REVIEW ARTICLE

Beyond GnRH, LH and FSH: The role of kisspeptin on hypothalalmic-pituitary gonadal (HPG) axis pathology and diagnostic consideration

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Abstract

Kisspeptin as a neuropeptide was established initially as regulator for gonadotropin-releasing hormone for pulse frequency and intensity. In the current review, initial search on PubMed was done with key word "Kisspeptin" with further filters to include reviews and trials from the last 10 years. Of the 313 articles shortlisted, 160(51%) dealt with kisspeptin pathology and diagnostic evaluation in various physiological conditions like puberty, while 57(18.2%) dealt with pathological conditions like hypogonadism, 53(17%) infertility, and 43(13.7%) with polycystic ovarian syndrome. This review explored existing data regarding understanding of the negative and positive influences on the kisspeptin hormone-release kinetics. It highlighted the recently identified ligands and pathways which could affect the gonadal steroids, including various metabolic alterations and environmental triggers. Also, the review highlighted the kisspepetin/G-protein coupled receptor-54 interaction which were influenced by neighbouring endocannabinoid system, Gamma aminobutyric acid (GABA)-ergic neuronal outputs and other chemical agents. It was also highlighted that the release of kisspepetin was identified as a group of neurons termed kisspeptin, neurokinin B, and dynorphin, or KNDy, in the arcuate nuclei. The data indicated the use of kisspepetin as a diagnostic marker for precocious puberty, puberty confirmation, hypogonadism, infertility and polycystic ovarian syndrome.

Keywords: Gonadotropic releasing hormone, GnRH, Kisspeptin, Kp, Hypothalamic-pituitary-gonadal axis, HPG, Luteinizing hormone, LH, Follicle stimulating hormone, FSH, Polycystic ovarian syndrome, PCOS, Hypogonadism.

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Introduction

With advancement in diagnostic biotechnological research and various newer scientific discoveries, there are many newer avenues for poorly understood mechanisms of reproductive tract abnormalities. The whistle-blower in inferring anomalies in the hypothalamic-pituitary-gonadal (HPG) axis was preliminary data on pituitary hormones and sex steroids followed by interactions of gonadotropin-releasing hormone (GnRH) from the hypothalamus.[1] Initial research and endocrine diagnostics mainly based decisions on data from the chemicals and hormones involving GnRH, luteinizing hormone (LH), follicle-stimulating hormone (FSH) steroids to decipher reproductive tract diseases.[2] Over the years, the researchers further explored multiple new actors, including hormones, receptors and intervening chemical signalling mechanisms, acting in physiological harmony to ensure optimal reproductive organ development and function.

One of the seminal discoveries over the last two decades is that of kisspeptin (Kp), a neuropeptide derived from Kiss-1 gene (chromosome-1) which not only interacts as hormonal driver of GnRH, but also has various autocrine and paracrine functions within hypothalamus and nearby neuronal cells.[3] Released primarily from the hippocampal dentate nuclei, arcuate (ARC) and antero-ventral periventricular (AVPV) nuclei, the major triggers leading to the release of Kp, remain under stress and depression and show some specific spatial behaviours.[4] The Kp receptor is G-protein coupled receptor54 (GPR54) in various neurons and across various locations.[5] GnRH is dispersed throughout the hypothalamic region in contrast to AVPV and ARC nuclei which are predominantly limited to preoptic and the mediobasal regions of the hypothalamus. This anatomical finding is important as Kp neurons in preoptic area cause GnRH surges, while those in ARC nuclei only regulate the pulsatile GnRH release.[5] Apart from KP's role in affecting GnRH secretion, it receives stimulatory and inhibitory inputs from neighbouring neurons from different regions of the brain through various endogenous stimuli and environmental triggers.[6]

The current narrative review was planned to bridge the understanding between Kp's role with regards to a master regulator to gain inputs from various internal and external sources and then to converge it down to affect GnRH

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surges and pulses. The review also planned to address the current data on Kp utility as a biomarker for various reproductive tract abnormalities.

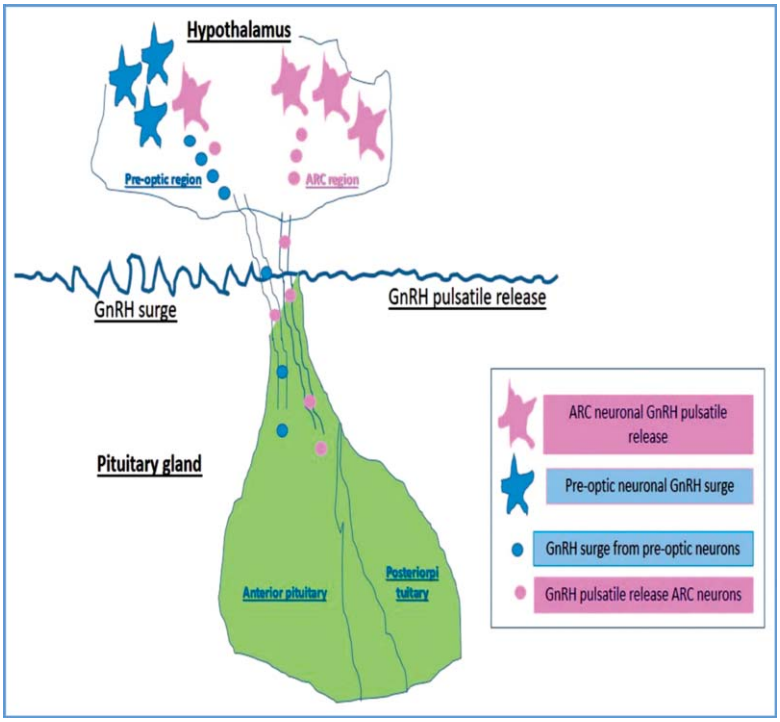
Review Methodology

Initial search on PubMed was done with key word 'Kisspeptin' and subsequently '10 years' filter was applied, followed by key words 'reviews' and 'trials'. The shortlisted articles dealing with Kp pathology and diagnostic evaluation in various physiological and pathological conditions were reviewed. Provided maximum attempt was made to incorporate human research over last decade, but overwhelming quality and proof concept studies from animal models were incorporated to address specific roles of Kp's functionality in the HPG axis, including inputs from the surrounding regions.

Results

The initial search yielded 2263 articles. Of them, 1866(82.5%) related to the preceding 10 years, and, of them, 313(16.8%) were shortlisted; 160(51%) dealing with Kp pathology and diagnostic evaluation in puberty, 57(18.2%) hypogonadism, 53(17%) infertility, and 43(13.7%) with polycystic ovarian syndrome PCOS).

Kisspeptin and HPG Axis: Kp's role in HPG axis is not simplistic in terms of providing stimulatory feedback to GnRH neurons in the hypothalamus, but overlaps or contributes at various other levels in managing the master regulator role of the hypothalamus. Both direct and indirect Kp's interactive role in case of HPG functioning trickles down on GnRH neurons to pace up or down the GnRH release and further downstream actions.[6] GnRH neurons along with Kp neurons in the hypothalamus remain pivotal, but still need support from various other signalling pathways to optimize the HPG axis function (Figure-1), which include the following:



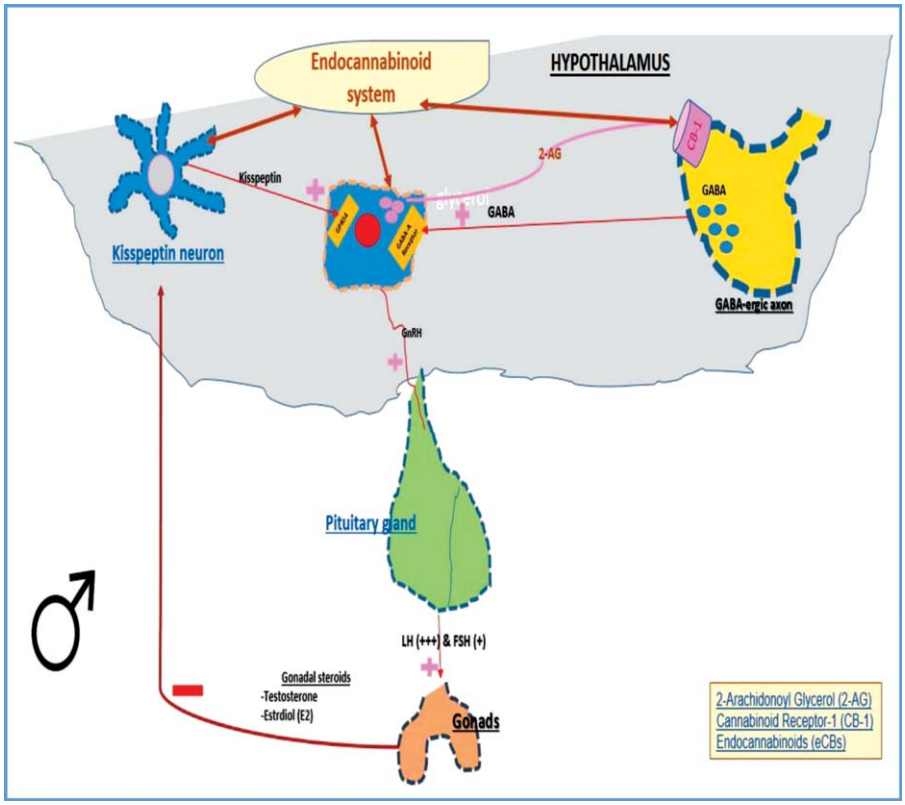
KP: Kisspeptin, GnRH: Gonadotropic releasing hormone, LH: Luteinizing hormone.

Figure-1: General anatomical relation of Kp/GnRH/LH/gonadal steroids.

i. GnRH Interacting Neurons: Apart from the Kp-secreting neurons, there are multiple known neuronal signal pathways which affect GnRH secretion directly or through affecting Kp secretion changes both in physiological ways and in relation to some pathology. Kp, neurokinin B, and dynorphin (KNDy) is a group of neuron in the ARC nuclei which also contain dynorphin (Dyn) and neurokinin-B (NK-B).[7] Alongside these ARC areas within the hypothalamus, recent data from animal research suggests plentiful presence of pro-opiomelanocortin (POMC), Gamma aminobutyric acid (GABA)-ergic and dopamine neuronal signalling pathways with probable function linked with environment, metabolic disorders, like PCOS, and lactation-related effects.[8] AVPV nuclei, apart from Kp cells, also contain rich supply of sexually dimorphic nuclei (SDN) which in males is associated with specific sexual behaviour.[9] Furthermore, there is also evidence about the presence of neurons in the hypothalamus which secrete a gonadotropin inhibitory hormone (GnIH) in median eminence and paraventricular nuclei.

ii. Receptor's Role in Hypothalamus: Though Kp-GnRH stimulatory interactions appear simplistic, Kp receives varying feedback from multiple sources, including ligands, signalling pathways and, most importantly, steroidal and non-steroidal hormones. Evidence to this originates from the fact that Kp signalling mechanisms have been discovered throughout the HPG axis and related areas.[6] Some of the pertinent effectors involved in Kp-GnRH interplay to cause variation in GnRH and downstream concentrations are being discussed below:

a) Hypothalamic GnRH Signalling: Considered pivotal and central to optimal functionality of the reproductive system, the theory is being redefined after Kp and associated hypothalamic factors. Kp affects



GABA: Gamma Aminobutyric Acid.

Figure-2: Overview of interactions and regulatory workup in hypothalmic-pituitary-gonadal axis along with role of GABA-ergic and endocannabinnoid (eCB) system in males.

GnRH through GPR54 receptors whose subtypes have now been cloned and well-characterised in animal studies during different stages of reproductive development and functions.[10] Moreover, oestrogen receptor-beta (ER-beta) and GnIH through GPR147 receptors have also been detected to induce negative feedback on Kp-GnRH pathway in males and females[11] (Figure-2, 3).

b) Arcuate Nucleus: Also termed the arcuate nucleus of the hypothalamus (ARH) or the infundibular nucleus, the region is located in the mediobasal area of hypothalamus. Though the physiological roles of ARC nuclei are diverse, focus will be maintained on its HPG effects. Multiple receptors on ARC nuclei are involved in negative feedback mechanism through GnRH signalling pathways involving dynorphin, glucocorticoids, ER-alpha and other neuropeptides.[7] Saedi et al. have reviewed various neuropeptides, including KNDy neurons and neuropeptides, like agouti-related protein, and neurotransmitters, like dopamine, to conclude significant interactions between GnRH signalling effects, but acknowledged the gaps in complete understanding.[12] The situation seems more perplexed once a study discovered endocannabinoid receptors (eCBs) followed by leptin receptors in ARC nuclei.[13] A very interesting study identified the positive feedback signal mechanism through Kp and NK-B receptor where loss of function mutation in the aforementioned receptor resulted in hypogonadotropic hypogonadism, showing the central role of Kp/GPR54 significance in reproductive system development and functions.[14]

c) Endocannabinoids Receptors (eCBs): Generally, eCBs are classified into two main types, CB-1 and CB-2, with presence for both demonstrated in the central nervous system (CNS) and the peripheral nervous system (PNS). While eCBs have been observed to have a role in male and female reproduction, the role has primarily been graded as inhibitory on GnRH function.[15]

d) Antero-Ventral Periventricular Nuclei (AVPV): Primarily associated more with the female reproductive system, they remain the key backdoor actors by housing receptors for ER-alpha and progesterone. These neuronal signalling pathways are responsible through ER-alpha receptor to cause the positive feedback stimulation event, leading to luteal-phase LH surge in females.[16] Their role in males is not appropriately understood.

e) Linked Receptors: GnRH neurons have been home to other receptors with probable role to regulate its tonic release for downstream HPG axis, like dopaminergic receptors (D1), growth hormone secretagogues receptors (GHS-R) and GABA receptors.[17]

f. Connecting Environment with Kp/GnRH Axis: We have seen the Kp signalling pathways being dispersed throughout the hypothalamus. In simplistic terms they gather internal and external information through various receptors and translate them into a desirable signal for GnRH to modify its downstream sequential actions. So how does the Kp neuron accumulate these signal for long-term and short-term changes in Kp signalling?

g) Body's Requirements: Animal's body faces various

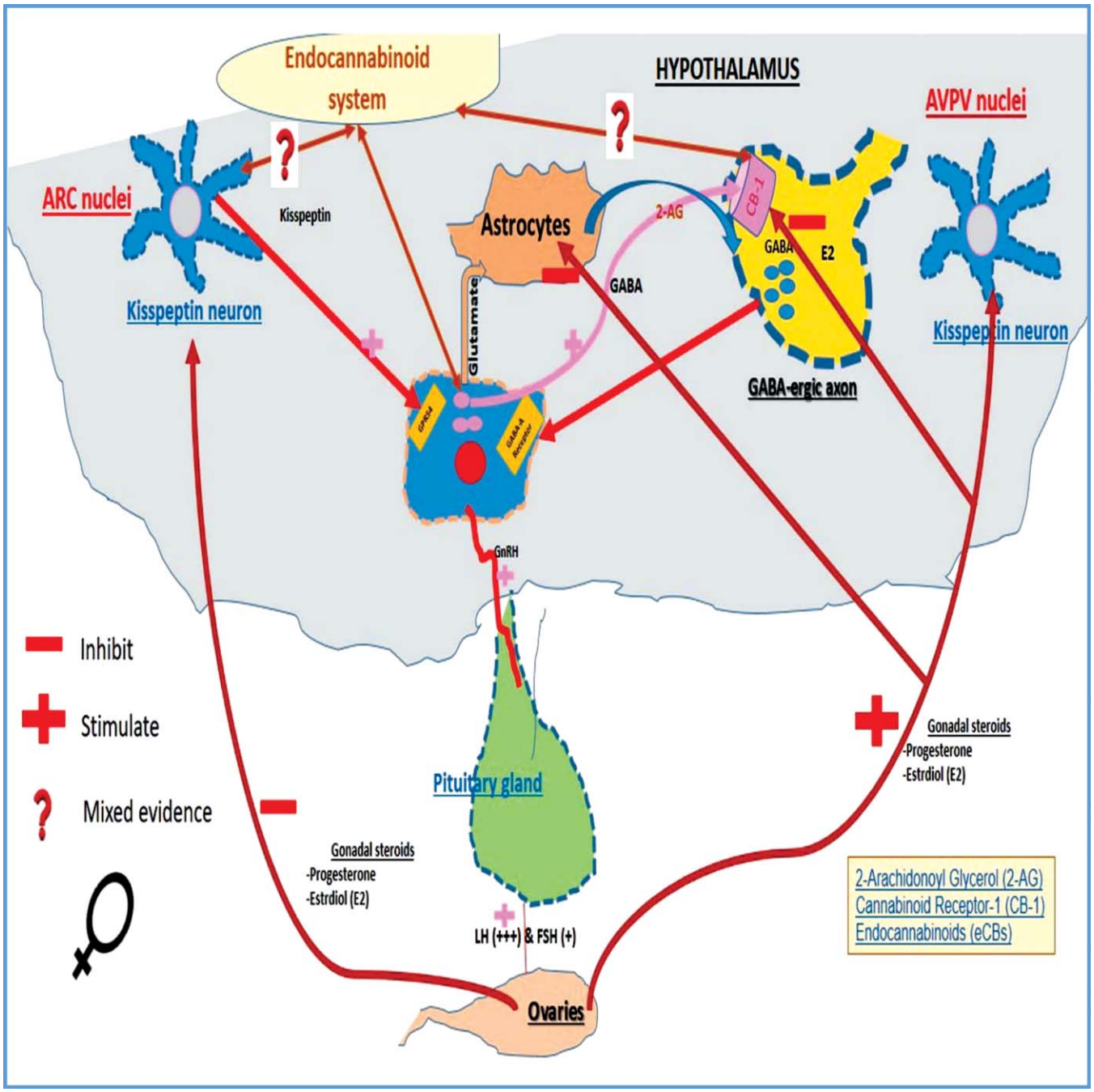


Figure-3: Overview of interactions and regulatory workup in the hypothalmic-pituitary-gonadal axis along with role of GABA-ergic and endocannabinnoid (eCB) system in females.

phases of reproductive requirements superimposed by physiological and pathological stresses from inside and outside. A study suggested link between food supply and GnRH release resulting because of Ghrelin hormone.[17] Similar to that, female's physiology varies dramatically from the non-reproductive phase to the debut of reproductive menstrual cycle, pregnancy, lactation and menarche which needs appropriate readjustment of various reproductive hormones, with all of them having very drastic adjustments evolving from the master gland's neuronal signalling pathway, like Kp/GnRH interactions.[18] Ageing has always been a fixed factor in declining reproductive function, but Neal-Perry et al. have shown that Kp administration improves reproductivity in animal models.[19]

h) Metabolic Adjustments and Kp/GnRH Function: There is evidence that low Kp levels are associated with decreased insulin sensitivity in human subjects. Rashad et al. have demonstrated that altered Kp actions are associated with PCOS.[20] Furthermore, animals fed with higher fat content in post-natal life experienced early onset of puberty and menstrual cycle disturbances associated with Kp expression up-regulation.

i. Role of Enviroment in Kp/GnRH Signalling: As acknowledged above, quality literature related to Kp and HPG axis is mostly available through animal studies. The impact of weather, chemicals, stress and lifestyles do affect Kp expression and thus have the potential to affect HPG axis. Shahjahan et al. have shown that anomalous environmental conditions do affect the Kiss gene expression to cause unfavourable reproductive outcomes.[21] Likewise, Loganathan et al. have identified darkness and high temperature associated with raised melatonin levels affecting Kiss gene expression along with other proteins to affect reproductive function.[22] Several endocrine-disrupting chemicals (ECDs) have been shown to affect the Kp/GPR54 receptor signalling to cause alteration in GnRH release from the hypothalamus. Psychological and physical stress also affects Kp release and related GnRH-based regulation of the HPG axis.[23] Furthermore, physical stress and self-induced starvation, like anorexia nervosa, are also associated with low Kp release and higher ghrelin levels.[24] Joining the dots from the shared evidence about environment and Kp, it can be stated that environmental conditions, like temperature, dark-light cycle, stress, Endocrine Disrupting Chemicals (ECDS) and may be other environmental factors, can play a major role in overall HPG axis functioning.

j. Kisspeptin's Downstream Actions: Though Kp mainstream actions originate from hypothalamic neurons as per convention, recent evidence indicates the presence of Kp receptors in various other tissues, including anterior pituitary cells, like gonadotropes and lactotropes. It appears from available evidence that Kp acts not just in the endocrine system, but autocrine and paracrine effects are also well-documented. Carcia-ortege et al. have been able to identify Kp and related reception in various reproductive organs.[26] With these new findings, it appears that Kp/GnRH in the HPG axis has a very broad-spectrum untapped roles throughout the HPG axis, starting from phenotypic sex determination, inhibiting premature puberty through mediation of ER-alpha receptor and finally letting off the brakes to puberty spurt involving ER-alpha, NK-B and, most importantly, GnRH upsurge to maturation.[26] Kp later in post-pubertal life probably acts as a gate-keeper and attempts to optimise downstream reproductive function through managing various hormones. In females, Kp levels rise prior to LH surge and ovulation.[27] Female subjects with Kp/GnRH axis defects have been identified in infertile females and PCOS.[9] Moreover, low level of Kp during early pregnancy have been associated with adverse pregnancy outcomes. Males with defects in Kp/GnRH axis in association with other hormones like B-Arrestin-mediated pathway lead to hypogonadotropic hypogonadism.[14] Beyond these

Table: Evidence highlighting possible diagnostic use of kisspeptin and its receptor GPR54.

Ser	Diagnostic study	Key end-points	Ref
1	Central precocious puberty (CPP) diagnosis Method: Radioimmunoassay (RIA), Phoenix Pharmaceutical, Inc. CA,USA)	Kp levels among CPP girls are raised	[29]
2	PCOS diagnosis Method: ELISA (Phoenix pharmaceuticals)	Eumenorrheic-PCOS: Normal Kp pulse Oligomenorrheic-PCOS: Increase Kp pulse frequency	[30]
3	CPP and premature thelarche diagnosis Method: ELISA	Kp, neurokinin-B and leptin were higher in subjects with CPP and premature thelarche	[31]
4	Kallamann syndrome diagnosis Method: Whole Exome Sequencing (WES)	Stop codon mutation (c.1195T>C) was discovered in KISS1-Receptor gene through whole exome sequencing	[32]
5	Diagnosis of obese precocious pubertal females Method: ELISA	Serum Kp in lean females with precocious puberty less than obese females with precocious puberty	[33]
6	Mutations in GnRH dependent precocious puberty Method: NAT/PCR	2 gain of function mutations of KISS-gene and KISS1-gene receptor associated with GnRH-dependent precocious puberty	[34]
7	Tumor marker Method: Review	Kp levels as tumour marker for urogenital carcinoma	[35]
8	Male infertility Method: ELISA (Cloud-clone, USA)	Kp concentrations in seminal plasma positively associated with male reproductive function	[36]

GPR54: G-protein coupled receptor-54, ELISA: Enzyme-linked immunosorbent assay, PCOS: Polycystic ovarian syndrome, GnRH: Gonadotropic releasing hormone, NAT: Nucleic Acid Test , PCR: Polymerase chain reaction.

anticipated roles of Kp, it has been shown by some authors that sperm maturation and release are linked with the needful presence of certain trace elements which are affected by Kp's role in testicular functions.[28]

Diagnostic and Therapeutic Utility: Currently, research is focussed more on understanding the physiological aspects of various neuronal functions and their secretions, especially in the hypothalamic region and how they vary with internal and external factors. However, studies have identified the use of Kp as a marker for various diseases (Table). Still, it is important to interpret the lack of characterisation of such markers with regards to gender, biological changes, reference ranges, and diagnostic parameters, like sensitivity, specificity and predictive value. Moreover, the exactness of results, remain variable due to constant changes, especially in females with regards to menstrual cycles, is essential.

Discussion

The physiology associated with Kp-GnRH-gonadal axis has been well studied over time. However, recent research has highlighted various chemicals, ligand receptors and pathways which affect the holistic depiction of Kp-GnRH-gonadal functions. In essence, the release of Kp from hypothalamus can be assumed as a trigger GnRH release from pre-optic and ARC nuclei. The process, though straightforward, is affected by regular and intermittent negative and positive feedbacks from neighbouring hypothalamic nuclei, including eCB system, GABA-ergic inputs, GnIH, dynorphin, neurokinin-B (NK-B) and others which affect GnRH release through interactions with its receptors GPR54.[6,10,11]

Hypothalamus acts as the prime interlink between the body's internal milieu and external environment. The release kinetics of Kp are affected directly by hunger (ghrelin hormone), leptin (insulin release), reproductive stimuli, age, EDCs, and consequences of any disease.[21,23] Kp and downstream actions remain the primary drivers for the smooth conduct of male and female pubertal progression, menstrual cycle in females, pregnancy, lactation and menarche which are mediated through associated hypothalamic nuclei.[17-20] With various body derangements and metabolic influences, the Kp-GnRH-gonadal axis faces their fallouts. Metabolic disease processes, like insulin resistance, obesity, PCOS, premature menarche and infertility, are usually associated with deranged Kp levels.[31-36] There is strong influence of metabolic disorders on the human reproductive system which seems to be derived through the involvement of Kp-GnRH axis.[19,20] Kp also seem to direct extra-hypothalamic effects, as indicated by animal model studies where Kp seems to directly derive testicular maturation, sex-determination and having a role in the development of female reproductive tract.[9,27,28,14]

Though preliminary and needing disease-specific validation, we presume that Kp can emerge as a potential biomarker for various diseases, like precocious puberty, diagnosis of PCOS, Kallaman's syndrome, tumour marker for certain cancers and evaluation of male infertility.[29-36] However, the role of Kp needs to be standardised and requisite reference intervals and methodology need to be validated for clinical use.

Future Dimensions

The current review highlighted some very interesting aspects of Kp/GnRH effects along with extra-gonadal effects. While most of the research is limited to animal models, need is there to replicate the findings in human subjects. We also feel that the complete interactive mapping of Kp pathways still needs to be explored and possible hypothalamic new roles in terms of interaction with environment may further shape our understanding. As a diagnostic biomarker, Kp has to undergo standardisation of methodology, needs disease-specific reference range validation, and further work-up for use as a biomarker in male infertility and as a tumour marker. Kp may also have enormous therapeutic potential which needs to be further evaluated.

Conclusion

The data review of Kp suggests quality interventional research has been carried out in animal models and has thus remained preclinical to date. The pathology on the subject has been explored in greater detail to correlate various metabolic, environmental and psychosocial feedback mechanism converging on Kp and GnRH neurons to define the outflow of trophic hormones from anterior pituitary and further downstream hormonal actions throughout the HPG axis. Thus, Kp has multi-dimensional roles, including the whistle-blower job for puberty which may get altered by various external and internal stimuli. Kp is not alone in the little galaxy of hypothalamic and non-hypothalamic neuronal chemicals that include KNDy, dopaminergic, glutaminergic and cannabinoid systems along with feedback from steroidal hormones. These hormones are well-connected and define the final output of GnRH-stimulated gonadotropins from anterior pituitary. Though many dots have been identified in the HPG axis functional roles in regulation with internal biological rhythms and external environmental impacts, it will take some time before the exact clinical utility of Kp hormone is established to enter into clinical usage as a diagnostic marker. For its

evaluation as a marker for hypogonadism, puberty, pregnancy and medical conditions, like infertility and PCOS, specific diagnostic characterisation is needed to define sensitivity, specificity and predictive values for which further research is warranted.

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