Neuroscience 346 (2017) 29–42

OLEATE INDUCES K_{ATP} CHANNEL-DEPENDENT HYPERPOLARIZATION IN MOUSE HYPOTHALAMIC GLUCOSE-EXCITED NEURONS WITHOUT ALTERING CELLULAR ENERGY CHARGE

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Abstract—The unsaturated fatty acid, oleate exhibits anorexigenic properties reducing food intake and hepatic glucose output. However, its mechanism of action in the hypothalamus has not been fully determined. This study investigated the effects of oleate and glucose on GT1-7 mouse hypothalamic cells (a model of glucose-excited (GE) neurons) and mouse arcuate nucleus (ARC) neurons. Whole-cell and perforated patch-clamp recordings, immunoblotting and cell energy status measures were used to investigate oleate- and glucose-sensing properties of mouse hypothalamic neurons. Oleate or lowered glucose concentration caused hyperpolarization and inhibition of firing of GT1-7 cells by the activation of ATP-sensitive K^+ channels (K_{ATP}). This effect of oleate was not dependent on fatty acid oxidation or raised AMP-activated protein kinase activity or prevented by the presence of the UCP2 inhibitor genipin. Oleate did not alter intracellular calcium, indicating that CD36/fatty acid translocase may not play a role. However, oleate activation of K_{ATP} may require ATP metabolism. The short-chain fatty acid octanoate was unable to replicate the actions of oleate on GT1-7 cells. Although oleate decreased GT1-7 cell mitochondrial membrane potential there was no change in total cellular ATP or ATP/ADP ratios. Perforated patch and whole-cell recordings from mouse hypothalamic slices demonstrated that oleate hyperpolarized a subpopulation of ARC GE neurons by K_{ATP} activation. Additionally, in a separate small population of ARC neurons, oleate application or lowered glucose concentration caused membrane depolarization. In conclusion, oleate induces K_{ATP}-dependent hyperpolarization and inhibition of firing of a subgroup of GE hypothalamic neurons without altering cellular energy charge.

Key words: glucose sensing, K_{ATP}, oleate, hypothalamus, mitochondria, fatty acid oxidation.

INTRODUCTION

The hypothalamus is critical for the continuous regulation of whole-body glucose, lipid and energy homeostasis. To perform this function, various hypothalamic nuclei (e.g. arcuate (ARC), ventromedial (VMN), lateral hypothalamic area and paraventricular) contain neuropeptide-expressing neurons that monitor circulating nutrients and hormone levels (Levin et al., 2004). Scattered throughout these nuclei are subpopulations of neurons that sense changes in glucose levels, resulting in altered neuronal firing and modified energy homeostasis (Levin et al., 1999). There are two main subtypes of glucose-sensing hypothalamic neurons that contribute to these homeostatic mechanisms: neurons excited (glucose-excited (GE)) and inhibited (glucose-inhibited (GI)) by increased levels of glucose. GE neurons utilize ATP-sensitive potassium (K_{ATP}) channels (in a manner similar to pancreatic beta cells (MacDonald et al., 2005) to modulate their electrical activity in response to changes in extracellular glucose concentration (Ashford et al., 1990; Wang et al., 2004; Kang et al., 2006). It is presently unclear which ion transport mechanism is responsible for transducing changes in glucose concentration to modify electrical activity in GI neurons (Gonzalez et al., 2009). These glucose-sensing neurons, in particular GE neurons, play important roles in the feeding response to glucoprivation (as suppression of glucokinase (GK) diminishes the glucoprivic stimulation of feeding (Dunn-Meynell et al., 2009), and liver glucose production (Parton et al., 2007) and have been strongly implicated in the detection of hypoglycemia and subsequent generation of counterregulatory responses (Beall et al., 2012). For example, loss of the K_{ATP} channel subunit, $K_{IR}6.2$, causes near complete suppression of glucagon responses to hypoglycemia, which is driven by the loss of K_{ATP} on neural cells (Miki et al., 2001).

Similarly, energy status (i.e. lipid level) is also communicated continuously to the hypothalamus. This

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Abbreviations: ACC, acetyl-CoA carboxylase; AMPK, adenosine 5′-monophosphate-activated protein kinase; AMP-PNP, 5′-adenylylimido diphosphate; ARC, arcuate nucleus; CPT1, carnitine palmitolytransferase-1; CSF, cerebrospinal fluid; GE, glucose-excited; GI, glucose-inhibited; K_{ATP}, ATP-sensitive potassium channel, oleate; POMC, proopiomelanocortin; UCP, uncoupling protein; VMN, ventromedial nucleus.

http://dx.doi.org/10.1016/j.neuroscience.2016.12.053
0306-4522/

is performed, at least in part, by hypothalamic neurons responding to changes in the concentrations of circulating hormones (e.g. leptin and insulin), the levels of which correlate with adipose tissue depot size. It is this latter communication system that is considered to become faulty in obesity (Frederich et al., 1995). However, circulating lipids, such as long-chain fatty acids, have also been demonstrated to directly act on hypothalamic centers to modulate feeding and hepatic glucose output (Obici et al., 2002; Lam et al., 2005). Circulating levels can be acutely elevated, such as during fasting or hypoglycemia, where lipolysis is elevated (De Feo et al., 1989), therefore it is plausible that some neurons have a capacity to detect both reduced glucose and elevated lipid levels. Indeed the long-chain fatty acid, oleate alters ARC neuron neuropeptide expression, electrical activity and within glucosensing neurons, can alter intracellular calcium signaling via CD36/fatty acid translocase (Obici et al., 2002; Morgan et al., 2004; Wang et al., 2006; Le Foll et al., 2009). Furthermore, long-chain fatty acids are activators of K_{ATP} channels in pancreatic beta cells (Larsson et al., 1996) and hypothalamic-delivered oleate suppresses hepatic glucose production in a K_{ATP}-dependent manner (Obici et al., 2002). A previous study suggested that oleate alters the excitability of ARC neurons in a glucose-concentration-dependent manner suggesting interaction at the level of cellular nutrient metabolism (Wang et al., 2006). In addition, the central effects of fatty acids on glucose homeostasis have been ascribed to neuronal fatty acid metabolism (Obici et al., 2003; Cruciani-Guglielmacci et al., 2004).

Alternatively, fatty acids can regulate the activity of mitochondrial uncoupling proteins (UCP) (Echtay et al., 2001), including the neuronal enriched UCP isoforms, UCP4 and UCP5 (Hoang et al., 2012). In the pancreatic beta cell, UCP2 has been implicated in regulating glucose-sensing behavior (Lameloise, 2001; Zhang, 2001; Parton et al., 2007). Indeed, oleate modulates the expression of UCP2 and alters glucose-dependent insulin secretion in pancreatic beta cells and beta cell lines (Medvedev et al., 2002; Oprescu et al., 2007). Importantly, UCP2 has been shown to regulate the glucose-sensing behavior of GE neurons and alter whole-body glucose homeostasis (Parton et al., 2007). Consequently, in order to explore the mechanisms by which oleate modifies neuronal excitability we have examined the interplay between nutrient-dependent pathways and oleate responses in mouse GE-type neurons.

EXPERIMENTAL PROCEDURES

Cell culture

Immortalized mouse hypothalamic GnRH secreting GT1-7 cells (Pamela Mellon, San Diego, California, USA (Lam et al., 2005) were maintained in Dulbecco's modified Eagle's medium (DMEM; Sigma–Aldrich, Gillingham, UK)) with 10% fetal bovine serum (PAA Laboratories, Yeovil, UK & Hyclone, Pasching, Austria) as described (Mirshamsi et al., 2004).

Immunoblotting

GT1-7 cells, seeded in 6-well dishes, were serum-starved for three hours with DMEM replaced by saline and then treated with nutrients (0–100 μM oleate or 2.5/0.1 mM glucose) for various times as described in the results section. We used oleate concentrations in the low micromolar (10–100 μM) as plasma concentrations of total free fatty acids have been estimated within the range of 350–500 μM under normal circumstances (Richieri and Kleinfeld, 1995; Mai et al., 2006; Abdul-Ghani et al., 2008) and, in type 1 diabetes, can reach levels of more than 1200 μM (Boden, 1998). In human CSF levels of oleate have been reported to be >10 μM in non-diabetic populations (Levi et al., 2013), and may be expected to reach much higher levels in type 1 diabetic patients. In rats, brain glucose levels have been estimated at ~2.5 and ~0.1 mM during euglycemia and hypoglycemia, respectively (Silver and Erecinska, 1994).

Protein isolation and immunoblotting procedures were as described previously (Mirshamsi et al., 2004). Briefly, protein lysates were subjected to SDS–PAGE, electrotransferred to nitrocellulose membranes, and probed with primary antibodies against p-AMPKα (Thr172; 1:1000), p-ACC (Ser79; 1:1000), actin (1:5000), UCP4 (1:1000) and UCP5 (1:1000). All antibodies were obtained from Cell Signalling Technology Inc. (New England Biolabs, Hitchin, UK) except UCP4 and UCP5, which were obtained from Acris Antibodies (Herford, Germany). Proteins were detected with horseradish peroxidase-conjugated Goat anti-Rabbit IgG and immunoreactive proteins identified by chemiluminescence. Gel protein bands were quantified by densitometry, where total density was determined with respect to constant area, background subtracted and average relative band density calculated.

Hypothalamic slice preparation

All animal procedures conformed to the UK Animals Scientific Procedures Act (1986) and were approved by the University of Dundee institutional ethics review committee. Wild-type male C57Bl/6 mice (6–20 weeks old) were killed by cervical dislocation and the brains rapidly removed and submerged in an ice cold slicing solution as described previously (Hisadome et al., 2009). Briefly, hypothalamic coronal slices containing the ARC (350 μm) were prepared using a Vibratome (St Louis, MO, USA) and stored at room temperature (22–25 °C) in an external solution containing (in mM): NaCl 125, KCl 2.5, NaH_2PO_4 1.25, $NaHCO_3$ 25, $CaCl_2$ 2, $MgCl_2$ 1, D-Glucose 10, D-Mannitol 15, ascorbate 1 and pyruvate 3, equilibrated with 95% O_2, 5% CO_2, pH 7.4. Immediately before use, brain slices were transferred to the recording chamber of an upright Zeiss Axioskop-2 FS plus microscope and continuously perfused with a modified external solution (containing 0.5 mM $CaCl_2$ and 2.5 mM $MgCl_2$, no ascorbate and pyruvate) at a constant flow rate of 5–10 ml min^{-1} and bath temperature of 33 °C.

Electrophysiology

GT1-7 cells were visualized by phase contrast and individual neurons of mouse hypothalamic slices by