

## Hypothalamus-Pituitary-Gonadal Axis: It is Time for Revision

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The structure and the function of the Hypothalamus-Pituitary-Gonadal (HPG) axis is an integral part of the knowledge on the physiology of the human body that every bioscientist receives during his/her education. Especially for those scientists who work in the fields of assisted reproduction and endocrinology, the function of HPG axis is of paramount importance.

The HPG axis drives reproduction: Hypothalamus secretes Gonadotrophin Releasing Hormone (GnRH), GnRH stimulates the gonadotroph cells of pituitary to secrete Follicular Stimulating Hormone (FSH) and Luteinizing Hormone (LH) and in turn these two hormones regulate the gonadal function in both sexes. Steroid hormones as well as inhibins and activins, produced by gonads, influence the secretion of gonadotrophins. It is also well known that internal and external cues influence the HPG axis. For example, stress hormones, leptin and the opioid system modulate the secretion of GnRH and gonadotrophins. However, it has become obvious that they are not main regulators. GnRH, FSH and LH, androgens, and oestrogens are the main players in this classical schema and until recently, GnRH was recognized as the neurohormone having the pivotal role in the physiology of reproduction. This is the basic knowledge on the function of HPG axis. However, during the last ten years, novel findings on the actions of RFamide peptides have challenged this knowledge. RFamides are small peptides possessing the motif Arg-Phe-NH<sub>2</sub> at C-terminus. Two groups of RFamide peptides proved to participate in HPG axis: Gonadotropin Inhibiting Hormone (GnIH) and its related peptides and the group of kisspeptins.

In 2000, Tsutsui et al. [1] discovered a new RFamide in the hypothalamus of Japanese quail that was found to decrease gonadotropin secretion in a dose-dependent manner and also to inhibit biosynthesis of gonadotropin α and β subunits [1-3]. This RFamide was designated as GnIH and subsequent studies revealed GnIH and similar peptides in the brain of other avian species, amphibians and fish [4-7]. In mammals, the existence of RFamide peptides similar to avian GnIH first was predicted by a search in gene databases [8] and soon after, two RFamide related peptides (RFRPs): RFRP-1 and RFRP-3 were isolated from bovine [9], rat [10], rhesus macaque [11] and human brain [12]. The RFRP-3 was found to inhibit LH release in rats [13], sheep [14] and cattle [15]. Thus, RFRP-3 seems to be the mammalian ortholog of GnIH. In human brain, neurons expressing RFRP-3 are located in the dorsomedial region and their fibers come in contact with GnRH neurons in the preoptic area and they also give projections to the median eminence [12].

The actions of GnIH and RFRPs are mediated through a G protein-coupled receptor (GPCR) that was first identified in the brain of Japanese quail [16]. In mammals, two putative receptors for GnIH and RFRPs were found: GPR-74 and GPR-147 [17]. In humans, GPR-147 is expressed in the hypothalamus and in the gonadotroph cells of pituitary [12].

As it was mentioned before, GnIH and RFRPs inhibit *in vivo* and *in vitro* gonadotrophin release from hypophysis in avian and mammal species. The projection of GnIH fibers in median eminence and hypophysis further document and explain this action. The existence

of close contacts between GnIH fibers and GnRH neurons indicates a regulatory action of GnIH on GnRH release, although the expression of GPR-74 and GPR-147 in mammalian GnRH neurons has not been observed.

Recently, Oishi et al. [18] reported that RFRP-3 and GPR147 are expressed in granulosa cell layer of human preovulatory follicles as well as in the human corpus luteum and they showed that RFRP-3 suppresses gonadotropin induced progesterone production in human granulosa cell cultures.

Studies on the regulation of RFRPs have shown that in hamsters, RFRP neurons express estrogen receptor-α and it was found that estrogen treatment increased FOS expression in these neurons [19]. This finding suggests an activation of RFRP neurons by estrogens and thus it can be postulated that these neurons participate in the feedback system between gonadal steroids and hypothalamus. Kirby et al. [20] have also presented findings suggesting a regulation of RFRPs by stress hormones in rats: Stress increased RFRP hypothalamic expression and this increase could be blocked by adrenalectomy.

The other groups of RFamides that draw the attention due to their actions on HPG-axis are kisspeptins, the products of the gene kiss-1 [21]. The gene encodes for a 145 amino acid precursor that after cleavage gives several peptides with a length of 54, 14, 13 or 10 amino acids. The receptor for kisspeptins is an orphan one known as GPR-54 [22]. It is notable that kisspeptins are highly conserved through the evolution of vertebrates: kiss-1 gene has been found in a plethora of vertebrate species, including mammals [23]. In 2003, it was reported that several patients suffering from idiopathic hypogonadotropic hypogonadism had mutations in the GPR-54 [24,25]. This finding fuelled the investigation on the role of kisspeptins and their receptor in reproductive physiology.

Today, it is known that in mammals, kisspeptins are expressed by hypothalamic neurons [26-32]. In primates, kisspeptin neurons are primarily located in the arcuate nucleus and the preoptic area giving also projections to the median eminence and, in humans, to the ventromedial nucleus as well [33-36]. Kisspeptin neurons establish synaptic contacts with GnRH neuronal bodies and particularly in the median eminence with terminals of GnRH neurons [34]. The majority of GnRH neurons express GPR-54 [27,37].

Kisspeptins stimulate GnRH secretion and subsequently they

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stimulate the secretion of LH and, in a less degree, of FSH. This was found in mouse [26], rat [38], sheep [39], cow [40], rhesus macaque [41] and humans [42,43].

Kisspeptin neurons express estrogen receptor  $\alpha$  (ER $\alpha$ ) and a large body of evidence suggests that kisspeptin neurons of the arcuate nucleus are responsible for the negative feedback of sex steroids on HPG axis [30,33-35]. Besides, in rodents, the kisspeptin neurons of the anteroventral periventricular area increase the expression of kiss-1 mRNA when estrogens are at high levels and decrease the expression in absence or low levels of estrogens [30]. This finding indicates that in rodents, the kisspeptin neurons of the anteroventral periventricular area are responsible for the positive feedback of sex steroids on HPG axis. In primates, who do not have kisspeptin neurons in the anteroventral periventricular area, it seems that different subpopulations of the arcuate nucleus and the preoptic area respond differentially to estrogen levels mediating both positive and negative feedbacks.

Furthermore, metabolic status is implicated in the regulation of kisspeptins: fasting decreases their expression [44], whereas a considerable number of kisspeptin neurons express leptin receptors [45].

The above findings outline a new, more complete perception of the HPG axis: the gonadotropin release in pituitary is positively regulated by GnRH and negatively by GnIH/RFRPs. GnRH release is under the control of kisspeptins that are subjected to negative and positive feedback from gonadal steroids. Kisspeptins expression is influenced by metabolic signals. GnIH/RFRPs are also subjected to feedback by gonadal steroids and they are influenced by stress hormones.

This new perception of the HPG axis suggests new therapeutic options. Already, the treatment with kisspeptins has proved to be successful in cases of functional hypothalamic amenorrhea [46]. It is reasonable to postulate that treatment with GnIH and RFRPs could be proved useful for the cessation of gonadotropin release.

Although further investigation is needed, particularly on the actions of RFRPs in humans, the new findings on the structure and the function of HPG axis enter us in a new era with a better knowledge of the physiology of reproduction and new options for the treatment of related disorders.

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