Physiological Changes in Glucose Differentially Modulate the Excitability of Hypothalamic Melanin-Concentrating Hormone and Orexin Neurons In Situ

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The physiological signaling mechanisms that link normal variations in body energy status to the activity of arousal- and metabolism-regulating brain centers are not well understood. The melanin-concentrating hormone (MCH) and orexin/hypocretin types of neurons of the lateral hypothalamus (LH) exert opposing effects on arousal and metabolism. We examined whether shifts in brain extracellular glucose that correspond to physiological changes in blood glucose can alter the electrical output of neurochemically and biophysically defined LH cells in mouse brain slices. Here, we show that physiologically relevant concentrations of glucose dose-dependently enhance the electrical excitability of MCH neurons by inducing depolarization and increasing membrane resistance. We also demonstrate that the same physiological shifts in glucose have the opposite effects on the electrical activity of orexin neurons. We propose that these direct actions of glucose on the arousal- and metabolism-regulating LH neurons play a key role in the translation of normal variations in body energy resources into appropriate changes in arousal and metabolism.

Key words: sleep; wakefulness; feeding; glucose; orexin; hypocretin; melanin-concentrating hormone

Introduction

In mammals, arousal is reduced after feeding and increased during fasting (Danguir and Nicolaidis, 1979; Dewasmes et al., 1989; Harnish et al., 1998; Yamanaka et al., 2003b), yet the physiological signaling mechanisms that couple body energy status to the activity of wakefulness- and metabolism-regulating brain centers are not well understood. The activity of neurons in the lateral hypothalamus (LH) is vital for normal sleep–wake behavior and body energy metabolism (Shimada et al., 1998; Saper et al., 2001; Willie et al., 2001; Sutcliffe and de Lecea, 2002; Taheri et al., 2002; Pissios and Maratos-Flier, 2003; Yamanaka et al., 2003b), and it has been recognized since the 1960s that some of these cells specifically respond to elevations in extracellular glucose with increased or decreased electrical activity (Oomura et al., 1969). However, the physiological significance of this potential arousal-modulating signaling pathway remained unsettled (Routh, 2002) because of the lack of knowledge about the functional identities of LH neurons whose electrical output is sensitive to physiologically relevant changes in ambient levels of glucose.

Two types of LH neurons, those expressing the neuropeptides melanin-concentrating hormone (MCH) and orexins (hypocretins), recently emerged as critical regulators of sleep–wake behavior and energy balance (Shimada et al., 1998; Willie et al., 2001; Sutcliffe and de Lecea, 2002; Pissios and Maratos-Flier, 2003; Verret et al., 2003; Yamanaka et al., 2003b; Burdakov, 2004), but it has not been established whether the electrical activity of these vital cells is sensitive to physiological changes in glucose. Although the firing of isolated LH orexin neurons is inhibited by glucose in the 5–30 mM concentration range (Yamanaka et al., 2003b), it is unknown whether this occurs in situ at physiologically relevant concentrations of brain glucose, which are <5 mM (Silver and Erecinska, 1994; de Vries et al., 2003). The impact of changes in extracellular glucose on the electrical output of MCH neurons has not been explored.

In this study, we analyzed the electrical responses of LH MCH and orexin neurons to physiological shifts in extracellular glucose in mouse brain slices, using patch-clamp recordings and postrecording immunocytochemistry.

Materials and Methods

Electrophysiology. Procedures involving animals were performed in accordance with the United Kingdom Animals (Scientific Procedures) Act of 1986. Brain slices containing the LH (bregma coordinates corresponding to between −1.2 and −2 mm in adult mice) were prepared from male C57BL/6 mice (postnatal days 13–16), and whole-cell patch-clamp recordings and data analysis were performed as described previously (Burdakov et al., 2003). Experiments were performed at room temperature (22–24°C). Extracellular solution was artificial CSF gassed with 95% O₂ and 5% CO₂ and contained the following (in mM): 118 NaCl, 25 NaHCO₃, 3 KCl, 1.2 NaH₂PO₄, 2 CaCl₂, 2 MgCl₂, and 0.2–5 glucose. When the extracellular glucose concentration was altered, osmolality was balanced with sucrose. Patch pipettes had tip resistances of 3–5 MΩ when filled with internal solution containing the following (in mM): 120...
Figure 1. Electrical responses of LH MCH neurons to physiological changes in glucose. A, Defining electrical signature of MCH neurons: absence of spontaneous firing (i), no H-current-mediated sag (ii), and spike-rate adaptation (iii). Current-clamp protocols used to elicit these responses are shown schematically below the corresponding traces. B, Immunofluorescence imaging of the cell shown in A, identified by Neurobiotin staining (green); the cell contains MCH (red) but not orexin-A (yellow). Scale bar, 20 μm. C, Glucose induced reversible depolarization and spiking in an MCH neuron. D, Glucose enhanced spiking evoked by depolarizing current injection (20 pA for 3 s; protocol shown schematically below the traces); this effect was reversibly after glucose washout. E, Dose–response relationship of glucose-induced depolarization of MCH cells (EC50 = 0.8 mM; h = 3.2; Vm0 = −43.5 mV; Vm = −54.5 mV; the general equation of the fit is given in Materials and Methods). Numbers of cells are indicated above corresponding points. F, In the presence of tetrodotoxin (TTX) (300 nM), glucose induced depolarization and increased membrane resistance (the latter effect is manifested as increased amplitude of membrane potential responses to hyperpolarizing current pulses; 40 pA for 500 ms at 30 s intervals).

Results
Identification of MCH and orexin neurons in brain slices
We identified MCH and orexin cells by their distinctive electrophysiological properties and postrecording immunocytochemistry. MCH neurons are electrically silent, do not display H-current-mediated depolarization, and exhibit pronounced spike-rate adaptation (Eggermann et al., 2003; van den Pol et al., 2004). Using postrecording triple-label immunocytochemistry to correlate electrophysiological signature with expression of MCH and orexin peptides (see Materials and Methods), we confirmed that all LH neurons that displayed this combination of properties (Fig. 1A) contained MCH and not orexin (n = 30 of 30) (Fig. 1B).

In contrast to MCH neurons, orexin neurons exhibit tonic spontaneous firing, H-currents, a low-threshold spike on recovery from hyperpolarization, and little spike-rate adaptation (Eggermann et al., 2003; Yamanaka et al., 2003a; Burdakov et al., 2004). We confirmed by postrecording immunocytochemistry that all LH neurons displaying the latter set of properties (Fig. 2A) contained orexin and not MCH peptides (n = 35 of 35)
Physiological concentrations of glucose excite MCH neurons

In vivo, extracellular glucose concentration in the brain varies between ~0.2 mM during systemic hypoglycemia and ~5 mM during hyperglycemia [measurements from rat brain (Silver and Erecinska, 1994)]. Changing extracellular glucose from 0.2 to 5 mM reversibly depolarized MCH neurons (n = 15 of 18) (Fig. 1C). This depolarization was accompanied by the appearance of spiking responses to depolarizing current pulses that failed to induce action potentials in 0.2 mM glucose (n = 15 of 15) (Fig. 1D) and by induction of spontaneous firing in 4 of 15 MCH neurons (Fig. 1C). Glucose-induced depolarization of MCH neurons was dose dependent with half-maximal effect at 0.8 mM (Fig. 1E) and was associated with an increase in membrane resistance (calculated from membrane potential changes in response to a series of 10–40 pA hyperpolarizing current pulses, such as those shown in Fig. 1F). Membrane resistance increased by 74 ± 18% in 5 mM glucose compared with control values in 0.2 mM glucose measured in the same cell (n = 8). The largest resistance increase observed was from 400 to 1100 MΩ, and the smallest was from 650 to 800 MΩ in 0.2 and 5 mM glucose, respectively. Reversible glucose-induced depolarization and resistance increase persisted when neurons were synaptically isolated with tetrodotoxin (n = 4) (Fig. 1F), consistent with a direct postsynaptic action of glucose on MCH neurons.

Physiological concentrations of glucose inhibit orexin neurons

The same physiological changes in glucose had the opposite effects on LH orexin neurons. Elevating glucose from 0.2 to 5 mM induced hyperpolarization and suppressed both spontaneous (Fig. 2C) and evoked (Fig. 2D) firing in 20 of 21 orexin neurons. Glucose-induced hyperpolarization of orexin neurons was concentration dependent with half-maximal response at 3.5 mM (Fig. 2E) and was accompanied by a pronounced decrease in membrane resistance (Fig. 2F). Membrane resistance fell by 66 ± 3% in 5 mM glucose compared with control values in 0.2 mM glucose measured in the same cell (n = 7). The largest resistance decrease was from 1000 to 250 MΩ, and the smallest was from 1160 to 530 MΩ in 0.2 and 5 mM glucose, respectively. The hyperpolarization and resistance decrease persisted in tetrodotoxin (n = 5) (Fig. 2F), indicating a direct postsynaptic action of glucose on orexin neurons.

Discussion

Effects of glucose on MCH and orexin neurons

In this study, we show for the first time that physiologically relevant concentrations of glucose directly enhance the electrical excitability of MCH neurons by causing depolarization and increasing membrane resistance but inhibit the firing of orexin neurons by triggering hyperpolarization and decreasing membrane resistance. Crucially, our concentration–response analysis (Figs. 1E, 2B). Our qualitative electrophysiological criteria (Figs. 1A, 2A) therefore provide a highly reliable way of identifying MCH and orexin neurons in our in situ preparation.

Figure 2.  Electrochemical responses of LH orexin neurons to physiological changes in glucose. A, Defining electrical signature of orexin neurons: tonic spontaneous firing (i), H-current-mediated sag (ii), low-threshold spike (iii), and little spike-rate adaptation (iv). Current–clamp protocols used to elicit these responses are shown schematically below corresponding traces. B, Immunofluorescence imaging of the cell shown in A, identified by Neurobiotin staining (green); the cell contains orexin-A (yellow) but not MCH (red). Scale bar, 20 μm. C, Glucose-induced reversible hyperpolarization and inhibited spiking in an orexin neuron. D, Glucose suppressed the spiking response to depolarizing current injection (40 pA for 3 s; protocol shown schematically below the traces); this effect was reversible after glucose washout. E, Dose–response relationship of glucose-induced hyperpolarization of orexin cells (IC50 = 3.5 mM; h = 1.8; Vmmax = −78.8 mV; Vh = −42.9 mV; the general equation of the fit is given in Materials and Methods). Numbers of cells are indicated above corresponding points. F, In the presence of tetrodotoxin (TTX) (300 nM), glucose induced hyperpolarization and decreased membrane resistance (resistance was monitored as described in Fig. 1F).
maintained in 10 mM glucose for a long time (throughout other recent reports indicate that after LH brain slices have been evoked electrical output in response to changes in ambient glucose neurons pathway remained controversial because inhibition of the electrical output caused by falling levels of glucose may mediate heri et al., 2002). It was proposed recently that disinhibition of orexin excites GABAergic neurons of the arcuate nucleus by activating the sodium–calcium exchanger. J Neurosci 23:4951—4957.


Silver and Erecinska, 1994; de Vries et al., 2003).

In summary, our data offer an integrated biophysical and neurochemical explanation for how LH glucose-sensing neurons translate subtle physiological variations in glucose levels into appropriate adaptive changes in arousal and energy metabolism. This may also be relevant to understanding the pathogenesis of narcolepsy and body-weight disorders.

References


