Peripherally administered amylin agonists reduce energy intake in rats on a free choice diet

Marja Lauren Dela Rosa*, Avery Hum*, Madeline Norton*, Tyler J. Gustafson, Elizabeth G. Mietlicki-Baase
Department of Exercise and Nutrition Sciences, University at Buffalo

Introduction

Amylin is a hormone made in the pancreas that has been shown in previous studies to reduce food intake and promote negative energy balance (Lutz et al., 1994; Mietlicki-Baase et al., 2017). However, it is not clear how amylin-mediated changes in food intake may be distributed when rats have access to different types of foods or if other amylin-based compounds produce the same results. One goal of our study was to examine meal patterns in rats with different food preferences, before any pharmacological intervention. Then, we hypothesized that amylin and pramlintide (an amylin analogue) would reduce energy intake in a free choice diet (FCD) model (la Fleur et al., 2010); we also assessed effects of amylin and pramlintide on meal patterns and food choice. If our results show that the feeding patterns and food consumption are also altered by amylin or pramlintide, this may help us understand how the amylin system impacts energy balance and food preference.

Baseline data

<table>
<thead>
<tr>
<th>Raw kcal</th>
<th>Measured wire vs. Feeder</th>
<th>Chow preference % kcal</th>
<th>Hanging Wire vs. Feeder</th>
<th>Sucreose preference % kcal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chow</td>
<td>Hanging wire</td>
<td>Chow</td>
<td>Hanging wire</td>
<td>Sucreose</td>
</tr>
</tbody>
</table>

A main effect of food was measured by selecting the meal pattern analysis showed that fat intake was significantly lower than intake of Chow or sucrose when comparing the raw and pramlintide groups. (*p<0.05 compared to all other food choice).

Amylin (50 mg/kg and 5 μg/kg) and pramlintide (5 μg/kg) significantly suppressed total energy intake at 1h compared to vehicle. At 6h, there was a significant difference in energy intake between amylin (5 μg/kg) and pramlintide (5 μg/kg). There was also a trend in the overall ANOVA at 24h. For 24h body weight change, there were no significant differences between groups. Collectively, this suggests that the energy balance effects of amylin and pramlintide at these doses are short-term. *p<0.05, *p<0.01 in overall ANOVA. *p<0.05 versus vehicle. *p<0.05 compared to 5 μg/kg pramlintide.

Trends for changes in chew meal size were observed at 1h and 6h. Meal size for fat was unaffected by amylin or pramlintide. For sucrose, amylin (50 mg/kg) and pramlintide (5 μg/kg) significantly suppressed sucrose meal size compared to vehicle at 1h. *p<0.05, *p<0.01 in overall ANOVA. *p<0.05 versus vehicle.

Conclusions

- Amylin (5 μg/kg) decreased total Chow intake at 1h, 6h, and 24h (p<0.05). Effects of this dose of amylin on meal-related Chow intake were similar, with significant effects on meal-related Chow intake at 24h (p<0.05) and a trend for suppression of meal-related Chow intake at 1h (p<0.08).
- All doses of amylin and pramlintide significantly suppressed total and meal-related sucrose intake at 1h. Sucrose intake was suppressed through a decrease in meal size rather than meal number.
- Many of the feeding effects of amylin and pramlintide occurred at the same time points, and there was no effect on 24h weight gain. This suggests that the energy balance effects of amylin and pramlintide at these doses are primarily short-term.
- Unexpectedly, we observed that many rats in this cohort preferred Chow in both the hanging wire and food pellets cages. This may be due to slight methodological changes from previous work (Mietlicki-Baase et al., 2017), such as the type of Chow available in the FCD. We plan to explore this possibility in future studies.
- The ability of peripherally administered amylin and pramlintide to change meal patterns and food intake in rats on a free-choice diet has given us insight on how the amylin system impacts energy balance when animals have a choice of foods available. Understanding these effects may help us to identify novel combination therapies to treat patients with obesity and produce long lasting weight loss.

Methods

Eight male Sprague Dawley rats (Charles River) were placed in hanging wire cages for a one week habituation period with access to food and water. After habituation, rats were given ad libitum access to a free-choice diet (FCD) consisting of standard rodent Chow (Teklad 2018, Envigo), sucrose (30% solution), and fat (vegetable shortening), along with water, for two weeks. Through manual food intake measurements, food preferences were determined based on percentage of kcal consumed from each food choice (Mietlicki-Baase et al., 2017). Rats were then transferred to automated food feeding system cages (Research Diet) and given a two week habituation period with ad libitum access to the FCD. Food intake was automatically and continuously monitored by the food measuring system.

Daily intake data were analyzed using variance (ANOVA) tests (Tukey’s Statistical) to see if their preferences for food choice remained the same by comparing the amount of kcal from Chow, Chows, and sucrose on day 5 of the five hungry animal cages to the amount of kcal from the last pre-treatment day in the automated feeder. After preferences were evaluated, the effects of amylin or pramlintide on FCD intake were tested.

Rats received five treatments, amylin (50 mg/kg and 5 μg/kg), pramlintide (50 μg/kg and 5 μg/kg), vehicle (3 mL/kg sterile 0.9% NaCl), control, on an in-sutential partial Latin square design. Effects of each treatment on intake of each food were observed for 24 hours after treatment. Relevant data were extracted from the food feeding system data, focusing on data measured at specific post-injection time points (1, 3, 6, 12, and 24 hours).

Food intake and meal pattern variables (mealsize, number of meals, and latency for each food) along with body weight gain were compared using ANOVA tests that account for the within-subjects experimental design. Student Newman Keuls posthoc analysis was used to differentiate any statistical significance (*p<0.05) between treatment conditions.

Acknowledgements

The authors thank Houda Nashawi, Lorin Perry, and Xingyung Xie for valuable technical assistance. * Indicates co-first authorship. This work was supported by the Undergraduate Research Award from UB’s Center for Undergraduate Research & Creative Activities (MD, AH, MN) and start-up funds from the University at Buffalo (IGM-8).

Literature cited

