation after anestrus (Bossis *et al.*, 2002). In post-partum beef cattle, the use of beta-adrenergic agonists reduces the response to an estrous induction program associated with a reduction of IGF-I serum concentration (Guzmán *et al*., 2009b).

The presence of IGFR-I in the hypothalamus (median eminence and MPO) and hypophysis (Bach and Bondy, 1992; Daftary and Gore, 2005) suggests a direct effect of IGF-I on the secretion of GnRH and

LH. In rodents, IGF-I is required for triggering the effects of positive feedback of estradiol on LH (Etgen *et al.,* 2006), while in the culture of neurons expressing GnRH, the addition of 10 ng/mL of IGF-I to the culture medium increases the mRNA of GnRH (Daftary and Gore, 2005), which shows that this growth factor has an important effect on the production of GnRH. On the other hand, it has been shown that IGF-I can increase the release of GnRH when stimulating the axons of GnRH neurons in the median eminence of the hypothalamus (Ojeda *et al.*, 2008). Although IGF-I is expressed in astrocytes and GnRH neurons, the larger part of this factor in the median eminence during puberty and the estral cycles comes from the general circulation (Ojeda *et al.*, 2008). The inactivation of IGFR-I in adult rats result in the abolition of the synaptic plasticity in the hypothalamus (Fernández- Galaz *et al.*, 1999). In the rat, IGF-I acts on the axons of GnRH neurons in the medial eminence to stimulate a dose-dependent release of GnRH (Hiney *et al.*, 1991). In rats close to puberty, small doses of IGF-I administered intraventricularly increase the secretion of LH. In addition, in late proestrus, the IGF-I serum levels and the expression of IGFR-I in the median eminence increase (Hiney *et al.*, 1996). Although it is clear that IGF-I directly regulates GnRH neurons, it has recently been shown that IGF-I is capable of increasing the expression of kiss-1 in female rats before puberty (Hiney *et al.*, 2009).

**Insulin**

**Structure and Mechanism of Action**

Insulin is a 51 amino acids peptide hormone produced as a pre-hormone by the beta cells of the pancreatic Langerhans islets. It is produced as a pre-prohormone. The pre-proinsulin is composed by an acid peptide chain (A) and a basic peptide chain (B) bound by a peptide called peptide C. Proinsulin is formed by the elimination of peptide C and the binding of two chains through a disulphide bridge. In this way, insulin is stored in secretory granules until a stimulus causes its release (Hayirli, 2006).

The insulin receptor is a tyrosine kinase receptor that is homologous to the IGF-I receptor in 85% (Corcoran *et al.,* 2007). Binding of insulin to the receptor´s α-subunit causes autophosphorylation of the kinase tyrosine domain present in the β subunits. This coincides with the internalization of the receptor which is one of the mechanisms by which insulin´s action is regulated (Hayirli, 2006). The activation of the kinase tyrosine domain in the insulin receptor phosphorilates the insulin receptor substrates (mainly IRS-I and IRS-2), and the Sch adaptor proteins. After the phosphorylation of the receptor, the Grb2 protein binds to the IRS-I and to Shc activating the *ras* complex and the MAPK cascade. These events stimulate the cell growth and the expression of genes. The IRS-I, and in a lesser proportion the IRS-2, recruit Src (homology 2 domain-containing proteins) and the p85 regulating subunit of PI3K for activation. The PI3K phosphorilates phosphatidylinositol-diphosphate to convert it to phosphatidylinositol-triphosphate (IP3) that activates IP3-dependent kinase proteins such as PKC and protein kinase B (PKB). The PKC stimulates the translocation towards the plasmatic membrane of the glucose-4 transporter (GLUT4) to stimulate the uptake of glucose, while PKB enters the nucleus for stimulating the transcription of genes protein synthesis and phosphorylation of glycogen synthetase to promote the synthesis of glycogen. Finally, the activation of PI3K is associated to lipogenesis in response to insulin (Hayirli, 2006; Corcoran *et al*., 2007; Gerozissis, 2008). An increase in nutrient intake, glucagon and parasympathetic stimulation triggers the synthesis and secretion of insulin whereas fasting, hunger, exercise, galanin, somatostatin, sympathetic stimulus, IL-6 and PGF2-α can inhibit its synthesis and secretion (Hayirli, 2006).

**Insulin and GnRH**

Insulin levels vary throughout the day. However, the amount of circulating insulin is in direct proportion to the amount of adipose tissue (León *et al.,* 2004; Crown *et al.*, 2007). The central infusion of insulin in sheep stimulates the secretion of LH (Miller *et al.,* 1995), while in sheep with an increase in food intake there is a positive correlation (0.73) between LH concentrations and insulin, however, this relationship is lost in feed restricted sheep (Miller *et al.,* 2007). The supply of nutrients to nutritionally anestrus beef cows, increases insulin levels and the animals recover ovarian activity (Richards *et al.,* 1989b). Similar results were reported in heifers (León *et al.,* 2004). Sinclair *et al.* (2002) showed that animals with more than 8 U/L of insulin tended to have a shorter interval between calving to first estrus than animals with a lesser concentration. In dairy cows, there seem to be a similar effect (Gong *et al.* 2002; Gutiérrez *et al.,* 2006).

Although evidence shows that insulin regulates reproductive function possibly by affecting the GnRH secretion, the presence of the insulin receptor in GnRH neurons has not been demonstrated *in vivo* (Hill *et al.,* 2008). *In vitro* studies with GnRH immortalized neurons show that insulin can activate these neurons through the MAPK pathway (Kim *et al.*, 2005; Salvi *et al.,* 2006). However, although these results suggest a direct effect of insulin on the GnRH neurons, it is possible that this hormone regulates GnRH indirectly by acting on other hypothalamic nuclei.

As previously mentioned, ARC is an important regulator of GnRH neurons since the axons of some neurons in this nucleus synapse directly to the bodies of the GnRH neurons in the MPO. The NPY and POMC neurons (Hill *et al.*, 2007) in ARC have receptors to insulin. In lactating rats, there is an increase in the expression of NPY mRNA and a decrease in the mRNA of POMC in ARC. However, the treatment with insulin inverts the expression pattern of these two neurotransmitters (Xu *et al.*, 2009b). Yang *et al* (2010) reports that insulin regulates $Ca^{+2}$ conductance channels activated by K+ channels in NPY neurons in ARC. The effect of insulin on these channels is via PI3K and can be the mechanism through which this hormone regulates the synthesis of NPY. In rats that did not express an insulin receptor in POMC exclusively, the metabolic and reproductive phenotype was not affected (Könner *et al.*, 2007) suggesting that POMC does not participate in the regulation of GnRH mediated by insulin. Fasting for 48 hours in rats decreases the expression of mRNA of GALP. However, the intracerebroventricular infusion of insulin increases GALP (Fraley *et al.*, 2004). Although insulin seems to regulate GALP, its receptor has not been reported in GALP neurons. However, GALP neurons have NPY receptors (Gottsch *et al.*, 2004b). By which NPY reduces production of GALP the same way as NPY reduces GnRH. Therefore it is possible that that as insulin reduce NPY synthesis, it reduction removes the inhibitory effect of NPY on GALP and in this way insulin may increase GALP expression. Finally, in male diabetic rats with hypogonadism, insulin does not affect kiss-1 mRNA concentrations (Castellano *et al.*, 2006).

**Leptin**

**Mechanism of Action**

Leptin is a hormone produced by the *ob* gene in adipose tissue (Zhang *et al.*, 1994). It is a polypeptide hormone of 167 amino acids and secreted by adipose tissue in direct proportion to fat tissue content, nutritional status and tissue location (Chilliard *et al.*, 2005). The leptin receptor (Ob-R) is a glycoprotein with a single transmembrane domain that belongs to the family of receptors linking to cytokines. There are six isoforms of the leptin receptor derived by the splicing alternative of immature mRNA. However, it is only the long isoform (Ob-Rb = 1162 amino acids) that translates the cell signal in response to leptin. The six isoforms of the leptin receptor possess the same binding domain to the ligand differing in the length of the intracellular dominion. The intracellular domain of Ob-Rb contains approximately 300 amino acids with several coupling sites for proteins essential in signal transduction. On the other hand, the intracellular domain of the short isoforms only have 30 to 40 amino acids and do not have these coupling sites (Robertson *et al.*, 2008).

The Ob-Rb does not have intrinsic enzymatic activity and should associate with janus kinase 2 (JAK2) in order for the signal to be translated. The Ob-Rb has two sites rich in proline (Box1 and Box2) that mediate the binding with JAK2. The binding of leptin to the receptor stimulates the autophosphorylation of JAK2 in two tyrosine residues. The phosphorylated JAK2 binds phosphate groups to Ob-Rb in three highly conserved tyrosine residues. Phosphorylated tyrosine residues 1077 and 1138 linked to signal transducers and activators of transcription (STAT3 and STAT5) to activate it by phosphorylation. The phosphorylation of the 985 tyrosine residue recruits SHP2 (SH2 domain with phosphatase 2 activity) and SOCS-3 (suppressor of cytokine signaling-3). The SHP2 activates the extracellular kinase regulators (ERK) or MAPK, while SOCS-3 blocks the translation of the signal by Ob-Rb. The Ob-Rb activates IRS 1 and 2 to phosphorylate IP3K (Zieba *et al.*, 2008; Robertson *et al.*, 2008).

**Leptin and GnRH**

Mice mutanr for the *ob* gene (*ob/ob*) or the receptor Ob-Rb (db/db) are obese and infertile but the administration of exogenous leptin in ob/ob rats reverts the phenotype but not so in db/db rats (Chilliard *et al.*, 2005). Leptin increases the releases of GnRH by the hypothalamus (Amstalden *et al.*, 2002). In beef heifers, the nutritional restriction decreases leptin concentrations and when the animals are re-fed, the hormone levels increase (León *et al.*, 2004). Two-day fasted cows had decrease concentrations of insulin and leptin, and the infusion of recombinant leptin normalizes insulin concentrations and causes hyperstimulation of LH secretion (Amstalden *et al.*, 2002). In heifers, fasting decreases concentrations of leptin, insulin and IGF-I, and LH pulsing frequency (Amstalden *et al.*, 2000). Multiparous Brahman cows with a short anestrous postpartum ($\leq$ 37 days) have greater leptin concentrations during the first 6 weeks postpartum than cows with long anestrus postpartum ($\geq$ 78 days). Similarly, there is a negative correlation between anestrous postpartum and leptin concentrations before calving, at calving and postpartum (Strauch *et al.*, 2003). The reduction of back fat by the use of a beta adrenergic receptor agonist, decreases leptin concentrations and the response to an estrous induction program in lactating beef cows (Guzmán *et al.*, 2009a).

Although leptin is involved in GnRH regulation, the presence of its receptor in GnRH neurons has not been shown. However, Ob-Rb is expressed in ARC where leptin may reduce NPY (orexigenic) expression and increase the expression of POMC (anorexigenic) to

decrease food intake, increasing energy expenditure (Friedman and Halaas, 1998; Coll *et al.*, 2007) and regulating GnRH secretion (Schneider, 2004). Just as insulin, the infusion of leptin in lactating rats decreases NPY expression and increases POMC (Xu *et al.*, 2009b). The infusion of leptin after 48 hours of fasting in rats increases the expression of mRNA for GALP (Juréus *et al.*, 2000). Similarly, in ob/ob or diabetic rats, the expression of GALP is decreased in comparison to the control and the phenomenon can be reverted by the infusion of leptin (Cunninham, 2004; Gottsch *et al.*, 2004b). Leptin seems to be the main regulator of kisspeptin under conditions of nutritional stress. It has been recently reported that leptin increases the expression of kiss-1 in ARC neurons (Hill et al, 2007). Castellano *et al.* (2009) reports that there at least three lines of evidence that show that leptin regulates kiss-1. First, thre expression of kiss-1 mRNA decreases significantly in both intact and gonadectomized leptin deficient mice (ob/ob). In both cases, the infusion of leptin reverts the reduction of kiss-1. Secondly, in diabetic rats where there is hypoleptinemia or hypoinsulinemia, the chronic infusion of leptin, but not insulin normalizes the levels of kiss-1 in the hypothalamus. Thirdly, *in vitro* studies with murine kiss-1 neurons, leptin is capable of increasing the expression of kiss-1 mRNA.

## CONCLUSION

The information presented here shows that under nutritional stress, the decrease in circulating concentrations of IGF-I, insulin and leptin is associated with reduced release of GnRH. Under low energy intake or reserves, IGF-I concentrations are low and has a low impact on kiss-1 and GnRH secretion. Similarly, the reduction in insulin and leptin concentrations allows an increase in NPY and a reduction in POMC and GALP, which will inhibit GnRH secretion. In addition, the reduction in leptin decreases kiss-1 and the positive effect of kiss-1 on GnRH neurons. In contrast, when energy balance is positive, IGF-1 increases and directly stimulates the synthesis and secretion of GnRH and kiss-1. Whereas the increase in leptin increases kiss-1 and leptin and insulin decrease NPY and increase POMC, GALP to reestablish the function of the hypothalamic-pituitary-gonadal axis (Figure 1).

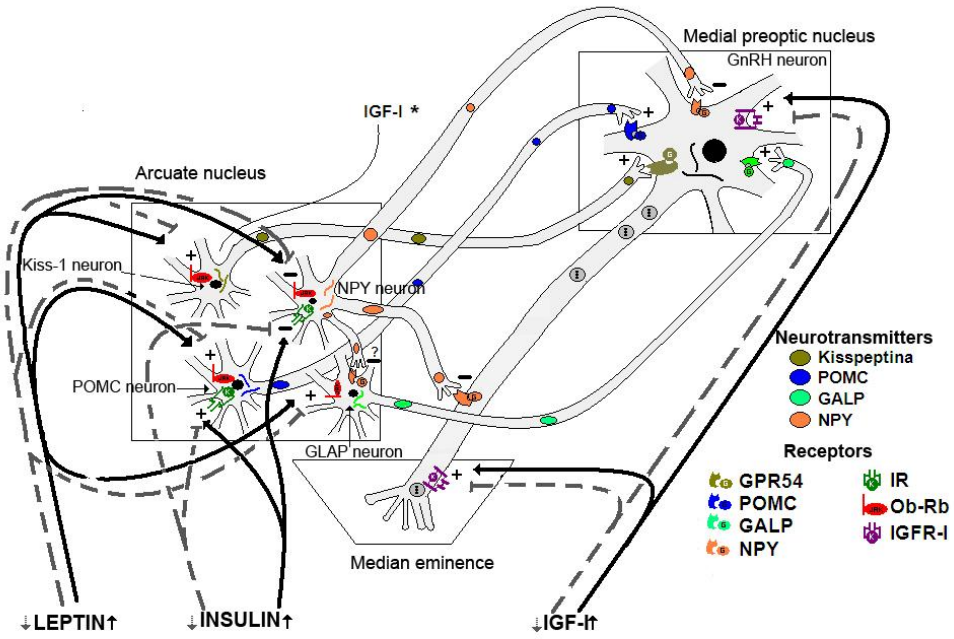


Figure 1. Neuronal integration of the pathway by which insulin, IGF-I and leptin regulate the functioning of the GnRH neurons.
+,- = Stimulating or inhibitory effect of the hormone on the synthesis or release of the neurotransmitter; ↓↑ = low or high peripheral concentrations of insulin, IGF-I and leptin;* = the IGF-I receptor has not been reported in kiss-1 neurons but IGF-I increases the synthesis of kiss-1. Dotted line = low concentrations of insulin, IGF-1 and leptin when they cannot exert their effect on neurons that have their receptor; Continuous line = when the balance of energy is positive, the concentrations of insulin, IGF-I and leptin increase and may then affect the neurons that have their receptors; POMC = proopiomelanocortin; GALP = galanin-like peptide; NPY = neuropeptide Y, GPR54= kisspeptin receptor, IR = insulin receptor, Ob-Rb = Leptin receptor, IGFR-I = insulin like growth factor receptor type I (IGF-I)

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