Hypoventilation and autonomic dysfunction in infant rats following orexin receptor blockade

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Introduction: Orexin (hypocretin) is a neuropeptide expressed by neurons in the lateral and perifornical hypothalamus that project widely to respiratory and autonomic regions of the brainstem. The activity of orexin neurons depends on vigilance state; they are most active in wakefulness, less active in quiet sleep, and silent during active sleep. Although there are well-described facilitatory effects of orexin in adult animals on the control of breathing and autonomic response to stress, its role in infancy has not been studied. This is an important issue because there is accumulating pathological evidence of orexinergic dysfunction in some cases of the Sudden Infant Death Syndrome (SIDS), a leading cause of death in infancy that is highly associated with abnormal respiratory and autonomic control during periods of sleep. We hypothesized that in infant (~2 week old) rat pups, orexin receptor blockade would: 1) lead to respiratory dysfunction, more so in wakefulness and quiet sleep than in active sleep, and 2) compromise the thermogenic response to mild environmental cooling.

Objective: To study the effects of orexin in infant rats on the control of breathing and autonomic response to stress.

Material and Methods: To test these hypotheses we used whole-body plethysmography to monitor breathing and metabolic O_2 consumption in rat pups treated with suvorexant, a selective orexin one and 2-receptor antagonist. Vigilance state was determined using high-definition video to monitor and confirm standard behavioral criteria associated with quiet sleep, active sleep, and arousal in infant rat pups. **Experiment 1:** Pups cycled through wakefulness, quiet and active sleep for 1hr at thermoneutral ambient temperature (T_A=31°C), at which point suvorexant (1mg/kg in 50% DMSO; n=3) or vehicle alone (n=2) was injected via an intra-abdominal cannula, and pups cycled between wakefulness and sleep for another 1 hr. The two groups were compared with respect to respiratory frequency (f), tidal volume (V_T), ventilation (V_E) and metabolic O_2

consumption (VO_2). **Experiment 2:** Pups were kept at thermoneutral T_A for 1 hr, then exposed to a ~2°C drop in T_A over the following 15 min, and then returned to baseline T_A. Pups were then injected with either suvorexant (1mg/kg; n=8) or vehicle alone (n=8), and after another 1 hr, the T_A challenge was repeated. In each animal, the change in metabolic O_2 consumption in response to cooling was measured before and after drug or vehicle injection.

Results: In wakefulness and quiet sleep, suvorexant reduced respiratory frequency by 48 ± 4 breaths/min (~30%; p=0.01), and V_E by 770 ± 254 ml/min/kg (~40%; p<0.001), with no effect on VO_2. These effects were not consistently observed during active sleep, and were absent in pups given vehicle alone. Suvorexant nearly abolished the metabolic response to cooling (p=0.01).

Conclusions: These data suggest that orexin dysfunction can severely compromise breathing and autonomic function in infancy during wakefulness and quiet sleep.

Keywords: orexin, infant rats, control breathing, autonomic response, stress