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THERMOSENSING MECHANISMS AND THEIR IMPAIRMENT BY HIGH-FAT DIET IN OREXIN NEURONS

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Abstract—In homeotherms, the hypothalamus controls thermoregulatory and adaptive mechanisms in energy balance, sleep–wake and locomotor activity to maintain optimal body temperature. Orexin neurons may be involved in these functions as they promote thermogenesis, food intake and behavioral arousal, and are sensitive to temperature and metabolic status. How thermal and energy balance signals are integrated in these neurons is unknown. Thus, we investigated the cellular mechanisms of thermosensing in orexin neurons and their response to a change in energy status using whole-cell patch clamp on rat brain slices. We found that warming induced an increase in miniature excitatory postsynaptic current (EPSC) frequency, which was blocked by the transient receptor potential vanilloid-1 (TRPV1) receptor antagonist AMG9810 and mimicked by its agonist capsaicin, suggesting that the synaptic effect is mediated by heat-sensitive TRPV1 channels. Furthermore, warming inhibits orexin neurons by activating ATP-sensitive potassium (KATP) channels, an effect regulated by uncoupling protein 2 (UCP2), as the UCP2 inhibitor genipin abolished this response. These properties are unique to orexin neurons in the lateral hypothalamus, as neighboring melanin-concentrating hormone neurons showed no response to warming within the physiological temperature range. Interestingly, in rats fed with western diet for 1 or 11 weeks, orexin neurons had impaired synaptic and KATP response to warming. In summary, this study reveals several mechanisms underlying thermosensing in orexin neurons and their attenuation by western diet. Overeating induced by western diet may in part be due to impaired orexin thermosensing, as post-prandial thermogenesis may promote satiety and lethargy by inhibiting orexin neurons.

Key words: orexin neurons, neuronal thermosensing, transient receptor potential vanilloid-1, western diet.

INTRODUCTION

The maintenance of a stable core body temperature is vital for optimal physiological function and survival in homeotherms (Nakamura, 2011). Changes in body temperature are not only countered by thermoregulatory mechanisms, but also often accompany adaptive changes in energy balance, sleep–wake state and locomotor activity (Yates, 1993). The hypothalamus is responsible for preserving a relatively constant body temperature despite environmental thermal challenges as well as inducing thermogenesis in response to events such as infection (Nakamura, 2011) or food intake (diet-induced thermogenesis) (Freeman and Wellman, 1987).While the preoptic area is well known as the thermoregulatory center where peripheral and central thermal signals converge onto thermosensitive neurons, there are other cell types in the hypothalamus that are also thermosensitive and play significant roles in thermal and non-thermal functions (Hori, 1991).

Our previous study has found that orexin neurons (also known as hypocretin neurons) are inhibited by rises in temperature (Parsons et al., 2012). These neurons are known to exert many functions including thermogenesis (Yoshimichi et al., 2001), sympathetic outflow (Ferguson and Samson, 2003), arousal and wakefulness (Chemelli et al., 1999), locomotor activity (Kotz, 2006), food intake and other motivated behaviors (Sakurai et al., 1998; Harris et al., 2005). Therefore, the thermosensitive property of orexin neurons may underlie diverse physiological responses to fluctuations in body temperature. Furthermore, these neurons are also sensitive to metabolic states such as fasting (Sakurai et al., 1998), obesity (Yamamoto et al., 1999) and high-fat diet (Wortley et al., 2003), suggesting that integration of metabolic and temperature signals may occur at orexin neurons. The metabolic status of an organism is known to have a significant impact on thermoregulatory processes. For instance, obese animals have reduced core body temperature (James and Trayhurn, 1981) and impaired thermogenic responses (Himms-Hagen, 1984). Orexin neurons may be involved in these interactions of energy balance and thermoregulation.

The thermosensing mechanism of orexin neurons involves the activation of ATP-sensitive potassium (KATP) channels, resulting in reduced firing activity

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Abbreviations: ACSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; EGTA, ethylene glycol tetraacetic acid; HEPES, 4-(2-hydro xyethyl)-1-piperazineethanesulfonic acid; KATP, ATP-sensitive potassium channel; LH/PFA, lateral hypothalamus and perifornical area; MCH, melanin-concentrating hormone; mEPSC, miniature excitatory postsynaptic current; TRPV1, transient receptor potential vanilloid-1; TTX, tetrodotoxin; UCP2, uncoupling protein 2; WD, western diet.

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during a rise in temperature (Parsons et al., 2012). However, it remains unknown whether warming has additional effects on synaptic transmission. Thus, in the present study we have characterized the effect of warming on excitatory transmission and investigated its relationship with the KATP-mediated response. In addition, to determine whether metabolic and temperature signals are integrated by orexin neurons, we investigated the impact of palatable high-fat diet on the thermosensing properties of these neurons.

EXPERIMENTAL PROCEDURES

Ethical information

All experimental procedures were reviewed and approved by Memorial University Institutional Animal Care Committee in accordance with the guidelines set by the Canadian Council on Animal Care. All efforts were made to minimize the number of animals used and their suffering.

Animals

Male Sprague–Dawley rats (3–4 weeks old) were obtained from a breeding colony at Memorial University or from Charles River. Animals were housed in a light- and temperature-controlled room (L:D = 12:12 h, lights on at 7:30 am, 22 ± 1 °C) and fed *ad libitum* chow (Prolab RMH 3000) or high-fat western diet (WD; TestDiet AIN-76A Western Diet, cat#1810060) for up to 11 weeks.

Electrophysiology

Whole-cell patch-clamp recordings were performed on acute brain slices. Rats were anesthetized with 4% isoflurane, sacrificed by decapitation, and brains were quickly removed and placed into ice-cold artificial cerebrospinal fluid (ACSF) composed of (in mM): 126 NaCl, 2.5 KCl, 1.2 NaH_2PO_4, 1.2 $MgCl_2$, 18 $NaHCO_3$, 2.5 glucose, 2 $CaCl_2$, pH 7.3–7.35. Coronal hypothalamic slices 250-μm-thick were obtained using a vibratome (VT-1000, Leica Microsystems, Richmond Hill, ON, Canada). Following dissection, slices were incubated in ACSF at 32–35 °C for 30–45 min, then at room temperature prior to recording. ACSF was continuously bubbled with O_2 (95%)/CO_2 (5%).

Patch-clamp recordings were performed using an infrared-differential interference contrast optics microscope (DM-LFSA, Leica Microsystems, Richmond Hill, ON, Canada), a Multiclamp 700B amplifier and pClamp 9 or 10 software (Molecular Devices, Sunnyvale, CA, USA). Slices were perfused with ACSF at a rate of 2–3 ml/min. Cells were chosen based on their location and morphology: orexin and melanin-concentrating hormone (MCH) neurons are localized in the lateral hypothalamus and perifornical area (LH/PFA), dorsal to the fornix, and are typically large in size (10–20 μm) and oval or triangular in shape.

Recordings were conducted using conventional whole-cell internal solution containing (in mM): 123 K-gluconate, 8 KCl, 2 $MgCl_2$, 10 HEPES, 0.2 EGTA, 5 Na_2ATP, 0.3 NaGTP, pH 7.3 with KOH. Biocytin (1–1.5 mg/ml) was added to the internal pipette solution for post hoc immunohistochemical phenotyping. Glass pipette electrodes had a tip resistance 3–5 MΩ when filled with internal solution. When whole-cell access with a series/access resistance of 5–20 MΩ was achieved, a series of depolarizing and hyperpolarizing current steps were applied (−200 to +200 pA in 50 or 100 pA increments, 300–600 ms). Orexin and MCH neurons have distinct electrophysiological characteristics, which in our experience are highly effective in differentiating phenotype (Alberto and Hirasawa, 2010; Parsons and Hirasawa, 2011). In particular, a combined presence of H-current, spontaneous firing and uniphasic after hyperpolarizing potential is a very reliable marker of orexin neurons (Linehan et al., 2015).

Whole-cell voltage-clamp recordings were conducted at a holding potential of −50 mV. Miniature excitatory postsynaptic currents (mEPSCs) were recorded with tetrodotoxin (TTX, 1 μM) in the bath. Throughout the recording, −20 mV square pulses were applied for 100 ms to monitor series resistance. Experiments in which the series resistance changed by >20% were excluded from analysis.

The bath temperature was controlled using a temperature probe placed in close proximity to the slice and an in-line heater (TC-324B, Warner Instruments, Hamden, CT, USA or HW-30, Dagan, Minneapolis, MN, USA). Experimental protocol involved increasing the bath temperature at a rate of approximately 3 °C/min, which was maintained at the peak for several minutes before returning to baseline temperature. The majority of the study was performed using a baseline and test temperature of 32 and 37 °C, respectively, as stable recordings were more easily attained at these temperatures than physiological temperatures (37–40 °C). For comparison purposes, control cells were always tested with the same temperature range as the treatment group.

Chemical compounds

Drugs were prepared as frozen aliquots of ×1000 stock solutions, diluted with ACSF to final concentrations immediately before use and applied in the bath using a perfusion system. Final concentration of DMSO, when used as vehicle, was 0.1%. Drugs were applied in the bath for a minimum of 5–10 min before manipulating the bath temperature.

TTX was obtained from Alomone Labs (Jerusalem, Israel), glibenclamide from Tocris Bioscience (Ellisville, MO, USA), tolbutamide and capsaicin from Sigma–Aldrich (St. Louis, MO, USA), genipin from Cayman Chemical Co. (Ann Arbor, MI, USA) and AMG9810 from Enzo Life Sciences, Inc. (Farmingdale, NY, USA).

Post-hoc Immunohistochemistry

Immediately following experimentation, slices were fixed in 10% formalin for >16 h at 4 °C. Slices were washed with PBS and treated with goat anti-orexin A (1:1000–3000; Santa Cruz Biotechnology, Dallas, TX, USA, sc-8070) and rabbit anti-MCH (1:1000–4000; Phoenix Pharmaceuticals, Burlingame, CA, USA, G-070-47) at