Orexin A-Induced Activity Is Inversely Correlated With Body Weight Gain And May Promote Weight Loss In Rats

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Introduction

Obesity is the result of a caloric imbalance, where energy intake exceeds energy expenditure in an individual over time. While this imbalance may be addressed by reducing intake, weight gain in response to caloric intake varies widely between individuals, in part due to differences in spontaneous physical activity (SPA) [1]. Clinical studies and animal models strongly suggest that non-exercise activity thermogenesis (NEAT) generated through SPA is an important component in defense against weight gain [1-3].

Orexin A and B (hypocretin 1 and 2) are neuropeptides primarily involved in the regulation of arousal and sleep-wake behavior, general activity, and ingestive behavior [4-8]. Recent studies indicate that orexin A (OXA) might play a role in determining SPA levels [2, 3, 9, 10]. Our lab has shown differences in both OXA-induced SPA and in hypothalamic orexin receptor expression in obesity-prone and obesity-resistant rats [2]. In this study we investigated whether OXA influences body weight by contributing to SPA levels in rodents. We hypothesized that (1) OXA responsiveness and orexin receptor expression are correlated to SPA and body weight gain; that (2) blocking endogenous OXA would reduce SPA and increase weight gain; and conversely (3) OXA injections would increase SPA and reduce body weight gain.

Methods

Experiment 1: Adult male Sprague-Dawley (SD) rats (n = 10; Charles River, Kingston, NY USA) were fitted with stainless steel guide cannulae aimed at the rostral lateral hypothalamic area (rLHA). Animals were subjected to a dose-response experiment over a 2 week period in which each was injected with artificial cerebrospinal fluid (aCSF) or one of four concentrations of OXA (31.25, 62.5, 125, and 250 pmol; American Peptides, Sunnyvale, CA USA) in a randomized Latin square design, such that all animals received each treatment once, with one day recovery between injections. Food intake and SPA were monitored for 2 hr following each injection; body weight change was tracked for the duration of the experiment.

Experiment 2: Six-week old male SD rats (n = 6) were individually housed and maintained on a low-fat diet (D12489B, Research Diets, New Brunswick, NJ USA) for 2 weeks. Weight gain during this period was tracked daily. After 2 weeks, animals were sacrificed, and microdissected rLHA tissue was subjected to RNA extraction. Relative orexin-1 (OX1R) and orexin-2 receptor (OX2R) mRNA levels were determined by real-time RT-PCR analysis. All data were corrected for rat ribosomal protein L32 (Rpl32) mRNA levels.

Experiment 3: Adult male obesity-resistant (OR) rats (Charles River) were fitted with rLHA guide cannulae as in Experiment 1. Rats were subjected to a chronic application of aCSF (n = 3) or 10 pmol / 0.5 µl / h OX1R antagonist SB 334867 (n = 4; Tocris Bioscience, Ellisville, MO USA) for 5 days using an osmotic minipump. 24-hour SPA was determined on the 5th day of the experiment.

Experiment 4: Adult male SD rats fitted with rLHA guide cannulae as in Experiment 1 were treated three times daily (1, 5, and 9 hours after lights-on) for 14 days with aCSF (n = 8) or 500 pmol OXA (n = 9). Final body weight was obtained on Day 15, but no treatment was given on this day.

Figure 1: OXA-Induced SPA, Dose-Response

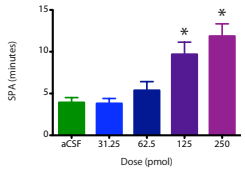

Figure 1: 2 hour SPA response to aCSF or 31.25, 62.5, 125, and 250 pmol dose OXA. Columns marked with a star (*) are significantly different than aCSF control ($p < 0.05$).

Figure 2: OXA-Induced SPA vs. Weight Gain

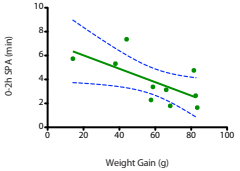

Figure 2: OXA-induced SPA vs. 2-week weight gain. SPA reflects response to 31.25 pmol dose OXA. Correlation $r^2 = 0.4342$; 95% confidence interval indicated by dashed lines.

Figure 3: Relative OX2R mRNA vs. Weight Gain

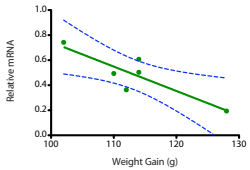

Figure 3: Relative hypothalamic OX2R mRNA level vs. 2-week weight gain. Relative OX2R levels determined by RT-PCR, corrected for Rpl32 mRNA. Correlation $r^2 = 0.7486$; 95% confidence interval indicated by dashed lines.

Figure 4: 24 h SPA in Obesity-Resistant Rats

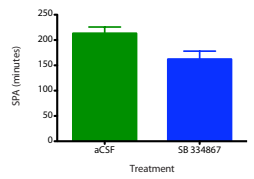

Figure 4: 24 h SPA in OR rats treated with aCSF (green) or 10 pmol / h OX1R antagonist SB 334867 (blue). SB 334867-treated rats show a strong trend for reduced SPA ($p = 0.0655$) but columns are not significantly different.

Figure 5: Light- and Dark-Period SPA

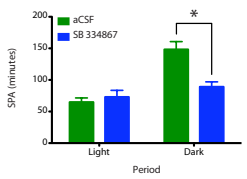

Figure 5: Light- and dark-period SPA in OR rats after 5 day chronic application of aCSF (green) or 10 pmol / h OX1R antagonist SB 334867 (blue). Columns marked with a star (*) are significantly different ($p = 0.0081$).

Figure 6: Weight Change in OXA-Treated Rats

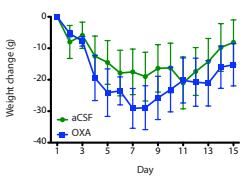

Figure 6: Weight change in rats treated 3 times daily with 500 pmol OXA (blue) or aCSF (green). OXA-treated animals show a trend for weight loss over the first week, but change in weight was short-lived and did not reach significance.

For all experiments, food was provided ad libitum. For Experiments 2–4, food intake and body weights were monitored daily. SPA (defined as time spent moving + time vertical) was recorded using Open Field Activity chambers and software (MED Associates, East Fairville, VT, USA); each SPA measurement was performed following a 1-day chamber acclimation period.

Results

Experiment 1: Treatment with OXA significantly increased 2 h SPA at 125 and 250 pmol doses only ($p < 0.05$; Figure 1). However, body weight gain over 2 weeks was significantly and inversely correlated with 2 h cumulative SPA for 31.25 pmol OXA ($r^2 = 0.4342$, $F = 6.150$, $p = 0.0382$; Figure 2). Correlations at higher OXA doses were also inverse but were non-significant (data not shown). Weight gain ranged from 14.1 to 83.4 g; average weight gain (mean ± SEM) was 59.40 ± 7.057 g.

Experiment 2: Weight gain over 2 weeks was significantly and negatively correlated with relative rLHA OX2R expression ($r^2 = 0.7486$, $F = 11.91$, $p = 0.0260$; Figure 3). Weight gain was similarly negatively correlated with relative rLHA OX1R expression, but results were not significant (data not shown). Weight gain ranged from 102 to 128 g; average weight gain was 113.3 ± 3.451 g.

Experiment 3: After 5 days of treatment, rats receiving the OX1R antagonist SB 334867 exhibited less 24 h SPA than did rats receiving aCSF (Figure 4), but the results did not reach significance ($p = 0.0655$). However, rats receiving SB 334867 showed significantly reduced SPA during the dark (active) period ($p = 0.0081$; Figure 4). No difference in light-period SPA was observed (Figure 5). SB 334867 did not significantly affect either body weight or food intake in this study (data not shown).

Experiment 4: Both aCSF- and OXA-treated rats showed an insignificant weight loss over the first 7 days of treatment (Figure 6), with the mean loss in OXA-treated rats being about 12 grams more than that of controls. Over the next 7 days rats in all groups gained weight (98.41 ± 1.36% and 97.11 ± 1.23% of starting weight for aCSF- and OXA-treated animals, respectively). Repeated-measures ANOVA (treatment x time) showed no significant effect of treatment between groups.

Conclusion

1. Rats showing a high SPA response to a low dose of OXA are less likely to become obese than those showing low response.
2. Rats that gain more weight have lower orexin receptor mRNA in the rLHA than do animals that resist weight gain.
3. Chronic blocking of orexin receptor using an antagonist significantly decreases SPA only during the dark (active) period in obesity-resistant rats.
4. Daily application of OXA is associated with a non-significant, transient decrease in body weight.

Experiments 1 and 2 suggest that OXA sensitivity and receptor expression level may predict body weight gain. Experiment 3 shows that chronically blocking orexin receptors reduces SPA at a time when animals are normally active. Experiment 4 suggests that OXA-induced SPA might have some influence on weight loss; in a more intensive program (such as constant infusions with agonists of longer duration of action), OXA therapy may help reduce body weight.

References

1. Levine, J.A., N.L. Eberhardt, and M.D. Jensen, Role of nonexercise activity thermogenesis in resistance to fat gain in humans. Science, 1999. 283(5399): p. 212-4.
2. Teske, J.A., et al., Elevated hypothalamic orexin signaling, sensitivity to orexin A, and spontaneous physical activity in obesity-resistant rats. Am J Physiol Regul Integr Comp Physiol, 2006. 291(4): p. R889-99.
3. Novak, C.M., C.M. Kotz, and J.A. Levine, Central orexin sensitivity, physical activity, and obesity in diet-induced obese and diet-resistant rats. Am J Physiol Endocrinol Metab, 2006. 290(2): p. E396-403.
4. Estabrooke, I.V., et al., Fos expression in orexin neurons varies with behavioral state. J Neurosci, 2001. 21(5): p. 1656-62.
5. Hagan, J.J., et al., Orexin A activates locus coeruleus cell firing and increases arousal in the rat. Proceedings of the National Academy of Sciences of the United States of America, 1999. 96(19): p. 10911-6.
6. Kunii, K., et al., Orexins/hypocretins regulate drinking behaviour. Brain Research, 1999. 842(1): p. 256-61.
7. Piper, D.C., et al., The novel brain neuropeptide, orexin-A, modulates the sleep-wake cycle of rats. Eur J Neurosci, 2000. 12(2): p. 726-30.
8. Kotz, C.M., et al., Feeding and activity induced by orexin A in the lateral hypothalamus in rats. Regulatory Peptides, 2002. 104(1-3): p. 27-32.
9. Kotz, C.M., et al., Orexin A mediation of time spent moving in rats: neural mechanisms. Neuroscience, 2006. 142(1): p. 29-36.
10. Kotz, C.M., Integration of feeding and spontaneous physical activity: role for orexin. Physiol Behav, 2006. 88(3): p. 294-301.

Acknowledgements

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