Central glucagon-like peptide 1 receptor-induced anorexia requires glucose metabolism-mediated suppression of AMPK and is impaired by central fructose.

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Burmeister MA, Ayala J, Drucker DJ, Ayala JE. Central glucagon-like peptide 1 receptor-induced anorexia requires glucose metabolism-mediated suppression of AMPK and is impaired by central fructose. Am J Physiol Endocrinol Metab 304: E677–E685, 2013. First published January 22, 2013; doi:10.1152/ajpendo.00446.2012.—Glucagon-like peptide-1 (GLP-1) suppresses food intake via activation of a central (i.e., brain) GLP-1 receptor (GLP-1R). Central AMP-activated protein kinase (AMPK) is a nutrient-sensitive regulator of food intake that is inhibited by anorectic signals. The anorectic effect elicited by hindbrain GLP-1R activation is attenuated by the AMPK stimulator AICAR. This suggests that central GLP-1R activation suppresses food intake via inhibition of central AMPK. The present studies examined the mechanism(s) by which central GLP-1R activation inhibits AMPK. Supporting previous findings, AICAR attenuated the anorectic effect elicited by intracerebroventricular (icv) administration of the GLP-1R agonist exendin-4 (Ex-4). We demonstrate that Ex-4 stimulates glycogenolysis and suppresses AMPK phosphorylation in a glucose-dependent manner in hypothalamic GT1-7 cells. This suggests that inhibition of AMPK and food intake by Ex-4 requires central glucose metabolism. Supporting this, the glycolytic inhibitor 2-deoxyglucose (2-DG) attenuated the anorectic effect of Ex-4. However, icv glucose did not enhance the suppression of food intake by Ex-4. AICAR had no effect on Ex-4-mediated reduction in locomotor activity. We also tested whether other carbohydrates affect the anorectic response to Ex-4. Intracerebroventricular pretreatment with the sucrose metabolite fructose, an AMPK activator, attenuated the anorectic effect of Ex-4. This potentially explains the increased food intake observed in sucrose-fed mice. In summary, we propose a model whereby activation of the central GLP-1R reduces food intake via glucose metabolism-dependent inhibition of central AMPK. We also suggest that fructose stimulates food intake by impairing central GLP-1R action. This has significant implications given the correlation between sugar consumption and obesity.

GLP-1 is secreted from intestinal L cells in response to nutrient intake and was identified for its ability to stimulate insulin secretion via activation of a pancreatic β-cell GLP-1 receptor (GLP-1R) (3). GLP-1 also regulates processes independently of its pancreatic effects, including gastric emptying and cardiac function (57). One of the first extrapancreatic effects identified for GLP-1 is its ability to suppress food intake. Intracerebroventricular (icv) and targeted hypothalamic or hindbrain administration of GLP-1R agonists reduces food intake (12, 20, 55). Conversely, central (i.e., brain) administration of GLP-1R antagonists increases food intake (56), demonstrating that endogenous GLP-1 is an anorectic factor. Some GLP-1-based therapies used for the treatment of type 2 diabetes elicit moderate weight loss (58). The available data (14) suggest that targeting the central GLP-1R may be useful as an anti-obesity strategy.

The mechanism(s) by which GLP-1 reduces food intake has not been fully elucidated. Prolonged fasting blunts the anorexic effect mediated by the GLP-1R agonist exendin-4 (Ex-4) (62). This suggests that nutrient status plays a critical role in reduction of food intake by the GLP-1R. Changes in nutrient status modulate the activity of AMP-activated protein kinase (AMPK) in various tissues. Hypothalamic AMPK is a key sensor of nutrient status and plays an integral role in regulating food intake (1, 42). Prolonged fasting stimulates hypothalamic AMPK activity and the subsequent drive to increase food intake (41). Direct pharmacological and genetic activation of hypothalamic AMPK increases food intake (25, 41), whereas its inhibition reduces food intake (23, 41). Moreover, anorectic signals such as leptin (54) and glucose (10, 36, 37) inhibit hypothalamic AMPK activity. Intracerebroventricular GLP-1 administration reduces hypothalamic AMPK Thr172 phosphorylation, a marker of AMPK activation (6, 49). Ex-4 also suppresses AMPK phosphorylation in the GT1-7 hypothalamic cell line (20). Inhibition of AMPK by GLP-1R activation is also observed in nonhypothalamic brain regions. Delivery of Ex-4 into the fourth ventricle reduces AMPK phosphorylation in the dorsal vagal complex in the hindbrain (20). Furthermore, administration of Ex-4 into the fourth ventricle suppresses food intake, and this anorectic effect is attenuated by the AMPK activator 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) (20). Taken together, these observations suggest that inhibition of AMPK is required for the anorectic effect mediated by central GLP-1R activation.

Delineating the signaling pathways mediating the anorectic effect of central GLP-1R activation may identify sites where obesogenic diets interfere. High-fat diets inhibit the anorectic effects of insulin, leptin, and GLP-1 (2, 8, 32, 63). Diets rich in sucrose and fructose also promote insulin and leptin resistance (50, 53), but the effects of sucrose or fructose on GLP-1 action remain largely unexplored. Increased consumption of sucrose...
and fructose has been implicated as a culprit in the obesity epidemic, particularly in children (34). Interestingly, icv administration of the sucrose metabolite fructose stimulates food intake and central (hypothalamic) AMPK activity (28). If inhibition of central AMPK is required for the anorectic effect of GLP-1R agonists, then activation of AMPK by fructose could attenuate the satiety effect of GLP-1. Assuming that dietary fructose is metabolized in the brain, this could explain reduced satiety associated with consumption of sucrose- and fructose-rich foods and beverages.

The present studies explored the mechanisms by which central GLP-1R activation inhibits AMPK and food intake. We employed lateral ventricle administration of AMPK activators and Ex-4 to corroborate previous findings that central GLP-1R activation suppresses food intake via inhibition of central AMPK (20). Time course measurements of food and water intake, locomotor activity, and energy expenditure were obtained following icv treatments. The hypothalamic GT1-7 cell line was used to determine a potential mechanism by which GLP-1R activation inhibits AMPK. Intracerebroventricular administration of fructose was used to test the hypothesis that this carbohydrate attenuates the anorectic effect mediated by the central GLP-1R.

MATERIALS AND METHODS

Animals. All procedures were approved by the Institutional Animal Care and Use Committee at the Sanford-Burnham Medical Research Institute at Lake Nona. Male GLP-1R knockout (Glp1r<sup>−/−</sup>) and wild-type (Glp1r<sup>+/+</sup>) mice on a C57Bl/6 background were fed a high-starch diet (D12328; Research Diets, New Brunswick, NJ). For diet intervention studies, some mice were fed an isocaloric high-sucrose diet (D12329; Research Diets). High-starch and high-sucrose diets contained 2.44 kcal/g corn starch and sucrose, respectively. Both diets were composed of 10.5, 73.1, and 16.4 kcal/g fat, carbohydrate, and protein, respectively. All experiments were performed on 4- to 5-mo-old mice maintained on a standard light-dark cycle (0600–1800 h).

Surgical procedures. Intracerebroventricular cannulae were implanted under isoflurane (2%) anesthesia using a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). The skull was exposed by an incision and leveled between lambda and bregma. Cannulae (Plastics One, Roanoke, VA) were implanted to target the lateral cerebral ventricle at the following coordinates: 0.3 mm caudal, 1.0 mm from midline, and 3.2 mm ventral. A guide cannula was inserted into the brain by drilling a burr hole through the skull and was fixed into position by two jeweler’s screws and cranioplastic cement. Verification of cannula position in the lateral cerebroventricle was made by observing spontaneous flow of cerebrospinal fluid from the tip of the cannula after removal of the obturator. Animals were housed individually after surgery and allowed to recover for 5 days before experimentation. Cannula placement in the lateral cerebroventricle was reverified by observing increased drinking in response to an icv administration of angiotensin II (1 g) on the final day of experimentation. Feeding, drinking, locomotor activity, and energy expenditure were measured continuously for 18 h (1800–1200 h) following icv treatments. Locomotor activity was converted to distance traveled by multiplying the number of beam breaks recorded for ambulatory activity by the distance between the beams. For pica tests, mice were housed individually and acclimated to the presence of kaolin clay in their home cages for 1 wk. Mice were given icv treatments of ACSF or Ex-4 at the doses and volumes indicated above or an intraperitoneal (ip) injection of lithium chloride (0.15 M) prior to the onset of the dark cycle and following a 5-h fast. Eighteen-hour kaolin clay consumption was measured manually using a precision scale.

Protein immunoblots in isolated hypothalami. Whole cell extracts from entire hypothalami were obtained by homogenizing tissue in 100 μl of tissue lysis buffer (1 M Tris-HCl, 1 M NaCl, 100 mM EDTA, 50 mM EGTA, 10% NP-40, 25 mM sodium pyrophosphate, 100 mM sodium orthovanadate, and 1 M NaF) supplemented with protease and phosphatase inhibitor cocktails (Sigma-Aldrich). Homogenates were centrifuged (15 min, 10,000 g, 4°C), pellets were discarded, and supernatants were retained for protein determination. Protein content was determined using a Coomassie (Bradford) protein assay kit (Pierce, Rockford, IL). Whole cell (30 μg) extracts were separated on 4–12% Bis-Tris SDS-PAGE gels (Life Technologies, Carlsbad, CA), followed by electrophoretic transfer to polyvinylidene fluoride membranes. Membranes were incubated with primary antibodies overnight at 4°C and with secondary antibodies conjugated to alkaline phosphatase at room temperature for 1 h. Imaging was performed using a Typhoon FLA9500 imager (GE Healthcare), and densitometry was calculated using Image J software (NIH). Antibodies for AMPK, Thr<sup>172</sup> phosphorylated AMPK, and β-actin were from Cell Signaling Technology (Danvers, MA).

Studies in hypothalamic GT1-7 neurons. Hypothalamic GT1-7 neurons, an immortalized mouse hypothalamic cell line expressing the GLP-1R [a generous gift from P. L. Mellon, (39)], were maintained in DMEM with 4.5 g/l glucose, l-glutamine, and sodium pyruvate (Mediatech, Manassas, VA), 10% fetal bovine serum (Sigma, St. Louis, MO), and 10 ml/l penicillin-streptomycin (HyClone, Rockford, IL) at 37°C in 5% CO<sub>2</sub>. Prior to treatments, cells were plated in 10-cm cell culture dishes and allowed to reach 70% confluence. For glucose/Ex-4 experiments, cells were washed twice with phosphate-buffered saline (PBS) and serum starved in media (Sigma D-5030 supplemented with sodium bicarbonate, l-glutamine, and sodium pyruvate) containing 25 mM glucose for 3 h. For fructose/Ex-4 experiments, cells were serum starved in media supplemented with 25 mM fructose for 2 h and then switched to serum-free medium containing 25 mM fructose (in place of glucose) for 1 h. For each experiment, vehicle (PBS) or Ex-4 (Abcam Biochemicals, Cambridge, MA) was then added to the plates at the appropriate concentrations (1, 10, or 100 nM) for 15 min. Medium was removed, and plates were immediately placed on liquid nitrogen. Plates were then placed on ice, and cells were scraped into freshly prepared lysis buffer for protein determination and subsequent immunoblot analysis, as described previously. For mannitol experiments, 25 mM mannitol was used in place of 25 mM fructose.
For analysis of glycolysis, GT1-7 cells were seeded in growth medium (1.5 x 10^5 cells/well) in a V3-PS 96-well plate (Seahorse Bioscience, Billerica, MA). The following day, cells were washed with PBS and incubated in XF assay medium (Seahorse Bioscience) supplemented with 1 mM pyruvate and 2 mM L-glutamine (pH = 7.4) for 1 h at 37°C in a CO₂-free incubator. Glucose (0 or 16.7 mM) and Ex-4 (100 nM) treatments were prepared in XF assay medium containing pyruvate and L-glutamine (pH = 7.38–7.42). Extracellular acidification rate (ECAR), an index of lactate production as a result of glycolysis, was measured before and after administration of glucose and Ex-4. Measurements were normalized to protein concentration as determined by Bradford protein assay, as described previously.

Statistical analysis. Data are presented as means ± SE. Differences between groups were determined by two-tailed t-test or by one- or two-way repeated-measures ANOVA followed by either Dunnett’s or Neuman-Keuls multiple comparison post hoc test as appropriate. Significance level was defined as P < 0.05.

RESULTS

Functional deletion of the GLP-1R is associated with elevated basal hypothalamic AMPK signaling and increased food intake. To determine whether endogenous basal GLP-1R signaling regulates central AMPK activity, AMPK phosphorylation was assessed in hypothalamic extracts from wild-type (Glp1r^+/+) and GLP-1R knockout (Glp1r^-/-) mice. As shown in Fig. 1A, protein levels of phosphorylated AMPK normalized to total AMPK were significantly higher (P < 0.05) in hypothalamus from 5-h-fasted Glp1r^-/- mice compared with Glp1r^+/+ mice. In agreement with previous studies (17), Glp1r^-/- mice exhibited significantly increased (P < 0.05) 24-h food intake (Fig. 1B).

Intracerebroventricular pretreatment with the AMPK activator AICAR attenuates Ex-4-mediated suppression of food intake. Pretreatment with AICAR significantly attenuated (P < 0.05) the anorectic effect of Ex-4 administered into the lateral ventricle (Fig. 2A). We chose a dose of AICAR that by itself does not stimulate food intake (Fig. 2A), as has been observed previously with higher doses (25). Indeed, a 10-fold higher dose of AICAR did stimulate 18-h food intake compared with vehicle (3.77 ± 0.37 vs. 2.61 ± 0.14 g, P < 0.05, n = 7). In agreement with previous reports (38), central GLP-1R activation suppressed (P < 0.05) water intake (Fig. 2B). Intracerebroventricular AICAR alone did not affect water intake. When administered as a pretreatment, icv AICAR attenuated (P < 0.05) the adipic effect of icv Ex-4 by ~40% (Fig. 2B).

Locomotor activity was measured to rule out the possibility that the observed effects of AICAR pretreatment are general and not specific to food intake. Supporting previous findings (9), central GLP-1R activation reduced locomotor activity (Fig. 2C). Unlike the effects on food and water intake, icv AICAR pretreatment did not attenuate the reduction of locomotor activity elicited by icv Ex-4 (Fig. 2C). Despite previous reports that GLP-1R agonists reduce energy expenditure (4, 29), icv Ex-4 had no significant effect on 18-h energy expenditure in the present studies (data not shown).

The anorectic effect elicited by Ex-4 at the chosen dose was not attributed to nausea. We measured kaolin clay consumption (i.e., pica test) as an index of nausea (43) and observed similar 18-h kaolin consumption following icv ACSF vehicle (0.21 ± 0.05 g) or Ex-4 (0.26 ± 0.10 g, n = 6). By comparison, administration of lithium chloride (0.15 M ip), a known inducer of nausea in rodents (27), stimulated 18-h kaolin consumption (1.15 ± 0.08 g, P < 0.05 vs. icv ACSF).

Ex-4 inhibits AMPK and stimulates glycolysis in the GT1-7 hypothalamic cell line. We next used the GLP-1R-expressing GT1-7 hypothalamic cell line (20, 46) to explore the mechanism by which Ex-4 inhibits AMPK activity. Ex-4 suppressed AMPK activation in these cells in a dose-dependent manner (Fig. 3A), demonstrating the validity of using this cell model. Interestingly, Ex-4 treatment did not suppress AMPK activation in GT1-7 cells cultured in the absence of glucose (Fig. 3B).

This demonstrates that the ability of GLP-1R activation to inhibit AMPK is dependent upon glucose availability. We tested the hypothesis that Ex-4 stimulates glucose metabolism in GT1-7 neurons. Using a Seahorse extracellular flux analyzer, we measured glycolytic rates as a surrogate for glucose metabolism in GT1-7 cells cultured in the absence of glucose (Fig. 3B).

Fig. 1. Glucagon-like peptide-1 receptor (Glp1r^-/-) mice display increased AMP-activated protein kinase (AMPK) activation in the hypothalamus and elevated 24-h food intake compared with Glp1r^+/+ littermates. A: representative protein immunoblots and group data for phosphorylated AMPK (p-AMPK), total AMPK, and β-actin in 5-h-fasted Glp1r^+/+ vs. Glp1r^-/- mice. Values are means ± SE and represent quantification of the ratio of p-AMPK to AMPK (each normalized individually to β-actin) for group data (n = 7/group). B: values are means ± SE and represent cumulative 24-h food intake in Glp1r^+/+ vs. Glp1r^-/- (n = 4/genotype) mice fed a high-starch diet. *P < 0.05, Glp1r^-/- vs. Glp1r^+/+.
metabolism. Figure 3C shows that Ex-4 increased (P < 0.05) glycolytic rates beyond the effect of glucose alone.

In vivo inhibition of central glycolysis attenuates the anorectic effect mediated by icv Ex-4. Based on our observations that Ex-4 inhibits AMPK in a glucose-dependent manner and stimulates glycolysis in GT1-7 cells, we tested whether inhibition of glycolysis in vivo attenuates the Ex-4-mediated suppression of food intake. Central glycolysis was inhibited via icv administration of the glycolytic inhibitor 2-DG. The ability of icv Ex-4 to reduce food intake was abolished (P < 0.05) by

Fig. 2. Intracerebroventricular (icv) pretreatment with the AMPK activator 5-aminimidazole-4-carboxamide-1-β-ribofuranoside (AICAR) attenuates exendin-4 (Ex-4)-mediated suppression of food intake. Values are means ± SE and represent cumulative food intake (A), water intake (B), and locomotor activity (C) in Glp1r−/− mice treated with icv artificial cerebrospinal fluid (ACSF), Ex-4, AICAR, or AICAR + Ex-4 (n = 8/treatment). *P < 0.05 vs. ACSF; †P < 0.05 vs. AICAR; ‡P < 0.05 vs. Ex-4.

Fig. 3. Ex-4 inhibits AMPK and stimulates glycolysis in the GT1-7 hypothalamic cell line. A: representative protein immunoblots and group data (n = 4/group) for p-AMPK, total AMPK, and β-actin in GT1-7 cells cultured in 25 mM glucose and treated with either PBS vehicle or Ex-4 at 1, 10, or 100 nM. Values are means ± SE and represent quantification of the ratio of p-AMPK to AMPK (normalized to β-actin). *P < 0.05 vs. vehicle. B: representative protein immunoblots and group data (n = 4/group) for p-AMPK, total AMPK, and β-actin in GT1-7 cells cultured in the absence of glucose and treated with either PBS vehicle or Ex-4 at 1, 10, or 100 nM. Values are means ± SE and represent quantification of the ratio of p-AMPK to AMPK (normalized to β-actin). C: extracellular acidification rate (ECAR) measured over 28 min in GT1-7 cells exposed to either 0 or 16.7 mM glucose and treated with either PBS vehicle or Ex-4 (100 nM; n = 3). Data are expressed as fold change in ECAR in vehicle- vs. Ex-4-treated cells relative to the time point at which vehicle or Ex-4 was injected (t = 0 min). Values are means ± SE. *P < 0.05 vs. 0 mM glucose. Inset: changes in ECAR following treatment with vehicle or Ex-4 in the presence of either 0 or 16.7 mM glucose measured over 28 min in the same cells for which data are presented in C.
pretreatment with icv 2-DG (Fig. 4A). Intracerebroventricular 2-DG alone had no significant effect on food intake. Pretreatment with icv 2-DG also prevented the adipic effect of Ex-4 by ∼50% (Fig. 4B). Neither icv Ex-4 nor icv 2-DG had a significant effect on 18-h energy expenditure (data not shown).

Intracerebroventricular glucose does not enhance the anorectic effect mediated by icv Ex-4. Our results indicate that central GLP-1R activation suppresses food intake via inhibition of AMPK and suggest that this occurs via increased central glucose metabolism. Therefore, we hypothesized that central administration of glucose would enhance the anorectic effect of Ex-4. Food intake was measured in mice receiving icv glucose prior to icv Ex-4 administration. At the chosen dose, icv glucose alone had no effect on food intake (Fig. 5A). When given as a pretreatment, icv glucose did not enhance the anorectic effect mediated by icv Ex-4 (Fig. 5A). Pretreatment with icv glucose also did not enhance the ability of Ex-4 to reduce water intake (Fig. 5B). Thus, contrary to our hypothesis, increasing central glucose availability does not enhance the anorectic effect mediated by activation of the central GLP-1R.

Intracerebroventricular fructose attenuates Ex-4-mediated reduction of food intake and AMPK activity. Mice fed a high-sucrose diet display increased (P < 0.05) food intake compared with mice fed an isocaloric starch diet (Fig. 6A). We explored whether fructose, a component of sucrose, could affect the anorectic response mediated by Ex-4. This is based on the observation that icv-administered fructose stimulates hypothalamic AMPK activity (28). The anorectic effects of Ex-4 were blunted (P < 0.05) by pretreatment with icv fructose (Fig. 6B). At the chosen dose, icv fructose alone had no significant effect on food intake. Intracerebroventricular fructose also attenuated (P < 0.05) the reduction in water intake elicited by icv Ex-4 (Fig. 6C). Similar to what was observed in the icv AICAR and 2-DG studies, neither icv Ex-4 nor icv fructose had a significant effect on 18-h energy expenditure (data not shown).

Fig. 4. In vivo inhibition of central glycolysis attenuates the anorectic effect mediated by icv Ex-4. Values are means ± SE and represent cumulative food intake (A) and water intake (B) in Glp1r+/+ mice treated with icv ACSF, Ex-4, glucose, or glucose + Ex-4 (n = 7–15/treatment). *P < 0.05 vs. ACSF; †P < 0.05 vs. 2-DG; ‡P < 0.05 vs. Ex-4.

Fig. 5. Intracerebroventricular glucose does not enhance the anorectic effect mediated by icv Ex-4. Values are means ± SE and represent cumulative food intake (A) and water intake (B) in Glp1r+/+ mice treated with icv ACSF, Ex-4, glucose, or glucose + Ex-4 (n = 10/treatment). *P < 0.05 vs. ACSF; †P < 0.05 vs. glucose; ‡P < 0.05 vs. Ex-4.

To determine whether fructose can interfere with the inhibition of AMPK by Ex-4, GT1-7 cells were cultured in the presence of fructose for 1 h prior to treatment with Ex-4. A 1-h fructose incubation was sufficient to markedly (P < 0.05) stimulate AMPK (Fig. 7A). Contrastingly, the ability of Ex-4 to reduce AMPK phosphorylation in the presence of glucose (Fig. 3A), Ex-4 did not suppress AMPK activation in GT1-7 cells exposed to an equal concentration of fructose (Fig. 7B). Ex-4 also did not suppress AMPK activation in the presence of 25 mM mannitol (Fig. 7C), demonstrating that inhibition of AMPK by GLP-1R activation requires glucose.

DISCUSSION

The ability of GLP-1R agonists to suppress food intake has been recognized for more than a decade, yet the mechanism by which this occurs remains largely unknown. Hayes et al. (20) showed recently that hindbrain Ex-4 administration suppresses food intake and inhibits AMPK in the dorsal vagal complex. Furthermore, pretreatment with AICAR blunted the anorectic effect mediated by hindbrain Ex-4 administration. The present studies support these findings and show that AICAR attenuates the anorectic effect mediated by Ex-4 administered into the forebrain lateral ventricle. We extend these findings by showing that the inhibition of AMPK and food intake by Ex-4 requires glucose metabolism. This is analogous to the dependence on glucose that is characteristic of pancreatic GLP-1 action (59). We demonstrate further that the nature of carbohydrates can influence the central actions of GLP-1R agonists. Our results show that the sucrose metabolite fructose impairs the anorectic effect mediated by centrally administered Ex-4. This is likely due to the ability of fructose to prevent the inhibition of hypothalamic AMPK by Ex-4. Assuming that dietary fructose is metabolized in the brain, this provides a potential mechanism for the increased food intake associated with sucrose consumption. This has significant clinical rele-
vance given the association between increased sucrose and fructose consumption and the obesity epidemic, particularly in children (34).

AMPK is a critical cellular energy sensor that regulates energy homeostasis by sensing changes in nutritional and hormonal signals (64). Modulation of central AMPK activity has direct effects on food intake. Activation of central AMPK by orexigenic factors (e.g., fasting, ghrelin, and hypoglycemia) stimulates food intake, whereas its inhibition by anorexigenic factors (e.g., feeding, leptin, insulin, and glucose) suppresses food intake (41). We and others have shown that GLP-1R agonists inhibit AMPK in the hypothalamus (6, 49) and hindbrain (20). In the present studies, we show that loss of basal GLP-1R activity in Glp1r−/− mice is associated with both increased food intake and hypothalamic AMPK activity. The observation that pretreatment with AICAR in either the fourth ventricle (20) or lateral ventricle, as in the present studies, attenuates the anorectic effect of Ex-4 demonstrates that inhibition of AMPK is a general mechanism by which central GLP-1R activation suppresses food intake. However, this does not rule out potential contributions of non-AMPK pathways to the anorectic effect of GLP-1R agonists. Hindbrain administration of Ex-4 also activates the cAMP-dependent protein kinase (PKA) and p44/42 MAP kinase, which are regulators of feeding-related genes via the CREB transcription factor (20). Future studies in mice with conditional disruption of brain AMPK will be needed to define the potential contribution of non-AMPK pathways to the anorectic effects of GLP-1R agonists.

The GLP-1R is expressed throughout the brain, including areas that play a role in the regulation of food intake, such as the hypothalamus and hindbrain (15, 40). Since compounds were administered into the lateral ventricle in the present studies, we cannot identify the specific brain region(s) in which inhibition of AMPK by Ex-4 results in decreased food intake. We also cannot exclude the possibility that modulation of GLP-1R activation and AMPK exert control on food intake via distinct brain regions. Nevertheless, administration of GLP-1R agonists into the lateral, third, or fourth ventricles, as well as targeted delivery of GLP-1R agonists into hypothalamic nuclei or hindbrain regions, reduces food intake (21, 45, 47, 55, 56) as well as AMPK phosphorylation (6, 20, 49). When administered into the lateral or the fourth ventricles, GLP-1R agonists reduce hypothalamic and hindbrain AMPK phosphorylation within 20 min (6, 20). Interestingly, the anorectic effect of hindbrain GLP-1R activation is delayed by several hours and is maximal at 24 h following Ex-4 administration (20). In the present studies, the anorectic effect of Ex-4 administered into the lateral ventricle was observed within the first hour of dosing, during which food intake was suppressed by ~75% compared with vehicle (data not shown). This is in agreement with previous studies demonstrating a rapid anorectic response to GLP-1R agonists delivered to the lateral or third ventricles or directly into the hypothalamus (45, 47, 55, 56). The apparent difference in the onset of anorexia between hindbrain and forebrain GLP-1R activation may reflect an effect of experimental design, such as the different doses of GLP-1R agonists used. Alternatively, this could be indicative of differences in the mechanisms by which forebrain and hindbrain GLP-1R signaling regulate food intake. However, whether such a discrepancy between forebrain and hindbrain GLP-1R signaling exists in mice remains to be determined. Studies using site-specific disruptions of the GLP-1R are required to fully elucidate the relative contributions of different brain regions to the regulation of food intake by GLP-1R agonists and to identify the signaling pathways downstream of the GLP-1R in distinct brain regions.

GLP-1 acts at multiple sites in both the central nervous system (CNS) and periphery to promote insulin secretion, maintain glucose homeostasis, and reduce food intake and body weight (60). GLP-1 is not only secreted from the gut but also synthesized in the nucleus of the tractus solitarius of the brainstem, a brain region that receives vagal inputs from visceral organs and extends axonal projections to other brain regions, including the hypothalamus (22, 26, 30, 40). This suggests that GLP-1 synthesized in the brainstem may be the principal mediator of the anorectic effects attributed to GLP-1. However, there is also evidence of peripheral...
GLP-1 action in the regulation of food intake (61). Thus, the source of GLP-1 that regulates feeding behavior as well as the relative contributions of peripheral vs. central GLP-1R-mediated effects on food intake remain to be clearly identified.

AMPK activity is regulated allosterically by changes in the AMP/ATP ratio and covalently via phosphorylation by various AMPK kinases (18). A decrease in the AMP/ATP ratio due to increased metabolism and ATP production causes a conformational change in AMPK that enhances its interaction with inactivating AMPK phosphatases. GLP-1R activation increases glucose uptake and ATP production in NSC-34 motor neurons (31). Therefore, we speculated that inhibition of AMPK by GLP-1R activation occurred via increased glucose metabolism. This was supported by the observation that, in the absence of glucose, Ex-4 was unable to suppress AMPK in hypothalamic GT1-7 cells. Furthermore, we show that Ex-4 increases glycolysis beyond the effects of glucose alone in GT1-7 cells. We observed a similar effect in another GLP-1R-expressing hypothalamic cell line, A2/28 cells (data not shown) (5). As immortalized cell lines, GT1-7 and A2/28 cells display high basal glycolytic rates, making it all the more significant that Ex-4 further stimulated glycolysis. This demonstrates a novel mechanism by which GLP-1R activation regulates central AMPK activity. It must be noted that inhibition of AMPK by Ex-4 in the hypothalamic cell lines was observed in the presence of 25 mM glucose, which exceeds the typical glucose concentrations in the brain. Nevertheless, physiological evidence for the proposed glucose-dependent properties of GLP-1R-induced anorexia is demonstrated by the fact that inhibition of central glycolysis via icv 2-DG administration attenuates the anorectic effect mediated by icv Ex-4 administration. Taken together, our findings propose a model whereby central GLP-1R activation enhances glucose metabolism, resulting in decreased AMPK activity and suppression of food intake. This provides a mechanism for feedback inhibition of food intake following a meal.

The proposed model indicates that glucose is not only a stimulus for GLP-1 secretion but also a component of the mechanism by which GLP-1 suppresses food intake. Therefore, we hypothesized that an increase in central glucose would mimic the fed state and enhance the anorectic effect of Ex-4. However, we did not observe such a cooperative effect. We did not measure CNS glucose levels, nor did we perform glucose dose response experiments, so it is not clear whether the changes in CNS glucose that occur within a fast-to-fed cycle in mice are sufficient to modulate the response to central GLP-1 action. Nevertheless, our studies using 2-DG suggest that glucose metabolism is a necessary component for the anorectic effect of Ex-4. Glucose was delivered centrally in the present studies to circumvent the effects of other anorectic factors normally secreted in response to oral nutrient intake. However, we cannot exclude the possibility that central GLP-1 action is coordinated with pathways activated by hormones such as insulin and leptin or even by the peripheral sensing of glucose.

Components of palatable foods, mainly fat and sugar, can impair anorectic mechanisms and stimulate weight gain. In rats chronically fed sugar solutions, caloric overconsumption and body weight gain occur from an activation of hunger signals and reward components and a depression of satiety signals (33). Moreover, food intake is increased in rats given an oral sucrose preload compared with a starch preload (13). When administered centrally, the sucrose metabolite fructose enhances hypothalamic AMPK activation and subsequently stimulates food intake (7, 24). We expand upon these observations and show that icv pretreatment with fructose attenuates the anorectic effect of icv Ex-4. This is likely due to the ability of fructose to prevent the inhibition of hypothalamic AMPK by Ex-4, as shown in GT1-7 cells. These findings suggest that sucrose-derived fructose can attenuate the

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**Fig. 7.** Fructose stimulates AMPK and prevents inhibition of AMPK by Ex-4 in GT1-7 cells. A: representative protein immunoblots and group data (n = 4/group) for p-AMPK, total AMPK, and β-actin in GT1-7 cells cultured in fructose for ≤60 min. *P < 0.05 vs. t = 0 min. B: representative protein immunoblots and group data (n = 4/group) for p-AMPK, total AMPK, and β-actin in GT1-7 cells cultured in 25 mM fructose for 60 min and treated with either PBS vehicle or Ex-4 at 1, 10, or 100 nM. Values are means ± SE and represent quantification of the ratio of p-AMPK/AMPK (normalized to β-actin). C: representative protein immunoblots and group data (n = 3/group) for p-AMPK, total AMPK, and β-actin in GT1-7 cells cultured in 25 mM mannitol for 60 min and treated with either PBS vehicle or Ex-4 at 1, 10, or 100 nM. Values are means ± SE and represent quantification of the ratio of p-AMPK to AMPK (normalized to β-actin).
satiation effect mediated by the central GLP-1R, resulting in the increased food intake associated with sucrose feeding. This hypothesis posits that the brain is a site for the metabolism of dietary fructose. The GLUT5 fructose transporter is expressed in the central nervous system, and its expression is increased in response to chronic fructose feeding (52). This is especially significant since fructose itself regulates GLUT5 expression, thus raising the possibility that fructose is taken up and metabolized in the CNS. Furthermore, proteins necessary for the metabolism of fructose, including fructokinase and aldolase, are expressed in the brain (11). However, it is generally believed that dietary fructose is significantly extracted in the liver (35), and the possibility that sucrose consumption impairs central GLP-1 action acutely via central fructose metabolism remains to be tested. We cannot exclude the possibility that central GLP-1 resistance results from chronic intake of sucrose. Chronic sucrose consumption results in leptin resistance (19, 51). Given the interaction between central leptin and GLP-1 action (16, 46, 48), it is possible that sucrose feeding impairs the anorectic effects of GLP-1 indirectly via modulation of leptin action.

Collectively, these findings implicate central AMPK as a critical signaling molecule in the anorectic effect following central GLP-1R activation. We provide mechanistic insight by demonstrating a role for glucose metabolism in the regulation of hypothalamic AMPK by GLP-1R activation. Glucose dependence is a characteristic of pancreatic GLP-1 action, and in the context of feeding behavior, it provides a safety mechanism ensuring that GLP-1 suppresses food intake only in the presence of nutrients. This has significant clinical implications given the increased use of GLP-1-based therapies as treatments for type 2 diabetes. Importantly, some GLP-1-based therapies are associated with moderate weight loss (58), highlighting the need to understand the potential anti-obesity mechanisms regulated by these compounds. We demonstrate further that fructose can significantly impair the anorectic response to GLP-1R agonists. The link between increased consumption of sugary foods and beverages and the obesity epidemic underscores the need to understand the mechanisms by which dietary carbohydrate composition influences satiety and feeding behavior.

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**DISCLOSURES**

The authors have no conflicts of interest, financial or otherwise, to declare.

**AUTHOR CONTRIBUTIONS**

M.A.B., Jennifer E. Ayala, and Julio E. Ayala contributed to the conception and design of the research; M.A.B. and Jennifer E. Ayala performed the experiments; M.A.B., Jennifer E. Ayala, and Julio E. Ayala analyzed the data; M.A.B., Jennifer E. Ayala, and Julio E. Ayala interpreted the results of the experiments; M.A.B. and Jennifer E. Ayala prepared the figures; M.A.B. drafted the manuscript; M.A.B., Jennifer E. Ayala, D.J.D., and Julio E. Ayala edited and revised the manuscript; Julio E. Ayala approved the final version of the manuscript.

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