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Evaluation of Orexin siRNA Effects On Food Intake, Body Weight And Activity In Rats

*Joshua P. Nixon[1], Claudio E. Pérez-Leighton[2] and Catherine M. Kotz[2,3]

[1]Veterans Administration Medical Center, Minneapolis, MN, USA 55417 [2]Department of Neuroscience, University of Minnesota, Minneapolis , MN, USA 55455

[3] Department of Food Science and Nutrition, University of Minnesota, St Paul, MN USA 55108

Introduction

Orexins are hypothalamic neuropeptides important in integrating a wide range of autonomic and behavioral functions, including feeding, sleep/wake behavior, arousal, and physical activity[1-3]. 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[2]. To further investigate this possibility, we used small interference RNA (siRNA), short double-stranded RNA sequences capable of post-translational knockdown of gene expression[5]. 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. Because orexin neurons are relatively sparse in the rostral hypothalamus and more numerous in caudal regions[6], siRNA was targeted at the caudal lateral hypothalamus (cLH) rather than the rLH in this pilot study to affect the greatest number of orexin neurons at once.

Methods

Adult male Sprague-Dawley rats received scrambled control (SCR), GAPDH positive control (GAP) or orexin-specific (ORX) siRNA treatments stereotaxically directed at the cLH using the following coordinates (mm relative to bregma): AP: -3.4; DV: -8.2; LM: ±1.2. ORX treatments were either single-sequence or a combination of three sequences targeting the prepro-orexin mRNA (Fig 1). Individual injections were 1 nmol total siRNA in a volume of 0.5 – 2 µl in 0.9% saline delivered over a 5 minute period using a Hamilton syringe attached to a Stoelting QSI automated programmable injector. Treatments and assessment methods were as follows:

Experiment 1: Rats received bilateral treatment with single-sequence ORX (n = 4), GAP (n = 3), or SCR siRNA (n = 4). For 10 days post-injection, body weights and food intake were monitored. After a 20-day wash-out period, rats were divided into two groups, administered a second unilateral injection of ORX (n = 6) or SCR siRNA (n = 5), and placed in an open-field activity chamber for 24 h SPA measurements.

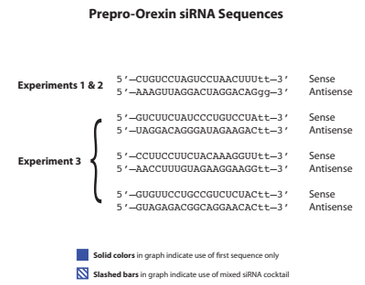

Figure 1: Sequences for the orexin-specific siRNA used in this study.

All data expressed as mean ± SEM.

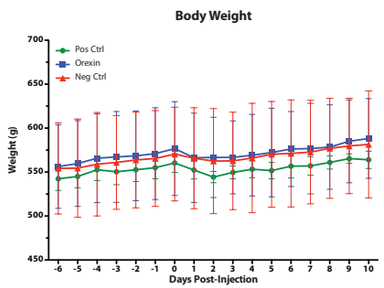

Figure 2: Pre- and post-surgery body weights for rats receiving bilateral single-sequence orexin-specific, scrambled control, or GAPDH positive control siRNA. Day 0 is day of surgery.

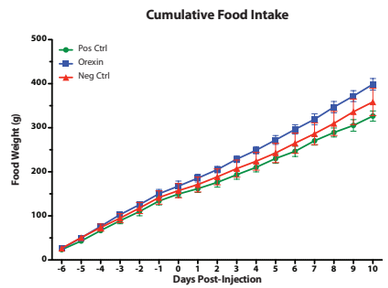

Figure 3: Cumulative food intake for siRNA-treated rats. Day 0 is day of surgery. Significant interaction between treatment and time; orexin siRNA rats ate more than positive controls ($p < 0.01$).

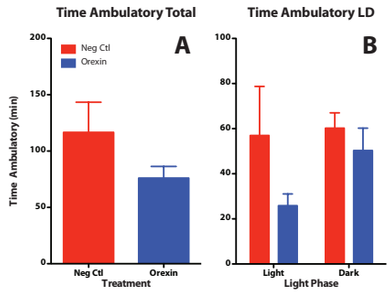

Figure 4: (A) Total 24 h locomotor activity (time ambulatory, in minutes) and (B) activity by light phase after unilateral single-sequence orexin-specific or scrambled control siRNA treatment.

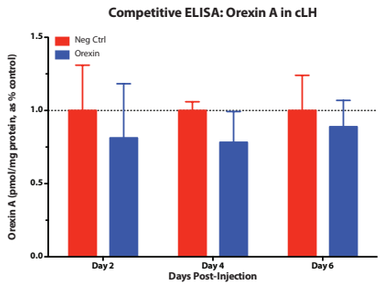

Figure 5: Orexin A protein levels (pmol/mg, normalized to control) in the cLH after bilateral single-sequence orexin-specific or scrambled control siRNA treatment.

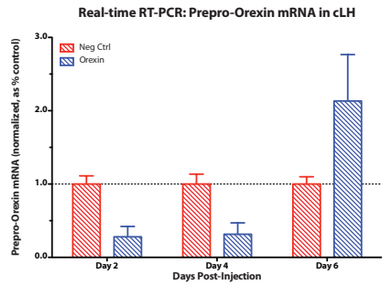

Figure 6: Prepro-orexin mRNA in the cLH after unilateral treatment with mixed orexin-specific and control siRNA. Time and interaction (Treatment x Time) both significant ($p < 0.05$).

Experiment 2: Rats received bilateral treatment with single-sequence ORX (n = 12) or SCR siRNA (n = 11). Rats were sacrificed at 2, 4 and 6 days post-injection, and brain punches were analyzed for orexin A protein levels by competitive ELISA.

Experiment 3: Rats (n = 16) received unilateral injections of mixed ORX siRNA, with SCR siRNA delivered contralaterally. Tissue was collected as in Experiment 3 and orexin mRNA levels were analyzed via real-time RT-PCR.

Results

Experiment 1: While no significant differences were found between groups for body weights (Fig 2) or average daily food intake (not shown), repeated measures ANOVA showed a significant interaction for treatment over time for cumulative food intake ($p < 0.0001$; Fig 3). Post-tests showed that ORX siRNA-treated rats ate more food than did GAP animals ($p < 0.05$). Activity data showed a trend ($p = 0.1639$) for decreased ambulatory movement in orexin siRNA animals, especially during the light phase (Fig 4).

Experiment 2: Bilateral ORX siRNA treatment resulted in a weak trend for decreased orexin A peptide in the cLH relative to SCR rats (Fig 5), but results were not significant.

Experiment 3: Unilateral ORX treatment reduced prepro-orexin mRNA relative to SCR on postinjection days 2 and 4 (Fig 6). Repeated measures ANOVA showed a significant effect of time ($p = 0.0033$) and a significant interaction between treatments over time ($p = 0.0033$).

Conclusions

While the results of this pilot experiment suggest that blocking orexin expression using siRNA might affect energy expenditure by reducing SPA, it is clear that the single application used here is insufficient to cause significant changes in either SPA or body weight. In 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. 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. Finally, the use of the combined siRNA cocktail appeared to prove more effective in reducing orexin expression than did the single sequence. We are currently planning a dose-response study using the siRNA cocktail to determine the optimal strategy for effective knockdown, and hope to use this approach to investigate the importance of specific orexin neuron subpopulations in food intake and energy expenditure.

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Acknowledgements

Funding for these experiments provided by the US Department of Veterans Affairs Rehabilitation Research & Development, Minnesota Craniofacial Research Training Program Grant T32DE007288 from NIDCR, NIDDK grant DK078985 and Minnesota Obesity Center Grant DK050456.

Research supported by NIDDK