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Metabolic regulation of lateral hypothalamic glucose-inhibited orexin neurons may influence midbrain reward neurocircuitry

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ABSTRACT

Lateral hypothalamic area (LHA) orexin neurons modulate reward-based feeding by activating ventral tegmental area (VTA) dopamine (DA) neurons. We hypothesize that signals of peripheral energy status influence reward-based feeding by modulating the glucose sensitivity of LHA orexin glucose-inhibited (GI) neurons. This hypothesis was tested using electrophysiological recordings of LHA orexin-GI neurons in brain slices from 4 to 6 week old male mice whose orexin neurons express green fluorescent protein (GFP) or putative VTA-DA neurons from C57Bl/6 mice. Low glucose directly activated ~60% of LHA orexin-GFP neurons in both whole cell and cell attached recordings. Leptin indirectly reduced and ghrelin directly enhanced the activation of LHA orexin-GI neurons by glucose decreases from 2.5 to 0.1 mM by 53 ± 12% ($n = 16$, $P < 0.001$) and 41 ± 24% ($n = 8$, $P < 0.05$), respectively. GABA or neurotensin receptor blockade prevented leptin's effect on glucose sensitivity. Fasting increased activation of LHA orexin-GI neurons by decreased glucose, as would be predicted by these hormonal effects. We also evaluated putative VTA-DA neurons in a novel horizontal slice preparation containing the LHA and VTA. Decreased glucose increased the frequency of spontaneous excitatory post-synaptic currents (sEPSCs; 125 ± 40%, $n = 9$, $P < 0.05$) and action potentials ($n = 9$; $P < 0.05$) in 45% (9/20) of VTA DA neurons. sEPSCs were completely blocked by AMPA and NMDA glutamate receptor antagonists (CNQX 20 μM, $n = 4$; APV 20 μM, $n = 4$; respectively), demonstrating that these sEPSCs were mediated by glutamatergic transmission onto VTA DA neurons. Orexin-1 but not 2 receptor antagonism with SB334867 (10 μM; $n = 9$) and TCS-OX2-29 (2 μM; $n = 5$), respectively, blocks the effects of decreased glucose on VTA DA neurons. Thus, decreased glucose increases orexin-dependent excitatory glutamate neurotransmission onto VTA DA neurons. These data suggest that the glucose sensitivity of LHA orexin-GI neurons links metabolic state and reward-based feeding.

1. Introduction

Most individuals in developed countries are exposed to an almost unlimited food supply largely consisting of highly palatable macronutrients such as sugar and fat. Intake of these macronutrients can trigger ingestion beyond homeostatic needs (reward-based feeding) and is one factor in the modern obesity and Type 2 Diabetes Mellitus epidemic (Fulton, 2010). The DA neurons of the ventral tegmental area (VTA) are an important part of the classical reward neurocircuitry for appetitive behavior and drug addiction (Fields et al., 2007). VTA DA neurons project to brain regions involved in motivation and positive reinforcement including the nucleus accumbens and the prefrontal cortex, which are assumed to be critical in reward-based feeding (Vittoz et al., 2008). The VTA also receives input from many brain regions involved in reward behavior including the prefrontal cortex, amygdala and lateral hypothalamic area (LHA) (Fields et al., 2007).

LHA orexin neurons activate VTA DA neurons through co-release of glutamate as well as by strengthening glutamatergic synaptic transmission (Borgland et al., 2006, 2009; Rosin et al., 2003). Thus, the LHA orexin neurons may modulate reward behavior, in part, via effects on VTA DA neurons. LHA orexin neurons are regulated by signals of metabolic status including circulating nutrients and nutrient-related hormones. The majority of orexin neurons are inhibited by glucose (i.e., glucose-inhibited or GI neurons) (Gonzalez et al., 2008). Hormonal signals of energy balance (e.g., leptin, ghrelin) also regulate the activity of LHA orexin neurons. The hormone leptin is secreted from adipose tissue in proportion to adipose tissue mass. Leptin levels increase after a meal or with weight gain (Ahima et al., 2000). In contrast, stomach secretion of the hormone ghrelin increases prior to a meal or after diet-induced weight loss (Cummings et al., 2002). Calcium imaging studies in isolated orexin neurons suggest that leptin inhibits while ghrelin activates these neurons (Yamanaka et al., 2003). Fasting (low leptin and high ghrelin) increases orexin mRNA; an effect blocked by leptin (Cai et al., 1999; López et al., 2000). Mice lacking orexin do not increase vigilance or exploration in response to a fast (Yamanaka et al., 2003). Thus, energy deficit activates LHA orexin neurons. On the

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other hand, hormonal (e.g., leptin) or nutrient (e.g., glucose) signals of energy sufficiency inhibit LHA orexin neurons. These data are consistent with a role for orexin in the enhanced reward-based feeding observed after food deprivation and weight loss.

We hypothesize that hormonal signals of energy status affect reward-based feeding, in part, by altering the glucose sensitivity of VTA projecting orexin neurons. Thus, LHA orexin GI neurons may link reward-based feeding with peripheral energy status. In order to test this hypothesis we first used electrophysiological techniques to determine whether ghrelin exacerbated while leptin attenuated activation of LHA orexin GI neurons by decreases in extracellular glucose concentration. We then determined whether glucose modulated putative VTA DA neurons in an orexin dependent manner using a novel horizontal brain slice containing the LHA and VTA.

2. Results

2.1. Low glucose directly excites LHA orexin neurons

LHA orexin-GFP neurons were defined by their location lateral to the fornix in coronal brain slices. Approximately 60% (48/82) of LHA orexin-GFP neurons were glucose inhibited (GI) neurons. In the whole cell current clamp recording mode decreasing glucose from 2.5 to 0.1 mM reversibly depolarized the orexin-GI neurons by 8.1 ± 0.5% (2.5 mM glucose: −56.9 ± 0.8 mV; 0.1 mM: −52.3 ± 0.8 mV; n = 48; P < 0.0001) and increased input resistance by 23 ± 2% (2.5 mM glucose: 744 ± 36 MΩ; 0.1 mM: 921 ± 47 MΩ; n = 48; P < 0.005); the latter indicating ion channel closure (Fig. 1A, B, C). Decreased glucose from 2.5 to 0.7 mM also significantly and reversibly depolarized orexin-GI neurons (3.6 ± 0.3%; P < 0.0001) and increased input resistance by 10.12 ± 0.8% (P < 0.0001; n = 6). The percent change in membrane potential and input resistance in response to decreased glucose from 2.5 to 0.7 mM was significantly smaller than that observed in response to a glucose decrease to 0.1 mM (P < 0.0001 for both variables) indicating that the effect of glucose is concentration-dependent. The reversal potential for the inhibitory effect of glucose on orexin GI neurons was −103 ± 7 mV (n = 4), which is close to the potassium equilibrium potential in our solutions (−99 mV). This suggests that low glucose excites these neurons by closing a potassium channel. The effect of glucose persisted in the presence of the sodium channel blocker, tetrodotoxin (TTX, 400 nM; n = 10; Fig. 1A, B, C), which blocks presynaptic action potentials. Thus, glucose directly inhibited these neurons.

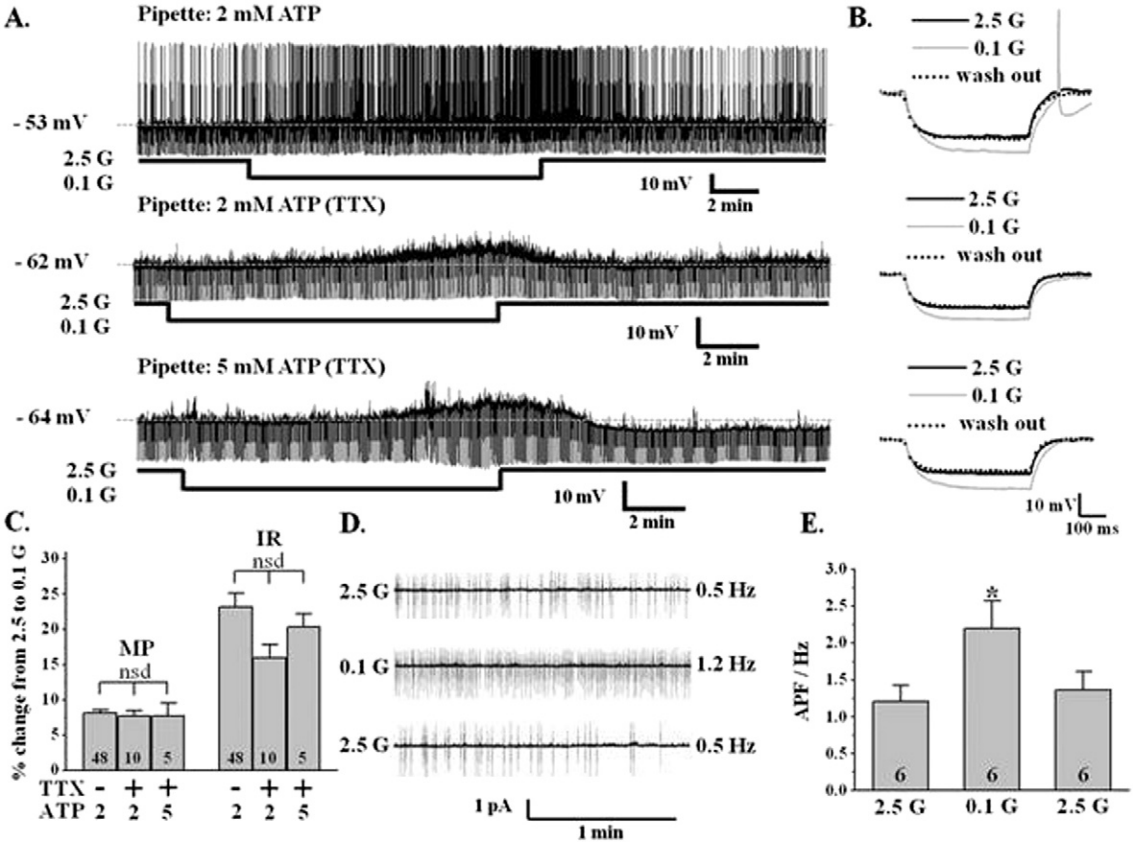

Fig. 1. *Verification of glucose sensing by orexin neurons.* A) Whole cell current-clamp recordings from representative LHA orexin-GI neurons in coronal brain slices with either 2 or 5 mM ATP in the recording pipette. For this and all subsequent current clamp traces the baseline membrane potential (MP) is indicated by a dotted line and given at the left of the trace. The upward deflections are action potentials and the downward deflections are the membrane voltage response to a constant hyperpolarizing current pulse. Input resistance (IR) is calculated according to Ohm's Law where voltage is equal to current times resistance. A change in IR is directly proportional to a change in the voltage response. Approximately 60% (48/82) of LHA orexin-GFP neurons were inhibited by glucose indicating that they were GI neurons. Decreasing glucose (G) from 2.5 to 0.1 mM reversibly depolarized this neuron and increased action potential frequency and IR (top trace). The effects of glucose persisted in the presence of tetrodotoxin (TTX; blocks presynaptic action potentials) in the extracellular recording medium (middle trace). The effects of glucose also persisted in the presence of TTX when 5 mM ATP was included in the recording pipette (bottom trace). B) Representative traces of the voltage response to a constant hyperpolarizing pulse from each of the traces in (A) in an expanded time scale. Decreasing glucose from 2.5 to 0.1 mM reversibly increased IR as indicated by an increase in the voltage response independent of the pipette ATP concentration or the presence of TTX. C) Data bars represent the % change in MP and IR in 0.1 mM glucose relative to 2.5 mM in the presence and absence of TTX. Decreased glucose depolarized LHA orexin-GI neurons by 8.1 ± 0.5% and increased their input resistance by 23 ± 2% (n = 48). There were no significant differences in the response to decreased glucose regardless of whether TTX was present in the recording media (n = 10) or whether 2 (n = 10) or 5 (n = 5) mM ATP was included in the pipette solution (Student's *t*-test, P > 0.05). Thus, decreased glucose directly excited LHA orexin neurons. D) Cell attached voltage-clamp recording of action potentials from an LHA orexin-GI neuron in a coronal brain slice. Decreasing glucose from 2.5 to 0.1 mM significantly increased action potential frequency. E) Data bars represent the action potential frequency in Hz. Decreased glucose reversibly increased action potential frequency by 82 ± 25% (Student's *t*-test, *P < 0.05, n = 6). nsd: no significant difference. N values are indicated within the bar graphs.