Models of Stress and Reproductive Dysfunction

Workshop

Kevin O’Byrne
Division of Endocrinology & Reproduction
School of Biomedical & Health Sciences
Hypothalamic amenorrhoea

Stress induces changes in the hypothalamic-pituitary-ovary axis, leading to a decrease in GnRH pulsatility and consequently decreased LH and FSH secretion. This results in anovulation and amenorrhea. The stress response also affects the hypothalamic-pituitary-adrenal axis, increasing cortisol levels, which further contributes to the suppression of reproductive function.
Monitor activity of GnRH pulse generator

**In vivo:**
- LH pulses
- GnRH pulses
  - Pituitary portal blood
  - Cerebrospinal fluid
  - Extracellular fluid (dialysis)
- MUA volleys

**In vitro:**
- GFP-identified GnRH
- Primary cultures or GT1-7
  - Electrical activity
  - Calcium oscillations
  - GnRH pulses
3 way solenoid valve

SALINE
HEPARIN

Dual channel swivel

PUMP

25 µl at 5 min interval for 6-10 hours

Blood travels along the pathways marked in red
Monitor activity of GnRH pulse generator: Human

**In vivo:**
- LH pulses ✓
- GnRH pulses
  - Pituitary portal blood
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- MUA volleys

**In vitro:**
- GFP-identified GnRH
- Primary cultures or GT1-7
  - Electrical activity
  - Calcium oscillations
  - GnRH pulses
Monitor activity of GnRH pulse generator: GnRH Pulse
Monitor activity of GnRH pulse generator: MUA Volleys

A

Arcuate Nucleus
Mammillary Body
LHRH
post.
Pituitary
ant.
LH

B

1 2

X-Ray

B


LH (ng / ml)

MUA (spikes / min)

Time (min)

0 60 120 180 240

2000 3000

1000 2000

25 35 45

Kisspeptin (green) and GnRH (red) neurones in the male rhesus monkey

K.T.O’Byrne et al, Endocrinology, 1991
Methodological strategies to manipulate the HPG axis

**Pharmacological tools**
- Classical stress neuropeptides and their antagonists:
  - CRF, Vasopressin, Opioids, CGRP, etc.
- Myriad other modulators:
  - Cytokines, prostaglandins, etc.

**Routes of administration**
- Peripheral:
  - Iv, ip, sc, etc.
- Central:
  - Intracerebroventricular
  - Intra-nuclear (unilateral vs bilateral)
Osmotic mini-pump – prepubertal rats

**Methods:**
- Post natal day 28 female rat (~65 g)
- 28 gauge icv cannula (Plastics One)
- Alzet mini-pump (~1g) (0.5µl/h for a 14-day)
- CRF (400 pmol/day) or CRF antagonist (4 nmol/day)
- Controls: Non-surgical or aCSF
- Vaginal opening and First Oestrus (Puberty markers)

**Results:** Time of puberty
- Non-surgical = aCSF controls: pnd 37
- CRF: pnd 40
- CRF antagonist: pnd 35
- (No change in body weight)
Problem: hierarchical control and integrative neurobiology?

Neuropeptide synapses

5-HT
DA
NA
GnRH
CRF
VP
GAL
NT
SP
NPY
Kiss1
POMC
GABA
Etc.
Physiological stressors?
Socially mediated infertility
Immunological stress

Endotoxin - LPS

Restraint stress

I thought I’d take the tube to avoid the congestion charge

Restraint 1 hour

LH (ng/ml)

Time (h)
Hypoglycemia
insulin iv

Chen et al. Neuroendocrinology, 1992
Fasting and re-feeding affects on pulsatile LH secretion

Schreinhofer DA et al., Endocrinology 1996, 137:3770
Exercise-induced amenorrhoea

Cynomolgus monkey

Clinically recognised forms of stress-induced reproductive dysfunction:

- Functional hypothalamic amenorrhoea: psychosocial
- Anorexia (bulimic) nervosa: nutritional compromise
- Exercise-associated: excessive exercise

Growing recognition that each of these syndromes develops in response to exposure to combinations of psychogenic and metabolic stressors.
Functional hypothalamic amenorrhoea

- **Mild indices of “psychological stress”**
  - Dysfunctional attitudes
  - Unrealistic expectations
  - Higher levels of perfectionism
  - Higher need for social approval
  - Difficulty coping with daily hassles

- **Mild metabolic deficits**
  - Higher incidence of subclinical eating abnormalities
  - Exercising
Schematic diagram of the experimental design (monkey model of FHA)

Mild Psychosocial stress: Move
- Group 1

Mild Metabolic stressors: Exercise + Diet
- Group 2
  - Exercise + diet

Move Exercise + Diet
- Group 3
  - Exercise + diet

Percentage of monkeys showing abnormal menstrual cycles (i.e., either cycles >44 days in length or anovulatory cycles) in E3+4 for each of the 3 experimental groups

[Subthreshold stressors synergise to compromise reproductive function]

Effects of a layered stress paradigm on GnRH, LH & cortisol in the ewe

Individual stress-sensitivity & reproductive dysfunction in female macaques

Schematic diagram of experimental design

High stress resilience (HSR)  Medium stress resilience (MSR)  Stress sensitive (SS)

Bethea CL et al., Mol Neurobiol 2008 38:199
An amazing array of models of stress-induced reproductive dysfunction

Molecular tools?

- In Situ
- qRT-PCT
- Transgenics
- Etc.
Cynomolgus monkeys: In the absence of stress

<table>
<thead>
<tr>
<th>Gene Expression</th>
<th>Brain Region</th>
<th>HSR &gt; MSR &gt; SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>GnRH expression</td>
<td>Hypothalamus</td>
<td>HSR &gt; MSR &gt; SS</td>
</tr>
<tr>
<td>Serotonin gene expression</td>
<td>Raphae</td>
<td>HSR &gt; MSR &gt; SS</td>
</tr>
<tr>
<td>Number of serotonin neurones</td>
<td>Raphae</td>
<td>HSR &gt; MSR &gt; SS</td>
</tr>
<tr>
<td>GABA expression</td>
<td>Hypothalamus</td>
<td>SS &gt; MSR &gt; HSR</td>
</tr>
<tr>
<td>CRH expression</td>
<td>Hypothalamus/amygdala</td>
<td>SS &gt; MSR &gt; HSR</td>
</tr>
</tbody>
</table>
Kisspeptin stimulates the neuroendocrine reproductive axis

Micropunch method of Palkovits *versus* whole hypothalamus
GFP labelled GnRH neurones

Suter, K. J. et al. Endocrinology 2000;141:3731-3736
Burst interval is altered to produce peaks and nadir in firing rate

Model of interacting rhythms in GnRH neurones (GnRH pulse frequency)

MUA recordings in the rhesus monkey

A

10μV
0.5 ms

B

MUA

Unit 1L

Unit 2L

Unit 3L

20 s
LH secretion in ovariectomized female mice of GnRH-GFP and non-transgenic controls