Transnasal Delivery of a Gonadotropin-releasing Hormone Analog In Miniature Swine

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Outline

Background

My Study

Results

Conclusions
Hypothalamus and Pituitary Gland

Figure 50-2 The hypothalamus regulates pituitary activity.

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### Vertebrate Family of Peptides

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**What is Gonadotropin-Releasing Hormone (GnRH)?**

Gonadotropin-Releasing Hormone (GnRH) is a small, peptide hormone that is synthesized in the hypothalamus and facilitates the release of gonadotropins from the anterior pituitary gland. GnRH plays a crucial role in the regulation of the reproductive system, including the stimulation of the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary gland. These hormones, in turn, regulate the production of sex steroids (estrogens and androgens) in the gonads (ovaries or testes), which are essential for the development, maturation, and function of the reproductive system in both males and females.

**Vertebrate Family of Peptides**

- **Vertebrate**: Mammal, Guinea Pig, Chicken - I, Rana, Seabream, Salmon, Medaka, Catfish, Herring, Chicken -II, Dogfish, Lamprey - III, Lamprey - I, Whitefish.

**Peptide Sequence**

- pGlu: Proline-glycine with amidated C-terminus
- His: Histidine
- Trp: Tryptophan
- Ser: Serine
- Tyr: Tyrosine
- Gly: Glycine
- Leu: Leucine
- Arg: Arginine
- Pro: Proline
- Asn: Asparagine
- Met: Methionine
- Glu: Glutamic Acid

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**Diagram Details**

- **CNS**: Central Nervous System
- **Hypothalamus**: Region of the brain that regulates body functions and homeostasis
- **GnRH**: Gonadotropin-Releasing Hormone
- **Anterior pituitary**: Gland that secretes hormones stimulating other glands
- **FSH**: Follicle-stimulating hormone
- **LH**: Luteinizing hormone
- **FSH-R**: FSH receptor
- **LH-R**: LH receptor
- **Sex steroids**: Estrogens and androgens
- **Activin, Inhibin**: Hormones involved in the regulation of fertility

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Mihael Freamat
Identification of novel GnRHs and Receptors can contribute to the development of novel GnRH therapeutics.
How Is GnRH Used Medically

10,000 Analogs Developed

GnRH Analog

Fertilization

Cancer Therapy

Contraception

Westside Pregnancy Resource Center

Breast cancer cell

http://perso.wanadoo.fr/pharma.rocheville/contraception_nouveautes.htm

http://www.news-medical.net/images/breast%20cancer%20cell.jpg
Delivery System of Insulin-2005
Why Miniature Swine?

Swine have been shown to have similar mucosal membrane properties to humans making them an ideal test subject (Bollen et al., 2000).
Figure 21-2 Cross-section of nasal cavity.
Objective

To determine if the GnRH analog DAla²Pro⁹NEt can successfully be delivered via a transnasal application.

Measure GnRH & LH

Biological Effect

GnRH Complex
Methods

Catheter Implantation to 4 Miniature Swine

Plasma Collection for GnRH Analysis
  - GnRH Extracted From Plasma
    - Samples Assayed Using a Specific Radioimmunoassay

Serum Collection for LH Analysis
  - Samples Assayed Using a Specific Radioimmunoassay

Data Analyzed

Dr. Roger Wells and Van Gould

Samples Sent to Dr. Richard Barb
This study was completed using 4 miniature swine aged for 4 months. They were cared for according to IACUC requirements.
The Treatments

- Pig 1: 0% CPE 0% GnRH Analog (Control)
- Pig 2: 2% CPE 1% GnRH Analog
- Pig 3: 0% CPE 1% GnRH Analog
- Pig 4: 0% CPE 2% GnRH Analog
Transnasal Drug Delivery
Sampling Times

Week 1 Days 1+2
- 0 Hours
- 30 Min
- 60 Min
- 90 Min
- 180 Min
- 24 Hours

Week 2 Days 1+2
- 0 Hours
- 30 Min
- 60 Min
- 90 Min
- 180 Min
- 360 Min
- 24 Hours

Week 1 Days 3+4
- 0 Hours
- 30 Min
- 60 Min
- 90 Min
- 180 Min
- 360 Min
- 24 Hours

Week 2 Days 3+4
- 0 Hours
- 30 Min
- 60 Min
- 90 Min
- 180 Min
- 360 Min
- 24 Hours
Tubes for Blood Collection
Steps for GnRH Analysis

- Centrifuge Blood
  - Extract
  - Centrifuge
  - Filter
  - Sep Pak
  - RIA
- Analysis
Centrifuge Blood
GnRH Extraction Using a Polytron
Centrifugation
Filtration
Separation by Sep Pak
Radioimmunoassay
Outline

Background

My Study

Results

Conclusions
Sampling Times

Week 1 Days 1+2
- 0 Hours
- 30 Min
- 60 Min
- 90 Min
- 180 Min
- 24 Hours

Week 2 Days 1+2
- 0 Hours
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- 0 Hours
- 30 Min
- 60 Min
- 90 Min
- 180 Min
- 360 Min
- 24 Hours

Week 2 Days 3+4
- 0 Hours
- 30 Min
- 60 Min
- 90 Min
- 180 Min
- 360 Min
- 24 Hours
These results show a significant increase in GnRH concentration in the blood using the Bentley Formulation with GnRH.
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Summary

These studies showed that GnRH combined with the Bentley formulation was successfully administered into the pig following transnasal application.
Implications

The results from this project can be eventually used by pharmaceutical industries in development of drug applications.
Acknowledgements

- Dr. Stacia Sower
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