

Kisspeptin: a central regulator of reproduction in the bitch




HERSHEY'S SWEET MILK CHOCOLATE KISSES

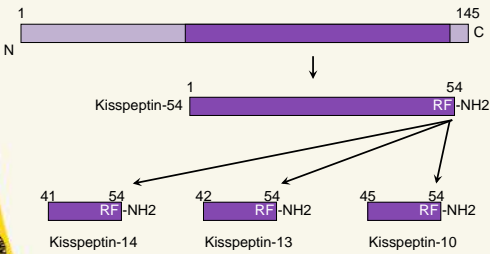
Utrecht University Karin Albers-Wolthers
DVM, PhD Candidate, Resident ECAR

Discovery of Kisspeptin

- 1996: Discovery of the *KISS1* gene and its metastasis inhibiting properties (Metastin)
- 2001: Ligand of (orphan) G-protein Coupled Receptor 54 (GPR54 or KISS1R)
- 2003: Role in (human) reproduction
 - Two independent research groups
 - Consanguineous families
 - Parents were first cousins
 - Male and female offspring
 - Idiopathic Hypogonadotropic Hypogonadism (IHH)
 - Low circulating gonadotropins
 - No puberty
 - Infertile



(human) *Kiss1*- gene products



1 N 145 C

↓

Kisspeptin-54 1 54 RF-NH₂

↙ ↘ ↘

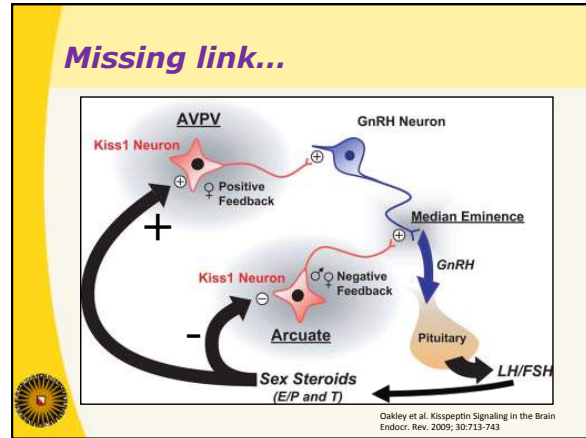
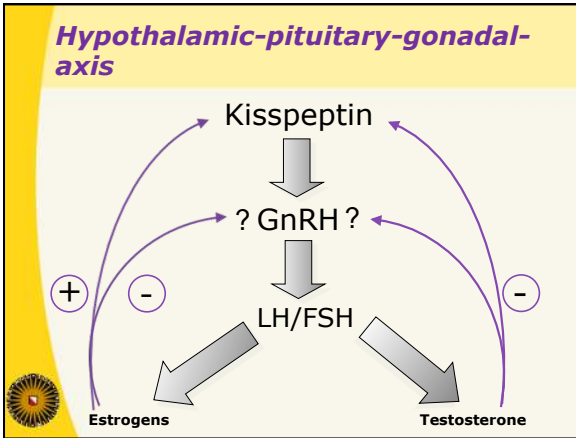
41 54 RF-NH₂ 42 54 RF-NH₂ 45 54 RF-NH₂

Kisspeptin-14 Kisspeptin-13 Kisspeptin-10

Role in reproduction

- *Kiss1* and *kiss1r* knock-out mice
 - Same symptoms as humans with IHH
 - Normal GnRH content in hypothalamic tissue
- Activating mutation of *KISS1R*
 - Precocious puberty
- Administration of kisspeptin (human KP10) stimulates gonadotropin secretion in different species

Kisspeptin → Strong stimulator of GnRH secretion
→ Increased secretion of LH and FSH



Kisspeptin antagonist

- Roseweir et al. produced kisspeptin antagonists
 - p234
 - p271 (=p234-penetratin)
- Administration of p234:
 - Delayed onset of puberty (female mice)
 - Prevented pre-ovulatory LH surge (female mice)
 - Castrated males: lower plasma [LH] (rats)
- Peripheral (IP) administration of p234-penetratin (p271)
 - Blunted LH response after KP10 administration (male rats)

→ inhibition of kisspeptin signaling results in less GnRH and thus less LH and FSH secretion

Hypothesis: Kisspeptin is a target for non-surgical contraception

Aims of the study

- To identify the canine *KISS1* gene
- To study the effect of peripheral administration of KP10 in the dog

KiSS1 Gene

- In silico identification:
 - Comparing human cDNA sequences using BLASTn software
- Two fragments on chromosome 38 of the dog displayed high similarity with human *KiSS1* cDNA.
- The region containing these fragments is flanked by orthologs of two genes, and these genes are the same genes that flank the human *KiSS1* gene

→ Large similarity with human alignment of *KiSS1* gene

→ *KiSS1* gene is present in the canine genome



KiSS1 gene product

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Canine  1  MNSLVSQQLMLLLCATSFRETLLIKVAPMETPGPAGQRLGAQALPAWERSPP-----
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Human   1  MNSLVSQQLLLFLCATHFGEPLIKVASVGNRSRPTGQQLSLGLLAPGQSLPCTERKPAAT

Canine  53  -----RAPQRHLPARRGA-----DLPAYNNWVFGRLRYG
          || || ||
Human   62  ARLSRRTSLSPFPESGSPQQPGLSAPHSRQIPAPQGAVLVQREKDLNYYNWSFGLRFG

Canine  82  RRRRAATPGLRGGTPSPRLRVFVGVGLGLRS
          || | | |
Human  123  KREAA-PGNHGRSAGRG
    
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- Preprokisspeptin: 111 amino acids (human 145)

- Canine KP10: Y N W N V F G L R Y
- Human KP10: Y N W N S F G L R F
- rat KP10: Y N W N S F G L R Y



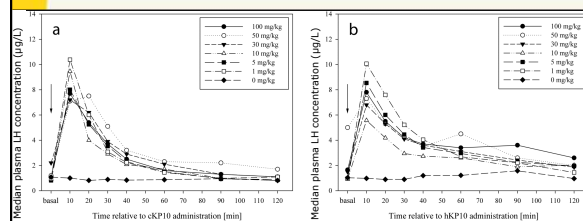
In vivo: materials & methods

- 6 adult Beagle bitches in anestrus (100 days after ovulation)
- KP10 administered intravenously at weekly intervals
- Doses: 0 (controls), 1, 5, 10, 30, 50, 100 µg/kg
- Blood sampling for determination of plasma [LH]
 - 40 and 0 minutes before KP10 (basal)
 - 10, 20, 30, 40, 60, 90 and 120 minutes after KP10

Pilot with human KP10 and canine KP10



In vivo: pilot (n=3)



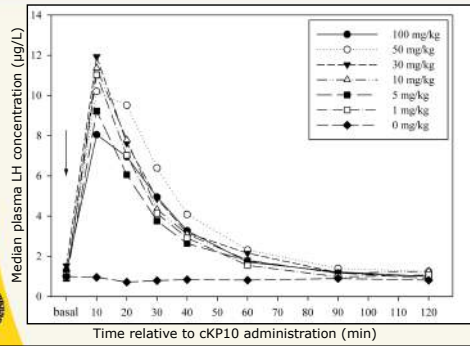
Canine KP10

Human KP10

→ Continue with canine KP10



Results: Canine KP10 (n=6)



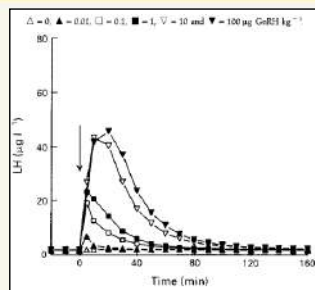
Discussion

- Dog: 10-fold increase of plasma [LH]
 - (humans: 3-fold)
- GnRH versus KP10



Discussion: GnRH stimulation test

- 6 Beagle bitches
- Dosages: 0, 0.01, 0.1, 1, 10 and 100 µg/kg
- Blood samples: Every 10 minutes
- 5 minutes after GnRH



From: Van Haften et al. Increasing sensitivity of the pituitary from early to late anestrus in the Beagle bitch. J Reprod Fert 1994; 101:221-225

Discussion

- Dog: 10-fold increase of plasma [LH]
 - (humans: 3-fold)
- GnRH → larger LH response
- No differences between dosages
- Additional study to complete dose response:
 - 0.1, 0.2, 0.3, 0.5 µg/kg (in progress...)



Conclusions

- Kisspeptin is present in the dog
- Sensitive to exogenous cKP10
- Basis for further research...

Kisspeptin is an interesting target for non-surgical contraception in the dog!



Work in progress...

- Sensitivity to kisspeptin varies among different cycle phases
- cKP10 stimulation tests during different stages of the estrous cycle and anestrus
- Kisspeptin antagonist administration in male and female dogs
- In vitro approach: Calcium-flux in celllines with KISS1 receptor
- Funded by Michelson Grant...



Research team

Auke Schaeffers-Okkens, DVM, PhD, dipl. ECAR
Hans Kooistra, DVM, PhD Dipl. ECVIM, Endocrinology
Jeffrey de Gier, DVM, PhD Dipl. ECAR
Peter Leegwater, PhD, Genetics
Victor Rutten, Prof. PhD, Immunology
Peter van Kooten, Msc, Immunology
Theo van Haften, PhD, Biochemistry and Cell Biology
Jan-Willem Hesselink, DVM, prof. PhD, Dipl. ECAR
Niels Lambalk, Prof. Human Reproductive Science
Bob Millar, Prof. Human Reproductive Science



Thank you!

