The Potential Effectiveness of UBP 302 as a KAR Antagonist in Suppressing ATPA-elicited Feeding in the LH

Students of Bio 254
Jamie Nichols
Ashley Carr
Cameron Simmons
Why Conduct This Study?

• Understanding more about how the brain regulates eating behavior

• High rates of obesity and comorbidities

• Expand treatment options for eating disorders
What Part(s) of the Brain Regulate(s) Eating?

- Lateral hypothalamus (LH), among others

(Hettes, et al., 2007)
What Neurotransmitters Elicit Eating in the LH?

Agonist: ATPA

Antagonist: UBP 302

Glutamate


ATPA’s solvent: aCSF = artificial cerebrospinal fluid

UBP 302’s solvent: DMSO = dimethyl sulfoxide
What Do We Know So Far?

- ATPA has been shown to induce eating in rats in LH
  – Hettes, et al., 2007

- *In vitro* studies have shown that UBP 302 acts as an antagonist of KA receptors.
  – Ireland, et al., 2008

- This is the first *in vivo* study of UBP 302 (that we know of).

(Hettes, et al., 2007)
Hypothesis

LH injection of the antagonist **UBP 302** will suppress eating elicited by subsequent injection of the receptor agonist **ATPA**.
Subjects and Conditions

• 17 male Sprague-Dawley Rats

• Standard Conditions
  – 12:12 hour light/dark cycle
  – Temperature
  – *ad libitum* rat chow and water

• Test Conditions
  – Lights on
  – Milk mash
  – *ad libitum* water
Procedures

<table>
<thead>
<tr>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3-8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgery and</td>
<td>Taming and</td>
<td>Experiment and Data</td>
</tr>
<tr>
<td>Recovery</td>
<td>Mock Injections</td>
<td>Collection</td>
</tr>
</tbody>
</table>

![Graph showing Injections and Behavioral Observations](image_url)

**Initial Feeding**

![Graph showing Time (min)](image_url)
Behaviors

- Eating
- Locomotion
- Resting
- Alert
- Grooming
- Drinking

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Eat</th>
<th>Drink</th>
<th>Grm.</th>
<th>Loc.</th>
<th>Alert</th>
<th>Rest</th>
</tr>
</thead>
<tbody>
<tr>
<td>-10</td>
<td></td>
<td>✔</td>
<td>✔</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-9</td>
<td></td>
<td>✔</td>
<td>✔</td>
<td></td>
<td>✔</td>
<td></td>
</tr>
<tr>
<td>-8</td>
<td></td>
<td>✔</td>
<td>✔</td>
<td></td>
<td>✔</td>
<td></td>
</tr>
<tr>
<td>-7</td>
<td></td>
<td>✔</td>
<td>✔</td>
<td></td>
<td>✔</td>
<td></td>
</tr>
<tr>
<td>-6</td>
<td></td>
<td>✔</td>
<td></td>
<td></td>
<td>✔</td>
<td></td>
</tr>
<tr>
<td>-5</td>
<td></td>
<td>✔</td>
<td>✔</td>
<td></td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>-4</td>
<td></td>
<td>✔</td>
<td>✔</td>
<td></td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>-3</td>
<td></td>
<td>✔</td>
<td>✔</td>
<td></td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>-2</td>
<td></td>
<td>✔</td>
<td>✔</td>
<td></td>
<td>✔</td>
<td>✔</td>
</tr>
</tbody>
</table>

Injection
1 Time =
Injection 2 Time =
Procedures

<table>
<thead>
<tr>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3-8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgery and Recovery</td>
<td>Taming and Mock Injections</td>
<td>Experiment and Data Collection</td>
</tr>
</tbody>
</table>

Injections and Behavioral Observations

- Initial Feeding
- Begin Behavioral Observations
- Injection Period
- Food Weight

Time (min)
Injections

At time 0…timeline halts

<table>
<thead>
<tr>
<th>1st Injection (UBP 302 or DMSO)</th>
<th>5 minute Diffusion Period</th>
<th>2nd Injection (ATPA or aCSF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO + aCSF (Control)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMSO + ATPA (1 nmol)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (3 nmol) UBP 302 + ATPA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High (9 nmol) UBP 302 + ATPA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (3 nmol) UBP 302 + aCSF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High (9 nmol) UBP 302 + aCSF</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Procedures

<table>
<thead>
<tr>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3-8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgery and</td>
<td>Taming and</td>
<td>Experiment and Data</td>
</tr>
<tr>
<td>Recovery</td>
<td>Mock Injections</td>
<td>Collection</td>
</tr>
</tbody>
</table>

#### Injections and Behavioral Observations

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Initial Feeding</th>
<th>Injection Period</th>
<th>End Behavioral Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>-60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>110</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Are the Rats Satiated Before Each Injection?
How Much Food Was Eaten After Each Injection?

* = p< 0.05 compared to DMSO + aCSF injection
How Much Time Was Spent EATING?

* = p< 0.05 compared to DMSO + aCSF injection

Legend:
- DMSO + aCSF
- DMSO + ATPA
- Low UBP302 + ATPA
- High UBP302 + ATPA
- Low UBP302 + aCSF
- High UBP302 + aCSF
Injections and Behavioral Observations

How Much Time Was Spent EATING?

% Time Spent EATING

* = p< 0.05 compared to DMSO + aCSF injection

Time (min)
How Much Time Was Spent EATING?

* = p< 0.05 compared to DMSO + aCSF injection
How Much Time Was Spent LOCOMOTING?

* = p< 0.05 compared to DMSO + aCSF injection

*Denotes statistical significance compared to the DMSO + aCSF injection group.
Time Spent LOCOMOTING

How Much Time Was Spent LOCOMOTING?

How Much Time Was Spent LOCOMOTING?

* = p< 0.05 compared to DMSO + aCSF injection

* = p< 0.05 compared to DMSO + aCSF injection

* = p< 0.05 compared to DMSO + aCSF injection
How Much Time Was Spent ALERT?

![Graph showing time spent ALERT vs time (min) with injections at different time points. The x-axis represents time (min) from -10 to 55, and the y-axis represents % Time Spent ALERT from 0 to 100. The graph compares DMSO + aCSF and DMSO + ATPA injections, with a comparison note of * = p<0.05 compared to DMSO + aCSF injection.]
How Much Time Was Spent RESTING?

* = p < 0.05 compared to DMSO + aCSF injection
How Much Time Was Spent RESTING?

* = p< 0.05 compared to DMSO + aCSF injection
What Can We Conclude About ATPA?

• Pre-injection eating was comparable across groups
  → all rats were satiated

• ATPA: (replicated results, Hettes, et al., 2007)
  – cumulative food intake
  – time spent eating
  – eating behavior through 15 min post injection

• ATPA elicits eating… presumably at kainate receptors
What Can We Conclude About UBP 302?

- UBP 302 did not suppress ATPA-elicited eating at the doses tested.
- UBP 302 either:
  - Is not effective at blocking enough receptors at doses tested or…
  - Is not effective as an antagonist *in vivo*
- No behavioral differences in response to the UBP 302 injection.
What’s Next?

- Doses of UBP 302
- Diffusion time between injections

UBP 302 = antagonist to kainate receptors in vitro

Support continuing research in vivo
Research conducted by Bio 254 Class 2008. Thanks Dr. Hettes
% Time Spent
Grooming or Drinking

Grooming

Drinking

Time (min)

% Time Spent GROOMING

% Time Spent DRINKING

-10  -5   0   5   10  15  20  25  30  35  40  45  50  55

-10  -5   0   5   10  15  20  25  30  35  40  45  50  55
% TIME SPENT RESTING

* = p< 0.05 compared to DMSO + aCSF injection
Latency to Feed Data

Latency to Feed

Latency to Feed- capped at 55 min

* = p< 0.05 compared to DMSO + aCSF injection
Why is this so important?

Based on van den Pol, et al., 1996
Figure adapted from Purves, et al., *Neuroscience*, 4th edition, 2007