The Value of Dynamic Testing in Non-Organic Hypogonadotropic Hypogonadism

Thesis

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List of Abbreviations

μL	Microliter
ABP	Androgen binding protein
AR	Androgen receptors
AVP	Arginine vasopressin
BA	Bone age
BMI	Body mass index
BNDF	Brain derived neutropic factor
cAMP	Cyclic adenosine mono-phosphate
CART	Cocaine and amphetamine-regulated transcript
CDGP	Constitutional delay of growth and puberty
CDP	Constitutional delay of puberty
CNS	Central nervous system
CRF	Corticotrophin-releasing factor
CRH	Corticotrophin releasing hormone
CST	Cortico-spinal tract
CT	Computerized tomography
DAX1	Dosage-sensitive sex reversal-adrenal hyperplasia congenita critical region on the X chromosome
E2	Oestradiol
ECLIA	ElectroChemiLuminescence ImmunoAssay
EDCs	Endocrine disrupting chemicals
ER	Estrogen receptors
FAS	Free alpha subunits
FDA	Food and drug administration
FGFR1	Fibroblast growth factor receptor type 1
FH	Final height
FSH	Follicle stimulating hormone
FT_4	Free thyroxine
GABA	γ-amino aminobutyric acid
GAP	GnRH-associated peptide
GFP	Green fluorescent protein
GH	Growth hormone
GnRH	Gonadotropin-Releasing Hormone
GnRH-a	Gonadotropin-releasing hormone-agonist
GPCR	G protein-coupled receptor
GPR54	G protein-coupled receptor54
hCG	Human chorionic gonadotropin
HH	Hypogonadotropic hypogonadism
HPG	Hypothalamic Pituitary Gonadal
hr	Hour
ICMA	Immunochemiluminometric assay
IFMA	Immunofluorometric assay
IGF	Insulin like Growth Factor

List of Abbreviations (Cont.)

IHH	Isolated hypogonadotropic hypogonadism
IRMA	Immunoradiometric assays
kg	Kilogram
Kiss	Kisspeptin
KS	Kallmann syndrome
LH	Leutenizing hormone
m^2	Meter square
MAPK	Mitogen-activated protein kinase
MCH	Melanin-concentrating hormone
mg	Milligram
min	Minute
MIS	Müllerian inhibiting substance
MRI	Magnetic resonant image
ng/mL	Nanogram/milliliter
NMDA	*N*-methyl-d,l-aspartate
NPY	Neuropeptide Y
ºC	Degree celzius
PC	Personal computer
PCOS	Polycystic ovarian syndrome
PK	Protein kinase
PKC	Protein kinase C
POMC	Pro-opiomelanocortin
PROP1	Prophet of pit-1
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SD	Standard deviation
SEMA3E	Semaphorin 3E
SF1	Steroidogenic factor 1
SGA	Small for gestational age
T	Testosterone
T4	Thyroxine
TGF-α	Transforming growth factor
TR-FIA	Time-resolved fluoroimmunoassay
TRH	Thyrotropin-releasing hormone
TSH	Thyrotropin releasing hormone
VIP	Vasoactive intestinal peptide
VMH	Ventromedial hypothalamus

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INTRODUCTION

Puberty is the result of increasing gonadotropin releasing hormone (GnRH) release by the hypothalamus followed by a complex sequence of endocrine changes with functioning of negative and positive feedbacks, and associated with the development of sex characteristics, a growth spurt and reproductive competence ***(Dellemarre-van de Waal, 2004).***

There are wide variations in the onset of puberty. It is considered to be delayed if the initial signs of sexual maturation don't appear by an age that is 2.5 SD beyond the mean for healthy boys or girls ***(Bhasin, 2007).***

Myriad conditions can delay the onset of pubertal maturation. If no underlying condition can explain pubertal delay i.e. functional hypogonadotropic hypogonadism, and if sexual maturation occurs before the age of 18 years a diagnosis of constitutional delay of growth and development is made ***(Sedlmeyer and Palmert, 2002).***

Idiopathic hypogonadotropic hypogonadism which may be associated with anosmia (the kallmann syndrome) or with normal sense of smell is a form of male infertility caused by a congenital defect in the secretion or action of gonadotropin releasing hormone (GnRH). Patients have absent or incomplete sexual maturation by the age of 18 ***(Raivio et al., 2007).***

At the time of referral it is often difficult to distinguish boys with constitutional delay of growth and puberty from those with idiopathic hypogonadotropic hypogonadism. Both conditions present effectively with similar clinical and hormonal features ***(Degros et al., 2003)*.**

AIM OF THE WORK

The aim of the study was to evaluate the pituitary gonadotropic reserve among patients with non-organic hypogonadotropic hypogonadism by administration of a standard subcutaneous 0.1 mg GnRH analogue.

GONADOTROPIN-RELEASING HORMONE AND THE HYPOTHALAMIC PITUITARY GONADAL (HPG) AXIS

Gonadotropin Releasing Hormone (GnRH) Chemistry and Evolution:

The hypothalamic neuropeptide that controls the function of the reproductive axis is GnRH. GnRH is a 10-amino-acid peptide that is synthesized as part of a larger precursor molecule and is then enzymatically cleaved to remove a signal peptide from the N-terminus and GnRH-associated peptide (GAP) from the C-terminus ***(Wierman et al., 2004)***.

Molecular cloning has identified more than 16 isoforms of GnRH represented among diverse organisms. At least two isoforms of GnRH have been identifies in the mammalian central nervous system; GnRH-I and GnRH-II. GnRH-I is the hypothalamic decapeptide responsible for LH and FSH secretion from the anterior pituitary ***(Densmore and Urbanski, 2003)***.

GnRH-II was intitially discovered as chicken GnRH-II and displays a diffuse pattern of localization in most tissues. In the CNS, GnRH-II has been hypothesized to play a role in the behavioral components of reproduction. Actions of GnRH II on limbic neurons may mediate sexual arousal, whereas those on granulosa-luteal cells may subserve steroidogenic inhibition.

GnRH III (first identified in lamprey) may release FSH preferentially over LH in the rat ***(Barnett et al., 2006)***.

The genes for GnRH-I and GnRH-II are on chromosomes 8 and 20 respectively. Both isoforms of GnRH are decapeptides that are characterized by post-translational modifications, including the pyro-glutamic acid at the amino termini and amidated glycine at the carboxy termini. GnRH-I is conserved throughout evolution and has been identified in both vertebrates and invertebrates ***(Harrison et al., 2004)***.

All GnRH genes have the same basic structure, with the pre-prohormone mRNA encoded in four exons. Exon 1 contains the 5′ untranslated region of the gene; exon 2 contains the signal peptide, GnRH, and the N-terminus of GAP; exon 3 contains the central portion of GAP; and exon 4 contains the C-terminus of GAP and the 3′ untranslated region. Among species, the nucleotide sequences encoding the GnRH decapeptide are highly homologous ***(Pawson et al., 2003)***.

Two transcriptional start sites have been identified in the rat GnRH-1 gene at +1 and -579, with the +1 promoter being active in hypothalamic neurons and the other promoter active in placenta. The first 173 base pairs of the promoter are highly conserved among species ***(Nelson et al., 1998)***. In addition, a variety of hormones and second messengers have been shown to regulate GnRH gene expression, and the majority of the *cis*-acting elements thus far characterized for hormonal control of GnRH transcription have been localized to the proximal

promoter region ***(Cheng and Leung, 2005)***. The 5′ flanking region of the rodent and human GnRH-1 genes also contain a distal 300-base-pair enhancer region that is 1.8 or 0.9 kb, respectively, upstream of the transcription start site. Other studies implicate the homeodomain transcription factors OCT1, MSX, and DLX in the specification of neuron expression and developmental activation (Fig 1) ***(Givens et al., 2005)***.

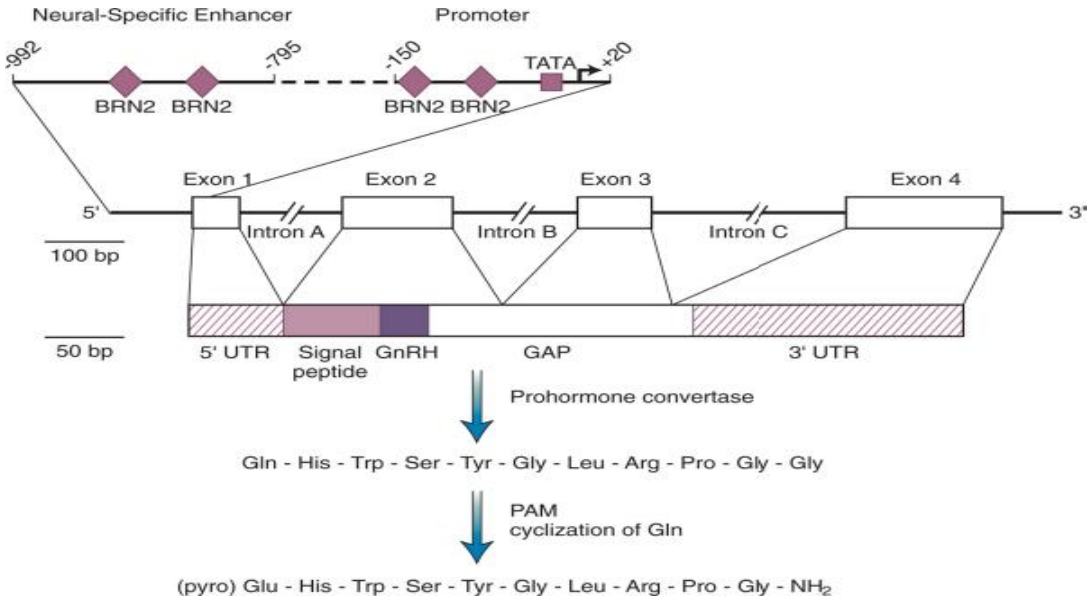

Figure 1: Schematic diagram of the human gene for (GnRH-I), the hypothalamic cDNA, and posttranslational processing of the GnRH peptide. A cluster of binding sites for the homeodomain transcription factor BRN2 is present in both the proximal promoter and a distal enhancer region and is important for neuron-specific expression of the gene. *GAP*, GnRH-associated peptide; *PAM*, peptidylglycine α-amidating monooxygenase; *UTR*, untranslated region ***(Cheng and Leung, 2005).***

Whereas deletion of the GnRH gene in the infertile knock-out mouse causes profound hypogonadotropism, this defect has not been described in the human ***(Veldhuis et al., 2006)***.

Anatomic Distribution:

Gonadotropin releasing hormone (GnRH) neurons are small, diffusely located cells that are not concentrated in a discrete nucleus. They are generally bipolar and fusiform in shape, with slender axons projecting predominantly to the median eminence and infundibular stalk. The location of hypothalamic GnRH neurons is species-dependent. In the rat, hypothalamic GnRH neurons are concentrated in rostral areas including the medial preoptic area, the diagonal band of Broca, the septal areas, and the anterior hypothalamus ***(Phoenix and Chambers, 2001).*** Immunohistochemical and radioimmunoassay data show that the median eminence of mammalian species contains the greatest amount of GnRH, this being the area in which the peptide is stored in neuronal terminals prior to release into hypophyseal portal blood ***(Clarke and Pompolo, 2005).***

In humans and non-human primates, the majority of hypothalamic GnRH neurons are located more dorsally in the medial basal hypothalamus, the infundibulum, and periventricular region. Throughout the hypothalamus, neurohypophyseal GnRH neurons are interspersed with non-neuroendocrine GnRH neurons, which extend their axons to other regions of the brain including other hypothalamic regions and various regions of the cortex ***(Phoenix and Chambers, 2001).***

Origin of GnRH Neurons:

There are fewer than 2000 GnRH-synthesising neurons in the adult human brain. In all animal species studied, these neurons have been shown to originate extracranially. They first appear in the human embryonic medial olfactory placode at 6 weeks gestation and begin to migrate penetrating the forebrain at 6.5 weeks medial and caudal to the developing olfactory bulbs. Migration is dependent on a scaffolding of neurons and glial cells along which the GnRH neurons move, with neural cell adhesion molecules playing a critical role in guiding the migration process ***(Quinton et al., 2003)***.

Gonadotropin releasing hormone (GnRH) neurons then proceed posteriorly in the submeningeal space bordering the interhemispheric fissure, before finally turning laterally to reach their ultimate position within the medio-basal hypothalamus. They are well established within the hypothalamus by 14 weeks, although the migratory process is still ongoing at 16 weeks, and is completed only by 19 weeks gestation. Intriguingly, GnRH immunoreactive neurons persist in the adult human olfactory epithelium ***(Quinton et al., 1997)***.

The migration pathway is not exclusive to GnRH neurons, but is shared with other neuroendocrine cells, including many that are immunoreactive for glutamate or neuropeptide Y (NPY). With leptin, corticotropin-releasing factor (CRF) and other neurotransmitter molecules ***(Dudás Merchenthaler, 2003)***, Glutamate and NPY are known to be involved in