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Effect of Dorsomedial Hypothalamic Nucleus Orexin Signaling on Body Weight, Food Intake, and Physical Activity in Rats

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Introduction

Our lab has previously shown that rats with lower orexin (hypocretin) response or sensitivity are more prone to obesity than those with higher response[1]. Prior studies have demonstrated that orexin is important in brown fat thermogenesis, energy expenditure (EE), and food intake[1–5]. Although most studies have shown that orexin increases thermogenesis, paradoxically, one study showed that systemic treatment with the orexin 1 receptor antagonist SB-334867 also resulted in increased thermogenesis[6].

The dorsomedial hypothalamus (DMH) is an important brain site for control of thermogenesis and EE[5], and neuropeptide Y (NPY) neurons in the DMH are thought to be involved as knockdown of NPY expression in the DMH leads to increased EE[7]. Because of the density of orexin fibers projecting into the DMH[8], the presence of orexin 1 and 2 receptors in the DMH[9,10], and the fact that orexin input is known to increase NPY mRNA and NPY neuronal activity in other brain regions[11,12], we hypothesized that orexin effects in the DMH may be important in regulating EE. Given the prior data showing systemic orexin antagonist can increase EE, we used site-specific intraparenchymal injection of SB334867 to test whether orexin signaling in the DMH is potentially important to this effect. As NPY is increased by orexin signaling elsewhere in the brain, and as increased NPY in the DMH reduces thermogenesis, we predicted that antagonizing orexin receptors in the DMH might increase EE in rats, leading to weight loss.

Methods

All studies were conducted with approval of the Minneapolis VAHCS IACUC. **Surgery:** Adult male Sprague-Dawley rats (n = 32) were surgically implanted with unilateral indwelling guide cannulae targeting the DMH (AP -2.8, LM -0.4, DV -8.5 relative to bregma). **Experiment 1:** Rats were singly housed in standard hanging wire rodent caging. On Day 0, all rats received vehicle (0.5 µl aCSF with 10% cyclodextran and 1% DMSO) and body composition was obtained using the EchoMRI system[13]. Rats then received vehicle or SB334867 (5 µg/0.5 µl in vehicle) once daily between 9:00 AM and 9:30 AM for 7 consecutive days. Body weight and food intake (food removed from hopper, minus spillage) was collected daily. On Day 8 EchoMRI endpoint measures were obtained. **Experiment 2:** Rats were placed in a MedAssociates physical activity cage (17" × 17"), treated with vehicle, and allowed to acclimate for 24 h. The following day, rats received vehicle or SB334867 and 24 h physical activity was measured, with data collection starting at 10:00 AM. **Experiment 3:** Rats were placed in a Columbus Instruments CLAMS indirect calorimetry cage and allowed to acclimate for three days. On first acclimation day all rats recieved vehicle, while on days 2, 3, and 4 rats received vehicle or SB334867. Indirect calorimetry data was recorded on Day 4, with data collection starting at 10:00 AM. At conclusion of study, rats were euthanized with CO_2, and ink was injected into the cannula to aid in placement verification. Only rats with correctly placed cannulas were included in analysis (SB334867: n = 9; Vehicle: n = 8).

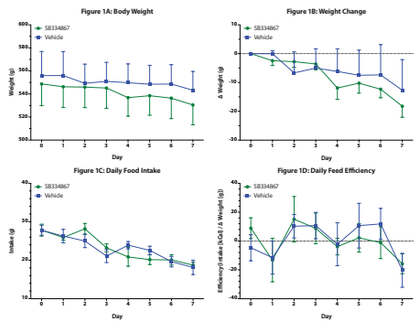

Figure 1: Seven day chronic treatment with SB334867 in the DMH had no significant effect on body weights (A), weight change from baseline (B), daily food intake (C), or feed efficiency (D).

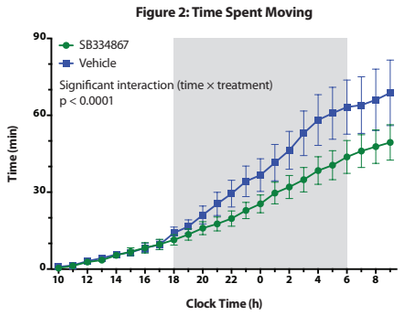

Figure 2: Rats treated with SB334867 had reduced 24 h physical activity (time spent moving + time vertical). *Shaded area indicates active phase (lights off). Two way repeated measures ANOVA (time × treatment); interaction F = 2.72, DFn = 23, DFd = 322, p < 0.0001.*

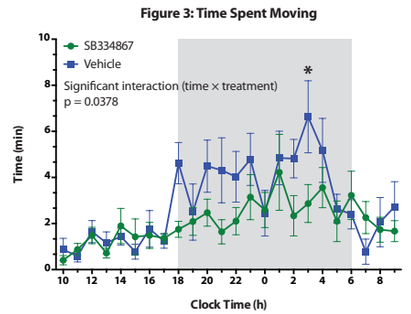

Figure 3: Hourly analysis showed that physical activity was reduced in treated rats mainly during the dark (active) phase (shaded area). *Two way repeated measures ANOVA (time × treatment); interaction F = 1.62, DFn = 23, DFd = 322, p = 0.0378.*

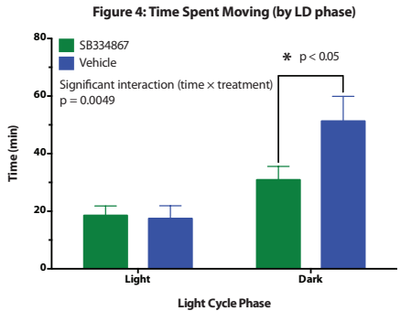

Figure 4: Analysis of activity by light:dark phase showed that SB334867 significantly reduced physical activity during the active phase. *Two way ANOVA (time × treatment) followed by Sidak's multiple comparison test.*

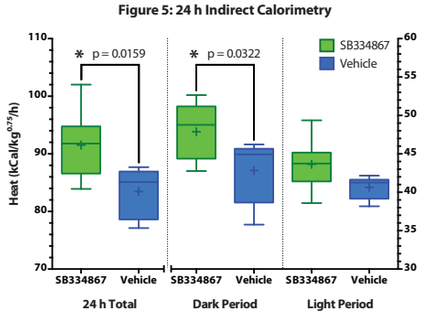

Figure 5: Twenty-four hour indirect calorimetry showed SB334867 significantly increased heat (corrected by body weight) in treated rats. *24 h: Two-tailed t test, t = 2.806, df = 12, p = 0.0159; Dark: Two-tailed t test, t = 2.422, df = 12, p = 0.0322.*

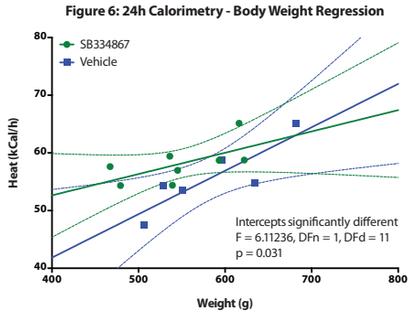

Figure 6: Regression of heat against body weight. While slopes did not differ, intercepts were significantly different; rats treated with SB334867 thus produced more heat per gram body mass. *Y intercept at X = 0: SB334867, 37.89 ± 10.29; vehicle, 11.75 ± 12.92.*

Results

Contrary to expectations, injection of SB334867 into the DMH did not affect food intake or body weight (**Figure 1**). However, treated rats showed decreased physical activity (**Figure 2**), with the greatest reduction during the active phase (**Figures 3–4**). SB334867 treatment also increased energy expenditure (heat kCal corrected for body weight; **Figures 5–6**), especially in the dark (**Figure 6**), suggesting that SB334867 increased thermogenesis.

Discussion

We conclude that in this study, increased thermogenesis prevented significant weight loss in these rats despite their reduced physical activity. It is important to note that the effect of orexin antagonist is site-specific, and that the outcome of orexin antagonism elsewhere in the brain is known (or predicted) to have different effects on body weight, activity, and energy expenditure. For example, the raphé pallidus, which is important in thermogenesis, receives direct synaptic input from both orexin neurons and the DMH[4,7]. It is possible that antagonizing orexin in the DMH might reduce inhibitory signals to the raphé, allowing thermogenesis, while orexin antagonism in the raphé itself would reduce energy expenditure.

Alternatively, we also note that at least one study suggests activation of DMH neurons is critical for orexin effects on thermogenesis[14]. However, data suggest sirtuins increase thermogenesis in part through upregulation of orexin 2 receptors in the DMH[15], and endogenous orexin 2 receptor may be more abundant than orexin 1 receptor in the DMH[9]. Pharmacological blockade of orexin 1 receptor using SB-334867 may thus shunt available orexin to the orexin 2 receptor, resulting in an increase in energy expenditure.

We are currently conducting studies to further evaluate the mechanisms proposed above and expand upon the findings presented here.

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