Characteristics and mechanisms of hypothalamic neuronal fatty acid sensing

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1Neurology Service, Department of Veterans Affairs Medical Center, East Orange, New Jersey; 2Centre National de la Recherche Scientifique-University of Paris Diderot, Paris, France; and 3Department of Neurology and Neurosciences, New Jersey Medical School, Newark, New Jersey

Le Foll C, Irani BG, Magnan C, Dunn-Meynell AA, Levin BE. Characteristics and mechanisms of hypothalamic neuronal fatty acid sensing. Am J Physiol Regul Integr Comp Physiol 297: R655–R664, 2009. First published June 17, 2009; doi:10.1152/ajpregu.00223.2009.—We assessed the mechanisms by which specialized hypothalamic ventromedial nucleus (VMN) neurons utilize both glucose and long-chain fatty acids as signaling molecules to alter their activity as a potential means of regulating energy homeostasis. Fura-2 calcium (Ca2+) and membrane potential dye imaging, together with pharmacological agents, were used to assess the mechanisms by which oleic acid (OA) alters the activity of dissociated VMN neurons from 3- to 4-wk-old rats. OA excited up to 43% and inhibited up to 29% of all VMN neurons independently of glucose concentrations. In those neurons excited by both 2.5 mM glucose and OA, OA had a concentration-dependent effective excitatory concentration (EC50) of 13.1 nM. Neurons inhibited by both 2.5 mM glucose and OA had an effective inhibitory concentration (IC50) of 93 nM. At 0.5 mM glucose, OA had markedly different effects on these same neurons. Inhibition of carnitine palmitoyltransferase, reactive oxygen species formation, long-chain acetyl-CoA synthetase and ATP-sensitive K+ channel activity or activation of uncoupling protein 2 (UCP2) accounted for only 40% of OA’s inhibitory effects. Inhibition of CD36, a fatty acid transporter that can alter cell function independently of intracellular fatty acid metabolism, reduced the effects of OA by up to 45%. Thus OA affects VMN neuronal activity through multiple pathways. In glucosensing neurons, its effects are glucose dependent. This glucose-OA interaction provides a potential mechanism whereby such “metabolic sensing” neurons can respond to differences in the metabolic states associated with fasting and feeding.

MATERIALS AND METHODS

Animals. All experiments were reviewed and approved by the Animal Care and Use Committee of the East Orange Department of Veterans Affairs Medical Center and were in compliance with the guidelines of the American Physiological Society (2). Male 3- to 4-wk-old Sprague-Dawley rats (Charles River Labs) were housed at 23–24°C on a 12:12-h light-dark cycle (lights on at 0700). Food (Purina rat chow no. 5001) and water were available ad libitum.

Preparation of dissociated VMN neurons and expression of fatty acid-sensing candidate mRNAs. The VMN was punched out of slices made through the ventrobasal hypothalamus, and single VMN neurons were dissociated by papain digestion and trituration. The freshly dissociated neurons were then lysed and analyzed by real-time quantitative PCR as previously described (21, 22, 34). Primer sets for each of the candidate mRNAs are provided in Table 1. Data are expressed as the ratio of the standardized amount of the gene of interest to the standardized amount of cyclophilin.

Measurement of glucose- and oleic acid-induced changes in intracellular Ca2+ concentration in dissociated VMN neurons. Evaluation of glucose-induced alterations in intracellular calcium concentration ([Ca2+]i) oscillations in individual VMN neurons derived from bilateral VMN punches was assessed with fura-2 acetoxymethyl ester (Molecular Probes, Eugene, OR) as previously described (22, 23). Neurons were classified as glucose excited (GE), glucose inhibited (GI), and nonglucosensing (NG) with previously established criteria for glucose-induced changes in [Ca2+]i, area under the curve (AUC) (22, 23), with [Ca2+]i, defined by the ratio of 340- to 380-nm emissions for fura-2. The lipophilic, anionic bis-oxonol dye from the fluorometric imaging plate reader (FLIPR) membrane potential assay kit (Molecular Devices, Sunnyvale, CA) was used in dissociated VMN neurons to assess OA- and glucose-induced changes in membrane potential as previously described (21).

Criteria for quantitating changes in [Ca2+]i, fluctuations in response to oleic acid. All experiments began with neurons held at 2.5 mM glucose unless otherwise specified. Changes in [Ca2+]i, fluctuations in response to glucose, OA, and/or drugs were assessed over 10-min periods after addition of each substance. Significant changes in [Ca2+]i, fluctuations were determined by first calculating the integrated AUC for every 10-min period with Origin 7.0 software (OriginLab, Northampton, MA). The neurons were then classified as OA excited

insulin secretion whereby long-chain acyl-CoA activates the ATP-sensitive K+ (KATP) channel to hyperpolarize the pancreatic β-cell (9). Additional postulated mechanisms for fatty acid sensing include inhibition of carnitine palmitoyltransferase I (CPT I) by malonyl-CoA with buildup of long-chain acyl-CoA intracellularly (28) or the production of reactive oxygen species (ROS) (5). In the present studies, we utilized calcium and membrane potential dye imaging to explore the mechanisms by which dissociated VMN neurons utilize oleic acid (OA) and physiological concentrations of hypothalamic glucose (11, 50) as a potential means of sensing and regulating energy homeostasis in the body.

DETECTION OF SUBSTRATES such as glucose and fatty acids allows the brain to modulate energy intake and expenditure and peripheral metabolic function as a means of controlling overall energy homeostasis. The hypothalamus is a major site of such functions (21, 28, 33, 48). In general, glucose is the primary energy substrate for neuronal metabolism (51), but specialized neurons in areas such as the ventromedial hypothalamic nucleus (VMN) also utilize glucose as a signaling molecule to regulate their activity (32, 35, 44). However, some hypothalamic neurons also utilize long-chain fatty acids as signaling molecules (39, 43, 56). In vivo, the ability of hypothalamic neurons to sense fatty acids affects insulin secretion, hepatic glucose production, and food intake (8, 39, 42). Obici et al. (42) hypothesized that the mechanism of neuronal fatty acid sensing was similar to the inhibitory effects of fatty acids on

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http://www.ajpregu.org
### Table 1. Sequences of primer pairs, their location in sequences cited in GenBank, and relative abundance of their mRNA transcripts compared with cyclophilin

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<td>NM103344</td>
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<td>0.10</td>
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**Data are means ± SE of triplicate determinations expressed relative to the amount of the mRNA expression of the housekeeping gene cyclophilin. Freshly dissociated ventromedial hypothalamic nucleus (VMN) neurons were harvested for quantitative real-time PCR.**

### Results

**Oleic acid-induced changes in [Ca\(^{2+}\)], oscillations of VMN neurons held at 2.5 mM glucose.** We first determined that the responses to OA were reversible at concentrations at or below 50 nM (data not shown). OA caused excitation (OAE; Fig. 1, A and C), inhibition (OAI; Fig. 1, B and D), or a biphasic response (OAB; data not shown) as defined above. Dissociated neurons (481) from 15 rats were analyzed at OA concentrations from 0.5 nM to 2 μM in the presence of 2.5 mM glucose. When considered independently of their glucosensing status, 43% were...
OAE, 29% were OAI, and 11% were OAB (Table 2). In an additional 234 neurons evaluated at 0.5 mM glucose, the percentages of OAE (39%) and OAI (22%) neurons were similar to those found at 2.5 mM glucose (Table 2). No biphasic neurons were identified in this group since they were tested at only a single concentration of OA (15 nM). Thus, when all VMN neurons were considered, 72% responded to OA with either excitation or inhibition at 2.5 mM glucose, while 61% responded at 0.5 mM glucose.

Next, the subset of glucosensing (GE and GI) VMN neurons was evaluated for their responses to OA at 2.5 and 0.5 mM glucose (Fig. 1, Table 2). Of the GE neurons held at 2.5 mM glucose (17% of the total tested), 35% were further excited in a concentration-dependent manner from 0.1 to 50 nM OA, with a threshold of 0.1 nM and a calculated EC50 of 13.1 nM ($R^2 = 0.89$; Fig. 2, A and B). The percentage of OAE neurons declined at OA concentrations above 50 nM and then increased in a concentration-dependent manner again from 100 to 500 nM (Fig. 2A). Above 500 nM OA, there was a 50% decrease in the percentage of neurons responding at 1 nM and no neurons responded to 2 nM OA (Fig. 2A). While OA concentrations up to 1 nM inhibited $[Ca^{2+}]_{i}$ oscillations in 38% of GE neurons held at 2.5 mM glucose, this response did not exhibit a concentration-dependent relationship (data not shown).

GI neurons (10% of the total tested) were also assessed for their sensitivity to OA at 2.5 mM glucose (Fig. 1, C and D), a concentration at which their activity is primarily inhibited (21). In 10% of these GI neurons, OA further reduced $[Ca^{2+}]_{i}$ oscillations in a concentration-dependent fashion, with a calculated IC50 of 93 nM ($R^2 = 0.88$), a threshold at 0.5 nM, and a maximal inhibition at 500 nM (Fig. 2, C and D, Table 2). No neurons were inhibited at or above 1 μM OA. On the other
Hand, while OA excited 68% of GI neurons held at 2.5 mM glucose, it did not do so in a concentration-dependent manner (data not shown).

Overall, GE, GI, and NG neurons differed significantly in their responsiveness to OA at 2.5 mM glucose ($\chi^2 = 17.06$, $P = 0.0019$; Table 2). The major difference was that GI neurons were predominantly excited by OA whereas GE neurons were almost equally excited and inhibited ($\chi^2 = 6.83$, $P = 0.009$; Table 2). Similarly, at 0.5 mM glucose there were significant differences among GE, GI, and NG neurons in their responses to OA ($\chi^2 = 20.85$, $P = 0.0003$; Table 2). However, in this case, GE neurons were predominantly excited while GI neurons were both excited and inhibited by OA, although these specific differences did not reach statistical significance. In summary, ambient glucose concentrations significantly affected the responses to OA only in glucosensing neurons.

**Comparison between OA-induced changes in [Ca$^{2+}$]**. Since it is possible that OA might alter [Ca$^{2+}$], oscillations with fura-2 calcium imaging without necessarily altering membrane potential or neuronal activity, 294 VMN neurons were also imaged with FLIPR membrane potential dye for their responses to 15 nM OA in the presence of 2.5 mM glucose (Fig. 3). Of these, 29% were OAE and 15% were OAI compared with 43% OAE and 29% OAI with calcium imaging. Therefore the relative proportions of OAE to OAI neurons identified by the two techniques were roughly comparable, while the differences in absolute percentages between the two techniques were most likely due to the reduced sensitivity and increased baseline noise of FLIPR dye images.

**Fatty acid sensing candidates.** Table 1 lists the various mRNAs for enzymes and transporters known to be involved in fatty acid metabolism in peripheral organs, as well as those utilized for neuronal glucosensing. Although all of the mRNA values are expressed relative to cyclophilin, these relative values are not comparable because of differences in amplification efficiency. Of all the candidate molecules assessed, only G protein-coupled receptor 40 (GPR40) was not detected in these neurons. Of particular note, VMN neurons expressed mRNA for two fatty acid transporters (fatty acid transport protein 1 and CD36), four variants of long-chain fatty acyl-CoA synthetase and CPT Ia and c, as well as enzymes involved in fatty acid synthesis from glucose (fatty acid synthetase, malonyl-CoA decarboxylase, and AMP kinase). Finally, mRNA for UCP2, a protein involved in ROS production (3), was also expressed in these neurons.

**Role of CPT I in mediating oleic acid effects.** In 1,017 VMN neurons held at 2.5 or 0.5 mM glucose and exposed to 15 nM OA, inhibition of CPT I by etomoxir had a small (11–21%) effect on OA neurons (Fig. 4) but did not affect OA responses in OAE neurons at 2.5 mM glucose (Table 3). On the other hand, etomoxir did decrease the excitatory effects of OA in a small (7%) proportion of OAE neurons at 0.5 mM glucose (Table 3).

**Role of ROS production in mediating effects of glucose and fatty acids on VMN neuronal activity.** Since supraphysiological glucose levels increase ROS production and alter the glucose responsiveness of hypothalamic neurons (30), we first evaluated the effects of ROS scavenging with Trolox on neuronal glucosensing within the physiological range of 0.5–2.5 mM glucose (21, 50). Of 352 VMN neurons, 12% were GE, 8% were GI, and 80% were NG. While there was no significant effect of ROS scavenging on GE neurons at 2.5 mM glucose, Trolox increased the activity of GI neurons by 47% ($P < 0.05$; data not shown). This suggests that at least half of the inhibitory effect of glucose in GI neurons is due to the generation of ROS.

Next, VMN neurons were held at either 2.5 or 0.5 mM glucose and assessed for the effects of ROS scavenging on responses to 15 nM OA. At 2.5 mM glucose, 28% of 315 neurons were OAE and 17% were OAI. ROS scavenging inhibited OA-induced excitation in 10% of OAE and reduced the inhibitory effect of OA in 19% of OAI VMN neurons (Table 3). While lowering glucose to 0.5 mM had no appreciable effect on the response to OA plus Trolox in OAE neurons, it did ablate the inhibition of OAI neurons seen at 2.5 mM glucose (Table 3). To further assess the potential role of ROS production, genipin, a UCP2 inhibitor that should inhibit ROS production (46, 57), was assessed in the presence of 2.5 mM glucose and 15 nM OA (Table 3). Although the percentages were somewhat different, genipin had the same general effects as Trolox on OAE and OAI neurons.

**Role of long-chain acyl-CoA formation and $K_{ATP}$ channel activity in mediating oleic acid’s effects.** As shown by calcium imaging, inhibition of acyl-CoA synthetase with triacsin C reduced the activating effects of OA only 18% of the OAE neurons and blocked the inhibitory effects of OA in 27% of OAI neurons ($P < 0.05$ vs. OA; Table 3). To ascertain that the

### Table 2. Effect of glucose and oleic acid on all VMN neurons and on VMN glucose-excited, glucose-inhibited, and nonglucosensing neurons

<table>
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<tr>
<th>Glucose Concentration</th>
<th>% of Total</th>
<th>OAE</th>
<th>OAI</th>
<th>OAN</th>
<th>OAB</th>
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<tr>
<td>2.5 mM Glucose</td>
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</tr>
<tr>
<td>GE</td>
<td>17</td>
<td>35</td>
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</tr>
<tr>
<td>GI</td>
<td>10</td>
<td>68</td>
<td>15</td>
<td>11</td>
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</tr>
<tr>
<td>NG</td>
<td>73</td>
<td>41</td>
<td>28</td>
<td>11</td>
<td>20</td>
</tr>
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Data are mean % of total neurons tested in each glucosensing category. At 2.5 mM glucose, neurons ($n = 481$) were classified by glucosensing categories by alterations in intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]) oscillations as glucose was changed from 2.5 to 0.5 to 2.5 mM. They were then held at 2.5 mM glucose and exposed to 0.5 mM to 2 mM oleic acid concentrations and classified as oleic acid excited (OAE), inhibited (OAI), biphasic (OAB), or nonresponsive (OAN). Other neurons were held at 0.5 mM glucose ($n = 234$) and classified by glucosensing category by sequential exposure to 0.5, 2.5, and 0.5 mM glucose and then characterized by their responses to 15 mM oleic acid in 0.5 mM glucose. GE, glucose excited; GI, glucose inhibited; NG, nonglucosensing; Total, total % of each category of neurons at each glucose concentration, irrespective of their glucosensing properties.

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effect of triacsin C was not due to intracellular calcium release without alterations in membrane potential or to a nonspecific effect of OA, additional studies were performed with FLIPR dye in the presence of triacsin C and OA. These results were essentially similar to those obtained with fura-2 calcium imaging, although 37% of the inhibitory effects of OA on OAI neurons was inhibited by triacsin C with this dye (Table 3). Next, VMN neurons were held at 2.5 mM glucose and exposed to tolbutamide to inactivate K_{ATP} channels in the presence of 15 nM OA. This channel inactivation inhibited the OA-induced excitation in only 8% of OAE neurons but blocked the inhibitory effects of OA in only 25% of OAI neurons (Table 3).

Role of fatty acid transporter CD36 in mediating oleic acid’s effects. Given the large proportion of OA-responsive neurons that were unaffected by inhibition of intracellular fatty acid metabolism, we next inhibited the binding of OA to CD36 with SSO to assess whether its role in VMN fatty acid sensing is similar to that observed in taste receptor cells (13) (Table 3). In fact, CD36 mRNA was present in the total pool of VMN neurons (Table 1) but was expressed at such low levels in single cells as to provide unreliable measures by quantitative real-time PCR (data not shown). We exposed VMN neurons to concentrations of 5 nM to 50 μM SSO and found that only at concentrations at or below 50 nM were there no nonspecific effects on [Ca^{2+}]_{i}. Thus, in VMN neurons held at 2.5 mM glucose, 50 nM SSO inhibited the excitatory effects of 15 nM OA on 46% of OAE and its inhibitory effects on 46% of OAI neurons (Table 3).

Combined pharmacological inhibition of oleic acid’s effects. In addition to inhibiting the individual steps of fatty acid transport and intracellular metabolism, we further assessed the effect of combined inhibition of acetyl-CoA synthetase (triacsin C), ROS production (Trolox), and CD36 (SSO) on the actions of 15 nM OA at 2.5 mM glucose. In 254 VMN neurons assessed at 2.5 mM glucose in the presence of 15 nM OA, 18% of VMN neurons were OAE and 12% were OAI. Of the OAE neurons, the combination of three drugs inhibited 81% of the excitatory actions of OA. Of the OAI neurons, the drug combination inhibited 90% of OA’s inhibitory effects. These data support the contention that the concentrations of drugs used in our studies were sufficient to inhibit and account for the majority of the activity of these three critical steps in fatty acid sensing.

DISCUSSION
The present studies were undertaken to identify the mechanisms by which fatty acids alter neuronal activity and the importance of ambient glucose concentrations in those responses. We assessed neurons in the VMN because of its critical role in the regulation of both energy and glucose homeostasis (12, 21, 22, 31, 44, 52, 54). First, we found that, while up to 43% of all VMN neurons were excited and 29% were inhibited by increasing concentrations of OA, only in glucosensing neurons, which utilize glucose as a signaling

Fig. 2. A: % of glucose-excited VMN neurons in which incremental concentrations of OA from 0.5 nM to 2 μM caused significant increases in AUC above that seen at baseline in 2.5 mM glucose. Each set of neurons of a total of 481 neurons was exposed to 3 different concentrations of OA within this range. B: concentration-response curve calculated for neurons in A for responses to 0.5 to 50 nM OA. EC_{50}, concentration of OA that increased [Ca^{2+}]_{i} fluctuations in 50% of neurons. C: % of glucose-inhibited VMN neurons in which incremental concentrations of OA from 0.5 nM to 2 μM caused significant decreases in AUC below that seen at baseline in 2.5 mM glucose. D: concentration-response curve for neurons in C was calculated for incremental doses of OA (0.5 nM to 2 μM) in the presence of 2.5 mM glucose. IC_{50}, OA concentration that inhibited [Ca^{2+}]_{i} fluctuations in 50% of neurons.
molecule (8), was the responsiveness to OA dependent upon ambient glucose concentrations. This means that these specialized "metabolic sensing" neurons, which were responsive to both OA and glucose (32), should theoretically be able to differentiate between the fed and fasted states. During fasting, fatty acid levels are raised, while they are usually considerably lower after a meal in association with elevated triglyceride levels (25). On the other hand, brain glucose levels are reduced during fasting and raised during feeding (11, 21, 50). Surprisingly, we also found that inhibition of acyl-CoA synthetase, the initiating step in fatty acid metabolism, or of any of the individual downstream steps involving CPT I, ROS formation, or K\textsubscript{ATP} channel activity, only reduced \textasciitilde 20\% of the excitatory and \textasciitilde 40\% of the inhibitory effects of OA on neuronal activity.

These results suggest that at least a portion of the remaining effects of OA might be due to a mechanism that is independent during fasting and raised during feeding (11, 21, 50).
Acid in the presence of (fura-2) and/or membrane potential

Effect of 15 nM of oleic acid and different drugs on freshly dissociated VMN neurons held at 2.5 mM of glucose

- The family consists of six fatty acid transporter proteins that are present in the general pool of VMN neurons. This suggests that fatty acid sensing does not fully inhibit at this concentration and that binding to CD36 is responsible for even more of the nonmetabolic effects of OA.

- We postulate that OA may be responsible for even more of the nonmetabolic effects of OA. Because SSO had nonspecific effects on neuronal function at concentrations >50 nM, we could not use higher doses. Thus it is possible that CD36 binding was not fully inhibited at this concentration and that binding to CD36 is responsible for even more of the nonmetabolic effects of OA. We postulate that binding of OA to CD36 alters neuronal activity in a manner analogous to that utilized for fat perception by taste receptor cells (13, 29). In those cells, long-chain fatty acids bind to CD36. This causes phosphorylation of protein tyrosine kinases, leading to generation of inositol 1,4,5-trisphosphate, recruitment of calcium from the endoplasmic reticulum, followed by influx of calcium via opening of store-operated calcium channels, membrane depolarization, and neurotransmitter release (13). Taken as a whole, our data suggest that VMN metabolic sensing neurons respond to glucose and fatty acid by at least two distinct, largely unrelated mechanisms within the same cell. A large part of fatty acid sensing may be mediated by binding to cell surface receptors with activation of downstream signaling cascades. In fact, while VMN neuronal glycosensing depends primarily on intracellular glucose metabolism and influx of calcium through voltage-dependent calcium channels (32), there is evidence that some glycosensing in the hypothalamus also occurs by mechanisms that do not require intracellular glucose metabolism (7, 40).

- Although this CD36-based mechanism, or one similar to it, may be responsible for the fatty acid sensing in the majority of VMN neurons, at least 20–40% of fatty acid sensing does depend on intracellular fatty acid metabolism. In such cases, transport of OA into the cell may be mediated by one of several fatty acid transporters such as the family of FATP transporters, which are present in the general pool of VMN neurons. This family consists of six fatty acid transporter proteins that are integral membrane proteins linked to acyl-CoA synthetase (17). After entry into the cell, long-chain fatty acids are converted to the acyl-CoA form. Previous studies demonstrated that centrally administered long-chain fatty acids reduced food intake and hepatic glucose production and increased glucose-induced insulin secretion (28, 39, 41, 42, 48). This was postulated to be due to activation of the KATP channel with hyperpolarization of neurons in a manner similar to the way in which long-chain fatty acid CoAs inhibit insulin secretion in pancreatic β-cells (9). However, it is notable that such a mechanism in the β-cells is dependent upon the presence of high glucose concentrations that would promote increased production of malonyl-CoA and accumulation of acyl-CoAs by inhibiting their mitochondrial uptake by CPT 1 (49). Here, inactivation of the KATP channel with tolbutamide blocked 25% of the inhibitory effects of OA on GI neurons, and inhibition of acyl-CoA synthetase antagonized the inhibition by OA in 37% of OAI neurons. Thus production of oleoyl-CoA and its activation of the KATP channel might be particularly important in GE neurons since two times more GE than GI neurons were inhibited by OA at 2.5 mM glucose, where the KATP channel is mainly inactivated, than at 0.5 mM glucose, where the channel is already primarily in the open state.

- In addition to the actions of fatty acids on the KATP channel, ROS formation plays an important role in neuronal fatty acid sensing. At 2.5 mM glucose, up to 20% of the inhibitory and excitatory effects of OA on the general population of VMN neurons and almost 50% of the inhibitory effects of glucose alone on GI neurons were mediated by ROS formation. On the other hand, there was little effect of ROS scavenging on either GE or GI neurons at 0.5 mM glucose, a level comparable to that seen in the hypothalamus during fasting (11). Thus these effects of ROS production should be most physiologically relevant under conditions simulating ingestion of a high-fat, high-carbohydrate meal. In fact, hypothalamic ROS production doubles when peripheral fatty acids are increased by infusions of a triglyceride emulsion (5).

A particular strength of our studies is that, by using dissociated neurons, we were able to assess the effects of OA directly on neurons without the presence of astrocytes. This is particularly important because astrocytes are responsible for...
the majority of fatty acid β-oxidation in the brain and may provide neurons with ketone bodies as an alternate energy source to glucose (14). On the other hand, glucose is the primary energy substrate for neurons (51). Thus, when assayed at 2.5 mM glucose, GE neurons were excited by OA with an EC50 of 13.1 nM, while GI neurons were inhibited by OA with an IC50 of 93 nM. These concentrations are much lower than those used to assess fatty acid sensing in slices of hypothalamic tissue (2 μM) (39, 55); these latter levels are comparable to in vivo levels of fatty acids (36). Although OA is one of the most abundant free fatty acids in the brain (45), the actual extracellular levels of free OA are not known. Thus we cannot be sure how closely our results mimic the levels of OA to which VMN neurons are actually exposed in vivo. However, the fact that there is a clear concentration responsiveness to low levels of OA for both OAE and OAI neurons suggests that these levels may be within their physiological response range. Given the low level at which they do respond, we postulate that, in slice preparations or in vivo, fatty acids would play a primary role as an energy substrate for astrocytes but act more specifically as a signaling molecule to alter neuronal activity in that select group of metabolic sensing neurons that are responsive specifically to fatty acids and glucose.

There is an important caveat to our assumption that the effects of drugs we used on neurons unresponsive to OA were “nonspecific” as regards the specific effects of extrinsic fatty acids on neuronal activity, i.e., fatty acid sensing. For our calculations of a specific effect of each drug, we subtracted the effect of that drug on non-fatty acid-sensing neurons from the comparable effect on OAE or OAI neurons. We believe that this assumption is valid for two reasons. First, scavenging ROS with Trolox reactivated almost half of the GI neurons held at 2.5 mM glucose in the absence of OA. This finding exemplifies the fact that neurons metabolize both glucose and fatty acids in a critically interlinked way. Thus all of the steps we inhibited with drugs, aside from CD36, are also steps by which glucose can both produce fatty acids and alter their intracellular metabolism. Here, we were interested only in how extrinsic fatty acids affect neuronal activity. Therefore, we subtracted out the effects of these drugs on neurons unresponsive to OA. Second, the combined blockade of CD36, acetyl-CoA synthetase, and ROS production accounted for up to 85% of the actions of OA with this same strategy of excluding nonspecific effects of the drugs on non-fatty acid-sensing neurons. Thus we feel that this approach is valid even though it provides only an approximation of the various components of neuronal fatty acid sensing.

A final issue is how to explain the apparent paradox that raising hypothalamic long-chain fatty acid levels, such as occurs during fasting, has been shown to reduce feeding (42). Obviously, such suppression would be counterproductive in a state of negative energy balance. On the other hand, plasma fatty acid levels fall during some meals (18), when fatty acid suppression of feeding would be most appropriate. Nevertheless, fatty acid levels are still maintained at significant levels in the postprandial state, and our finding of hormone-sensitive lipase mRNA in VMN neurons raises the theoretical possibility that triglycerides might provide an additional source of fatty acids to the hypothalamus in the fed state. However, it is likely that differences in free fatty acid and glucose levels during fasting and feeding provide the most direct substrate inputs to regulate the activity of resident hypothalamic metabolic sensing neurons. In addition, hormones such as leptin and insulin, to which these metabolic sensing neurons respond (20, 53), vary markedly between the fed and fasted states and thus provide additional inputs from the periphery to regulate the meal-related activity of VMN neurons involved in the regulation of both energy homeostasis (12, 21, 22, 31, 44, 52, 54) and glucose-induced insulin release (10). Thus the ability of metabolic sensing neurons in the hypothalamus to integrate inputs from a variety of metabolic substrates and hormones from the periphery is a critical attribute that allows them to monitor and potentially to regulate energy and glucose homeostasis in the body.

Perspectives

The finding that some neurons can respond to exogenous fatty acids by altering their activity is not a new one. Unfortunately, because neurons and astrocytes form an integral metabolic unit, it has always been difficult to assess the relative contributions of astrocytes to changes in neuronal activity resulting from addition of exogenous substrates such as glucose and fatty acids in vivo or in vitro. This is a particular problem when assessing the effects of fatty acids because, unlike neurons, astrocytes readily take up and utilize fatty acids as a major source of energy production (14). Also, because neurons make multiple synaptic connections with each other, it is often difficult to determine whether changes in the activity of a given neuron are in direct response or are a postsynaptic response to adding such metabolites. Although there are many caveats to utilizing freshly dissociated neurons to study fatty acid sensing, the major advantage of this method is that it tells us what neurons can do independently of their astrocytic companions. However, the issue of neuronal fatty acid sensing is further complicated by the fact that neurons can produce and utilize fatty acids as an energy source and also inhibit the oxidation of fatty acids via inhibition of CPT I by production of malonyl-CoA (19, 41). This is undoubtedly why the responses of glucosensing neurons to fatty acids are particularly affected by ambient glucose levels. It has generally been assumed that, like the pancreatic β-cell, long-chain fatty acids are transported into neurons, where their oxidation, production of ROS, and activation of the KATP channel are initiated by the formation of long-chain acyl-CoA to alter neuronal activity (5, 9, 49).

The importance of the present set of studies is that they demonstrate the multiple ways in which isolated hypothalamic neurons can be activated by exogenous fatty acid. Most importantly, they point to a novel mechanism that may not involve intracellular fatty acid metabolism at all. As in taste receptor cells on the tongue (13), it appears that VMN neurons utilize CD36 as a receptor to activate or inhibit activity in a select set of hypothalamic neurons. Furthermore, although we do not know the true levels of extracellular fatty acids in the brain, our studies demonstrate that hypothalamic neurons are responsive to very low concentrations of fatty acids. Again, this suggests that exogenous fatty acids play a predominantly signaling, rather than metabolic, role in neurons. Fatty acids probably play their most important role in neurons as building blocks for membranes and organelles (26). The primary target of exogenous fatty acids, after their transport across the blood-
brain barrier, is the astrocyte, which can both oxidize and utilize fatty acids to produce ATP and ketone bodies (14). Whereas lactate is the primary product of glucose metabolism exported from astrocytes to neurons (6, 47), it may be that ketone bodies play a similar role in fatty acid metabolism as an energy source and possible signaling molecule in the astrocyte-neuron dialogue (15, 24). While this idea is speculative, our present data demonstrate conclusively that neurons are capable of utilizing exogenous fatty acids as signaling molecules to alter their activity. However, it is clear from the long list in Table 1 of enzymes and transporters found in VMN neurons that there remain a number of unexplored possibilities for the ways in which neurons utilize fatty acid for their signaling versus metabolic needs.

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