Orexin siRNA Effects On Food Intake And Activity In Rats

*Joshua P. Nixon[1] and Catherine M. Kotz[2,3]

[1]Minnesota Craniofacial Research Training Program, University of Minnesota, Minneapolis , MN, USA 55455

[2]Department of Food Science and Nutrition, University of Minnesota, St Paul, MN USA 55108 [3]Veterans Administration Medical Center, Minneapolis, MN, USA 55417

Introduction

Orexins are hypothalamic neuropeptides important in integrating a wide range of autonomic and behavioral functions, including feeding, sleep/wake behavior, arousal, physical activity, nocioception, respiratory, trigeminal motor, neuroendocrine and cardiovascular systems[1-5]. The focus of study in our laboratory is on the role of orexin in promotion of physical activity and energy expenditure in a rodent model of obesity. In rats, delivery of orexin to the rostral lateral hypothalamus (rLH) results in increases in both food intake and in energy expenditure through increased spontaneous physical activity (SPA)[4]. Importantly, in the rLH, the SPA-inducing component of orexin appears to be stronger than the ingestive response. Previous data suggests that increased orexin sensitivity in obesity-resistant rats contributes to defense against weight gain by increasing SPA; likewise, reduced SPA response to orexin in obesity-prone rats might contribute to obesity[6]. To further investigate this possibility, we used small interference RNA (siRNA), short double-stranded RNA sequences capable of post-translational knockdown of gene expression[7]. We present here the preliminary results of a pilot study using an orexin-specific siRNA to test the hypothesis that reduced orexin signaling will decrease food intake and physical activity in rats. Although most work in our lab focuses on the rLH, because orexin neurons are relatively sparse in the rostral hypothalamus and more numerous in caudal regions[8], siRNA was targeted at the perifornical lateral hypothalamus (PeF) in this pilot study to affect the greatest number of orexin neurons at once.

Predictions:

1. Orexin siRNA-treated rats will exhibit less SPA than control rats
2. Orexin siRNA rats will eat less than controls
3. Orexin siRNA rats will show increases in body weight and/or fat mass due to reduced energy expenditure

Methods

Adult male Sprague-Dawley rats received 1 nmol bilateral pressure injections of orexin-specific (n = 4), positive control (n = 3), or scrambled-sequence negative control siRNA (n = 4) stereotaxically directed at the PeF using the following coordinates (in mm relative to bregma): AP: -3.4; DV: -8.2; LM: ±1.2. Individual injections were in a total volume of 0.5 µl 0.9% saline delivered over a 2 minute period using a Hamilton syringe attached to a Stoelting QSI automated programmable injector. For 10 days post-injection, daily body weights were recorded, and food intake was monitored using a BioDaq automated feeding system. Non-invasive measures of body composition (fat and lean mass) were obtained every two days using an EchoMRI quantitative magnetic resonance imaging system. After a 20 day wash-out period to allow recovery from siRNA effects, rats were divided into two groups, administered a second unilateral injection of orexin-specific (n = 6) or scrambled-sequence siRNA (n = 5) delivered as described above, and placed in an open-field activity chamber for SPA measurements. Locomotor activity (time ambulatory, time vertical, and total time spent moving) was recorded for 24 h after this second injection. All animals were sacrificed 48 h after the second injection for tissue analysis.

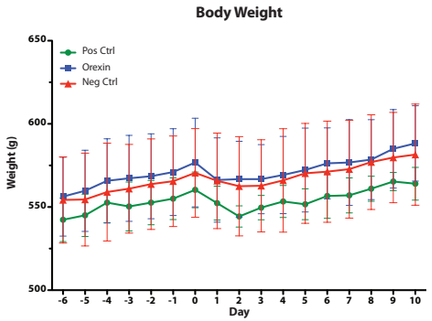

Figure 1: Pre- and post-surgery body weights (g ± SEM) for rats receiving bilateral orexin-specific, scrambled sequence, or GAPDH positive control siRNA. Day 0 is day of surgery.

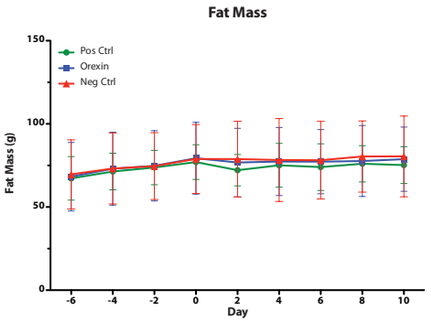

Figure 2: Pre- and post-surgery fat mass measures (g ± SEM) for siRNA-treated rats. Fat mass measured every two days using an MRI-based system. Day 0 is day of surgery.

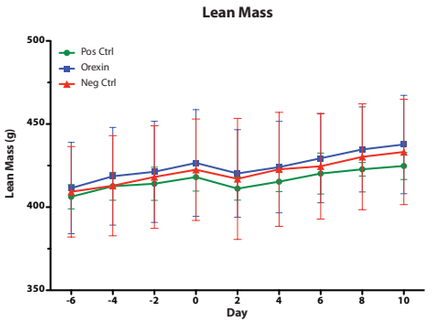

Figure 3: Pre- and post-surgery lean mass measures (g ± SEM) for siRNA-treated rats. Lean mass measured every two days using an MRI-based system. Day 0 is day of surgery.

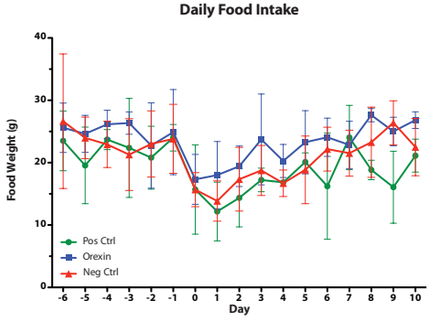

Figure 4: Average daily food intake (g ± SEM) for siRNA-treated rats. Food intake measured daily using an automated feeding system. Day 0 is day of surgery.

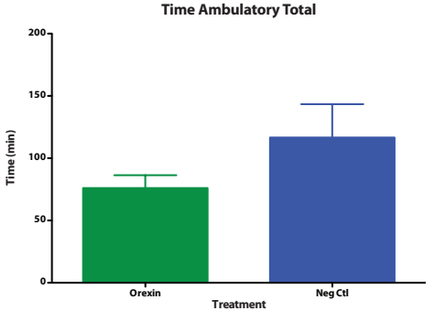

Figure 5: Twenty-four hour cumulative locomotor activity (time spent ambulatory, in minutes) after unilateral orexin-specific or scrambled control siRNA treatment.

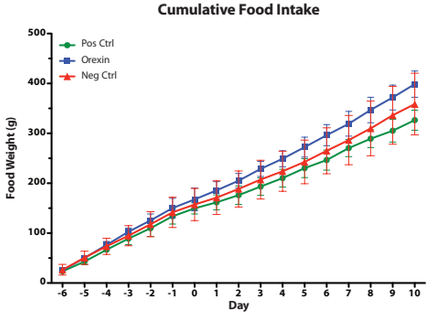

Figure 6: Cumulative food intake for siRNA-treated rats. Day 0 is day of surgery. Significant interaction between treatment and time; orexin siRNA rats ate more than positive controls ($p < 0.01$).

Results

No significant differences were found between groups for body weights (Fig 1), body composition measures (Fig 2–3), or average daily food intake (Fig 4). Activity data showed a trend for decreased ambulatory movement in orexin siRNA animals (Fig 5), but effects did not reach significance ($p = 0.1639$). **Repeated measures 2-way ANOVA showed a significant interaction for treatment over time for cumulative food intake ($p < 0.0001$; Fig 6). Contrary to expectations, post-tests showed that orexin siRNA-treated rats ate more food than did positive control animals ($p < 0.05$).**

Conclusions

With respect to SPA, the results of this pilot experiment do suggest that blocking orexin expression using siRNA are capable of reducing physical activity, as predicted; however, it is clear that the single application used here is insufficient to cause significant changes in either SPA or on body weight as a result of changes in energy expenditure. In sharp contrast to our predictions, food intake in orexin siRNA-treated animals was higher than in control rats, rather than lower. An increase in food intake after blocking orexin in the perifornical LH is surprising as all data from previous studies in our lab show orexin antagonists in the rLH decrease food intake. While we would not have predicted this outcome, we feel that this finding highlights an important point: **Specifically, that the action of a given peptide depends in part upon the specific brain region in question.** Although conclusions are limited by the small group sizes in this study, this site-specific difference in response to reduced orexin signaling suggests that **subpopulations of orexin neurons may play different roles in regulating autonomic and behavioral functions.** We are currently planning studies to investigate the importance of specific orexin neuron subpopulations in food intake and energy expenditure.

References

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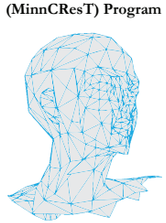

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