Effects of hindbrain orexin A signaling on brown adipose tissue thermogenesis and physical activity

RE Lee[1], CM Duffy[2–3], TA Butterick[2-3*], and JP Nixon[2–3*]

[1]Department of Integrative Biology and Physiology, University of Minnesota, Minneapolis, MN USA 55455 ◦ [2]Minneapolis VA Health Care System, Minneapolis, MN USA 55417

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

Introduction

Obesity (body mass index (BMI) ≥ 30) and overweight (BMI 25 –29) are major health concerns in the United States, affecting nearly one third of the US population[1]. Previous work has emphasized the importance of neuropeptides (protein neurotransmitters) in the control of behaviors associated with feeding, activity, and energy expenditure (EE). The orexins are a family of neuropeptides important in promoting physical activity, and have recently been shown to increase EE through effects on thermogenesis, specifically via the raphe pallidus (RPa) and dorsomedial hypothalamic nucleus[2-6].

The aim of this project is to explore brain-mediated defense against obesity via control of EE, specifically focusing on brown adipose tissue (BAT) thermogenesis as regulated by orexin A (OXA) via the RPa. While this pathway has been studied in a model using anesthetized rats, similar findings have not yet been duplicated in freely moving animals[3]. As orexin injected into the fourth ventricle (4V) has been shown to induce neuronal activation in the RPa[4], we chose to target the RPa indirectly via 4V injection.

Hypothesis: 4V orexin will increase EE via activation of RPa pathways controlling BAT thermogenesis.

Predictions: **1)** Rats receiving 4V orexin will show increased EE and decreased weight gain. **2)** EE increase will be driven by BAT thermogenesis, with little or no effect on physical activity. **3)** Increased thermogenesis will correlate with an increase in uncoupling protein-1 (UCP1; a marker of BAT thermogenesis) expression.

Methods

All studies were conducted with approval of the Minneapolis VAHCS IACUC. Animals: Adult male Sprague-Dawley rats (n = 16) were surgically implanted with unilateral stainless steel guide cannulae targeting the 4V (AP: -12.0; L/M: ±0.0; DV: -9.8 mm relative to bregma). For all studies, rats received vehicle (aCSF) or OXA (300 pmol/0.5 µl in aCSF). Treatments were delivered between 0900 and 0930 h, with data collection starting at 1000 h. Activity and indirect calorimetry was conducted using standard (7 ⅜" w × 12 ⅛" l × 5 ¾" h) test chambers (Columbus Instruments) equipped with infrared beam arrays (Med Associates) for simultaneous indirect calorimetry and activity data collection.

Experiment 1: Rats were placed in test chambers, treated with vehicle, and allowed to acclimate for 72 h. On the 4th day, rats received aCSF or OXA, and 24 h indirect calorimetry and physical activity was recorded.

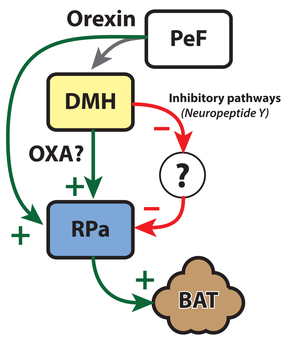

Figure 1: Schematic illustrating pathways and mechanisms of thermogenesis. Orexin from perifornical region (PeF) or dorsomedial hypothalamus (DMH) activates RPa and BAT thermogenesis.

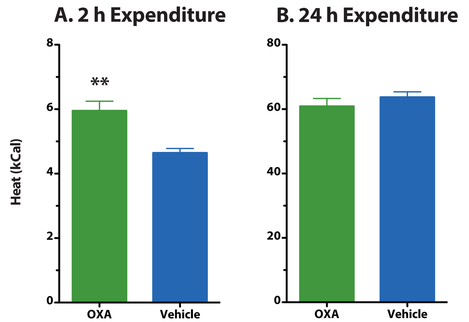

Figure 2: **(A)** OXA (300 pmol/0.5 µl) significantly increased EE in the first 2 h following treatment (p = 0.0019). **(B)** OXA did not alter 24 h energy expenditure.

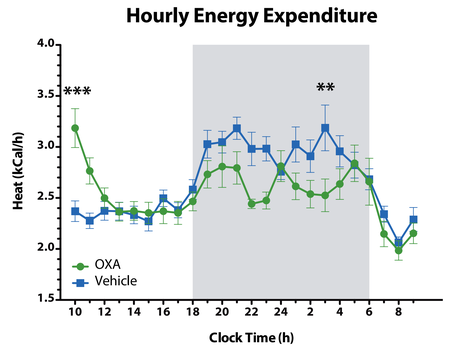

Figure 3: Significant interaction (treatment × time, p < 0.0001) was found in hourly EE. OXA significantly increased EE in the first 1 h (p < 0.001), and significantly decreased EE at 0300 h (p < 0.01).

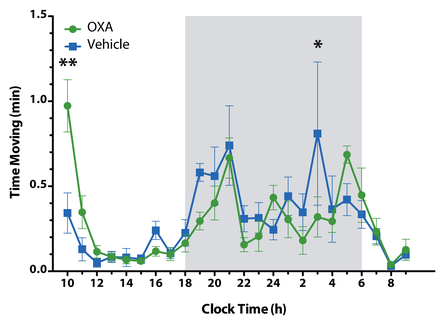

Figure 4: Significant interaction (treatment × time, p = 0.0374) was observed in ambulatory data. OXA significantly increased ambulatory time in first 1 h post-treatment (p < 0.01) and decreased ambulation at 0300 h (p < 0.05).

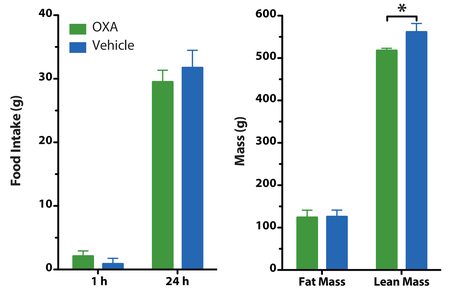

Figure 5: **(A)** No significant difference in food intake was observed. **(B)** Fat mass did not differ between OXA and control animals, though OXA animals had slightly but significantly less lean mass than controls (p = 0.0463).

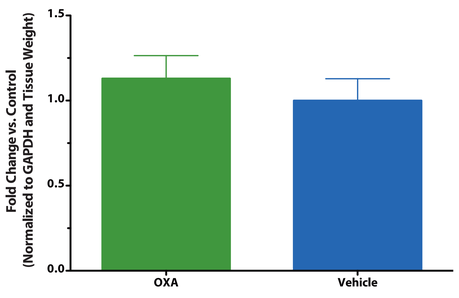

Figure 6: While there was a trend for increased BAT UCP1 expression in OXA-treated animals, this difference was not significant.

Experiment 2: Rats were kept in their standard home cages and treated with either aCSF or OXA. Food intake was measured at 1 h and 24 h post-injection.

Experiment 3: Rats received aCSF or OXA, and were then euthanized 90 min post-injection. Brains and BAT samples were collected to verify cannula placement and to test for UCP1 mRNA expression, respectively. Body composition (fat and lean mass) data for each animal was measured via noninvasive MRI (Echo Medical Systems) 24 h prior to euthanasia. Only rats with correctly placed cannulas were included in the analysis (OXA, n = 7; aCSF, n = 7).

Analysis: Data from calorimetry and activity studies were analyzed using 2-way repeated measures ANOVA (treatment × time) followed by Bonferroni posttests. Food intake and body composition was analyzed using unpaired *t* test. mRNA data analyzed by qRT-PCR.

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Results

- OXA significantly increased EE and ambulatory activity in the first 2 h post-treatment, and significantly reduced EE and ambulation at 0300 h. OXA did not significantly affect 24 h energy expenditure (Figures 1-3).
- No significant difference in food intake was observed between treatment or control animals at either 1 h or 24 h post-injection (Figure 4).
- While fat mass did not differ between groups, OXA-treated animals had less lean mass than controls (Figure 5).
- UCP1 expression in BAT was slightly (but not significantly) higher in treated animals (Figure 6).

Discussion

We show that 4V OXA increases short-term but not 24 h EE in rats. While ambulatory activity did increase post-treatment, we do not feel that the difference (1 min versus 30 s over 1 h) is large enough to fully explain the difference in EE during the first hour. Lack of difference in food intake suggests that BAT (rather than diet-induced) thermogenesis is contributing to the difference in EE. While short-term EE increased, animals compensated by reducing EE late in the active phase, resulting in no net change.

Understanding the neural mechanisms mediating EE can elucidate whether these mechanisms may be exploited to protect against obesity. Interest in BAT as a therapeutic target for obesity and related metabolic disorders is growing [7-8]. Known activators of BAT thermogenesis include stimuli such as cold exposure, thiazolidinediones, natriuretic peptides, thyroid hormone, and orexin, but many of these mechanisms are poorly understood [9-10]. Because our data did not demonstrate a significant effect of OXA on UCP1 expression, direct targeting of the RPa may be necessary for OXA stimulation of BAT thermogenesis. Future studies would need to be done, with direct cannulation of the RPa, to further investigate. Ultimately, data from this and ongoing studies aim to contribute to the development of orexin-based therapies to increase EE and reduce body weight in obese humans.

leex5040@umn.edu