Effects of Oleic Acid on Distinct Populations of Neurons in the Hypothalamic Arcuate Nucleus Are Dependent on Extracellular Glucose Levels

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Wang, R., C. Cruciani-Guglielmacci, S. Migrenne, C. Magnan, V. E. Cotero, and V. H. Routh. Effects of oleic acid on distinct populations of neurons in the hypothalamic arcuate nucleus are dependent on extracellular glucose levels. J Neurophysiol 95: 1491–1498, 2006. First published November 23, 2005; doi:10.1152/jn.00697.2005. Pharmacological manipulation of fatty acid metabolism in the hypothalamic arcuate nucleus (ARC) alters energy balance and glucose homeostasis. Thus, we tested the hypotheses that distinctive populations of ARC neurons are oleic acid (OA) sensors that exhibit a glucose dependency, independent of whether some of these OA sensors are also glucose-sensing neurons. We used patch-clamp recordings to investigate the effects of OA on ARC neurons in brain slices from 14- to 21-day-old Sprague–Dawley (SD) rats. Additionally, we recorded spontaneous discharge rate in ARC neurons in 8-wk-old fed and fasted SD rats in vivo. Patch-clamp studies showed that in 2.5 mM glucose 12 of 94 (13%) ARC neurons were excited by 2 μM OA (OA-excited or OAE neurons), whereas six of 94 (6%) were inhibited (OA-inhibited2.5 or OAI2.5 neurons). In contrast, in 0.1 mM glucose, OA inhibited six of 20 (30%) ARC neurons (OAI0.1 neurons); none was excited. None of the OAI0.1 neurons responded to OA in 2.5 mM glucose. Thus OAI2.5 and OAI0.1 neurons are distinct. Similarly, in seven of 20 fed rats (35%) the overall response was OAE-like, whereas in three of 20 (15%) it was OAI-like. In contrast, in fasted rats only OAI-like response was observed (three of 15; 20%). There was minimal overlap between OA-sensing neurons and glucose-sensing neurons. In conclusion, OA regulated three distinct subpopulations of ARC neurons in a glucose-dependent fashion. These data suggest that an interaction between glucose and fatty acids regulates OA sensing in ARC neurons.

INTRODUCTION

A number of recent studies have shown that pharmacological manipulation of hypothalamic fatty acid metabolism can alter energy balance and glucose homeostasis. Loftus and colleagues (2000) showed that systemic and intracerebroventricular (icv) injection of the fatty acid synthase (FAS) inhibitor, C75 (4-methylene-2-octyl-5-oxo-tetrahydro-furan-3-carboxylic acid), decreased food intake and body weight in mice. C75 also decreased hypothalamic neuropeptide Y (NPY) expression, suggesting that fatty acid signaling plays a role in the hypothalamic regulation of energy balance. This observation has since fueled considerable research on the topic of hypothalamic sensing of fatty acids. The central effects of fatty acids are not limited to food intake and body weight regulation. Intracarotid or icv infusion of a lipid emulsion increases glucose-induced insulin secretion and decreases hepatic gluconeogenesis (Clement et al. 2002; Cruciani-Guglielmacci et al. 2004). Inhibition of carnitine palmitoyl transferase 1 (CPT1), which transports long-chain fatty acids (LCFAs) into the mitochondria for β-oxidation, blocks these effects. This suggests that neuronal fatty acid metabolism is necessary for the effects of fatty acids on glucose homeostasis. Interestingly, icv glibenclamide increased glucose production in the presence of elevated LCFA, suggesting a role for adenosine triphosphate (ATP)–sensitive K+ (KATP) channels (Lam et al. 2005; Pocai et al. 2005). Thus it is now accepted that fatty acids act centrally to modulate not only food intake and body weight but glucose homeostasis as well.

Although multiple brain regions are likely to be involved in the central effects of fatty acids, the NPY and proopiomelanocortin (POMC) neurons of the hypothalamic arcuate nucleus (ARC) stand out as a primary site of action for fatty acid signaling. Several groups show that FAS inhibition alters ARC neuropeptide expression. C75 increases c-Fos expression in the lateral ARC, paraventricular nucleus (PVN), hindbrain, and the central nucleus of the amygdala, whereas inhibiting the fasting induced increases in c-Fos in the medial ARC, magnocellular PVN, lateral hypothalamus (LH), and dorsomedial nucleus (Miller et al. 2004). Cerulein, another FAS inhibitor, increased c-Fos expression in ARC POMC neurons and inhibits fasting-induced increases in c-Fos in the medial ARC (Shu et al. 2003). The effects of FAS inhibition are leptin independent. That is, the C75 or cerulein induced increased c-Fos expression in the POMC and decreased c-Fos expression in the NPY neurons, occurred in the presence of reduced leptin levels, which would normally have the opposite response (Loftus et al. 2000; Makimura et al. 2001). Finally, a third ventricular injection of CPT-ribo (which inhibits CPT1 expression) decreased food intake, endogenous glucose production, and NPY expression. In this study, CPT-ribo specifically decreased CPT1 in the ARC, but not PVN or LH (Obici et al. 2003).

Although pharmacological manipulation of fatty acid metabolism in the ARC alters peripheral glucose and energy homeostasis, the mechanism(s) by which this occurs are not clear. Oomura et al. (1975) showed that LCFAs alter neuronal activity in the lateral hypothalamus. Furthermore, there is a large body of literature indicating that LCFAs regulate the conductance of a wide variety of ion channels, including chloride channels [γ-aminobutyric acid type A (GABA_A)] (Witt and Nielson 1994), CIC-2 (Tewari et al. 2000)], potassium channels [KCa (Zheng et al. 2005), KATP (Branstorn et al. 2000)].
and calcium channels (Honen et al. 2003). Additionally, LCFAs inhibit the Na\(^+\)\-K\(^+\)-ATPase (Oishi et al. 1990). These effects of LCFAs may be directly on ion channels, or through the actions of metabolic intermediates. Because there is a reciprocal relationship between glucose and fatty acid metabolism (Randle 1998), flux through these metabolic pathways may interact to regulate neuronal activity in the ARC.

We recently characterized the glucose sensitivity of glucosensing neurons (GSNs) in the ARC and ventromedial hypothalamic nucleus (VMN). We found that the neuronal activity of both glucose-excited (GE) and glucose-inhibited (GI) neurons is finely tuned to changes in extracellular glucose levels within the physiological range (Silver and Erecinska 1998; Song and Routh 2005; Wang et al. 2004). GSNs are also modulated by hormones (e.g., insulin), as well as other central fuels (e.g., lactate) (Silver and Erecinska 1998; Song and Routh 2005). The present study was designed to test the hypothesis that the LCFA, oleic acid (OA), would modulate the activity of ARC neurons. Furthermore, because there is evidence for an interaction between glucose and fatty acid metabolism (Randle 1998), we hypothesize that the effects of OA will be dependent on extracellular glucose concentrations independent of whether ARC OA-sensing neurons are also GSNs. To test these hypotheses, we used in vitro patch-clamp recordings in ARC brain slices as well as in vivo extracellular recordings within the ARC to test the effects of OA on the activity of ARC neurons.

METHODS

In vitro studies

PREPARATION OF BRAIN SLICES. The experimental protocol was approved by the institutional animal care and use committee at the New Jersey Medical School, Newark, NJ. Male 14- to 21-day-old Sprague–Dawley rats were obtained from colonies at the VA Medical Center in East Orange, NJ. Animals were housed with their dams on a 12-h light/dark cycle at 22–23°C and fed with unrestricted access to low-fat diet (Purina rat chow #5001) and water. On the day of the experiment, rats were anesthetized with ketamine/xylazine (80:10 mg/kg, administered intraperitoneally) and transcardially perfused with ice-cold oxygenated (95% O\(_2\)-5% CO\(_2\)) perfusion solution composed of the following (in mM): 2.5 KCl, 7 MgCl\(_2\), 1.25 NaH\(_2\)PO\(_4\), 8 NaHCO\(_3\), 0.5 CaCl\(_2\), 7 glucose, 1 ascorbate, and 3 pyruvate (osmolality adjusted to about 300 mosm with sucrose; pH 7.4) as described previously (Song and Routh 2005). Brains were rapidly removed and placed in ice-cold (slushy) oxygenated perfusion solution. Sections (350 \(\mu m\)) through the hypothalamus were made on a vibratome (Viessolis, Camden Instruments). The brain slices were maintained at 34°C in oxygenated high-Mg\(^2+\)/low-Ca\(^2+\) artificial cerebrospinal fluid (ACSF) composed of the following (in mM): 126 NaCl, 1.9 KCl, 1.2 KH\(_2\)PO\(_4\), 26 NaHCO\(_3\), 2.5 glucose, 9 MgCl\(_2\), and 0.3 CaCl\(_2\) (osmolality adjusted to about 300 mosm with sucrose; pH 7.4) with 0.2 mM 2,3-butanedione monoxime for 30 min and allowed to come to room temperature. Slices were then transferred to normal oxygenated ACSF (composition as described above with the following exceptions: 2.4 mM CaCl\(_2\), 1.3 mM MgCl\(_2\)) for the remainder of the day. High-Mg\(^2+\) ACSF solutions (3.1 MgCl\(_2\), 0.3 CaCl\(_2\) or 11 MgCl\(_2\); 2.4 CaCl\(_2\)) were used to block presynaptic input as described previously (Song and Routh 2005; Wang et al. 2004).

ELECTROPHYSIOLOGY. Whole cell recordings in brain slices. Immediately before use, brain slices were placed in an RC-27 chamber (Warner Instruments, Hamden, CT) with a bath volume of 400 \(\mu l\) (Warner Instruments) at 33–34°C. During recording, brain slices were perfused at 6 ml/min with normal oxygenated ACSF. Viable neurons were visualized and studied under infrared differential interference contrast (DIC-IR) microscopy as described previously (Wang et al. 2004). Current- and voltage-clamp recordings (perforated-patch whole cell recording configuration) from neurons in the ARC were made using an Axopatch 1D amplifier (Axon Instruments), low-pass filtered at 1 kHz, and monitored using Axoscope software (Axon). Data were simultaneously digitized at 5 kHz (Digidata 1320A, Axon Instruments) and analyzed using pClamp9 software (Axon). The junction potential between the patch pipette and the bath solutions was neutralized before the gigaohm seal formation. Borosilicate pipettes (1.5–3.5 MΩ; Sutter Instruments) were filled with an intracellular solution containing (in mmol/l): 128 K-glucuronate, 10 KCl, 10 KOH, 10 HEPES, 4 MgCl\(_2\), 0.05 CaCl\(_2\), 0.5 EGTA, 2 Na\(_2\)ATP, and 2 Lucifer yellow, pH 7.2; osmolality was adjusted to 290–300 mosm with sucrose. Amphotericin was also included in the patch pipette (final concentration: 240 μg/ml; stock 60 mg/ml DMSO). Membrane potential and action potential frequency (APF) were allowed to stabilize for 10–15 min after the formation of a gigaohm seal. Only neurons with access resistance <40 MΩ were used. No differences in glucose or OA sensitivity were observed between standard and perforated patch recording configurations. Glucose and other chemicals (Sigma Chemicals) were added to the ACSF perfusion solution as described in the figures. Input resistance (IR) was calculated from the change in membrane potential in response to small 500-ms hyperpolarizing pulses (−10 or −20 pA) given every 3 s as described previously. The reversal potential was calculated from the change in membrane current in response to voltage steps from −120 to 0 mV from a holding potential of −60 mV. Steady-state currents were determined by measuring data points within the last 5 ms of the 200-ms voltage pulses. Neurons were defined as glucose or OA sensing if they altered their membrane potential, APF, and/or IR to glucose or OA, respectively. Data were analyzed using a Student’s t-test with P < 0.05 considered significant.

In vivo studies

ANIMALS. The experimental protocol was approved by the institutional animal care and use committee at the University of Paris 7. Two-month-old male Sprague–Dawley rats were obtained from Charles Rivers (Lyon, France). Rats weighed 230 ± 8 g. They were housed individually in stainless steel cages in a room maintained at 24 ± 3°C, with lights on from 7:00 AM to 7:00 PM, and had free access to water. Two series of experiments were performed at the University of Paris 7 either with fed (n = 20 rats) or overnight-fasted rats (n = 15 rats).

GENERAL SURGICAL PROCEDURES. Rats were anesthetized with 50 mg/kg of pentobarbital sodium (Roche et Dessingue, Vincennes, France). A thin catheter (OD 0.7 mm) was inserted into the left carotid artery toward the brain. The rats were then placed in a stereotaxic apparatus and a midline incision was made. The skull was drilled at 3.1 mm posterior to the Bregma point on the midline. The dura was removed to permit electrode insertion. ARC stereotaxic coordinates were obtained according to the Paxinos stereotaxic atlas.

MULTIUNIT RECORDING. ARC multiunit recordings were made using a monopolar platinum electrode. Action potentials were displayed and saved on a computer after initial amplification through a low-noise amplifier (BIOAmplifier, AD Instrument, Rabalot, France). Data were digitized with digitizer PowerLab/4sp. Signals were amplified 10⁵ and filtered at low- and high-frequency cutoff of 100 and 1,000 Hz, respectively, and monitored with computer program Chart 4. Multiunit recordings were made in response to a single intracarotid injection (200 μl) of either saline or OA (35 μM, Sigma, Paris, France) in the same rat. Baseline unit activity was recorded for 10 min before infusion of a compound.
RESULTS

In vitro experiments

Perforated-patch whole cell recording techniques were used to characterize 114 neurons from 53 rats in the lateral ARC with respect to their responsiveness to OA. Of these, 94 were evaluated for OA responsiveness in the presence of 2.5 mM glucose and 20 for OA responsiveness in the presence of 0.1 mM glucose. Three distinct subtypes of OA-sensing neurons were found under these conditions. These three subtypes of OA neurons were divided into two major categories: oleic acid excited (OAE) and oleic acid inhibited (OAI). The OAI neurons were further subdivided as OAI_{2.5} or OAI_{0.1} according to whether they were observed in 2.5 or 0.1 mM glucose. As implied from the preceding statement, the response to OA was dependent on extracellular glucose level. That is, in 2.5 mM glucose 12 of 94 (13%) ARC neurons were defined as OAE neurons when 2 μM OA was added to the perfusate, whereas six (6%) were OAI_{2.5} neurons. In contrast, when extracellular glucose levels were lowered to 0.1 mM, six of 20 (30%) ARC neurons were defined as OAI_{0.1} when 2 μM OA was added to the perfusate, whereas none was excited under these conditions. Thus OAE and OAI_{2.5} neurons were observed only in the presence of 2.5 mM glucose, whereas OAI_{0.1} neurons were found only when extracellular glucose levels were lowered to 0.1 mM. Eight of the 24 OA-sensing ARC neurons were evaluated for their response to OA in both 2.5 and 0.1 mM glucose. Of these, each neuron responded to OA in either 2.5 or 0.1 mM glucose, but none responded to OA in both glucose concentrations, suggesting that these three subtypes of OA-sensing neurons are distinct. Finally, only a very small percentage of lateral ARC neurons (three out of 23) changed their action potential frequency in response to both OA and glucose. That is, out of six ARC neurons identified as GE (excited when glucose levels increase from 0.1 to 2.5 mM), only three were also OA sensing and of these two were of the OAI_{0.1} subtype and the last was of the OAE subtype. Furthermore, out of 11 ARC neurons defined as OAE, only one was GE. Finally, out of seven ARC neurons defined as OAI_{0.1} only two were GE. None of the categories of OA-sensing neurons contained neurons defined as GI neurons (those that are inhibited when glucose increases from 0.1 to 2.5 mM). These subtypes of OA-sensing neurons are further described below and shown in Figs. 1–5. In these figures, the action potentials were truncated and the timescale greatly compressed. This procedure provides an overview of the basic firing pattern of a neuron in response to various compounds. Final classification of a cell into one of several categories was made using this data set (plotted on an expanded timescale).

FIG. 1. A: whole cell current-clamp recordings in an oleic acid excited (OAE) neuron in 2.5 mM glucose (traces are not consecutive). Resting membrane potential indicated by the dotted line is noted to the right of each trace in this and in Figs. 2 through 5. Similarly, downward deflections represent the membrane voltage response to a constant hyperpolarizing pulse. Action potentials were truncated, and the timescale was compressed in this and subsequent figures (2–5). Oleic acid (OA, 2 μM) reversibly depolarized and increased action potential frequency (APF) and input resistance (IR, top). Changes in glucose had no effect (bottom). B: single action potential from the trace in A drawn in an expanded time frame to illustrate standard shape and amplitude. C: voltage–current relations indicate that the OA-sensitive conductance reverses at about −50 mV. For this and Figs. 2 through 5, the vertical scale bar represents 20 mV and the horizontal scale bar represents 1 min.

OAE neurons

The addition of 2 μM OA in the presence of 2.5 mM glucose increased the APF of OAE neurons by 145 ± 25% (n = 12; P < 0.05). This is illustrated for one OAE neuron in Fig. 1A, top trace. A change in extracellular glucose from 2.5 to either 0.1 or 10 mM had no effect on 11 of 12 OAE_{2.5} neurons (Fig. 1A, bottom trace). The latency of the OA effect was 220 ± 25 s. Input resistance was increased by 21 ± 5% (n = 10; P < 0.05), indicating that OA closed an ion channel. The reversal potential for the OA effect was −59 ± 4 mV (n = 7; Fig. 1B), close to the theoretical reversal potential for Cl\. The effect of OA persisted in high-Mg^{2+} ACSF, which blocks synaptic transmission (n = 5), suggesting that the OA effect was direct (example in Fig. 2).

OAI_{2.5} neurons

OA decreased APF of these OA-sensing neurons by 71 ± 14% (n = 6; P < 0.05). This is illustrated for one OAI_{2.5} neuron in Fig. 3, top trace. The inhibitory effect of OA did not reverse during the 10-min experimental period for five out of six OAI_{2.5} neurons studied (example in bottom trace). Subsequent exposure to 0.1 mM glucose had no effect (bottom trace). The response to OA persisted in high-Mg^{2+} ACSF for the one OAI_{2.5} neuron that showed a washout of the OA effect, suggesting that at least in this neuron the effect of OA was direct. For the OAI_{2.5} neurons, there were no consistent changes either in reversal potentials or in IR, suggesting that multiple mechanisms are involved in the effects of OA on these neurons.

J Neurophysiol • VOL 95 • MARCH 2006 • www.jn.org
that the OAI2.5 and OAI0.1 subtypes of OA-sensing neurons are the effects of OA differ depending on whether the OAI 0.1 GE neurons (example in Fig. 4, OAI2.5 neurons were regulated by changes in extracellular glucose levels. That is, the addition of OA in the presence of 0.1 mM glucose caused a further decrease in APF, hyperpolarized the membrane potential, and increased IR of both of the GE neurons. The effect of OA on both of these GE neurons reversed at -43 mV and washed out after return to ACSF. In contrast, although OA also decreased the APF and hyperpolarized the membrane potential in 0.1 mM glucose in the non-GE neurons, the inhibitory effect did not wash out in four out of five of these neurons. The response to OA persisted in high-Mg2+ ACSF for the one OAI0.1 neuron that showed a washout of the OA effect, suggesting that at least in this neuron the effect of OA was direct. Like OAI2.5 neurons, there were neither consistent changes in reversal potentials nor changes in IR, suggesting that multiple mechanisms are involved in the effects of OA on the non-GE OAI0.1 neurons. Finally, OAI0.1 neurons responded to OA in the presence of 0.1 mM glucose but not 2.5 mM glucose (n = 6; example in Fig. 5), indicating that the OAI2.5 and OAI0.1 subtypes of OA-sensing neurons are distinct.

In vivo experiments

In fed rats, whose plasma glucose levels were 6.2 ± 0.3 mM, both excitatory and inhibitory changes in ARC neuronal activity were observed. That is, the number of discharges per second in the ARC was increased by 75 ± 8% in seven (35%) and decreased by 45 ± 6% in three (15%) of the 20 fed rats tested in response to OA infusion (examples in Fig. 6). In contrast, in rats that were fasted overnight (plasma glucose 3.8 ± 0.4 mM) the number of discharges per second in the ARC in response to OA infusion was decreased by 52 ± 7% in three (20%) of the 15 rats (example in Fig. 7). The discharge rate was not observed to increase in the ARC in response to OA in any of the 15 fasted rats.

**DISCUSSION**

Over the past 5 yr it has become apparent that pharmacological manipulation of hypothalamic fatty acid signaling alters the regulation of glucose and energy homeostasis (Clement et al. 2002; Cruciani-Guglielmacci et al. 2004; Kim et al. 2004; Lam et al. 2005; Loftus et al. 2000; Miller et al. 2004; Obici et al. 2003; Thupari et al. 2002; Tu et al. 2005). There was minimal overlap between OA-sensing neurons and GSNs. However, the present results indicate that there is a direct interaction between fatty acid-sensing and glucose metabolism on the regulation of ARC neuronal activity. We show here that the LCFA OA regulates the activity of distinct populations of ARC neurons. Like GSNs, there are both OAE and OAI neurons. As we hypothesized, the ability of ARC neurons to sense OA is dependent on extracellular glucose levels. That is, using in vivo recordings in fed rats or whole cell patch-clamp recordings in brain slices exposed to 2.5 mM extracellular glucose, we observed both excitatory and inhibitory changes in neuronal activity. However, in fasted rats or when extracellular glucose levels were lowered to 0.1 mM, only inhibitory responses were observed. Finally, the neurons that were inhibited by OA in 0.1 mM glucose were not responsive to OA in 2.5 mM glucose. This led us to designate them as OAI1.5 and OAI0.1 neurons, indicating that they are separate subpopulations of ARC OA-sensing neurons. The observation that their basic electrophysiological properties in 2.5 mM glucose differ, as shown in Table 1, provides further support for the presence of three distinct populations of OA-sensing neurons.

The fact that similar subtypes of OA-sensing neurons were obtained in the in vitro brain slices and the in vivo multiunit recordings in fed and fasted rats is very important in terms of the physiological relevance of these studies. Here, we show that in fed rats an overall excitation in response to OA infusion was observed in the ARC in seven of 20 rats, whereas an overall inhibition was seen in three of the 20 rats. Similarly, in

**FIG. 2.** A: whole cell current-clamp recordings in an OAE neuron in 2.5 mM extracellular glucose. Excitatory effect of 2 μM OA (top) persisted in high-Mg2+ artificial cerebrospinal fluid (ACSF), which blocks presynaptic input (bottom). B: single action potential from the trace in A drawn in an expanded time frame to illustrate standard shape and amplitude.

**FIG. 3.** A: consecutive whole cell current-clamp recordings in an oleic acid inhibited (OA) neuron in 2.5 mM extracellular glucose (OAI1.5). OA (2 μM) hyperpolarized and decreased the APF. This effect persisted after the washout of OA (top). There was no response to decreased glucose (bottom). B: single action potential from the trace in A drawn in an expanded time frame to illustrate standard shape and amplitude.
brain slices exposed to 2.5 mM glucose (analogous to 6 mM glucose in the periphery of fed rats; Silver and Erecinska 1998), we found both OAE versus OAI neurons when OA was added to the perfusate. In contrast, in the fasted rats (where VMH glucose has been shown to be significantly decreased; DeVries et al. 2003), an inhibition of ARC neuronal activity was observed in three of the 15 rats, whereas increased neuronal activity in response to OA was never observed in these animals. Similarly, only OAI neurons were observed in low glucose in vitro. Finally, although there are no studies in the literature that describe the exact range of concentrations for OA in the brain, there is precedence in the literature for the use of 2 mM OA in our studies. OA concentration varies from 0.2 to 1.2 mM in cortical superfusates (Phillis et al. 1999). Furthermore, the minimum OA concentration for activation of astrocytic aminopeptidases was between 1 and 10 μM (Ramirez-Exposito et al. 2001). Thus we felt that using a 2-μM concentration of OA was reasonable. The similarity between the changes in ARC neuronal activity observed in fed and fasted animals in response to OA and the types of OA-sensing neurons observed in brain slices in moderate and low glucose provides strong support for the physiological relevance of the subtypes of OA-sensing neurons described herein using this in vitro paradigm.

The mechanisms by which OA sensing occurred were varied. OA excitation of OAE neurons was associated with an increase in IR, which reversed at the theoretical Cl\(^{-}\) equilibrium potential, suggesting that the effect was mediated by closure of a chloride channel. Although reversal potential is only suggestive of the nature of the conductance involved, these data are consistent with those showing that OA rapidly (within 2 min) inhibits volume-sensitive Cl\(^{-}\) conductance in gastric myocytes (Xu et al. 1997). Interestingly, Best et al. (1997, 2004) suggest that this channel plays a role in the metabolic sensitivity of the pancreatic α- and β-cells. Finally, the latency of the effect of OA on OAE neurons was signifi-

**FIG. 4.** A: consecutive whole cell current-clamp recordings in a glucose-excited (GE)–OAI neuron in 0.1 mM glucose (GE-OAI\(_{0.1}\)). Decreasing glucose hyperpolarized and decreased APF and IR (top). OA (2 μM) further hyperpolarized and decreased APF and IR (top). OA effect washed out in 0.1 mM glucose alone (evidenced by increased APF in 0.1 mM glucose), indicating that the inhibitory affect of OA did not arise from glucose deprivation (bottom). APF recovered in 2.5 mM glucose (bottom). B: single action potential from the trace in A drawn in an expanded time frame to illustrate standard shape and amplitude. C: voltage–current relations indicate that the OA-sensitive conductance reverses at −43 mV.

**FIG. 5.** A: consecutive whole cell current-clamp recordings in a nonglucosensing (NG)–OAI neuron in 0.1 mM glucose (NG-OAI\(_{0.1}\)). Decreasing glucose had no effect (top). However, 2 μM OA caused hyperpolarization and decreased APF (middle). This effect washed out very slowly, as seen in the bottom trace recorded approximately 30 min after OA washout. OA did not inhibit this neuron in 2.5 mM glucose (bottom). B: single action potential from the trace in A drawn in an expanded time frame to illustrate standard shape and amplitude.
To determine the precise mechanisms mediating OA’s effects on neuronal activity.

The mechanisms by which OA may regulate neuronal activity are not fully understood. OA may affect a plethora of ion channels and pumps (Branstrom et al. 2004). It is possible that the KATP channel in pancreatic β-cells (Branstrom et al. 2004). The KATP channel in the GE neurons was maximally open in 0.1 mM glucose and thus could not be opened further by OA. Extracellular glucose levels may also explain the contradiction between our results and reports that OA causes nonspecific neuronal activation (Takahashi et al. 2004) and increases in presynaptic glutamate activity (Landree et al. 2004). Both of these studies were performed in the presence of 10 mM extracellular glucose. Thus it is likely that their results would differ significantly from the present study performed in much lower glucose levels.

The present study suggests that an interaction between glucose and fatty acid metabolism regulates neuronal activity. This interaction is clearly not simply summation of ATP because the OA 

The mechanisms by which OA may regulate neuronal activity are not fully understood. OA may affect a plethora of ion channels and pumps (Branstrom et al. 2004; Honen et al. 2003; Oishi et al. 1990; Oz et al. 2005; Tewari et al. 2000; Witt and Nielson 1994) and OA interacts with the G-protein–coupler receptor GPR40 (Fujiwara et al. 2005). Moreover, fatty acid metabolism alters the levels of AMP-kinase (AMPK) as well as cellular redox potential (Landree et al. 2004), both of which are capable of regulating ion channels and neuronal activity (Hallows et al. 2000; Hepp et al. 2005; Lui and Gutterman 2002). Interestingly, the two GE-OA 

In contrast, in neurons that have substantial levels of FAS, malonyl CoA would be quickly used for fatty acid synthesis and would not accumulate in the cell (Kim et al. 2002). Thus under physiological conditions, malonyl CoA may not play a critical role as a fuel sensor in the brain. However, this does not negate the Randle hypothesis concerning reciprocity between glucose and fatty acid metabolism, or that other sites of interaction between these pathways may serve as fuel sensors. For example, other sites of interaction between glucose and fatty acid metabolism include changes in AMPK and/or redox potential, both of which regulate ion channel conductance (Hepp et al. 2005; Liu and Gutterman 2002). The observation that FAS inhibition by C75 regulates energy homeostasis seems to support the malonyl CoA hypothesis (Loftus 2000). However, recent data from this group indicate that C75 also directly stimulates CPT1 and thus β-oxidation. This results in dramatic changes in cellular AMPK and ATP levels in primary cultures of cortical neurons (Landree et al. 2004). CPT1 stimulation does not occur by displacement of malonyl CoA binding (Yang et al. 2005). Clearly, further studies are needed to determine the precise mechanisms mediating OA’s effects on neuronal activity.

Finally, the reason that it would be advantageous to have evolved multiple populations of OA- and glucose-sensing neurons is a matter for speculation. One might hypothesize that excited and inhibited neurons exist for each nutrient because...
different systems need to be activated to compensate for nutrient excess and deficit, respectively. The more interesting question relates to the glucose dependency of the OA-sensing neurons. We have recently demonstrated glucose-dependent effects of insulin (Wang et al. 2004). It is our hypothesis that this will be the case for many other hormones (e.g., leptin) or neuropeptides (e.g., NPY or POMC), which are involved in the regulation of food intake and energy balance, suggesting that overall neuronal activity within the ARC, a critical region for energy homeostasis, reflects a balance of all indices of peripheral and central energy status. It stands to reason that the brain would perform very differently under the different metabolic conditions associated with the fed and fasted states, or during obesity and/or diabetes. To fully understand the role of the ARC in the regulation of energy balance our data suggest that it is crucial to evaluate the effects of hormones and peptides involved in energy regulation under a variety of conditions that mimic these metabolic states.

In conclusion, OA directly regulates the activity of three distinct subpopulations of ARC neurons both in vivo and in vitro. Although there is little overlap between GSNs and OA-sensing neurons, the effects of OA are dependent on ambient glucose levels. Thus our data suggest that glucose and OA metabolism interact in the ARC to produce an integrated regulation of energy balance. This is consistent with the observation that the counterregulatory response (CRR) to hypoglycemia is reduced in the presence of increased circulating lipids (Evans et al. 1998). Thus our findings provide a further mechanism by which the altered glucose and fatty acid levels in diabetes may cause the dysfunctional central regulation of glucose homeostasis seen in this disease. Finally, although the focus of this study was the ARC, we do not wish to rule out contributions from other brain regions. Clearly the hindbrain is important to glucose sensing (Sanders and Ritter 2000). Moreover, although C75 altered c-Fos expression in the forebrain after 24 h, acute (3 h) increases were noted in hindbrain regions (Miller et al. 2004). Furthermore, although the CRR was reduced, the cognitive deficits in response to hypoglycemia were unaffected by circulating lipids (Evans et al. 1998). This suggests regional differences between glucose sensing related to the CRR and that related to cognitive dysfunction and the development of hypoglycemia unawareness. This further supports the involvement of multiple brain regions in the overall nutrient-sensing network in the CNS.

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