Differential effects of hypothalamic long-chain fatty acid infusions on suppression of hepatic glucose production

R. A. Ross, L. Rossetti, T. K. T. Lam, and G. J. Schwartz
Departments of Medicine and Neuroscience, Albert Einstein College of Medicine, Bronx, New York; Toronto General Hospital, Toronto, Ontario, Canada; and Merck, Rahway, New Jersey
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Ross RA, Rossetti L, Lam TK, Schwartz GJ. Differential effects of hypothalamic long-chain fatty acid infusions on suppression of hepatic glucose production. Am J Physiol Endocrinol Metab 299:E633–E639, 2010. First published July 20, 2010; doi:10.1152/ajpendo.00190.2010.—Our objective was to investigate whether the direct bilateral infusion of the mono-unsaturated fatty acid (MUFA) oleic acid (OA) within the mediobasal hypothalamus (MBH) is sufficient to reproduce the effect of administration of OA (30 nmol) in the third cerebral ventricle, which inhibits glucose production (GP) in rats. We used the pancreatic basal insulin clamp technique (plasma insulin ~20 mU/ml) in combination with tracer dilution methodology to compare the effect of MBH OA on GP to that of a saturated fatty acid (SFA), palmitic acid (PA), and a polyunsaturated fatty acid (PUFA), linoleic acid (LA). The MBH infusion of 200 but not 40 pmol of OA was sufficient to markedly inhibit GP (by 61% from 12.6 ± 0.6 to 5.1 ± 1.6 mg·kg⁻¹·min⁻¹) such that exogenous glucose had to be infused at the rate of 6.0 ± 1.2 mg·kg⁻¹·min⁻¹ to prevent hypoglycemia. MBH infusion of PA also caused a significant decrease in GP, but only at a total dose of 4 nmol (GP 5.8 ± 1.6 mg·kg⁻¹·min⁻¹). Finally, MBH LA at a total dose of 0.2 and 4 nmol failed to modify GP compared with rats receiving MBH vehicle. Increased availability of OA within the MBH is sufficient to markedly inhibit GP. LA does not share the effect of OA, whereas PA can reproduce the potent effect of OA on GP, but only at a higher dose. It remains to be determined whether SFAs need to be converted to MUFA to exert this effect or whether they activate a separate signaling pathway to inhibit GP.

NUTRIENT EXCESS HAS BEEN SHOWN TO CONTRIBUTE TO THE DEVELOPMENT OF MULTIPLE ASPECTS OF THE METABOLIC SYNDROME in animal models and in humans (14, 41, 57). Excessive dietary fat has historically been blamed as the major culprit in the development of obesity, inflammation, cardiovascular disease, hypertension, and diabetes, although current opinion favors total caloric excess rather than fat alone (5, 10, 17, 58, 62). The quality of fat may also have an impact on the cardiometabolic risk factors that result from nutrient excess. It has been shown that a switch from a diet high in saturated fat to one high in monounsaturated fat can actually improve insulin sensitivity and decrease diastolic blood pressure in patients suffering from these aspects of the metabolic syndrome (40, 60). Similarly, dietary supplementation with polyunsaturated ω-3 fatty acids has been shown to decrease inflammation in otherwise healthy humans (33).

Fatty acids provide more than nutritive value. They have been shown to act as signaling molecules themselves by directly activating various nuclear receptors and transcription factors to induce expression of genes involved in energy homeostasis and both glucose and fat metabolism (4, 46, 47, 59). Many of these proteins, such as AMP-activated protein kinase and sterol-CoA desaturase 1 (SCD1), have been implicated in the biochemical mechanisms that translate excess dietary fat of different types into overweight, obesity, and their sequelae (18, 45, 54). With prolonged nutrient excess, these sensing mechanisms become less effective.

Dietary and hormonal signals from the major organs involved in energy homeostasis are integrated through various networks that converge in part in the hypothalamus. The hypothalamus is sensitive to the adiposity hormones insulin and leptin, although this responsiveness is decreased in both older and obese humans and animal models given a high-fat diet (11, 16, 27, 48, 61). Furthermore, the hypothalamus is directly responsive to macronutrients such as glucose and long-chain fatty acids (LCFA) (22, 36). In lean young animals, third ventricle (icv) infusion of glucose, fatty acids, insulin, and leptin has been shown to decrease food intake and hepatic glucose production (12, 22, 36, 39). Thus far, only the mono-unsaturated long-chain fatty acid oleic acid has been shown to inhibit hepatic glucose production in lean, young animals via icv infusion, and diet-induced obesity impairs this hypothalamic sensitivity (30).

It is possible that all three nutrient-signaling pathways converge at some level within individual neurons, since insulin and leptin have been shown to activate the KATP channel in neurons, and blockade of this channel disrupts downstream effects of insulin, glucose, and fatty acids in the brain (22, 38, 51, 52).

LCFA are a major component of the diet. The nutritional differences between LCFA of different lengths and saturation states make up a great body of (conflicting) literature (3, 7, 32, 44, 53, 56) that has only grown in recent years with the increased study of the metabolic syndrome in animal models (6, 20, 29, 43). Historically, saturated fatty acids (SFA) have been thought to be the most detrimental form of LCFA insofar as inducing insulin resistance and other facets of the metabolic syndrome (9, 42). However, polyunsaturated fatty acids (PUFA) have been shown to have the opposite effect, since several of these, such as linolenic acid and conjugated linoleic acid, are protective against the harmful effects of obesity (1, 26, 44, 64).

Given that there may be distinct contributions of saturated and unsaturated fatty acids to insulin resistance and the metabolic syndrome, we wanted to investigate the possibility that the ability of hypothalamic nutrient sensing to alter glucose production might vary as a function of LCFA chain length and/or saturation.

Address for reprint requests and other correspondence: G. J. Schwartz, Depts. of Medicine & Neuroscience, Albert Einstein College of Medicine, 1300 Morris Park Ave., Golding 501, Bronx, NY 10461 (e-mail: gary.schwartz@einstein.yu.edu).

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For LCFA to exert their effects on glucose homeostasis, they must be converted to long-chain fatty acyl-CoA (23). The present series of experiments was designed to evaluate the degree to which mediobasal hypothalamic LCFA infusion is sufficient to suppress hepatic glucose production. Furthermore, we examined the degree to which length and saturation state of mediobasal hypothalamus (MBH) LCFA infusions determined their impact on glucose homeostasis.

**MATERIALS AND METHODS**

Animal preparation. Ten-week-old male Sprague-Dawley rats served as subjects in all experiments (Charles River Breeding Laboratories). Indwelling stainless steel bilateral cannulae were stereotaxically placed into the MBH (31) 3 wk before experiments to target the arcuate nucleus. Catheters were placed in the internal jugular vein and the carotid artery, and 1 wk later, basal insulin euglycemic pancreatic clamp procedures were performed (35–37). All study protocols were approved by the Institutional Animal Care and Use Committee of the Albert Einstein College of Medicine.

Clamp procedure in rats. Infusion studies lasted a total of 360 min. At 0 min, a primed, continuous, intrahypothalamic (IH) infusion of the various study solutions was initiated and maintained at a rate of 0.33 ml/h. These consisted of various doses of the cyclodextrin-complexed long-chain fatty acids oleate, palmitate, and linoleate at doses of 4 nmol, 200 pmol, and, for oleate only, 40 pmol. Cyclodextrin was used, because this had been identified as an appropriate vehicle for the central delivery of fatty acids, and cyclodextrin alone has been shown to be without effect on hepatic glucose metabolism at 10- to 100-fold doses used in the present study (28). Cyclodextrin was complexed to individual fatty acids by Cyclodextrin Technologies Development (www.cyclodex.com; High Springs, FL) to form a water-soluble complex with 2.36% water content and 37–38 mg fatty acid/g of complex by spectrophotometry. Each fatty acid complex was then solubilized in water to a final concentration of 17 mM, as described previously (28). The resulting 4-hydroxy-1-(3-pyridyl)-1-butanol compound-fatty acid solution was diluted in artificial cerebrospinal fluid to the appropriate concentration used for each MBH injection. Based on the final nanomolar fatty acid concentrations used, the calculated osmolarities of the complexes are at physiological levels determined by the osmolarity of the artificial cerebrospinal fluid solvent (290–300 mOsm; Harvard Apparatus). The current fatty acid concentrations are consistent with or lower than those shown to nontoxically excite or inhibit hypothalamic neurons in slice preparations. The osmolarity of the artificial cerebrospinal fluid to the appropriate concentration used for each MBH injection. The current fatty acid concentrations are consistent with or lower than those shown to nontoxically excite or inhibit hypothalamic neurons in slice preparations. The osmolarity of the artificial cerebrospinal fluid solvent (290–300 mOsm; Harvard Apparatus). The current fatty acid concentrations are consistent with or lower than those shown to nontoxically excite or inhibit hypothalamic neurons in slice preparations. The osmolarity of the artificial cerebrospinal fluid solvent (290–300 mOsm; Harvard Apparatus). Based on the final nanomolar fatty acid concentrations used, the calculated osmolarities of the complexes are at physiological levels determined by the osmolarity of the artificial cerebrospinal fluid solvent (290–300 mOsm; Harvard Apparatus).

We next examined whether MBH administration of comparable 0.2- and 4-nmol doses of palmitic acid (C16:0; as a model SFA) and linoleic acid (C18:2; as a model PUFA) would affect hepatic glucose production. During the basal period, MBH oleic acid administration significantly reduced plasma glucose (P < 0.001 for the 4-nmol dose and P < 0.05 for the 0.2-nmol dose), whereas palmitic and linoleic acid infusions were without effect (Fig. 2).

During the clamp, both high and medium doses of oleic acid significantly increased the glucose infusion rate required to maintain euglycemia. This was replicated only by the 4-nmol dose of palmitic acid, with a significant but not quite as marked glucose infusion rate required to maintain euglycemia (5 mg·kg⁻¹·min⁻¹, P < 0.05; Fig. 3A). Although the net decrease in plasma glucose levels resulting from MBH palmitic acid infusion was not significant, the percent suppression of glucose production compared with basal rates was significant for both the 4- (58%, P < 0.05) and 0.2-nmol (43%, P < 0.05) doses. In fact, the high dose of palmitic acid fully replicated the percent suppression seen in animals treated with the high dose of oleic acid (57%; Fig. 3C). The 0.2-nmol dose of palmitic acid, which caused a significant suppression of glucose production compared with basal rates, did not reach the degree of suppression seen in animals treated with the 0.2-nmol dose of oleic acid (60%; Fig. 3, B and C). In contrast, neither dose of linoleic acid tested required more than a marginal amount of exogenous glucose to be infused to maintain euglycemia (Fig. 3A), and...
neither dose suppressed glucose production at all (Fig. 3, B and C). Glucose uptake was not significantly affected by any dose of palmitic, linoleic, or oleic acid compared with the control group (Fig. 3D).

Thus, MBH SFA infusion during basal pancreatic insulin clamp studies suppresses glucose production, but not as potently as the monounsaturated fatty acid. As was the case for oleic acid, SFA altered glucose homeostasis exclusively by

Table 1. General characteristics of experimental groups before intrahypothalamic infusions and during the pancreatic basal insulin clamp studies

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>High OA</th>
<th>Medium OA</th>
<th>Low OA</th>
<th>High PA</th>
<th>Medium PA</th>
<th>High LA</th>
<th>Med LA</th>
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<td>Basal</td>
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<tr>
<td>Body weight, g</td>
<td>313 ± 3</td>
<td>300 ± 4</td>
<td>307 ± 4</td>
<td>294 ± 9</td>
<td>304 ± 2</td>
<td>308 ± 8</td>
<td>310 ± 8</td>
<td>308 ± 11</td>
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<tr>
<td>Glucose, mmol/l</td>
<td>8.2 ± 0.1</td>
<td>8.0 ± 0.3</td>
<td>8.7 ± 0.4</td>
<td>8.7 ± 0.7</td>
<td>8.9 ± 0.2</td>
<td>8.7 ± 0.2</td>
<td>8.6 ± 0.2</td>
<td>8.5 ± 0.3</td>
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<tr>
<td>Insulin, ng/ml</td>
<td>1.0 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>0.7 ± 0.02</td>
<td>0.7 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.8 ± 0.1</td>
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</tr>
<tr>
<td>FFA, mmol/l</td>
<td>1.2 ± 0.1</td>
<td>0.8 ± 0.02</td>
<td>0.7 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>1.0 ± 0.02</td>
<td>0.8 ± 0.001</td>
<td>0.6 ± 0.02</td>
<td>0.7 ± 0.1</td>
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<td>Clamp</td>
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<tr>
<td>Glucose, mmol/l</td>
<td>7.8 ± 0.5</td>
<td>7.6 ± 0.3</td>
<td>7.7 ± 0.8</td>
<td>8.6 ± 0.2</td>
<td>7.7 ± 0.8</td>
<td>7.8 ± 0.4</td>
<td>8.5 ± 0.2</td>
<td>8.6 ± 0.7</td>
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<tr>
<td>Insulin, ng/ml</td>
<td>1.3 ± 0.1</td>
<td>1.2 ± 0.2</td>
<td>1.3 ± 0.3</td>
<td>1.5 ± 0.2</td>
<td>1.2 ± 0.2</td>
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<tr>
<td>FFA, mmol/l</td>
<td>1.0 ± 0.1</td>
<td>0.7 ± 0.2</td>
<td>0.6 ± 0.02</td>
<td>0.9 ± 0.05</td>
<td>0.7 ± 0.06</td>
<td>0.4 ± 0.1</td>
<td>0.6 ± 0.07</td>
<td>0.5 ± 0.07</td>
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Data are means ± SE. OA, oleic acid; PA, palmitic acid; LA, linoleic acid; FFA, free fatty acids. Values during the clamp studies are steady-state levels averaged from at least 6 plasma samples during the course of the study.
reducing glucose production without increasing peripheral glucose uptake.

Surprisingly, MBH linoleate administration had no effect on glucose infusion rate or hepatic glucose production and did not alter glucose uptake relative to vehicle infusion. These results show that the w-6 PUFA linoleic acid cannot reproduce the effects of centrally infused monounsaturated fatty acid on peripheral glucose metabolism.

To further understand the mechanism of action of intrahypothalamic fatty acids on glucose metabolism in the liver, we studied the in vivo hepatic glucose flux through glucose-6-phosphatase (G-6-Pase; also called futile glucose cycling) and the relative contributions of this along with gluconeogenesis and glycogenolysis to total glucose output for animals treated with the 0.2-nmol dose of each of the fatty acids.

The fluxes through G-6-Pase, gluconeogenesis, and glycogenolysis were all significantly decreased in response to MBH treatment with oleic acid, with gluconeogenesis being most depressed (Fig. 4, A–D). MBH infusion of oleate significantly reduced gluconeogenesis (0.7 ± 0.2 mg·kg⁻¹·min⁻¹, P < 0.05; Fig. 4A) and markedly reduced glycogenolysis compared with vehicle (5.2 ± 1.3; Fig. 4B). MBH palmitate had no effect on gluconeogenesis (1.8 ± 0.7 mg·kg⁻¹·min⁻¹; Fig. 4A) but did reduce glycogenolysis to a similar extent as MBH oleate (5.0 ± 1.2; Fig. 4B). Interestingly, IH linoleate did markedly suppress gluconeogenesis compared with vehicle (0.9 ± 0.3 mg·kg⁻¹·min⁻¹; Fig. 4A) but had no effect on glycogenolysis compared with vehicle (Fig. 4B). Compared with vehicle, only MBH oleic acid significantly reduced the futile cycling of glucose, by ∼50% (P < 0.05), although IH palmitic acid showed a trend toward decreased glucose cycling (Fig. 4C). The flux through G-6-Pase was decreased mostly by IH oleic acid, by ∼30% compared with vehicle. It was also decreased, albeit less so, by palmitic acid. As with glucose cycling, IH linoleic acid had no effect on the flux through G-6-Pase (Fig. 4D). Overall, at the 0.2-nmol dose, only the monounsaturated fatty acid oleic acid had a significant effect on glucose fluxes in the liver, thereby explaining its potent effects on glucose production seen in the clamp studies.

**DISCUSSION**

These results identify a role for mediobasal hypothalamic LCFA in the control of hepatic glucose metabolism. The mode of LCFA entry into neurons remains a subject of active investigation. LCFA may enter neurons by flip-flop mechanisms (19, 21) or, alternatively, rely on the neuronal expression of fatty acid-binding proteins (25), which are regulated by transcription factors activated by LCFA (50). Fatty acid-binding proteins function as transcription factors themselves as well to help activate peroxisome proliferator-activated receptor-activated receptors, which are known to upregulate lipid metabolic genes (15, 55). Because circulating free fatty acids have easy access to the central nervous system, there is likely a physiological role for LCFA in the hypothalamic control of peripheral glucose homeostasis.

The present results demonstrate that the monounsaturated fatty acid oleic acid is the most potent among the various types of fatty acids tested. This is consistent with demonstrations that monounsaturated fatty acids 1) have beneficial metabolic effects, 2) activate the K_ATP channels implicated in hepatic glucose metabolism, and 3) abrogate the effects of SFA and high glucose on pancreatic β-cells (24, 28, 36). Results from the present studies do not support a role for the w-6 polyunsaturated fatty acid linoleic acid in the hypothalamic control of glucose homeostasis. This was somewhat unexpected, although it may be a function of the number or placement of double bonds in the molecule. Recent literature has shown that different types of polyunsaturated fatty acids have different effects,
specifically that the two classes of dietary essential fatty acids, the ω-3 and ω-6, can have radically different nutritional and metabolic outcomes. A diet enriched in ω-6 fatty acids promotes increased risk of cancer and autoimmune and cardiovascular disease, whereas a diet enriched in ω-3 PUFA exerts a suppressive effect on those conditions (8, 49). Studies of ω-3 polyunsaturated LCFA will be important to identify the degree to which they share the effects of ω-6 stimuli. The role of polyunsaturated fatty acids may be more relevant for cellular integrity and maintenance rather than performing roles in

Fig. 3. MBH palmitic acid, but not linoleic acid, enhances insulin action and inhibits glucose production, albeit not as potently as oleic acid. A: effect of high- and low-dose palmitic and linoleic acids on glucose infusion rate compared with the same doses of oleic acid. B: effect of MBH palmitic and linoleic acids on the rate of glucose production during the clamp. The rates of glucose production were similar in all groups before the start of the pancreatic insulin clamp studies. C: effect of IH palmitic and linoleic acids on %decrease of glucose production during the clamp. D: effect of palmitic and linoleic acids on the rate of glucose uptake. *P < 0.05, **P < 0.01, ***P < 0.001.

Fig. 4. MBH oleic and palmitic acids decrease hepatic glucose fluxes, but linoleic acid does not. Data shown are from animals treated with the medium dose, 200 pmol, of each fatty acid. A: gluconeogenesis. B: glycogenolysis. C: glucose cycling. D: total glucose output. *P < 0.05.
nutrient signaling important in the central control of peripheral glucose homeostasis.

The present results reveal that palmitic acid acts in the arcuate nucleus of the mediobasal hypothalamus to decrease hepatic glucose production in an acute injection study but does not fully recapitulate the significant suppression of hepatic glucose production seen in response to central administration of the same doses of oleic acid. Two possible reasons for this are 1) the fact that these fatty acids have different saturation states and 2) the fact that they have different chain lengths. Either explanation would implicate the involvement of a number of enzymes known to play roles in the pathway of fatty acid biosynthesis, namely SCD and LCFA elongase (Elovl). These two proteins work together in a stepwise fashion to convert palmitic acid to oleic acid in mammals. Certain isoforms of each protein, SCD1 and Elovl6, have already been shown to be involved in mediating insulin resistance in the liver (13, 18, 29, 34). They may also be involved in the regulation of liver glucose metabolism by the brain. This makes them intriguing proteins to target for study in the continued work to elucidate the nutrient-based sensing pathway of the hypothalamus.

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DISCLOSURES

The authors have no competing financial interests to disclose.

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