Roles Of Connexin43 In Testicular Cells: Germ Cell Development And Endocrine Function

By

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Introduction

• Impact of Infertility
  – male factor
  – Testis
  – Cells of the testes (communication)
    • Gap junctions

• Gap junctions
  – Channels linking two cells
  – Made of proteins called connexins
  – Some protein defects lead to lack of communication
  – Disease conditions

• Connexins in the testis
• Most abundant connexin 43
• Absence or defects - infertility
• It defect due to failure to form gametes
• Or lack of androgen support
INFERTILITY

- 25% couples infertile; 186 million women with desire for motherhood

Based on the Demographic Health Survey (1994–2000), World Health Organization, Geneva
GAP JUNCTION INTERCELLULAR COMMUNICATION

- Gap junction channel
- Connexon
- Connexin

Extracellular space

Cytosol
Gene mutation in humans

(gene mutation Mice)
CONNEXINS IN THE TESTES:

- Cx43
- Cx33
- Cx37
- Cx26
- Cx32
HYPOTHESIS:
Deficiency Of Connexin43 Alters Androgen Production By The Leydig Cells
SPECIFIC OBJECTIVES:

Does complete deletion of Cx43 encoding gene (**Gja1**) from the whole mouse affect:

1. Androgens synthesis?
2. Regulation of androgen production?
3. Communication between Leydig cells?
**GENERAL APPROACH**

Cx43 encoding Gene (Gja1) → Connexin43

MODEL for postnatal studies

Gja1 +/- Mate → Genotype term fetuses

Gja1+-/ - Testes

Total deletion

DEATH at neonatal stage (heart defects)
GENERAL APPROACH

At 3 Weeks

\[ \text{Gja1}^{+/+} \quad \text{Gja1}^{-/-} \]

Genotype
LEYDIG CELL NUMBERS

Gja1+/+

Gja1−/−

Circulating testosterone

![Bar chart showing Androgen ng/ml for Gja1+/+ and Gja1−/− genotypes.]

-genotype

Gja1+/+

Gja1−/−

Androgen ng/ml

0
0.2
0.4
0.6
0.8

(14)

(13)

Genotype

Gja1+/+

Gja1−/−

Genotype

Leydig cells / section

Seminiferous tubules / section

0
500
1000
1500
2000
0
40
80
120
160
200

(14)

(13)

Gja1+/+

Gja1−/−

Genotype

Gja1+/+

Gja1−/−

Genotype

Gja1+/+

Gja1−/−

Genotype
LEYDIG CELL ENZYME ACTIVITIES

- **Cholesterol**
  - 20,22 Desmolase
  - 3β OH-Steroid Dehydrogenase
  - Progesterone

- **Progesterone**
  - 17-Hydroxylase
  - 17,20 Desmolase
  - Androstendione

- **Androstendione**
  - 17β OH-Steroid Dehydrogenase
  - (17βHSD)

- **Testosterone**
  - 5α Reductase
  - 3α OH-Steroid Dehydrogenase
  - Androstandiol

- **Androsterone**
  - Dihydrotestosterone
  - Estradiol

- **Estradiol**
  - Aromatase

- **Androstandiol**
  - (3αHSD)

**Enzyme Activity Chart**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Activity (pg/mg/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17βHSD</td>
<td></td>
</tr>
<tr>
<td>5α R</td>
<td></td>
</tr>
<tr>
<td>3α HSD</td>
<td></td>
</tr>
</tbody>
</table>
SENSITIVITY TO LH STIMULATION.

METHODS

Graft testes

At 3 weeks
Retrieve grafts

Culture 4hrs
+/- LH

RIA (medium)

Androgens & Sensitivity assay

Grafted testes

% of control ng/ml

Gja1+/+
Gja1-/-

Lh pg/ml

0 25 50 100 150 200 250 300

25 50 100 400

% of control ng/ml

Lh pg/ml
INTERCELLULAR COMMUNICATION

3βHSD

Hoechst

A

B

C

D

E

F

Gja1+/+

G

H

Gja1−/−

Negative Control

*
CONCLUSIONS:

• Androgens are produced.
• No difference in amounts, type or regulation
• Cells coupled despite the Cx43 deficiency.
  – Although the deletion causes decreased fertility due to the reduced germ cell numbers, this can not be due to functional failure in the Leydig cells.
IMPLICATIONS

• Cells functional despite the deficiency.

• Cx43 is not required for androgen production

• There could be other gap junctions in Leydig cells


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