The Role of Glutamate Receptor Activation Within The Hypothalamus In The Stimulation Of Eating

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Eating

“To take food into the mouth and swallow for nourishment”

• Why is eating important?
  – Social Activity
  – Emotional Aspect
  – Survival
Why Study Eating?

• **Obesity**
  – Incidence = $\uparrow 24\%$ over past 25 years
  – Hypertension
  – Diabetes
  – Stroke
  – Coronary Heart Disease

• **Anorexia and Bulimia**
  - 8 million in US (2007)
  - 4% of college women
• Multifactorial
  – Genetic
  – Environmental
  – Neurobiological Basis

• Neurobiological Approach
  – Study Neurotransmitter Systems ➔ Glutamate
  – Glutamate Receptor Activation ➔ Neuronal Excitation ➔ Behavioral Change (EATING)
  – Agonists = \( \uparrow \) Receptor Activation
  – Antagonists = \( \bigcirc \) Receptor Activation

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Synaptic vesicle
Voltage-gated Ca++ channels
Post-synaptic density
Neurotransmitters
Neurotransmitter re-uptake pump
Neurotransmitter receptors
Glutamate Receptors and Eating

• Previous studies: Glutamate receptor activation in the lateral hypothalamus (LH) regulates eating (Stanley et al., 1993, 1996, Hettes et al., 2003, 2007)

• 3 Glutamate Receptor Sub-types
  – Already studied: NMDA & AMPA receptors
  – Our Focus: Kainate (KA) receptors
  – KA receptor agonist = ATPA
  – KA receptor antagonist = UBP 296
A) Based on previous findings, we expect the agonist, ATPA, to elicit feeding when injected into the LH of satiated rats by activating kainate receptors.

ATPA injection $=$ \uparrow\text{ eating}

B) Treatment of rats with the kainate receptor antagonist, UBP 296, prior to ATPA administration will inhibit feeding by blocking access to kainate receptors.

UBP 296 Injection $=$ \downarrow\text{ ATPA- elicited eating}
Materials and Methods- Accessing the LH

- Tame rats for 1 week
- Stereotaxic surgery
  - Insert cannula into LH under general anesthesia
- Injections delivered directly to LH
- Week-long recovery
Materials and Methods- Pharmaceutical Agents

Kainate Receptor Antagonists and Agonists:

- **Antagonist**
  - **UBP 296**
    - (RS)-1-(2-Amino-2-carboxyethyl)-3-(2-carboxybenzyl) pyrimidine-2,4-dione

- **Agonist**
  - **ATPA**
    - (RS)-2-amino-3-(3-hydroxy-5-tert-butylisoxazo-4-yl) propanoic acid

- **Vehicles (Control Injections)**
  - **DMSO** = dimethyl sulfoxide
    - Solvent for UBP 296
  - **aCSF** = isotonic artificial cerebrospinal fluid
    - Solvent for ATPA
Materials and Methods- Prepare for Testing
Materials and Methods- Injection Days

- Feed rats 1 hour prior to injection
- Observe baseline behavior 10 minutes prior to injection
- Record initial food weight
- Inject UBP 296 → 5 minute latent period → inject ATPA
- Observe behavior every minute for 55 minutes post-injection
- Record food weight at 30, 60 and 90 minutes post-injection
Materials and Methods- Data Collection

• Injections
  – randomized treatment order
  – (♯) = nmol dose of drug

  – Weeks 1-6: injections
    • DMSO + aCSF
    • DMSO + (1) ATPA
    • (3) UBP 296 + (1) ATPA
    • (9) UBP 296 + (1) ATPA
    • (3) UBP 296 + aCSF
    • (9) UBP 296 + aCSF
Materials and Methods- Data Analysis

• Cumulative Food Intake – One way Repeated Measures ANOVA followed by Student-Newman-Keuls post-hoc testing

• Behavioral Analysis- paired t-tests compare to DMSO + aCSF (control) at matched times for matched behaviors
  – Microsoft Excel and Sigma Stat
Prior to injections, there were no significant differences between treatment groups, except for [UBP 296 + aCSF].
Cumulative Food Intake (grams)

Cumulative Food Intake

DMSO + aCSF

Cumulative Food Intake (grams)

Time Post-Injection

30 min  60 min  90 min

(*) p<0.01 compared to DMSO + aCSF food intake for matched time

(#) = nmol dose of drug
Injection of the agonist ATPA significantly increased food intake compared to [DMSO + aCSF].

(*) p<0.01 compared to DMSO + aCSF food intake for matched time
Injection of UBP 296 prior to injection of ATPA does not decrease food intake compared to [DMSO + ATPA].
Injection of any dose of UBP 296 + aCSF does not elicit an eating response.
Results: Behavioral Analysis

- Eating
- Alert
- In Locomotion
- Drinking
- Resting
- Grooming
The percent of time spent eating appears similar across most treatment groups.
When compared to the control, (DMSO + aCSF), the DMSO + (1) ATPA treatment group spent significantly more time eating at time 5.
The percent of time spent alert was similar across the treatment groups.
When compared to the control, (DMSO + aCSF), the DMSO + (1) ATPA treatment group spent significantly less time alert at time 5, 10, 15, 45, and 50.
The percent of time spent in locomotion appears similar across most of the treatment groups.
When compared to the control, (DMSO + aCSF), the DMSO + (1) ATPA treatment group spent significantly more time in locomotion at time 5, 10, and 15.
There were no significant differences between [DMSO + aCSF] and [DMSO + (1) ATPA] in any of the treatment groups in the percent of time spent resting, grooming, and drinking.
Summary – Food Intake

• Pre- Injection Cumulative Food Intake
  – Significant increase in (9) UBP 296 + aCSF

• Post- Injection Cumulative Food Intake
  – When injected into the LH, ATPA significantly increases feeding.
  – When injected into the LH prior to ATPA, UBP 296 does not suppress ATPA- elicited feeding at the dosages tested.
Summary – Behavioral Data

• A significant difference in the amount of time spent eating, alert, and in locomotion at specific times during the behavioral analysis was observed when rats received [DMSO + ATPA].
• No significant difference in the amount of time spent grooming, drinking, or resting was observed.
Conclusions

- Hypothesis A: ATPA injection = eating
  - When injected into the LH, ATPA activates kainate receptors without inducing general hyperactivity.

- Hypothesis B: UBP 296 Injection = ATPA- elicited eating
  - The concentrations tested of UBP 296 does not sufficiently block kainate receptors to suppress ATPA- elicited feeding.
Future Directions

• Improve Methodology
  – Pre-injection food intake baseline satiety
  – Video monitoring instead of minute by minute analysis
  – Measure cumulative time spent eating
  – Define additional behaviors (i.e. gnawing, rearing)

• Additional Experiments
  – Test other antagonists for the kainate receptors.
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References

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