The Effects of Oxytocin on the PIP2 Pathway in Response to Progesterone and/or Estrogen

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Statistics

* 215000 sheep in Oregon as of January 2011
* Over $560 million of national gross income in 2010

http://upload.wikimedia.org/wikipedia/commons/2/2c/Flock_of_sheep.jpg
Significance

- Progesterone (P4) → Prepares uterus for implantation of zygote and supports gestation.
- Estradiol (E2) → placental development
Background: OT the Hormone

- Oxytocin (OT) high during labor.
- Has a role in contractions
- Regulation by P4 and E2

More Background

* Phosphatidylinositol-4,5-bisphosphate (PIP2) is a phospholipid in the plasma membrane.
* PIP2 promotes cell signaling.
  * Secondary Messengers: DAG, IP3, and Ca^{2+}

Important Pathway

Key:
OT = Oxytocin
GPCR = G-Protein Coupled Receptor
PLC = Phospholipase C
DAG = Diacylglycerol
I = Inositol
P = Phosphate
Question

* Is OT more effective in the PIP2 breakdown when under a purely E2 environment or an E2 + P4 environment?

* **Hypothesis:** OT will stimulate PIP2 breakdown in the ovine endometrium more when exposed to just E2 compared to E2 + P4
# Modified Estrous Cycle

<table>
<thead>
<tr>
<th>Day</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group1</td>
<td>E2</td>
<td>E2</td>
<td>P4</td>
<td>P4</td>
<td>P4</td>
<td>P4</td>
<td>P4</td>
<td>E2</td>
<td>E2</td>
</tr>
<tr>
<td>Group2</td>
<td>E2</td>
<td>E2</td>
<td>P4</td>
<td>P4</td>
<td>P4</td>
<td>P4</td>
<td>P4</td>
<td>E2+P4</td>
<td>E2+P4</td>
</tr>
</tbody>
</table>

**Key:**
- E2- Estradiol 17β
- P4- Progesterone
Day 10

- Laparotomy– extracting endometrial tissue from the uterus.
Procedure: Incubation

* First, a series of incubations that include myo-[2^3H]-inositol
* Introduction of LiCl inhibits the pathway.
Procedure: Extraction

* Column chromatography — separates into fractions:
  * Including: I, IP, IP2, & IP3
  * Liquid scintillation is used to analyze
Predicted Results

* More $[2^3\text{H}]-\text{inositol}$ signifies more breakdown.

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>More breakdown</td>
<td>Less breakdown</td>
</tr>
</tbody>
</table>
Results

- Using a Repeated Measure Analysis of Variance

- IP3 is significant at the 10% level

- All others were not significant→ still showed an increase in I, IP, and IP3 in E2 compared to E2+P4
OT increases the activity of IP3

![Graph showing the effect of different treatments on CPM (counts per million) of tissue. The x-axis represents the treatments: E2, E2+P4, E2+OT, and E2+P4+OT. The y-axis represents CPM [3H]·IP3/g of tissue (mean ± SE). The graph shows that E2+OT has the highest CPM, followed by E2+P4, E2, and E2+P4+OT.]
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