Stimulation of feeding by three different glucose-sensing mechanisms requires hindbrain catecholamine neurons

Ai-Jun Li, Qing Wang, Thu T. Dinh, Bethany R. Powers, and Sue Ritter

Programs in Neuroscience, Washington State University, Pullman, Washington

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Li AJ, Wang Q, Dinh TT, Powers BR, Ritter S. Stimulation of feeding by three different glucose-sensing mechanisms requires hindbrain catecholamine neurons. Am J Physiol Regul Integr Comp Physiol 306: R257–R264, 2014. First published December 31, 2013; doi:10.1152/ajpregu.00451.2013.—Previous work has shown that hindbrain catecholamine neurons are required components of the brain’s glucoregulatory circuitry. However, the mechanisms and circuitry underlying their glucoregulatory functions are poorly understood. Here we examined three drugs, glucosamine (GcA), phloridzin (Phl), and 5-thio-d-glucose (5TG), that stimulate food intake but interfere in different ways with cellular glucose utilization or transport. We examined feeding and blood glucose responses to each drug in male rats previously injected into the hypothalamic paraventricular nucleus with anti-dopamine-β-hydroxylase conjugated to saporin (DSAP), a retrogradely transported immunotoxin that selectively lesions noradrenergic and adrenergic neurons, or with unconjugated saporin (SAP) control. Our major findings were 1) that GcA, Phl, and 5TG all stimulated feeding in SAP controls whether injected into the lateral or fourth ventricle (LV or 4V), 2) that each drug’s potency was similar for both LV and 4V injections, 3) that neither LV or 4V injection of these drugs evoked feeding in DSAP-lesioned rats, and 4) that only 5TG, which blocks glycolysis, stimulated a blood glucose response. The antagonist of the MEK/ERK signaling cascade, U0126, attenuated GcA-induced feeding, but not Phl- or 5TG-induced feeding. Thus GcA, Phl, and 5TG, although differing in mechanism and possibly activating different neural populations, stimulate feeding in a catecholamine-dependent manner. Although results do not exclude the possibility that catecholamine neurons possess glucose-sensing mechanisms responsive to all of these agents, currently available evidence favors the possibility that the feeding effects result from convergent neural circuits in which catecholamine neurons are a required component.

Hindbrain catecholamine neurons are required components of the brain’s glucoregulatory circuitry. Pharmacological (29), chemical (26, 43), or immunotoxin (35, 40) disruption of these neurons impairs or abolishes key protective responses to glucose deficiency induced by hypoglycemic doses of insulin or by central or peripheral blockade of glycolysis using the antimetabolic glucose analogue 2-deoxy-D-glucose (2DG). Dissection of the hindbrain catecholamine system using the retrogradely transported immunotoxin anti-dopamine-β-hydroxylase conjugated to saporin (DSAP) has revealed distinct responses to glucoprivation that are dependent on hypothalamically and spinally projecting norepinephrine (NE) and/or epinephrine (E) neurons. Specifically, some of those NE and E neurons with processes that innervate the medial hypothalamic region are required for feeding (35), corticosterone (40), and reproductive responses (15) to glucoprivation. Spinally projecting E neurons that innervate adrenal medullary preganglionic neurons are required for the adrenal medullary response to glucoprivation (35). These catecholamine-mediated responses are diverse array of behavioral, endocrine, and autonomic responses, all of which are involved in prevention of and recovery from glucose deficit.

Despite the demonstrated importance of hindbrain NE and E neurons for eliciting key responses to glucose deficit, the glucose-sensing mechanisms and circuitry underlying their glucoregulatory functions are poorly understood. Furthermore, a number of drugs that reduce glucose availability or metabolism, but by different mechanisms, have been shown to increase food intake, suggesting the possibility (or likelihood) of multiple glucose-driven feeding systems. Therefore, we address these issues in the present set of experiments, focusing on control of food intake and blood glucose response. We examine three drugs, glucosamine (GcA), phloridzin (Phl), and 5-thio-d-glucose (5TG), all known to produce glucose deficit by different mechanisms, to determine whether the signals they generate increase both feeding and blood glucose and whether they require NE or E neurons to do this.

5TG is an antimitabolic glucose analogue that reduces glucose utilization in all cells. Its most potent antiglycolytic actions are inhibition of phosphoglucuronate and glucose-6-phosphate dehydrogenase, but it also inhibits hexokinase (8). GcA inhibits glucokinase (GK), a hexokinase that selectively phosphorylates only glucose, is saturated at high glucose concentration ($S_{0.5}^G = 5–10$ mM glucose) and is not inhibited by its product glucose-6-phosphate (9, 16). GK is an endogenous glucose-sensing molecule that plays a central role in stimulating insulin secretion when glucose levels rise. GK is distributed sparsely in the brain but has been detected in areas important for control of food intake (9, 18, 27), including in glucose-excited (GE) and glucose-inhibited (GI) neurons of the hypothalamus (9) and nucleus of the solitary tract (NTS) (2). Phl is the classic sodium-linked glucose transporter (SGLT) antagonist (10). SGLTs transport glucose across cell membranes against the glucose concentration gradient by coupling with downgradient transport of Na$^+$. Phl reduces glucose entry only into cells that express the SGLT receptor. The latter include some hypothalamic neurons of both the GE and GI type (6, 12, 31). These neurons respond to changes in extra-cellular glucose but are not responsive to GcA, alloxan, or N-acetyl-D-glucosamine and do not express GK. Because the response of SGLT-expressing neurons to antimitabolic glucose analogues mimics the effect of glucose, this transporter has been described as a nonmetabolic glucose sensor, possibly...
serving to inform the brain about ambient extracellular glucose levels (6, 12).

In the experiments reported here, we examine the importance of hindbrain catecholamine neurons for GcA-, Phl-, and STG-induced feeding and the adrenal medullary hyperglycemic response using animals in which hypothalaminally projecting NE and E neurons were retrogradely lesioned by medial hypothalamic injection of DSAP. Since both lateral and fourth ventricle (LV and 4V, respectively) injections of GcA, Phl, and STG have been demonstrated to increase food intake (11, 34, 44, 45), suggesting the possibility that these drugs may act at multiple sites to produce their glucoregulatory actions, we tested both LV and 4V injections of these agents in this study. Finally, we examined the dependence of GcA-, Phl-, and STG-induced feeding on the mitogen-activated protein kinase (MAPK)/ERK1/2 signaling cascade, which has been shown to be essential for transducing NE-mediated corticotropin-releasing hormone transcription and release in response to glucoprivation (19).

RESEARCH DESIGN AND METHODS

**Animals.** Male Sprague-Dawley rats were purchased from Simon-sen Laboratories (Gilroy, CA) and housed individually in an animal care facility approved by the Association for Assessment and Accreditation of Laboratory Animal Care. Rats were maintained on a 12-h light/12-h dark cycle (lights on between 7 AM and 7 PM) and with ad libitum access to pelleted rodent food (F6 Rodent diet; Harlan Teklad, Madison, WI) and tap water. All experimental procedures were approved by Washington State University Institutional Animal Care and Use Committee, which conforms to National Institutes of Health guidelines.

**Microinjection of immunotoxin.** As previously described (35, 40), DSAP (82 ng/200 nl; Advanced Targeting Systems, San Diego, CA) or unconjugated saporin (SAP) control solution (17.2 ng/200 nl), dissolved in 0.1 M phosphate buffer (PBS, pH 7.4), was infused bilaterally in rats anesthetized using chloropent solution in water [4.25% chloral hydrate, 0.89% pentobarbital sodium, 2.12% magne-sium sulfate, 14.3% (vol/vol) ethanol, 33.8% (vol/vol) propylene glycol]. Injections were made using a Picospritzer through a pulled glass capillary pipette (30-μm tip diameter) positioned just dorsal to the targeted site in the paraventricular nucleus of hypothalamus (PVH) using the following coordinates: 1.8 mm caudal to bregma, 0.4 mm lateral to midline, and 7.4 mm ventral to the dura mater. The amount of unconjugated SAP in the control solution was equal to the amount of SAP present in the DSAP conjugate (21%), as indicated in the manufacturer’s product information. Previous work comparing SAP and noninjected controls demonstrated that SAP, used in this way, does not produce behavioral or histological signs of toxicity (35, 40). To permit retrograde transport of the toxin and complete degeneration of lesioned neurons, a 3-wk interval was allowed between the DSAP injections and further experimentation.

**Screening test for lesion effectiveness.** As an initial screening test to assess effectiveness of the DSAP lesion, SAP and DSAP rats were tested for 2DG-induced feeding, a response that is impaired by this lesion (35). 2DG (200 mg/kg body wt in 0.9% sterile saline; Sigma-Aldrich, St. Louis, MO) or 0.9% saline (control) was injected subcu-taneously at ~9:00 AM and food intake was measured during the subsequent 4 h. Any DSAP rat that ate more that 2.0 g of food after 2DG injection was eliminated from the study. At the end of experiments, the lesion was analyzed further by quantifying dopamine β-hydroxylase (DBH)-immunoreactive cell bodies in hindbrain cate-cholamine cell groups, as described below. To be included in the data analysis, DSAP rats were required to have a reduction of at least 70% in DBH cell numbers in A1, A1/C1, and C1m compared with the respective averages in SAP rats. Overall about 90% of rats had successful lesions.

**Cannula implantation and intraventricular injections.** Cannulas were implanted into the 4V or LV 3 wk after DSAP or SAP treatment and following the screening test for efficacy of the DSAP lesion. For cannula implantation, rats were anesthetized with 1.0 ml/kg body wt of ketamine-xylazine-acepromazine cocktail in 0.9% saline solution (50 mg/kg ketamine HCl, Fort Dodge Animal Health, Fort Dodge, IA; 5.0 mg/kg xylazine, Vedco, St. Joseph, MO; and 1.0 mg/kg acepromazine, Vedco) and placed in a stereotaxic device. A 26-gauge cannula was implanted into the 4V or left LV. Stereotaxic coordinates were 1.55 mm rostral to the occipital suture, 0 mm lateral to midline, and 6.3–6.4 mm ventral to the skull surface for 4V cannulation. For LV cannulation, coordinates were 1.0 mm caudal to bregma, 1.5 mm lateral to midline, and 3.9 mm ventral to the brain surface (33). Beginning 1 wk later, feeding and blood glucose tests were conducted after delivery of drug or vehicle into the 4V or LV over a 5-min period using a Gilmore micrometer syringe (Cole-Parmer, Vernon Hills, IL).

The potency and correct placement of 4V cannulas were assessed by measuring tail blood glucose levels before and 60 min after 4V STG (135 μg/3 μl) (34) using a FreeStyle glucose monitor. LV cannulas were assessed using angiotensin II (50 ng/3 μl per rat; Sigma-Aldrich)-induced water intake (17). Only rats that increased blood glucose levels to at least 150% of control within 60 min after 4V STG injection or drank at least 6 ml within 15 min of LV angiotensin II and that had cannula placements verified histologically as being correct were included in the data analysis. Overall about 90% of 4V and LV cannulas were correctly positioned.

**Measurements of food intake and blood glucose.** Food intake was measured in DSAP-lesioned rats and SAP controls in 4-h tests after injection of STG, GcA, Phl, or the respective vehicle into the LV or 4V. GcA (Sigma-Aldrich) was dissolved in artificial cerebral spinal fluid (aCSF) and injected into the LV and 4V at 0.2 or 0.6 mg in 3 μl or 1.0 mg in 5 μl (32). The composition of aCSF (in mM) was 128 NaCl, 3.1 KHCO3, 1 MgCl2, 21.4 NaHCO3, 1.2 CaCl2, and 2.8 glucose. Phl was dissolved in 50% DMSO in saline and injected at 56.25, 112.5, and 224.0 μg/3 μl per rat. STG (135 μg) was injected in 3 μl of aCSF. In the same rats on separate test days, blood glucose concentrations were measured in the absence of food before and during a 2- or 5-h period after intraventricular drug injection. Glucose was measured from tail blood using a FreeStyle glucose monitor (Abbot Diabetes Care, Alameda, CA). Body weights were measured on the test days. Average body weight differed in the experimental groups ranged from 410 to 478 g, and there were no significant differences between treatment groups (P > 0.09). Testing was begun 1 wk after LV or 4V cannula implantation and completed 3–4 wk later. Drug tests were separated by at least 1 wk.

To examine the involvement of the MEK/ERK signaling cascade in responses to these different glycemic challenges, an inhibitor of this pathway, U0126 (1,4-diamino-2,3-dicyano-1,4-bis[2-aminophenylthio]-butadiene, 1.0 or 6.0 μg/2 μl per rat) or 2 μl of the solvent (50% DMSO in saline), was injected into the 4V 30 min before injection of GcA (0.6 mg/rat), STG (135 μg/rat), Phl (112.5 μg/rat), or vehicle solutions into the 4V. Feeding and blood glucose tests were performed on separate test days, as described.

**Immunohistochemistry.** After completion of testing, rats were euthanized by deep isoflurane-induced anesthesia (Halocarbon Products). Just before cessation of the heartbeat, rats were perfused transcardially with cold phosphate-buffered saline (PBS, pH 7.4) followed by freshly made cold 4% formaldehyde in PBS. After perfusion, brains were rapidly removed and placed in 4% formaldehyde/PBS at 4°C overnight, then transferred to 12.5% and 25% sucrose in PBS for 24 h each, then sectioned coronally on a cryostat at 40 μm thickness and collected into serial sets for immunohistochemical processing and for examine of cannula placement. Brain sections were stained using standard avidin-biotin-peroxidase immunohistochemical
techniques to detect DBH (35). They were incubated with mouse monoclonal anti-DHB (1:20,000; Millipore, San Jose, CA), washed and sequentially incubated in biotintyalted donkey anti-mouse IgG (1:500; Jackson ImmunoResearch Laboratories), ExtrAvidin-Peroxidase (1:1,500; Sigma-Aldrich), and reacted with nickel-intensified diaminobenzidine to produce a gray/black reaction product. Catecholamine cell groups are defined as in The Rat Brain in Stereotaxic Coordinates (33). However, we refer here to the middle portion of C1 as C1m, which extends from −12.98 to −12.5 mm caudal to bregma. DBH-positive cells were counted bilaterally in three consecutive coronal sections for each region for each rat. Hypothalamic areas were examined to detect presence or absence of DBH terminals in established DBH terminal areas, but these were not quantified. However, all DSAP rats with significant loss of catecholamine neurons in the areas described above also had significant loss of DBH terminals in PVH and medial hypothalamic areas (Fig. 1), as reported previously (35, 40).

Statistical analysis. All results are presented as means ± SE. For statistical analysis of data, we used a t-test, one-way ANOVA, two-way repeated measures ANOVA, as appropriate. After significance was determined by ANOVA, multiple comparisons between individual groups were tested using a post hoc Fisher’s LSD test. *P < 0.05 was considered to be statistically significant.

RESULTS

Effects of GcA on feeding and blood glucose. Both 4V and LV GcA stimulated food intake (Fig. 2A) in normal rats in a dose-dependent fashion during the 4-h test. Intake was increased significantly after both 4V (n = 6–9 rats/group) and LV (n = 7–8 rats/group) GcA at 0.6 and 1.0 mg/rat (P < 0.05 for both 4V and LV compared with their respective aCSF control injections), but was not significantly increased at 0.2 mg/rat. The two higher GcA doses appeared to be nearly equipotent for stimulation of feeding. PVH SAP controls also increased feeding in response to both 4V (n = 8–9 rats/group) and LV (n = 6 rats/group) injections of GcA (0.6 mg/rat) compared with their intake after aCSF injection (P < 0.01 vs. SAP control; Fig. 2B). PVH DSAP abolished the feeding responses to both 4V and LV GcA injections (P > 0.7 vs. DSAP control; n = 7–8, and 8 rats/group for 4V and LV, respectively).

GcA injection (0.6 mg/rat) had no effect on blood glucose levels in rats (Fig. 3) during the 5 h after injection into the 4V (P > 0.8 between groups; n = 5 rats per group) or into the LV (P > 0.2 between groups; n = 6 rats per group).

Effects of Phl on feeding and blood glucose. Injection of Phl into either the 4V (n = 10–11 rats/group) or LV (n = 8–12 rats/group) dose-dependently enhanced food intake in rats. Four-hour food intake was increased significantly after 112.5 and 225 μg/rat, but not after 56.25 μg/rat (P < 0.01 vs. solvent control for both 4V and LV) and the amount consumed was similar in both LV and 4V groups (Fig. 4A). PVH SAP controls also increased feeding in response to both LV (n = 6 rats/group) and 4V (n = 8 rats/group) injections of Phl (Fig. 4B) compared with their intake after solvent injection (P < 0.001 vs. SAP control). In contrast, PVH DSAP rats failed to increase food intake in response to Phl injection into either LV or 4V (P > 0.7 vs. DSAP control; n = 8 or 9 rats/group for LV, 4V injection, respectively).
Blood glucose levels were not altered by injection of Phl (225 g/rat) into either the LV (P > 0.8, between treatments; n = 6 rats per group) or 4V (P > 0.2 for 4V and LV injections, respectively; n = 5, 6 rats per group) during 5 h after the injection (Fig. 5).

**Effects of 5TG on feeding and blood glucose.** Both LV and 4V 5TG (135 g/rat) increased feeding significantly in a 4-h test in SAP rats compared with intake after aCSF injection (P < 0.001 SAP 5TG vs. SAP saline for both LV and 4V rats; n = 5–6 and 6 rats per group for 4V and LV injections, respectively) but failed to increase feeding in PVH DSAP rats (P > 0.3 vs. SAP controls; Fig. 6). However, blood glucose levels in both SAP and DSAP rats were significantly elevated 1 h after 5TG injection compared with their respective aCSF control injection (P < 0.001 vs. aCSF; n = 11, and 6–8 rats per group for 4V and LV injections, respectively)

**Effects of U0126 on feeding and blood glucose.** The MEK inhibitor U0126 was injected into the 4V 30 min before 4V injection of 5TG (135 μg/rat), GcA (0.6 mg/rat), and Phl (112.5 μg/rat). As shown in Fig. 7A, prior injection of U0126 (1 μg/rat) completely blocked GcA-induced food intake (P > 0.5, vs. control; n = 8–9 rats per group) but did not reduce the feeding in response to Phl (Fig. 7B; P < 0.01 vs. control; n = 7–10 rats per group). Even a higher dose of U0126 (6 μg/rat) failed to block 5TG-induced feeding (Fig. 7C) or hyperglycemia (P < 0.001 vs. control; n = 6 rats per group). U0126 was also tested at lower doses and was ineffective in reducing the feeding and hyperglycemic effects of 5TG (not shown).

**DISCUSSION**

Results of these experiments show that GcA, Phl, and 5TG all stimulated feeding when injected into either the LV or 4V and, at the doses used here, the potency of each drug was similar at both injection sites. Furthermore, feeding responses to both LV and 4V injections were abolished by retrograde destruction of NE and E neurons innervating the medial hypothalamus, indicating that the feeding responses evoked by all three agents require these NE or E neurons. Earlier pharmacological and biochemical evidence has demonstrated a crucial role for NE and E neurons in glucoprivic feeding (3, 4, 20, 41). Fos and gene expression data have shown that specific subpopulations of catecholamine neurons are potently activated by 2DG-induced glucoprivation (21, 24, 37). Gene silencing results have indicated that neuropeptide Y/catecholamine coexpressing neurons are required for the glucoprivic feeding response (22). Previous work has also shown that food intake can be evoked by third ventricle injections of GcA (45), third ventricle (44) and 4V (11) injections of Phl, and both LV and 4V injections of 5TG (34). However, these are the first data indicating that the signals generated by the actions of these drugs interact with and require hindbrain catecholamine neurons for their orexigenic effects.

It could be argued that loss of feeding in response to 5TG, Phl, and GcA in PVH DSAP-lesioned rats results from nonse-
lective behavioral suppression arising from the lesion. However, this possibility is extremely unlikely because repeatedly replicated experimental observations indicate that this DSAP lesion of hindbrain catecholamine neurons does not impair feeding induced by nonglucoprivic stimuli, such as overnight food deprivation or administration of \( \text{H}_{2} \text{S}_{2} \text{O}_{3} \), an inhibitor of fatty acid oxidation (14, 35). Furthermore, we recently measured circadian distribution of feeding and activity in rats with this same DSAP lesion and found no differences between DSAP and control groups (23). Hence, this DSAP lesion appears to impair the glucoprivic feeding response without producing nonspecific impairment of feeding or other behaviors.

Together these results show that a variety of mechanistically distinct glucose-sensing mechanisms contribute to control of food intake, but those tested here all require intact hindbrain catecholamine neurons. However, an unanswered question highlighted by the present work is whether catecholamine neurons themselves possess or respond to the glucose-sensing mechanisms activated by these drugs or whether cells sensitive to these drugs alter catecholamine neurons through their neural connectivity. Catecholamine neurons are not homogeneous in phenotype or function and only limited work has been done to evaluate the various subtypes for their glucose-sensing capabilities or their expression of known glucose-sensing mechanisms.

Evidence currently in hand regarding 5TG, GcA, and Phl favors the view that their sites of action differ. 5TG, a glucose analogue, which uses cellular glucose transporters and antagonizes glycolysis at a number of steps, has the capability of reducing glucose utilization in all cells. Nevertheless, the brain sites where intraparenchymal nanoliter injections of 5TG are effective in stimulating feeding and counterregulatory responses are limited to specific sites within the hindbrain parenchyma (1, 36). Also, although both LV and 4V injections of 5TG are effective in stimulating feeding, acute aqueduct occlusion eliminates the response to LV, but does not diminish the feeding response to 4V injections (34), indicating that LV injections are effective due to their diffusion through the ventricular system to receptive sites in the hindbrain. Studies similar to those localizing 5TG’s sites of action have not been done for either GcA or Phl. However, both are more limited than 5TG in the cellular mechanisms they target, which also suggests that their anatomical sites of action may differ and may be more limited. GcA blocks glucose utilization only in the subset of cells that express GK (7). GK immunoreactivity has been identified in periventricular ependymocytes, endothelial cells, serotonin neurons (28), tanyocytes and periventricular glial cells (42), hypothalamic neurons (9, 12, 18, 27), and in both GE and GI neurons in the NTS (2). In addition, GK may be expressed by some catecholamine neurons. GK transcript has been detected in laser catapult microdissected tissue from...
The NTS containing A2 and C2 catecholamine cells and the GK message was inducible by hypoglycemia (5). Phl reduces glucose entry only into cells that express the SGLT receptor. Blockade of SGLT has been shown to inhibit activity of GE neurons and to activate SGLT-expressing GI neurons in the hypothalamus (6, 12). The response of these neurons to anti-metabolic glucose analogues mimics the effect of glucose. They respond to changes in extracellular glucose but are not responsive to GaA, alloxan, or N-acetyl-D-glucosamine and do not express GK, suggesting that, at least in some sites, GaA and Phl activate different cells. There is no evidence to date that NE or E neurons express SGLTs.

Although the DSAP lesion data reported here suggests that 5TG, GaA, and Phl all evoke feeding via a circuit that includes hindbrain catecholamine neurons, it should be noted that medial hypothalamic DSAP injections presumably destroy multiple subpopulations of functionally distinct catecholamine neurons with projections to the hypothalamus. Thus it may be that each of these agents either directly or indirectly activates different catecholamine subpopulations. Indeed, utilization of several different mechanisms of glucose sensing would seem adaptive for controlling food intake over a wide range of glucose concentrations. For example, Phl may stimulate feeding by suppressing activation of SGLT-expressing GE neurons whose normal role perhaps is to inhibit feeding or by activating GI neurons. Similarly, the fact that GK has a low affinity for glucose (7, 16), requiring glucose concentrations for activation that are much higher than normal brain glucose levels, suggests that GK may control food intake during daily meals, most of which occur at blood glucose levels that are within the normal range, or that it may sustain feeding after glucose levels have risen during a feeding bout. Control of feeding by GK-expressing neurons thus might be important under normal conditions in averting hypoglycemia by stimulating feeding before glucoprivic emergency. In contrast, rapid and profound glucoprivation would appear to require a fail-safe system with strong activation of neurons capable of eliciting feeding responses to repair glucose deficit and must do this without regard to availability of metabolic fuels that cannot be utilized by the brain. Research addressing such questions will significantly enhance our understanding of the overall role of glucose in controlling food intake.

AMP-activated protein kinase (AMPK) is a highly conserved kinase that is activated (phosphorylated) in response to increased ADP/ATP ratio, a characteristic that enables it to act as an energy sensor in some cells. Food deprivation activates AMPK and food intake can be decreased or increased by drugs that enhance or block AMPK activation (13). Therefore, our previous studies of AMPK (25) are of interest in the present context. We found that 2DG increased phosphorylation of AMPKα subunits at Thr172 in micropunches from the ventrolateral medullary area containing catecholamine cell groups A1 and C1, but not in punches from immediately adjacent noncatecholaminergic regions. However, 2DG did not increase AMPK phosphorylation in this same tissue site in rats with PVH DSAP lesions that destroyed the catecholamine neurons located there. These results suggest yet another energy-sensing mechanism that may activate hindbrain catecholamine neurons and contribute to their effects on food intake.

In contrast to food intake, of the three agents tested in the present study, only 5TG triggered a hyperglycemic response, suggesting that the glucose-sensing mechanisms controlling blood glucose responses differ from those controlling food intake and, in addition, that the blood glucose response depends specifically on sensing of glycolysis. The fact that GaA and Phl stimulated food intake, but did not alter blood glucose, is consistent with early reports. In studies using central administration of alloxan, a GK inhibitor and pancreatic β cell toxin, we demonstrated that a low 4V dose of alloxan evoked feeding, but did not trigger hyperglycemia (39). In addition, we found that high LV or 4V doses impaired subsequent 2DG-induced feeding, presumably due to alloxan’s toxic effects, but did not impair the hyperglycemic response to 2DG (30, 38). Similarly, Phl injections into 4V have been shown to stimulate feeding but not hyperglycemia (11). Moreover, we have reported that 4V drug-induced activation of AMPK stimulated feeding and that inhibition of AMPK significantly delayed the onset of systemic 2DG-induced feeding (25), but in unpublished results from this same study, we found that neither stimulation nor blockade of AMPK phosphorylation altered blood glucose levels (Li AJ, Wang Q, Ritter S, unpublished data). Finally, the mobilization of glucose and the stimulation of feeding are responses to glucose deficit that have been shown to require separate populations of catecholamine neurons (35). Therefore, it would be surprising to find that these functionally distinct catecholamine neurons...
cell populations are controlled by different glucose-sensing mechanisms and circuitry.

Data reported here indicating a differential effectiveness of U0126 in blocking the catecholamine-dependent feeding responses to GcA, but not to Phl or 5TG, is interesting in several respects. First, the blockade of GcA-induced feeding by U0126 suggests that the postsynaptic effects of catecholamine neurons required for GcA-induced feeding, like those required for secretion of corticotrophin-releasing hormone in response to 2DG and hypoglycemia (19), may be transcended by the MEK/ERK1/2 signaling cascade. Second, differential dependence of 5TG, GcA and Phl on MEK signaling for the feeding effect may indicate that these substances exert their effects on different catecholaminergic cell populations, as discussed above. Alternatively GcA-activated neurons may have inputs to NE and E neurons that are chemically distinct from inputs activated by 5TG or Phl.

Perspectives and Significance

We found that both 4V and LV injections of GcA, Phl, and 5TG increased food intake, but only 5TG triggered a hyperglycemic response. We conclude from our results that food intake is controlled by multiple glucose-monitoring mechanisms some of which do not depend on monitoring of glycolysis. By contrast, central control of blood glucose appears to depend exclusively on monitoring of glycolysis. However, despite differences in their cellular mechanisms of action, the effects of all three agents on food intake were abolished by lesion of hindbrain catecholamine neurons that project to the medial hypothalamus, the same lesion that abolishes stimulation of feeding by systemic 2DG and by insulin-induced hypoglycemia. It is not yet clear whether the control of food intake by multiple glucose-sensing mechanisms depends on convergence of mechanistically distinct glucose-sensing cells onto hindbrain catecholamine neurons or whether hindbrain catecholamine neurons themselves express multiple distinct glucose-sensing mechanisms. Further study of neuronal responses to agents such as GcA, Phl, and 5TG will yield important information regarding the localization, circuitry, and specific roles of the multiple glucose sensors that participate in control of food intake and other functions important for maintenance of local and overall glucose homeostasis.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


