Neurokinin B: A Novel Regulator of Reproductive Functions

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Normal H-P-G Axis

HYPOTHALAMUS

PITUITARY

GnRH

FSH    LH

GONAD

Steroids    Gametes
“GNRH pulse generator”

• a functionally interconnected and synchronized network of GNRH neurons
• inhibited throughout the childhood following a period of pubertal level activity during fetal life and early infancy
• release of this inhibition at the early years of the second decade marks the beginning of puberty
What triggers puberty?
Nutrition—including that received in utero—seems to help set this mysterious biological clock, but no one knows exactly what forces childhood to end.
Gene(s) taking role in initiating human puberty may be identified via autozygosity mapping in consanguous human families with two or more affected siblings with Normosmic Idiopathic Hypogonadotropin Hypogonadism
TÜBİTAK proje no: 106S276

NORMOSMİK İDİOPATİK HİPOGONADOTROPİK HİPOGONADİZMLİ OLGULARDA MOLEKÜLER GENETİK ANALİZLER YOLUYLA İNSANDA PÜBERTE SüRECİNDE ROL ALAN YENİ GENLERİN TANIMLANMASI.

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Inclusion criteria

• Male >14 Female>13 y
• Bone age>11.5y
• Tanner stage 1 breast in girls
• Testicular volume <4 ml in boys
• Prepubertal levels of sex steroids and FSH/LH
• Prepubertaal response to LHRH stimulation
• Normal hypothalamo-pituitary anatomy on MRI
• Normal olfactory bulbus and sulci on MRI
Exclusion criteria

- Anosmia/hyposmia (Kallmann syndrome)
- Inflammation, infection, tumor etc at the hypothalamus, pituitary
- Multiple pituitary hormone def (e.g. PROP1, HESX1)
- Chronic systemic diseases e.g. uremia, diabetes, IBD etc
- Extreme thinnes, athletes, anorexia nervosa, malnutrition
- Obesity (Leptin, Leptin receptor def)
- Syndromes e.g P. Wili, Bardet Biedl etc
Study cohort

- 9 consanguous families with at least 2 affected sibs
Genes cleared

- KAL1
- FGFR1
- GNRHR
- GNRH1
- GPR54
- KISS1
- PROK2
- PROK2R
- NELF
A genome-wide 250K NspI Affymetrix SNP microarray
SNP microarray gene chip data analyzed by AutoSNPa software (http://dna.leeds.ac.uk/autosnpa/)
Combined images of GnRH (green) and NK3R (red)-immunofluorescence show punctate colocalization of NK3R on GnRH fibers (yellow, arrows). (Krajewski J Comp Neurol 2005)
Schematic diagram of relationship between Neurokinin B and ER and GNRH (Rance Peptides 2008)
Kisspeptin neurons in the arcuate nucleus of the ewe express both dynorphin A and neurokinin B.


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Kisspeptin is a potent stimulator of GnRH secretion that has been implicated in the feedback actions of ovarian steroids. In ewes, the majority of hypothalamic kisspeptin neurons are found in the arcuate nucleus (ARC), with a smaller population located in the preoptic area. Most arcuate kisspeptin neurons express estrogen receptor-alpha, as do a set of arcuate neurons that contain both dynorphin and neurokinin B (NKB), suggesting that all three neuropeptides are colocalized in the same cells. In this study we tested this hypothesis using dual immunocytochemistry and also determined if kisspeptin neurons contain MSH or agouti-related peptide. To assess colocalization of kisspeptin and dynorphin, we used paraformaldehyde-fixed tissue from estrogen-treated ovariecctomized ewes in the breeding season (n = 5). Almost all ARC, but no preoptic area, kisspeptin neurons contained dynorphin. Similarly, almost all ARC dynorphin neurons contained kisspeptin. In experiment 2 we examined colocalization of kisspeptin and NKB in picric-acid fixed tissue collected from ovary intact ewes (n = 9). Over three quarters of ARC kisspeptin neurons also expressed NKB, and a similar percentage of NKB neurons contained kisspeptin. In contrast, no kisspeptin neurons stained for MSH or agouti-related peptide. These data demonstrate that, in the ewe, a high percentage of ARC kisspeptin neurons also produce dynorphin and NKB, and we propose that a single subpopulation of ARC neurons contains all three neuropeptides. Because virtually all of these neurons express estrogen and progesterone re-ceptors, they are likely to relay the feedback effects of these steroids to GnRH neurons to regulate reproductive function.

PMID: 17823266 [PubMed - indexed for MEDLINE]
**Neurokinin B orthologs**

- Human: DMHDFVGLM-NH₂
- Frog: DMHDFVGLM-NH₂
- Mouse: DMHDFVGLM-NH₂
- Cow: DMHDFVGLM-NH₂
- Rat: DMHDFVGLM-NH₂

**Neurokinin A orthologs**

- Human: HKTDSFVGLM-NH₂
- Python: HKTDSFVGLM-NH₂
- Mouse: HKTDSFVGLM-NH₂
- Rat: HKTDSFVGLM-NH₂
- Cow: HKTDSFVGLM-NH₂
- Lamprey: HF-DEFVGLM-NH₂
- Chicken: HKTDSFVGLM-NH₂

**Substance P orthologs**

- Human: RPKPQFFGLM-NH₂
- Tree shrew: RPKPQFFGLM-NH₂
- Guinea Pig: RPKPQFFGLM-NH₂
- Mouse: RPKPQFFGLM-NH₂
- Cow: RPKPQFFGLM-NH₂
- Alligator: RPRPHQFFGLM-NH₂
- Goldfish: RPRPHQFFGLM-NH₂
In summary

we have identified loss-of-function mutations in either neurokinin B or its receptor in four out of nine multiplex families affected by nIHH.

These findings establish that NKB action via the NK3R is necessary for the central neuroendocrine control of human reproduction. These families represent the first examples of inherited defects of tachykinin signalling in any human disorder.

NKB signaling system may provide a novel avenue for the pharmacological manipulation of human fertility and the treatment of sex steroid-related diseases.
Letter abstract

Nature Genetics 41, 354 - 358 (2008)
Published online: 11 December 2008 | doi:10.1038/ng.306

TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for Neurokinin B in the central control of reproduction

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The timely secretion of gonadal sex steroids is essential for the initiation of puberty, the postpubertal maintenance of secondary sexual characteristics and the normal perinatal development of male external genitalia. Normal gonadal steroid production requires the...
Implications and the future

- NKB action is required for normal HPG function both *in utero* and peripubertally in humans
- Yet, Tacr3 knockout mice are fertile (Kung et al 2004) AND
- Central infusion of a potent NK3R agonist in rodents inhibits gonadotropin secretion (Sandoval-Guzman & Rance NE 2004).
Implications and the future

primates (but not rodents) exhibit true centrally-mediated suppression of GnRH secretion in the prepubertal period (Plant 2006).

divergence between rodents and humans is likely

testing NKB in a primate model may be very informative
Implications and the future

Although kisspeptin and now Neurokinin B pathways appear to be a prerequisite for human puberty, it is likely that there are many more actors yet to be discovered.

It is extremely premature to assign a “master controller” for puberty.
Implications and the future

As data accumulate (with a perceived large input from autozygosity mapping in multiplex nIHH families) the organization of the GNRH pulse generator will be characterized in a more detailed way including its functional hierarchy and the factor(s) that reactivate the system around the expected age of human pubertal onset.