The role of kisspeptin in neuroendocrine disorders.

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### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>Arc</td>
<td>Arcuate nucleus</td>
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<td>AVPV</td>
<td>Anteroperiventricular nucleus</td>
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<tr>
<td>cAMP</td>
<td>Cyclic adenosine monophosphate</td>
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<tr>
<td>CNS</td>
<td>Central Nervous System</td>
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<tr>
<td>CXCR4</td>
<td>Chemokine (C-X-C Motif) Receptor 4</td>
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<tr>
<td>DHT</td>
<td>Dihydrotestosterone</td>
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<tr>
<td>E2</td>
<td>Estradiol</td>
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<tr>
<td>FSH</td>
<td>Follicle-stimulating hormone</td>
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<tr>
<td>GPR</td>
<td>G-protein coupled receptor</td>
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<tr>
<td>GTP</td>
<td>Guanosine-tri-phosphate</td>
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<tr>
<td>hCG</td>
<td>Human chorionic gonadotropin</td>
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<tr>
<td>HH</td>
<td>Hypogonadotropic Hypogonadism</td>
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<tr>
<td>IP3</td>
<td>Inositol-(1,4,5)-triphosphate</td>
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<tr>
<td>IPP</td>
<td>Idiopathic Precocious Puberty</td>
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<td>IVF</td>
<td>In vitro fertilization</td>
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<tr>
<td>Kiss1r</td>
<td>Kisspeptin Receptor (Rodents)</td>
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<td>KISS1R</td>
<td>Kisspeptin Receptor (Human)</td>
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<td>KO</td>
<td>Knock Out</td>
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<td>KP</td>
<td>Kisspeptin</td>
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<tr>
<td>LH</td>
<td>Luteinizing hormone</td>
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<td>OHSS</td>
<td>Ovarian hyperstimulation syndrome</td>
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<td>P</td>
<td>Progesterone</td>
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<td>PCOS</td>
<td>Polycystic ovary syndrome</td>
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<tr>
<td>PKC</td>
<td>Phosphokinase C</td>
</tr>
<tr>
<td>PLC</td>
<td>Phospholipase C</td>
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<tr>
<td>PP</td>
<td>Precocious Puberty</td>
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<tr>
<td>RT-PCR</td>
<td>Reverse transcription polymerase chain reaction</td>
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<tr>
<td>T</td>
<td>Testosterone</td>
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<tr>
<td>T2DM</td>
<td>Type 2 Diabetes Mellitus</td>
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<td>WT</td>
<td>Wild Type</td>
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Abstract
The kisspeptin system possesses a role in the neuroendocrine control of the reproductive axis. The localization of kisspeptin protein, as well as the localization of its receptor is widespread: high levels in the placenta and the central nervous system; particularly in the hypothalamus (arcuate nucleus and antero-periventricular nucleus) and in the limbic system (hippocampus and medial amygdala). This permits to assert that kisspeptin evokes the GnRH pulses which govern the reproductive function. The system is implicated in menstrual cycle, puberty, and metabolism by the leptin hormone, among others. Furthermore, kisspeptin has been linked to several disorders: loss or gain of function mutations in kisspeptin gene or KISS1R can occur, leading to pathologies such as hypogonadotropic hypogonadism, PCOS, hypothalamic amenorrhea, or precocious puberty, and metabolic diseases such as obesity or diabetes (T2DM). In conclusion, data on the kisspeptin system points to its pivotal role in neuroendocrinology and, as such, bears a therapeutic potential that needs further investigation.

Introduction
The kisspeptin system has been one of the most promising discoveries of the last twenty years. First discovered by its metastasis suppressor activity, its regulation of the neuroendocrine axis soon appeared to be a major center of interest. We concentrate our attention on kisspeptin’s implication in the reproductive axis, therefore the ovarian cycle and puberty. In order to understand better those physiological processes, we have investigated the question of sexual dimorphism of the brain, and kisspeptin’s involvement in this condition. But more importantly, after thinking about physiological mechanisms, its possible participation in different neuroendocrine disorders arise. Therefore we have reviewed the actual data on the activity of the kisspeptin system in polycystic ovary syndrome, hypogonadotropic hypogonadism, precocious puberty, obesity and diabetes. To conclude, we will discuss the potential therapeutic role of kisspeptin in those diseases.

I. The kisspeptin system: kisspeptin protein and KISS1R
1) KISS-1 gene and Kisspeptin protein
   a. Discovery of kiss-1 gene.
Kiss-1 gene was first isolated in 1996, by subtractive hybridization in melanoma cell lines with different metastatic capacities. The gene was overexpressed in metastasis-suppressed tumour cells, and so, considered as a metastasis suppressor gene. Further experiments have shown that
KISS-1 gene was expressed in melanoma, breast carcinoma, pancreas cancer, and papillary thyroid carcinoma. Low levels of the gene were of poor prognosis factor in tumour proliferation and metastasis in oesophageal squamous cell carcinoma, gastric carcinoma, and bladder cancer, among others [13].

As the discovery occurred in Hershey, Pennsylvania, the protein encoded by the KISS-1 gene and the gene were named after the hometown firm Hershey’s Kiss’ chocolates [13].

b. Structure of KISS-1 gene and kisspeptin protein.

Human KISS-1 gene is located on chromosome 1 (1q32). It possesses three exons: the first one (111 bases) is not transcripted, the second one (141 bases) contains a start codon at position 39, the third one (332 bases) contains the stop codon and the polyadenylation sequence [13]. The deduced sequence of the precursor KISS-1 protein is composed of 145 amino-acids (Figure 1). The major product of KISS-1 gene appears to be composed of 54 amino-acids (kisspeptin-54, also called metastatin). Then, other kisspeptin proteins have been identified: kisspeptin-14, kisspeptin-13, and kisspeptin-10 [13].

![Kisspeptin gene and its protein products](http://d6igaq6njxgjh.cloudfront.net/content/physrev/92/3/1235/F2.large.jpg)

In mice, KISS-1 gene is located on chromosome 1 too, but not for the rat (chromosome 13q13). The structure of the gene is highly homologous (over 90%) between the two rodent species, considering the cDNA sequence and the genomic organization. [13]

Human kisspeptin proteins present an Arg-Phe-NH2 motif, which includes them in the RF-amid family. It is a large family of regulatory peptides. They are involved in nociceptive transduction, feeding behaviour, regulation of pituitary hormones secretion, among other actions. They are widely represented in the central nervous system. [13]
c. Localization of KISS-1 mRNA and kisspeptin.

In humans, screening analyses showed prominent expression of KISS-1 gene mainly in the placenta, and at lower levels in the periphery. KISS-1 mRNA was prominently detected in the human brain, breast, and pancreas [12]. In the central nervous system (CNS), KISS-1 gene particularly expresses the mRNA in the hypothalamus and in the basal ganglia. Another study proved that in human brain, using metastatin-like immunoreactivity (irMT), high expressions of KISS-1 mRNA were detected in the infundibular nucleus [12] and the hippocampus (Hi), suggesting a potential role of kisspeptin on emotions, memory and neuroendocrine responses [13]. In mice, further experiments with in situ hybridization indicated that the arcuate nucleus (Arc), the periventricular nucleus (PeN) and the antero-periventricular nucleus (AVPV) were high expressing regions of the kisspeptin mRNA. In addition, KiSS-1 mRNA expressing cells were also detected in the antero-dorsal preoptic nucleus, the medial amygdala (Amy), and the bed nucleus of the stria terminalis (BST) [13]. In the rat, traces of KiSS-1 mRNA were found in the colon and cecum, with moderate to low expression levels, in different peripheral tissues, including the placenta. Moreover, KiSS-1 gene expression was demonstrated in rat brain (and specifically in the hypothalamic regions such as Arc and AVPV areas) (Figure 2) [12].

![Anatomical distribution of Kiss1 mRNA (blue dots), kisspeptin immunoreactivity (red dots) and GPR54 mRNA (black dots), in the different brain areas of rodent brain](image)

**Figure 2:** Anatomical distribution of Kiss1 mRNA (blue dots), kisspeptin immunoreactivity (red dots) and GPR54 mRNA (black dots), in the different brain areas of rodent brain [13].

2) KiSS-1 receptor

a. Discovery, structure and signalling pathways of the KiSS-1 receptor.

The receptor of kisspeptin was detected first in 1999 as an orphan receptor (GPR54); the proper ligand of it (KiSS-1 receptor (KISS1R)) was discovered independently by three groups two years later, in 2001. It was found in the brain area of rats. It was also brought to light, that human brain contains the above-mentioned receptor, which consists of 396 (398?) amino acids and is a heptahelical transmembrane protein. It is a typical G-protein coupled receptor: it connects to an intracellular molecule named after guanine, because G-protein needs guanosine-tri-
phosphate (GTP) to be activated. G-protein-coupled receptors could be differentiated by their signalling pathways. Indeed, those pathways may either cause activation an increase of cAMP or a Ca\textsuperscript{2+} release, for example. The latter plays the main role in the kisspeptin inducing signalling pathway, so the receptor could be determined as G\textsubscript{q/11}. A secondary intracellular messenger called inositol-(1,4,5)-triphosphate (IP3) enhances the level of the calcium; it is derived from the hydrolysis of a phospholipid located in the cell membrane (phosphatidylinositol 4,5-bisphosphate) by phospholipase C (PLC). This particle binds to its receptor in the membrane of the endoplasmic reticulum to elicit the calcium ions from stores [12]. However the entire process might be described as a biphasic increase of calcium-level: firstly there is a rapid enhance, then a slower one. This process is maintained for a quite long while [16]. A lot of functions are attributable to kisspeptin considering the known signalling pathways influenced by receptor-ligand interaction like phosphokinase C (PKC), arachidonic acid, mitogen activated protein kinases (MAPK). Moreover, as the original name of kisspeptin refers to it, the peptide has metastasis suppressor effects by activation of focal adhesion kinase, and inhibition of chemotaxis (CXCR4, calcineurin etc.) [12].

\textit{b. Localization of KISS1-R}

A lot of studies published results about the localization of the molecule in some species: rat, mouse, sheep, monkey and humans. In humans, using RT-PCR, Northern blotting and Immunohistochemistry, the KISS1R mRNA was detected in several human brain areas and in peripheral tissues like placenta, pancreas, heart etc [12]. In mice, the Kiss1r was found in several regions of CNS by X-gal histochemistry, which uses the β-galactosidase enzyme to demonstrate the activity of certain genes. CNS regions were as follow: dentate gyrus of hippocampus, GnRH neurons, periventricular region of the posterior hypothalamus, but not the rostral part of third ventricle or the arcuate nucleus [12]. In rats, the kisspeptin receptor was discovered in brain areas such as pons, midbrain, striatum, hypothalamus, amygdala, frontal cortex, thalamus, diagonal band of Broca, preoptic area, pituitary, among others, and also in peripheral tissues (liver, intestine) (Figure 2) [12].
II. **Physiology of the KISS-1 system**

1) Roles of kisspeptin on the ovarian cycle

The ovarian cycle could be divided in two phases by ovulation: the pre- and postovulatory phase also called respectively follicular and luteal phase (Figure 3) [3].

![Figure 3: The ovarian cycle. (http://biologyforums.com/gallery/33_01_08_11_10_36_29_1261447.jpeg)](http://biologyforums.com/gallery/33_01_08_11_10_36_29_1261447.jpeg)

**a. Follicular phase**

At this point it is important to know that the level of LH increases continuously, but very slowly, until the occurrence of the LH surge, which happens because of the high estradiol concentration and its positive feedback to GnRH. The FSH surge is less marked. The LH surge is followed by ovulation. In summary, both the positive and the negative feedback are determined by the amount of estrogen secreted from the follicles. In the early follicular phase, more follicles are recruited, but later, when the blood concentration of FSH is reduced enough, only one – the dominant – follicle is able to develop and produce estrogen continuously [3].

**Kisspeptin’s role:** To assess if kisspeptin influences the progress of LH surge, kisspeptin antagonist via central infusion was administered to adult females after of which they failed to develop an LH surge [11]. However, when kisspeptin-54 was taken by women regularly, twice-daily, the LH-surge appeared earlier and the menstrual cycle was shortened [17].

To estimate the effect of kisspeptin particularly in the early follicular phase, exogenous kisspeptin was administered to women, and the response to the treatment was observed. As the results of this investigation suggested, the level of endogenous kisspeptin during the early follicular phase of fertile women is likely higher than by postmenopausal ones, because the influence of exogenous kisspeptin was restricted during the above-mentioned period, and it did not lead to the development of an LH surge [17].

**b. Luteal phase**

After the ovulation, the neighbouring cells of the follicle form the corpus luteum. The corpus luteum is responsible for the secretion of progesterone (P) in the luteal phase and after fertilization within the early weeks of pregnancy. Therefore, the level of P and estrogen increases, but the amount of LH and FSH is reduced. Furthermore, the corpus luteum stops producing P in the absence of pregnancy, and the level of these hormones decreases. Finally, the ovarian cycle could be initiated again [3].
Kisspeptin’s role: An interesting experiment was performed in which women treated with contraceptives, and women without contraceptives, were administered KP. The sensitivity to KP differed between the two groups. This observation could also prove that the absolute and relative level of sex-steroids play a key role in the regulation of kisspeptin system [17]. Based on the above-mentionned data, clearly, KP regulates the reproductive axis. This is also supported by localization data, previously mentioned.

The Arc and AVPV/PeN regions of the hypothalamus contain Kiss1 neurons. KISS1 neurons bear receptors for E2 and T. It has been proved, that in the AVPV, E2R stimulate the KP release, whereas they inhibit it in the Arc. This provides an explanation for the positive and negative feedback mechanisms on the reproductive axis. Moreover, KISS1 neurons project on GnRH neurons, but at different places. The KISS1 neurons of the AVPV/PeN project on the body cell, whereas the KISS1 neurons of the Arc project on the termination of GnRH neurons in the median eminence (Figure 5) [11].

Figure 4: Variations of the gonadotropic and ovarian hormones along the days of cycle.
http://biology-forums.com/gallery/33_01_08_11_10_36_29_1261447.jpeg

Figure 5: KISS1 neurons project to and stimulate GnRH neurons, acting at either cell body (AVPV/Pen neurons) or GnRH fiber terminals in the median eminence (Arc neurons) [12]
2) The relationship between the sexual differentiation of the brain and kisspeptin.

The sex differences may be regulated by kisspeptin primarily in the sensitive periods of perinatal life. The kisspeptin neurons display sexual dimorphism in the hypothalamus where the two groups of Kiss1r neurons may be discriminated, as previously mentioned. In addition to several differences between the above-mentioned subtypes: the number of KISS1 neurons and the expression of kisspeptin per cell are different in females and males (female brain contains more kisspeptin in AVPV), because of the distinct perinatal exposure to certain hormones: testosterone (T) and estradiol (E2). The latter is converted from testosterone by aromatase enzyme. If sex steroids are present during the sensitive period, the brain becomes male-like. On the contrary, in the absence of E2 and T, a female-like brain develops [11].

On a functional level, sexual dimorphism is well characterized by the menstrual cycle. LH surge is the most important feature of this. Normally, the LH-peak is created by positive feedback of estrogen. Kisspeptin relays this effect to the GnRH neurons, and thus to the LH cells. Furthermore, as we already mentioned, kisspeptin neurons display sexual dimorphism. Naturally the question arises: is there a relationship between kisspeptin and the male/female-like development [11]?

More experiences were carried out to answer this question. One of them is likely to prove the role of kisspeptin. Kiss1r KO male mice were created, in which GnRH neurons do not express kisspeptin receptor selectively. Hereupon, the differentiation of the brain was not complete, and the testosterone surge, which appears after birth, did not develop. This suggests that KP may be responsible for the perinatal activation of GnRH neurons [3].

Besides, female mice lacking alpha-fetoprotein in the crucial period (which molecule is bound to E2 to obstruct its activity), the number of Kiss1 neurons in AVPV were reduced and missed the LH surge in adulthood [11].

To sum it up, kisspeptin is the key element of the ovarian cycle: without the increased level of it in AVPV, the LH surge is not possible. Obviously, KP expression depends on E2 as well, since E2 via positive feedback enhances the amount of KP in AVPV in the late follicular phase, which corresponds to the increase of GnRH and LH surge [11].

3) Puberty.

a. Normal physiology of puberty

Puberty is not a single definite event that makes an infant suddenly become a person capable of reproduction. It is a dynamic process, generally defined as the activation of the previously
dormant neuroendocrine reproductive axis. Starting around the age of ten years old, puberty stimulates gametogenesis and the development of secondary sex characteristics. In females, puberty leads to the apparition of the oestrous cycle and ovulation, indicating the ability to procreate. However, the molecular and neural mechanisms inducing puberty are still an endocrinal enigma. GnRH pulses were first thought to be the cause of puberty, but it is now known that the increase of GnRH and its pulsatile release are only the results of an upstream regulation. Considering, as explained previously, that KP exerts its effect on GnRH neurons during the menstrual cycle, it is plausible that it has a role in puberty as well [6].

Moreover, the onset of puberty could also be influenced by fat mass and bone development. Indeed, in the 1940s a new contention was introduced by Rose Frisch: she proposed that instead of reaching a critical age, girls had to reach a critical fat mass, or fat/lean ratio, for menarches to occur. Later on, more data accumulated that link puberty with fat mass (leptin) [21].

b. Kisspeptin’s implication in puberty
As discussed earlier, GnRH neurons bear KISS1R. Indeed, KO mice for the Kiss1r showed no more GnRH secretion. But, studies interested in its implication in puberty started in 2003. Two independent groups discovered inactivating mutations on KISS1R associated with impaired pubertal development in some patients with hypogonadotropic hypogonadism [16]. Later on, in 2008, an “activating” mutation on KISS1R gene occurred in a girl with precocious puberty [19]. So, mutations either of the gene encoding kisspeptin or its receptor can cause pubertal developmental defect.

During puberty, in rodents and primates, the hypothalamic expression of Kiss1 and Kiss1r mRNA is up-regulated. But, more interestingly, in mice, the percentage of GnRH neurons depolarization in response to KP stimulation increases from juvenile (25%) to prepubertal (50%) to adult mice (>90%). This suggests that GnRH neurons become more and more sensitive to kisspeptin across pubertal development [17]. Therefore, the KP/KISS1R signalling has undoubtedly a key role in the initiation and progression of puberty.

c. Kisspeptin’s link with metabolism
Human reproductive function is altered by two extreme states: undernutrition and obesity. In rodents and pubertal monkeys who were subjected to fasting, the level of Kiss1 mRNA decreased, as well as the gonadotropin level [16]. KP could therefore provide a link between the energy stores and the reproductive system. In 2006, leptin receptors were discovered on kisspeptin neurons in mice (40% in Arc). Also, leptin-deficient mice showed a decrease of KISS-1 mRNA expression [17]. Furthermore, humans diagnosed with hypogonadism showed
mutations in leptin, or leptin receptor [16]. Therefore, kisspeptin seems to be partially up-regulated by leptin.

III. Neuroendocrine disorders:

1) Polycystic ovary syndrome (PCOS)

Infertility is very frequently associated with PCOS that affect 100 million women worldwide. The symptoms are the following: hyperandrogenism, anovulation, enlarged polycystic ovaries, irregular cycle, elevated leptin level, and insulin resistance [22].

The chief cause of the disease may be hyperandrogenism, supported by the experiment in which female monkeys were exposed to testosterone; after of which they demonstrated the signs of PCOS [22]. In addition, Brown et. al developed an animal model of PCOS. They treated prepubertal rats with dyhidrotestosteron (DHT). These rats suffered from a similar disorder as girls with PCOS. Thus, not only the key role of androgens is suggested, but also the timing of the exposure is emphasized; the prepubertal (or maybe fetal) period could have importance in the development of the disease. Hereupon the research group raised the question: how will the kisspeptin level alter after exposure to DHT in central (hypothalamus) and peripheral tissue (ovary, fat, pituitary)? The measurements were carried out 26 and 60 days after the treatment. The researchers observed the following: increase in body weight, clitoral enlargement; cystic follicles on the surface of the ovary were detectable and the corpora lutea were absent from the ovaries of treated rats [22].

Furthermore, staining methods were used to reveal the changes of kisspeptin level after exposure to DHT. In hypothalamus - both in the ARC and in AVPV – the expression of kiss1 mRNA after 26 days decreased (almost completely absent), but after 60 days, mRNA expression returned to normal. In contrast, the kisspeptin protein was not detectable after 60 days (Figure 6). It could be explained either by the excretion of DHT pellets: the mRNA level could return to the normal level, but inhibitory effect on translation remained; or the cells might have become tolerant to DHT. Nevertheless, it seems to be likely that the kisspeptin level in the hypothalamus is regulated by androgens [22].

In addition, the level of kisspeptin was elevated in the ovaries. Since the amount of E2 was not affected, the reduced level of P could be the reason for it: the inhibitory effect of P on KP ceased [22]. Furthermore, in the adipose tissue, KP level increased; it could be possible, that both KP and DHT play a role in the adipogenesis. Furthermore, the level of KP is elevated in obese girls, and that may derive from the adipose tissue [22].
Based on these facts, it is supposed that PCOS may be characterized with alterations in KP signaling. It is necessary to mention, that one characteristic symptom of PCOS was not reproduced in this animal model: the enhanced pulsatile of LH is unconceivable to appear because of the decreased level of KP. Thus, further investigations are needed to clarify the relationship between kisspeptin and PCOS [22].

2) Hypogonadotropic Hypogonadism
   
   a. The disease

Hypogonadotropic hypogonadism is diagnosed by the absence of spontaneous sexual maturation as well as low blood levels of sexual steroids. Its prevalence is higher in males, and is around 1/100000. It can be either congenital or acquired. The normosmic HH, representing about 1/3 of congenital cases, called idiopathic hypogonatropic hypogonadism (IHH) will be the only type discussed [7].

Figure 6: Effect of DHT exposure on kiss1 expression [22].
b. Implication of the kisspeptin system

In 2003, Seminara et al., first found a defect in KP system in a Saudi consanguineous family. The patients showed a complete or partial absence of LH pulse. Their response to exogenous GnRH was successful, indicating the localization of the abnormality on the GnRH and consequently LH & FSH synthesis, secretion or activity. The research team identified a mutation in the KISS1R gene by screening the candidate gene. Then, several more mutations were detected in unrelated patients. The recurrent mutations are located at codon 148 (L148S), 331 (R331X) or 339 (X399R) [16]. Those mutations are likely responsible for the inhibition of the catalytic activation of Gq, so the inhibition of phospholipase C and its signalling pathway, leading to the decrease of IP3 (Figure 7) [21].

![Figure 7: Dose–Response Curves for the Ligand-Stimulated Production of Inositol Phosphate in Mutant Constructs. [16]](image)

3) Low levels of steroids in type 2 diabetes and obesity

Diabetes and obesity are two main diseases concerned about hypogonatropic hypogonadism (concerning ¼ to ½ of men with type 2 diabetes (T2DM)). Besides, rats with diabetes showed reduced levels of hypothalamic Kiss1 mRNA, with subsequently low levels of GnRH and sex-steroids. In obese and type-2 diabetes men, low levels of sex steroids and of GnRH were detected. This could raise the possibility of the involvement of kisspeptin and its diminished secretion in hypogonadism [17]. To summarize, kisspeptin acts downstream to metabolic signals and conveys information about energy stores to GnRH neurons, thereby regulating puberty, and consequently, reproduction [17].

For now, experiments and data were only collected on animals and especially on mice, in order to discover the physiopathological mechanisms of the KP system in metabolic diseases. One of the most important study done on this topic is the one made by Tolson et al. in 2014: they have considered the body weight (BW), leptin levels, adiposity and glucose-tolerance in KO mice for Kiss1r, and then compared those to wild type (WT) mice. The results are outstanding: only Kiss1r KO female mice gained BW, adiposity, and developed an impaired glucose-tolerance. Kiss1r KO males and females both showed increased leptin levels.
Moreover, female mice ate less than the WT ones, although they were 30% more obese (Figure 8). This can be explained by the higher leptin level, since leptin centrally decreases feeding behaviour [20].

The Kiss1r KO female mice were not hyperphagic, but still obese, either the metabolism or the energy expenditure were reduced. In fact, the oxygen consumption, the respiratory exchange ratio and the locomotor activity were decreased. Moreover, those Kiss1r KO female mice had lower E2 and sexual steroids levels. In order to know if the cause of this was the decrease in E2, Kiss1r KO female mice were ovariectomized and their development compared to ovariectomized female WT mice. Ovariectomized Kiss1r KO female mice still gained more weight (17%) than WT mice, and still showed obesity and impaired glucose regulation, despite the same absence of E2 in both mice. Hence, the Kiss1r KO mice phenotype is not caused solely by absent pubertal or adulthood E2 secretion. Concerning the glucose-tolerance, Kiss1r KO female mice displayed higher basal glucose levels as well as an impaired glucose-tolerance. Male mice didn’t undergo any of those changes. (Figure 9) [20].

These experiments show that kisspeptin influences the metabolism in a sexual dimorphic and partially steroid-independent way. The discoveries on mice are very promising regarding disorders occurring in human obesity and diabetes [18].
4) Precocious puberty

Since 2008, precocious puberty has been strongly linked to the kisspeptin metabolism. PP is defined by the development of secondary sexual characteristics, before the age of 7 for girls and 9 for boys [19]. There are two types of PP: first, the central PP (CPP) characterized by high levels of GnRH and LH, so called gonadotropin-dependent. Second, the gonadotropin-independent type. We will focus on CPP.

In 2008, Teles et al., found an “activating mutation” on the KISS1R gene, in an 8 year old girl. Clinically, the little girl showed breast and pubic hair growth but the other additional signs of puberty, such as oily skin, acne, axillary hair, were absent [19]. Her bone age was 11 years old. LH as well as GnRH levels were as in the prepubertal ones, but her serum E2 was above the norm [19]. Since the imaging of her CNS, as well as the pelvic ultrasonography indicated no abnormalities, ICPP was diagnosed. DNA analysis then indicated a mutation on the KISS1R gene: the replacement of a cytosine by a guanine, leading to the apparition of a proline instead of an arginine in position 386 (Arg396Pro) [17].

The mutation’s consequences on KISS1R are a prolonged intracellular signalling (increased levels of IP3 and of phosphorylated extracellular signal-induced kinase). Moreover, KISS1R stays longer on the cell-surface [20]. In addition, the plasma level of KP compared before and after treatment (by GnRH analogs) in IPCC girls significantly decreased during this treatment period, and the KISS1 gene expression returned to pre-pubertal one [23].

![Figure 10: KP level of girls with ICPP at the time of diagnosis and after and those of girls in PT group and control group. CPP, idiopathic central precocious puberty; PT, premature thelarche. [23]](image)

**IV. Potential therapeutic role of kisspeptin**

As we discussed earlier, KP system deficiency (either of KP or its receptor) is involved in many reproductive diseases, as well as metabolic ones. Those diseases imply potential targets for
treatments. Since most of the pathology presented above include an abnormal LH pulse, its correction could be either enhanced or diminished by administration of agonists or antagonists of KP [4]:

- In T2DM, patients undergoing infusion of KP showed stimulated LH levels and testosterone levels. Moreover, the LH pulse was restored [8].
- In PP, injections of KP activated puberty in monkeys and rodents, suffering from the disease [4].
- In hypothalamic amenorrhea: LH pulse deficiency were cured by high doses of KP-54. However, chronic administration causes desensitization. Therefore, there is a dose-dependent therapeutic window of kisspeptin’s action which needs to be further investigated [9].

Moreover, nowadays, in vitro fertilization (IVF) may be an essential opportunity to conceive for infertile women. Besides the obvious advantages, IVF also has some disadvantages. One of them is the ovarian hyperstimulation syndrome (OHSS), which could be life threatening. OHSS may develop because of the human chorionic gonadotropin (hCG), which is used by IVF in order to trigger the ovulation. To avoid the side-effect of hCG, it is suggested to employ another, safer medication without adverse effect. GnRH agonists are available as an alternative drug to elicit ovulation, although pregnancy, by women treated with GnRH agonist, could be observed less frequently; it is less harmful compared to hCG. However, experiments are underway to find a more physiological, and effective molecule to play the role as elicitor of ovulation. KP may be a candidate [1].

Since KP has a leading role in the induction of LH surge and ovulation, and the level of it is increased also during a normal pregnancy, it seems to be a promising drug in IVF [10]. To estimate the effect of KP in IVF, 53 subfertile women were randomly chosen: following the administration of GnRH antagonist and FSH, they received subcutaneous injection of KP-54 [10].

Egg maturation occurred in all participants, and the number of mature egg depended on the dose. In addition, egg fertilization and transfer of embryos was successful in 92% of the patients [10]. To measure the concentration of hormones, blood samples were collected from some patients. The results provided information about the alteration of hormone levels after KP-54 injection. Five hours after the administration the LH peak was [10].
Considering the ten live births after the IVF using kisspeptin treatment, it could be supposed that kisspeptin can contribute to successful fertilization. However, adverse effects and the most effective dose need to be investigated in the future [10].

References


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