Central lactate metabolism regulates food intake

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The incidence of obesity and type 2 diabetes mellitus has reached epidemic proportion. It is estimated that more than 180 million people worldwide have diabetes and approximately 400 million adults are obese. These numbers are projected to rise exponentially; more than 700 million are expected to be obese by 2015, and the number of people with diabetes is expected to double by 2030. Many laboratories worldwide have dedicated major efforts to elucidate the mechanisms responsible for the development of these metabolic disorders, with particular emphasis on the mechanistic regulation of energy and nutrient homeostasis. To unveil the pathways that regulate both energy and nutrient homeostasis, the central nervous system (CNS), or more specifically the hypothalamus, has received much attention recently. The hypothalamus detects a rise in nutrients or fat/gut-derived hormones to regulate both energy (7, 9–11, 19, 21, 23, 24, 28, 35) and nutrient (2, 3, 5, 8, 12, 14, 15, 17, 18, 25, 27, 28, 30, 31) homeostasis. Importantly, obesity and diabetes are associated with disruptions in hypothalamic sensing mechanisms, leading to an elevation of food intake (FI), body weight (BW), and glucose levels (12, 13, 17, 33, 34). Thus the dissection of CNS sensing mechanisms that regulate energy and nutrient homeostasis in normal and pathological settings is critical and could eventually be proven useful in treating obesity and type 2 diabetes mellitus.

Nutrients such as fatty acids, amino acids, and glucose signal the hypothalamus to regulate energy balance (9, 11, 24, 29). The underlying mechanisms of CNS nutrient sensing have been evaluated (4, 9, 21, 23, 28, 29, 38), but the associated regulatory mechanisms remain largely unknown. Neuronal uptake of lactate provides fuel for neurons (22). Furthermore, hypothalamic lactate metabolism has been shown to regulate peripheral glucose and lipid homeostasis in vivo (17, 18). On the basis of these independent yet parallel findings, we postulated that CNS lactate metabolism regulates energy homeostasis.

We first tested whether a selective increase in central lactate levels regulates FI and BW (Fig. 1A). We then inhibited central lactate metabolism to pyruvate with the pharmacological lactate dehydrogenase inhibitor oxamate to examine the role of lactate metabolism in the regulation of energy balance (Fig. 1A). We next increased central pyruvate levels to examine whether pyruvate could recapitulate the effects of central lactate (Fig. 1A). Finally, we tested whether a selective rise in circulating lactate levels regulates FI and BW, and whether such regulation is mediated through central lactate metabolism to pyruvate (see Fig. 4A). We found that activation of central lactate metabolism to pyruvate by either central injections of lactate or intravenous elevation of lactate reduced FI and BW. Furthermore, central injections of pyruvate recapitulated the effects of lactate to lower FI and BW. Together, these data indicate that activation of central lactate metabolism to pyruvate lowers FI and BW.

METHODS

Animal Preparation

Eight-week-old male Sprague-Dawley (SD) rats weighing between 260 and 280 g (Charles River Laboratories, Montreal, QC, Canada) were used for our studies. Rats were housed in individual cages and maintained on a standard light-dark cycle with access to standard rat chow and water ad libitum. Rats were stereotaxically (David Kopf Instruments, Tujunga, CA) implanted with indwelling intracerebroventricular single catheters (2.5 mm posterior of bregma, 0.0 mm lateral from midline, 8.0 mm below skull surface) as previously described (5). Animals receiving systemic circulating lactate then underwent intravenous catheterization, 5 days after stereotaxic surgery. Recovery from surgery was monitored by measuring daily FI and BW to ensure that animals had stable baseline FI and BW before in vivo experiments were performed. All study protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the University Health Network.

Feeding Protocol

Intracerebroventricular administration. Five days after intracerebroventricular surgery, animals whose daily FI and BW had recovered back to baseline and had been stable for a minimum of three consecutive days underwent the feeding protocol. Baseline measurements of FI and BW were taken on day 0 (10 AM, time = 6 h). Water and food were then withdrawn from the animals. Animals received treatments 1 h before the dark cycle (time 0 h). Animals were injected intracerebroventricularly with 3 μl of the following substances over a period of 30 s: 1) saline, 2) α-lactate (5 mM), 3) t-lactate (5 mM), 4) D-lactate (5 mM), and 5) oxamate (5 mM). The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Fig. 1. Central injections of lactate lower food intake (FI) and body weight (BW). A: schematic model for FI and BW regulation by central nervous system (CNS) lactate metabolism. Oxamate is an inhibitor of lactate dehydrogenase (LDH), which blocks the interconversion between L-lactate and pyruvate. B: intracerebroventricular (icv) 3rd ventricle stereotaxic surgery was performed on male Sprague-Dawley (SD) rats, and they were given 5 days of recovery for FI and BW to stabilize before icv injections. Baseline FI and BW were measured (time 0 h). Rats were injected at time 0 h (i.e., 1 h before dark cycle) with 3 μl of saline, t-lactate (5 mM), t-lactate (5 mM), t-lactate (2.5 mM), t-lactate (1 mM), or t-lactate (0.5 mM). FI and BW were monitored for 2 subsequent days. C and D: effect of icv saline (n = 10), t-lactate (5 mM, n = 7), and t-lactate (5 mM, n = 9) on FI (C) and %change in BW (D). E and F: effect of icv t-lactate (0.5 mM n = 4, 1 mM n = 5, and 2.5 mM n = 5) on FI (E) and %change in BW (F). *P < 0.05, icv lactate vs. icv saline/t-lactate; †P < 0.01 icv lactate vs. icv saline/t-lactate; ♂♂P < 0.05, icv 2.5 mM lactate vs. basal.

Results

Central Administration of Lactate Lowers Food Intake and Body Weight

We first subjected male SD rats to intracerebroventricular surgeries and allowed them 1 wk of recovery (Fig. 1B). Recovered rats only underwent intracerebroventricular injections if their baseline FI (∼26–30 g/day) and BW (∼260–280 g) were stable for a minimum of 3 days. To develop the protocol for FI and BW monitoring in response to a single bolus intracerebroventricular injection, we first injected intracerebroventricular saline (3 μl over 30 s) in rats 1 h before the dark cycle (6 h after the initial FI and BW measurements at 10 AM) and monitored their FI and BW for two subsequent days (Fig. 1B). We found that intracerebroventricular saline injections had minimal effects on FI and BW at 18 (1 day) or 42 (2 days) h (Fig. 1, C and D). Similarly, intracerebroventricular t-lactate (5 mM; nonactive isofrom of lactate) injections had minimal effects on FI and BW (Fig. 1, C and D). In contrast, intracerebroventricular injections of equimolar concentrations of 5 μl t-lactate 1 h before the dark cycle reduced FI from 29 ± 1 to 12 ± 3 g (a 58 ± 9% decrease) at 18 h (Fig. 1C). FI was not significantly different after 42 h of intracerebroventricular lactate injections (Fig. 1C). This is consistent with previous studies indicating that a single bolus intracerebroventricular injection of another nutrient, t-leucine, reduces FI up to 24 h (9), but in contrast to the ability of intracerebroventricular...
oleic acid to lower FI up to 48 h (29). Intracerebroventricular lactate reduced BW by 28 \% (or 10 \% of BW) after 18 h (Fig. 1D). BW remained significantly lower 42 h after intracerebroventricular lactate injections (Fig. 1D). Thus we report for the first time, to our knowledge, that a selective rise in central lactate levels reduced FI, which was accompanied by weight loss.

We next performed a dose-response curve for the anorectic effect of a single bolus intracerebroventricular L-lactate injection. We found that intracerebroventricular L-lactate injected at both 0.5 mM and 1 mM had minimal effects on FI and BW at 18 or 42 h (Fig. 1E and F). Intracerebroventricular L-lactate injected at 2.5 mM lowered FI from 28 to 22 g (P < 0.05) at 18 h, and FI returned to baseline at 42 h (Fig. 1E). Intracerebroventricular L-lactate injected at 2.5 mM did not significantly affect BW at 18 h (Fig. 1F). To further characterize the underlying mechanisms of CNS lactate sensing, we decided to focus on using intracerebroventricular L-lactate injected as a single bolus at 5 mM. This is because the anorectic effect of L-lactate at 5 mM was strongest in accordance with the dose-response curve and this will ensure a relatively maximal stimulation on the downstream biochemical/signaling pathway(s) of CNS lactate sensing.

Central Lactate Metabolism Mediates Lactate to Lower Food Intake and Body Weight

Neuronal lactate serves as an important energy source for neurons (22). Furthermore, activation of hypothalamic lactate metabolism regulates peripheral glucose and lipid homeostasis (17, 18). To examine whether central lactate metabolism mediates central lactate in the regulation of energy balance, we first injected the lactate dehydrogenase competitive inhibitor oxamate intracerebroventricularly to inhibit central lactate metabolism to pyruvate (Figs. 1A, 2A). Central injections of oxamate (50 mM) alone did not affect FI and BW up to 42 h (Fig. 2B and C). In contrast, intracerebroventricular oxamate coinjected with L-lactate completely abolished the 18-h effect of central lactate on FI (Fig. 2B), as well as both the 18- and 42-h effects on BW (Fig. 2C). These data indicate that central lactate metabolism to pyruvate is required for lactate to regulate FI and BW.

Central Administration of Pyruvate Lowers Food Intake and Body Weight

If central lactate metabolism to pyruvate represents an important biochemical pathway to lower FI and BW, direct intracerebroventricular pyruvate injections should recapitulate the anorectic effects of central lactate. Indeed, intracerebroventricular pyruvate (5 mM; 1 molecule of lactate generates 1 molecule of pyruvate) injections lowered FI from 27 to 12 g (Fig. 3A), with an accompanying 18-h BW reduction of 6 \% (Fig. 3B). The anorectic effects of intracerebroventricular pyruvate closely resemble those observed with intracerebroventricular lactate injections. Together, these data suggested that hypothalamic lactate → pyruvate metabolism regulates FI and BW.
Central Lactate Metabolism Is Required for Circulating Lactate to Lower Food Intake and Body Weight

Direct sensing of glucose (11), fatty acids (29), amino acids (9), and lactate (demonstrated in this study) by the brain regulates FI and BW. However, it is unclear whether these CNS nutrient-sensing mechanism(s) mediate circulating nutrients to regulate FI and BW. We here tested whether a selective and sustained doubling of plasma lactate levels for 3 h regulates FI and BW, and whether the associated effects are dependent on CNS lactate metabolism (Fig. 4A). We subjected rats to intracerebroventricular surgeries on day −12 and then intravascular surgeries on day −7. FI and BW returned to baseline by day −3. After consistent baseline readings for both FI and BW for three extra days, we performed in vivo infusion experiments on day 0 (Fig. 4B). Intravenous lactate (100 μmol·kg⁻¹·min⁻¹) was infused for 3 h to increase plasma lactate levels from 0.9 ± 0.1 mM (n = 5, iv saline) to 2.1 ± 0.4 mM (n = 6, iv lactate), in keeping with previous studies (17). Infusion was terminated 1 h before the dark cycle (Fig. 4B). Intravenous lactate infusion reduced FI from 27 ± 2 to 1 ± 0.5 g (a 98 ± 2% decrease) at 18 h, and FI returned to baseline after 42 h (Fig. 4C). In parallel, intravenous lactate significantly reduced BW by 43 ± 6 g (or 15 ± 3%) at 18 h, which returned to baseline by 42 h (Fig. 4D).

In a second set of rats, intracerebroventricular oxamate was injected to inhibit central lactate metabolism to pyruvate in the presence of intravenous lactate infusion (Fig. 4, A and B). Rats that received central oxamate administration in addition to intravenous lactate only reduced FI from 28 ± 3 to 18 ± 2 g (a 35 ± 7% decrease) after 18 h, returning to slightly higher than baseline after 42 h (Fig. 4C). BW was unchanged (Fig. 4D). Of note, central administration of oxamate did not fully abolish the anorectic effects of circulating lactate (Fig. 4C), indicating that other regions of the brain or other parts of the body could be involved in this appetite regulation. Thus we have demonstrated that central lactate metabolism to pyruvate is required for circulating lactate to regulate FI and BW.

DISCUSSION

The hypothalamus senses nutrients or hormones to exert physiological responses and maintain energy (7, 9–11, 19, 21, 23, 24, 28, 35) and nutrient (2, 3, 5, 8, 12, 14, 15, 17, 18, 27, 28, 30, 31) homeostasis. The associated hypothalamic mechanisms are potentially disrupted in obesity and diabetes, leading to an elevation of appetite and plasma metabolite level (12, 13, 17, 33, 34). Although neuronal networks have been demonstrated to regulate energy and nutrient homeostasis, much work is needed to probe the CNS biochemical and signaling path-
ways that are responsible for these regulations. Research with these particular focuses will reveal new molecular targets to lower FI and plasma metabolite level in obesity and type 2 diabetes mellitus.

The present study extends the quest to uncover novel CNS biochemical pathways and molecules in the regulation of energy homeostasis. We have provided the first direct evidence, to our knowledge, that activation of central lactate metabolism to pyruvate with two independent intracerebroventricular or intravenous lactate-infused approaches reduced FI and BW. This is further supported by the fact that direct central delivery of pyruvate fully recapitulated the anorectic effect of lactate. These studies position the biochemical metabolism of lactate to pyruvate in the hypothalamus as a novel biochemical target to reverse obesity.

Intravenous administration or peripheral injections of nutrients such as lactate, glucose, lipids, or amino acids negatively regulate FI and BW (20, 26, 36, 37, 39). However, the site(s) of the anorectic actions have not been investigated. Here we report that central lactate metabolism to pyruvate is required for circulating lactate to lower BW and FI in conscious, unrestrained rodents. We first established an in vivo model that illustrated that systemic elevation of plasma lactate levels by ~2.5-fold for 3 h lowered FI and BW. In the presence of this systemic elevation of lactate, we then negated central lactate metabolism to pyruvate with central injections of the lactate dehydrogenase inhibitor oxamate at the same dose that abolished the anorectic effects of central lactate injections. Central administrations of oxamate were sufficient to reverse the anorectic effects of circulating lactate. These data suggest that, similar to the regulation of glucose and lipid homeostasis (17, 18), CNS lactate metabolism is required for circulating lactate to regulate energy homeostasis.

The present study as a whole is pharmacological in nature and is designed to evaluate the role of the biochemical metabolism of lactate to pyruvate in the regulation of FI. The physiological relevance of CNS lactate metabolism to the regulation of FI remains to be clarified. Furthermore, the study does not distinguish the types of neurons [i.e., the agouti-related peptide (AgRP)/neuropeptide Y (NPY) and proopiomelanocortin (POMC) neurons] that are involved in CNS lactate metabolism. Genetic approaches that target selective populations of neurons and manipulate associated molecules involved in neuronal lactate sensing could be important tools to achieve this goal. In fact, emerging studies are showing that genetic disruptions of CNS nutrient and hormonal sensing mechanisms in AgRP or POMC neurons impair energy and glucose homeostasis (1, 6, 15, 16, 31, 32).

In conclusion, our data suggest that activation of central lactate metabolism to pyruvate lowers FI and BW. In light of the fact that CNS lactate metabolism controls plasma glucose and lipid levels (17, 18), therapeutic strategy designed to modulate CNS lactate metabolism may prove useful to lower BW and glucose and lipid levels in obesity and type 2 diabetes mellitus.

**REFERENCES**


