**PRESENTATION SUMMARY & POWERPOINT**

**Kisseptins and GnIH**

D. Fellmann, C. Pralong and P.Y. Risold

In both males and females, multiple signals are integrated in the hypothalamus to set the reproductive axis to the favorable conditions necessary for fertility and successful mating. Photoperiod, food availability, temperature, stress, and hormonal cues are some of the varied signals used by mammalian species to activate or suppress gonadotropin-releasing hormone (GnRH) secretion from the hypothalamus. Through its stimulation of LH and FSH from the pituitary, GnRH represents a critical step in the reproductive cascade. Thus, the GnRH neuronal system represents the final common pathway in the neural regulation of reproduction. GnRH neurons are located in anterior and mediodlateral areas of the hypothalamus. They project to the median eminence, but also to brain areas involved in the control of reproductive behaviors. Feedback control of the GnRH system is mainly accomplished by sex hormones acting either directly on GnRH neurons or on gonadal-steroid-responsive systems upstream of GnRH. However, despite 30 years of intensive research, little is understood of the mechanisms influencing the physiologic controls of GnRH neurons, and analyses of their neural connections mainly revealed interactions with common, widely distributed neuron populations.

The recent discovery of two peptides with specific and opposite effects on GnRH secretion clearly highlighted a new pathway for reproductive control. These unexpected findings provide us with a novel standpoint to study the physiological regulation of reproduction, as well as its pathology. They also open new avenues for pharmacological or vaccinal control of fertility.

Kisseptins, the peptide products of the KiSS-1 gene, were identified in 2001 as natural ligands of the previously orphan G protein-coupled receptor, GPR54, by using a reverse pharmacology screening technology. The first identified biological functions of kisseptins was their ability to suppress tumour metastasis, hence the name of metastin. However, in 2003, two groups reported that loss-of-function mutations of the GPR54 gene are linked to absence of puberty onset and hypogonadotrophic hypogonadism in humans. GPR54-null mice reproduced the same phenotype. These observations revealed a totally unexpected role of the KiSS-1/GPR54 system in control of reproduction and boosted investigations for the characterization of these novel functions of kisseptins. Indeed, in the last two years, the hypothalamic KiSS-1/GPR54 system has been proven as an essential gatekeeper of GnRH neurons, involved in their activation at puberty and their regulation by gonadal steroids. Studies in several mammalian species have shown that kisseptins stimulate the secretion of gonadotropins from the pituitary by stimulating the release of GnRH from the median eminence, after the activation of GPR54, which is expressed by GnRH neurons. Kisseptins are expressed in neurons of the arcuate nucleus and the anteroventral periventricular nucleus of the hypothalamus. These neurons synaptically contact GnRH neurons and they express steroid hormone receptors. Their responses to gonadal steroids suggest that kisseptin neurons in the arcuate nucleus are involved in the negative feedback regulation of gonadotropin secretion, whereas
kisspeptin neurons in the anteroventral periventricular nucleus may contribute to generating the preovulatory gonadotropin surge in the female.

In addition, the presence of the kisspeptin-GPR54 system was demonstrated in rat ovaries. The ability of the LH surge to induce ovarian expression of KiSS-1 at the pre-ovulatory period strongly suggests a role of locally produced kisspeptins in the control of ovulation. In the male, recent results suggested a down regulation of the hypothalamo-pituitary testicular axis response to kisspeptin following continuous administration.

These findings clearly demonstrate that kisspeptins are a novel tool for the manipulation of the gonadotropic axis and gametogenesis. However, possible therapeutic uses of agonists, or antagonists, of these peptides should not neglect the fact that they present other important physiological roles: Their metastasis suppressor effects mediated by GPR54 correspond in part to cell cycle arrest and induction of apoptosis in malignant cells; impacts on motility, chemotaxis, adhesion and invasion have also been documented. The GPR54/KiSS-1 system is also expressed in the endocrine pancreas, where it influences beta cell secretory function, but has no significant effect on glucagon secretion. These observations suggest an important role for this system in the normal regulation of islet function. Finally, KiSS-1 and GPR54 mRNAs were detected in the placenta, suggesting that kisspeptin-GPR54-signaling may participate in implantation of the mammalian embryo, placenta formation, and maintenance of pregnancy.

In 2000, a novel hypothalamic neuropeptide inhibiting gonadotropin release at the level of the pituitary was discovered in quail and named gonadotropin-inhibitory hormone (GnIH). It belongs to the vast family of RFamide peptides, and presents a characteristic « LPLRFamide » C-terminal peptide sequence. The receptor for GnIH was further identified and its expression and binding activity were characterized. It was found in the pituitary and several brain regions, including the hypothalamus. These results suggest that GnIH acts directly on the pituitary via GnIH receptors to inhibit gonadotropin release. GnIH may also act on the hypothalamus to inhibit GnRH release.

Through gene database searches, mammalian homologues similar in structure to GnIH were identified. They encode two RFamide peptides (RFRP-1 and -3). Their existence was known for several years and they had been found to participate in regulating prolactin, oxytocin, CRH release, and to take part in feeding behavior, but their role as regulators of gonadotropin secretion had not been discovered.

In the mammalian brain, RFRP cell bodies are located in the medial hypothalamus. GnRH cells receive important RFRP projections, suggesting the potential for direct inhibitory control. The gonadotropic role of mammalian RFRP was further confirmed in vivo: RPRF administration rapidly and markedly inhibits LH secretion. RFRP neurons express estrogen receptor-α and respond to estrogen, suggesting activation by gonadal steroids. Several studies also suggested that mammalian RFRP may mediate negative feedback effects of gonadal steroids at both the hypothalamic and pituitary levels. Thus, RFRP clearly appears as the mammalian homologue of avian GnIH.
Kisspeptins and RFRP have recently emerged as important regulators of the reproductive axis, underscoring the importance of further investigations into the neural, cellular, and molecular mechanisms by which these peptides act. Their potential for the manipulation of the gonadotropic axis and gametogenesis deserves a very particular interest.
Endogenous GnRH control:

Kisspeptins and GnIH

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GnRH summary

70s

Peptide discovery:

LH-RH in ovine hypothalamic extracts

Neuron distribution in the brain

Gonadotropic properties unraveled:

LH and FSH release

→ LH-RH became GnRH

Reproductive behavior

Pulsatile secretion pattern
GnRH neurons in human diencephalon

Medial - basal hypothalamus (3 - 4)
- Neuroendocrine effects
- « tonic » regulation

Anterior hypothalamus / Preoptic area (2)
- Neuroendocrine effects
- « cyclic » regulation

Other sites: telencephalon (1), posterior hypothalamus (5), mesencephalon ...

Other effects, behavioral regulations
GnRH summary

80s Control of GnRH secretion

steroids:
* evidence for positive and negative feedbacks
* lack of ERα receptors in GnRH neurons
  ➡ interneurones relaying gonadal status

neurotransmitters:
many « unspecific » stimulators or inhibitors
Glu NE DA GABA
Gala SP NPY Endo Enk CRH …
Innervation of GnRH neurons in human brain

Inputs from estrogen-sensitive neurones:
NPY
SP
Endorphins
Enkephalins
CRH
Galanin
TH

But also Glu, GABA, NE, DA, NO ...

Dudas & Merchenthaler, J Neuroendocrinol
GnRH neurons and steroids
GnRH summary

80s  Extra CNS locations and effects
     gonads, placenta, tumors, immune system ...

     Gene identification
     mutation results in hypogonadism

     Origin of GnRH neurons (olfactory placode)

90s  Receptor and intracellular signalling

Second GnRH system:

<table>
<thead>
<tr>
<th>GnRH1 / GnRHR1</th>
<th>Forebrain</th>
<th>Neuroendocrine functions</th>
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<tbody>
<tr>
<td>GnRH2 / GnRHR2</td>
<td>Post CNS</td>
<td>Reproductive behavior</td>
</tr>
<tr>
<td>or 1</td>
<td>Periphery</td>
<td>Local regulations</td>
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Life is what happens to you while you are busy making other plans

John Lennon
Kisspeptin story
First steps

1996  **KiSS-1** mRNA selectively overexpressed in metastasis-suppressed melanoma

2001  Gene product identified: **Kisspeptins (metastin)**

- Several proteolytic forms mainly 54 and 10 residues
- Belong to the « RF amide » peptide family
Kisspeptin story
First steps

1999  Cloning of GRP54: « orphan » G Protein-coupled Receptor of the galanin receptor family

2001  Kisspeptins are the natural ligands of GPR54
- High levels of KiSS1 expression in placenta and brain but also in testis, endocrine pancreas and intestine
- GPR54 expressed in placenta, CNS, pituitary, pancreas (suggesting endocrine functions)
- Gq signalling: PLC activation, IP3 accumulation, Ca++ mobilisation, kinase activation
Kisspeptin story  
First steps

2003  Loss-of-function mutations of the GPR54 gene linked to hypogonadotrophic hypogonadism in humans

Same phenotype in GPR54-null mice

GPR54 stimulates the release of gonadotropin-releasing hormone at the level of the hypothalamus
KiSS-1 as a **metastasis-suppressor gene** in several cancers and cell lines:

- melanoma
- breast cancer
- ovarian carcinoma
- oesophageal carcinoma
- papillary thyroid carcinoma
- pancreatic cancer cells
- gastric carcinoma
- bladder cancer …

Paracrine and autocrine mechanisms: inhibition of cell migration and pro-apoptotic effects

**Actual relevance in cancer progression to be defined**
Kisspeptin functions

Placental expression
In trophoblast giant cells
Dramatic increase of serum kisspeptin during pregnancy ($x10^3$ to $10^4$)

Probable endocrine and paracrine effects

Relevance in implantation of the mammalian embryo, placenta formation, and maintenance of pregnancy to be determined
Kisspeptin functions

Endocrine pancreas expression

Kisspeptins and GPR54 expressed in insulin and glucagon islet cells

Exogenous kisspeptin stimulates glucose-induced insulin secretion from mouse islets

Lack of detectable effect on glucagon secretion

Probable KISS1/GPR54 regulation of the insulin secretory responses to circulating nutrients
Gonadotropin effects of kisspeptins

*In vivo*:
Kisspeptins elicit robust stimulation of LH release
High activity: effect beginning at 0.1nmol iv (rat)
Desensitization of LH release by continuous infusion

Stimulation of FSH release

Stimulation of GnRH release

LH surge blocked by infusing kisspeptin mAB in the preoptic area

Precocious puberty induced in immature females by central infusions
Gonadotropic effects of kisspeptins

In vitro:

Kisspeptins stimulate GnRH release in hypothalamic explants

No or little direct effect on pituitary cells (although some of them express GPR54)
Morphofunctional correlates of kisspeptin effects

Expression of kisspeptins in areas containing GnRH neurons:
- Arcuate nucleus
- Anterior hypothalamus and septal regions

Other locations:
- Shell of the VMH and DMH
- Mesencephalon, parabrachial area

Kisspeptin fibers contact GnRH neurons (and OCT neurons)

GnRH neurons express GPR54 gene

GnRH neurons express c-Fos under kisspeptin stimulation
Morphofunctional correlates of kisspeptin effects

Kisspeptin expression strongly increases in castrated animals
Kisspeptin expression varies during the estrous cycle
Kisspeptin expression is sexually dimorphic
Kisspeptin neurons express ERα
Estradiol inhibits kisspeptin expression in the arcuate nucleus
Estradiol increases kisspeptin expression in anterior areas
Kisspeptin neurons in the rat hypothalamus

Example of location in the shell of the ventromedial nucleus and in the dorsomedial nucleus
Kisspeptin neurons express ERα receptors

In the ovine arcuate nucleus, more than 90% kisspeptin neurons express ERα (negative feed-back) vs about 40% in the preoptic area (positive feed-back)

Kisspeptin fibers in the basal hypothalamus

- Kisspeptin perikarya are observed in the arcuate nucleus (*).
- Kisspeptin fibers are abundant in the arcuate nucleus (ARH), but scarce in the median eminence (ME).
- Their distribution is sexually dimorphic.
- This SD is cycle-dependent (preovulatory period).
Kisspeptin innervation of neuroendocrine neurons

Kisspeptin fibers contact neuroendocrine neurons in the AVPV (●) and in magnocellular nuclei (▲)
Kisspeptin neurons in the cat hypothalamus

The distribution of kisspeptin perikarya is similar to that observed in the rat brain (here in the DMH)
GnRH and Kisspeptin in the cat median eminence

Very scarce kisspeptinergic innervation shows that this peptide is not a canonical neurohormone
GnIH story:

1
Unraveling RFamide family

1977 Mollusc cardioexcitatory neuropeptide FMRFamide potential neuromodulatory role in mammalian brain

1983 Chicken LPLRFamide lowers arterial blood pressure in rats

1985 Mammalian neuropeptide FF (NPFF) and neuropeptide AF (NPAF) reverse the analgesic actions of morphine.

1998 PrRP: Prolactin releasing peptide potent stimulator of CRH peptide mediating oxytocin release feeding regulation
GnIH story: Unraveling RFamide family

2000  Avian GnIH
       inhibits gonadotropin release from quail pituitaries
       suppression of female sexual behavior
       LPLRFamide is a part of the same precursor

2002  RFRP (1 and 3)
       mammalian homologues of avian GnIH

2006  Reproductive axis regulation role for RFRP 1 and 3
       mediate the negative feedback effects of sex steroids

NB: additional members: Kisspeptins and QRFP
In the rat brain, RFRP perikarya are located in the DMH/VMH area. They project to anterior and mediobasal areas containing GnRH perikarya. Only sparse innervation of the median eminence.

Kriegsfeld, Horm. Behav.
RFRP neuron distribution

RFRP in the DMH/VMH area

RFRP fibers contacting GnRH perikarya (±40%) and processes

Kriegsfeld, Horm. Behav.
RFRP/GnIH function

RFRP neurons deliver the gonadal steroid negative feed-back to GnRH system

RFRP/GnIH inhibits LH secretion

RFRP/GnIH-ir cells express sex steroid receptors

RFRP/GnIH-ir cells respond to gonadal steroids with increased c-Fos expression

Kriegsfeld, Horm. Behav.
Kisspeptins, GnIH, GnRH neurons and steroids (bis !)
Conclusion

Huge possibilities offered by Kisspeptins/GPR54 and by GnIH/OT7T022 systems in order to inhibit the gonadotropic axis:

- Activating GnIH system
- Suppressing Kisspeptin
- Suppressing GPR54 signalling

- Agonists
- Immunization (?)
- Agonists
- Antagonists
- Immunization (?)

Caveats: Large peptide family, not well known yet ...

Pleiotropic effects
Suppressing Kisspeptins ... or Activating GnIH?

1. What are the potential effects on sexual behaviors? SUPPRESSION ?
2. Will this approach work on both males and females? YES YES
3. Will the approach require more than one treatment? PROBABLY PROBABLY
4. How long does this approach produce infertility? ? ?
5. Does this approach have patent protection? NO NO