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

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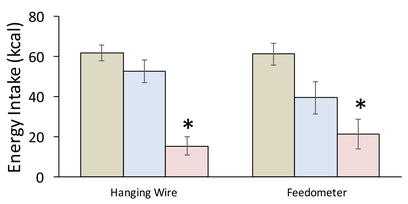
Introduction

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 tested the hypothesis 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.

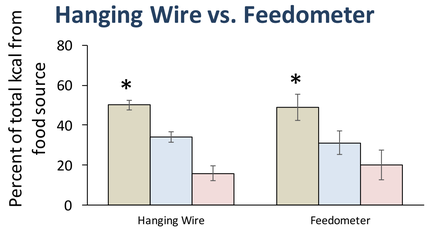
Baseline data

Raw kcal
Hanging Wire vs. Feedometer



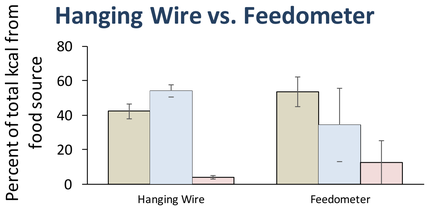
A main effect of food was revealed by ANOVA. Post hoc analyses showed that fat intake was significantly lower than intake of chow or sucrose when comparing the raw kcal consumed by all rats (n=6; *, p<0.05 compared to all other food choices).

Chow Preferring % kcal
Hanging Wire vs. Feedometer



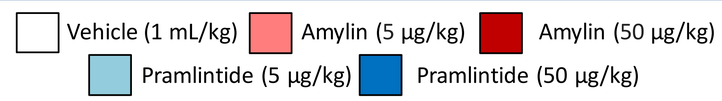
The food preference of each rat was determined by examining which food they consumed the most kcal from on the last day in the hanging wire cages. When the percent of kcal consumed from each food choice was evaluated in chow-preferring rats (n=4), a main effect of food was observed; chow-preferring rats ate the highest percent of kcal from chow (*, p<0.05). Although the percent of kcal from sucrose was numerically highest in rats that preferred sucrose in hanging wire cages, no statistically significant effects for food choice were observed in sucrose-preferring rats (n=2), perhaps due to the small sample size. There was no significant interaction between cage type and food type in either chow-preferring or sucrose-preferring rats (p>0.05).

Sucrose Preferring % kCal
Hanging Wire vs. Feedometer

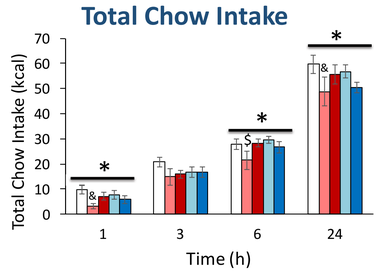


Chow Sucrose (30%) Fat (Vegetable Shortening)

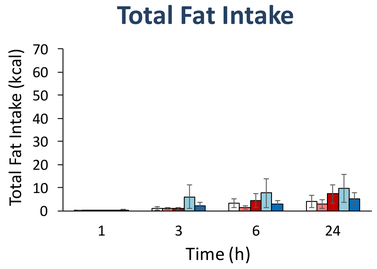
Results



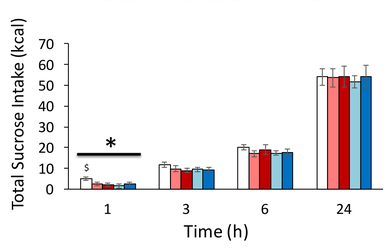
Total Chow Intake



Total Fat Intake

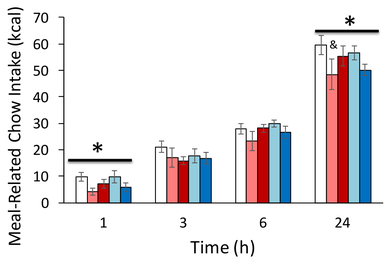


Total Sucrose Intake

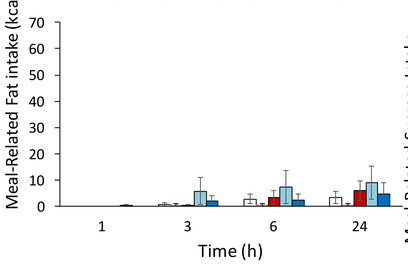


Total chow intake was decreased by amylin (5 µg/kg) treatment at 1h, 6h, and 24h. There were significant differences only between amylin (5 µg/kg) and vehicle at 1h and 24h; amylin at this dose was different from all other groups at 6h. No changes in total fat intake were observed at any point. For total sucrose intake, all doses of amylin and pramlintide tested significantly reduced intake at 1h compared to vehicle treatment. *, p<0.05 in overall ANOVA. &, p<0.05 versus vehicle. $, p<0.05 versus all other groups.

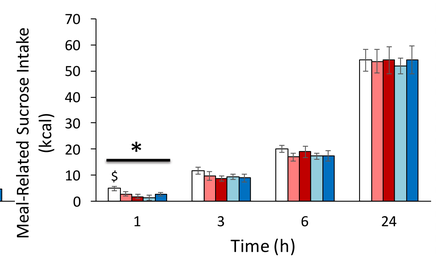
Meal-Related Chow Intake



Meal-Related Fat Intake

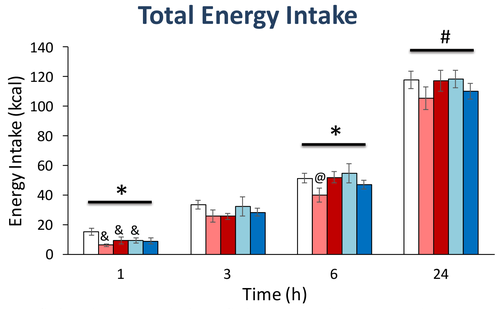


Meal-Related Sucrose Intake

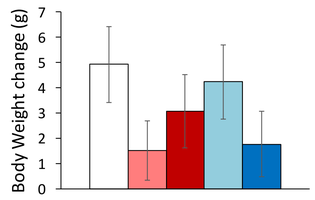


Meal-related chow intake was significantly decreased by amylin (5 µg/kg) at 24h. No changes in meal-related fat intake were observed at any point. At 1h, all doses of amylin and pramlintide tested significantly reduced meal-related sucrose intake compared to the vehicle treatment. *, p<0.05 in overall ANOVA. &, p<0.05 versus vehicle. $, p<0.05 versus all other groups.

Total Energy Intake

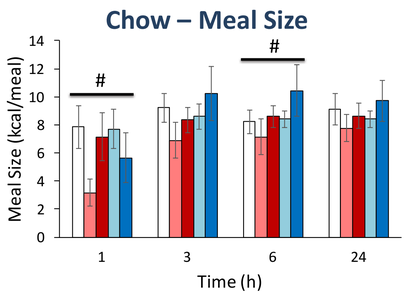


24 Hour Body Weight Change

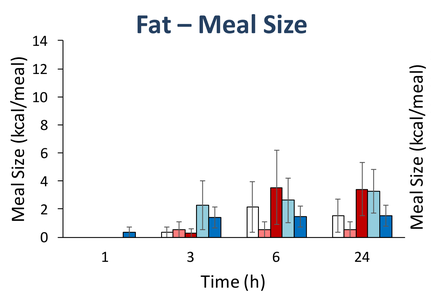


Amylin (50 µg/kg and 5 µg/kg) and pramlintide (5 µg/kg) significantly suppressed total energy intake at 1h compared to vehicle. At 6h, there was a significant difference in energy intake between amylin (5 µg/kg) and pramlintide (5 µg/kg). There was also a trend in the overall ANOVA at 24h. For 24h body 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 shorter-term. * p<0.05, # p<0.1 in overall ANOVA. &, p<0.05 versus vehicle. @, p<0.05 compared to 5 µg/kg pramlintide.

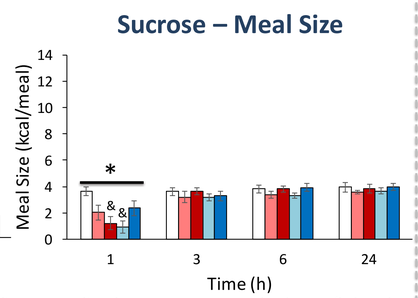
Chow – Meal Size



Fat – Meal Size

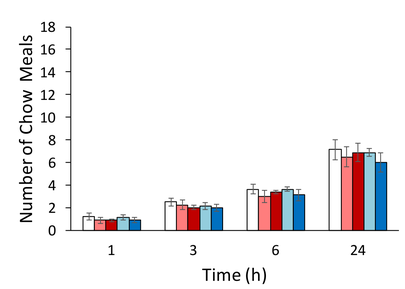


Sucrose – Meal Size

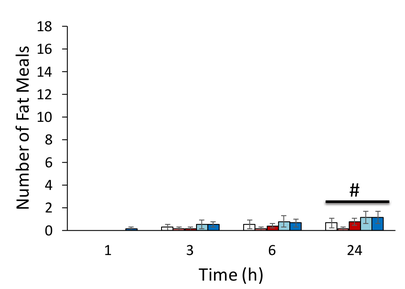


Trends for changes in chow meal size were observed at 1h and 6h. Meal size for fat was unaffected by amylin or pramlintide. For sucrose, amylin (50 µg/kg) and pramlintide (5 µg/kg) significantly suppressed sucrose meal size compared to vehicle at 1h. * p<0.05, # p<0.1 in overall ANOVA. &, p<0.05 versus vehicle.

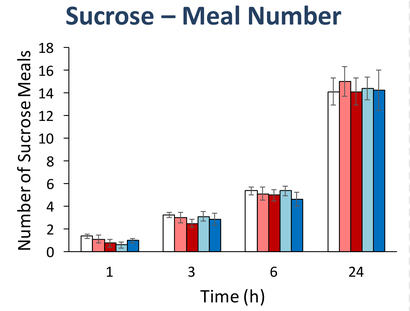
Chow – Meal Number



Fat – Meal Number



Sucrose – Meal Number



Amylin and pramlintide did not significantly affect number of meals for sucrose or chow. However, there was a trend for a change in the number of meals for fat at 24h. #, p<0.1 in overall ANOVA.

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 early 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 shorter-term.
- Unexpectedly, we observed that many rats in this cohort preferred chow in both the hanging wire and feedometer 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 feedometer system cages (Research Diets) and given a two week habituation period with *ad libitum* access to the FCD. Food intake was automatically and continuously monitored by the feedometer system.

Daily intake data were analyzed using analysis of variance (ANOVA) tests (TIBCO Statistica), to see if their preferences for food choice remained the same by comparing the amount of kcal of fat, chow, and sucrose on the last day of the hanging wire cages to the amount of kcal from the last pre-treatment day in the automated feedometer. After preferences were evaluated, the effects of amylin receptor activation on FCD intake were tested.

Rats received five treatments, amylin (50 µg/kg and 5 µg/kg), pramlintide (50 µg/kg and 5 µg/kg), and vehicle (1 mL/kg sterile 0.9% NaCl) via IP injections, in a within-subject 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 feedometer system data, focusing on data measured at specific post-injection time points (1, 3, 6, and 24 hours).

Food intake and meal pattern variables (meal size, 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.

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