RESEARCH ARTICLE | Obesity, Diabetes and Energy Homeostasis

Glucose elicits cephalic-phase insulin release in mice by activating $K_{\text{ATP}}$ channels in taste cells

John I. Glendinning,¹ Yonina G. Frim,¹ Ayelet Hochman,¹ Gabrielle S. Lubitz,¹ Anthony J. Basile,¹,2 and Anthony Sclafani³

¹Department of Biology, Barnard College, Columbia University, New York, New York; ²Institute of Human Nutrition, Columbia University, New York, New York; and ³Department of Psychology, Brooklyn College of City University of New York, Brooklyn, New York

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Glendinning JJ, Frim YG, Hochman A, Lubitz GS, Basile AJ, Sclafani A. Glucose elicits cephalic-phase insulin release in mice by activating $K_{\text{ATP}}$ channels in taste cells. Am J Physiol Regul Integr Comp Physiol 312: R597–R610, 2017. First published January 27, 2017; doi:10.1152/ajpregu.00433.2016.—The taste of sugar elicits cephalic-phase insulin release (CPIR), which limits the rise in blood glucose associated with meals. Little is known, however, about the gustatory mechanisms that trigger CPIR. We asked whether oral stimulation with any of the following taste stimuli elicited CPIR in mice: glucose, sucrose, maltose, fructose, Polycose, saccharin, sucrose-1-phosphate, AceK, SC45647, or a nonmetabolizable sugar analog. The only taste stimuli that elicited CPIR were glucose and the glucose-containing saccharides (sucrose, maltose, Polycose). When we mixed an $\alpha$-glucosidase inhibitor (acarbose) with the latter three saccharides, the mice no longer exhibited CPIR. This revealed that the carbohydrates were hydrolyzed in the mouth, and that the liberated glucose triggered CPIR. We also found that increasing the intensity or duration of oral glucose stimulation caused a corresponding increase in CPIR magnitude. To identify the components of the glucose-specific taste-signaling pathway, we examined the necessity of Calhm1, P2X2+P2X3, SGLT1, and Sur1. Among these proteins, only Sur1 was necessary for CPIR. Sur1 was not necessary, however, for taste-mediated attraction to sugars. Given that Sur1 is a subunit of the ATP-sensitive $K^+$ channel ($K_{\text{ATP}}$) channel and that this channel functions as a part of a glucose-sensing pathway in pancreatic $\beta$-cells, we asked whether the $K_{\text{ATP}}$ channel serves an analogous role in taste cells. We discovered that oral stimulation with drugs known to increase (glyburide) or decrease (diazoxide) $K_{\text{ATP}}$ signaling produced corresponding changes in glucose-stimulated CPIR. We propose that the $K_{\text{ATP}}$ channel is part of a novel signaling pathway in taste cells that mediates glucose-induced CPIR.

DURING AND AFTER A MEAL, nutrients surge into the body and disrupt the internal milieu. To limit this homeostatic challenge, mammals activate a variety of autonomic and endocrine responses called cephalic-phase responses (CPRs) (51, 69). The best-characterized CPR is cephalic-phase insulin release (CPIR). It is elicited preabsorptively and attenuates postprandial hyperglycemia in humans (10, 57), rats (6, 25, 36, 44, 52), and mice (19). Orosensory input is thought to activate preganglionic parasympathetic neurons within the dorsal motor nucleus of the vagus (DMNV) in the medulla oblongata, resulting in the release of neurotransmitters (e.g., acetylcholine) onto pancreatic $\beta$-cells and secretion of insulin (6, 17). Little is known, however, about the nature of the orosensory pathways that elicit CPIR.

Taste input from sugars and artificial sweeteners is sufficient to elicit CPIR in humans (31) and rats (59). It is possible that these sweeteners elicit CPIR by binding to the T1r2+T1r3 sweet taste receptor. In response to sweeteners, T1r2+T1r3 activates a signaling pathway involving inositol 1,4,5-trisphosphate and a voltage-gated ATP-release channel (calcium homeostasis modulator 1 or Calhm1) (12). The released ATP binds to the P2X2+P2X3 receptor on primary taste neurons, initiating gustatory nerve input to the brain (15). Many investigators infer that this is the only taste signaling pathway for sweeteners because genetic deletion of T1r2+T1r3, Calhm1, or P2X2+P2X3 eliminates the taste-mediated attraction of mice to sugars and artificial sweeteners (19, 56, 63, 71). However, there is evidence that mice lacking the T1r3 subunit of T1r2+T1r3 display a normal taste-mediated CPIR to glucose (19).

Mammalian taste cells have at least two T1r2+T1r3-independent mechanisms for sensing glucose: the Na+-glucose cotransporter 1 (SGLT1) and the ATP-gated K+ ($K_{\text{ATP}}$) channel. SGLT1 functions as a glucosensor in some enterococcal crine cells (24). Because it transports Na+ together with glucose, it produces an electrogenic signal that increases in direct proportion to glucose transport. Likewise, the $K_{\text{ATP}}$ channel functions as part of a glucosensor in pancreatic $\beta$-cells (4). It consists of an inwardly rectifying K+ channel (Kir6) and a regulatory sulfonylurea receptor (Sur1). Pancreatic $\beta$-cells use glucokinase to link glucose influx to ATP production and $K_{\text{ATP}}$ channels to link ATP production to membrane depolarization (3). Given that SGLT1 and the $K_{\text{ATP}}$ channel are both expressed in T1r2+T1r3-positive taste cells of mice (40, 61, 68), either mechanism could activate glucose-dependent signaling in taste cells and ultimately generate CPIR.

We reported previously that oral stimulation with sucrose elicits CPIR in mice (19). This observation would appear to contradict a role of SGLT1 and $K_{\text{ATP}}$ channels in taste-mediated CPIR. However, given that $\alpha$-glucosidases are expressed in taste cells (54), it is possible that these enzymes hydrolyze sucrose into glucose and fructose within the oral
Cavity. If so, then the liberated glucose could activate SGLT1 or \( K_{ATP} \) channels in taste cells and thereby trigger CPIR.

Here, we investigated the gustatory mediation of CPIR in mice. We found that 1) the early rise in plasma insulin concentration following a glucose drink precedes the rise in blood glucose; 2) oral stimulation with glucose is both necessary and sufficient to elicit CPIR; 3) CPIR magnitude increases with intensity and duration of oral glucose stimulation; 4) three taste signaling components (SGLT1, Calhm1, and the P2X2+P2X3 receptor) are not necessary for taste-mediated CPIR; 5) functional \( K_{ATP} \) channels are necessary for taste-mediated CPIR but not for taste-mediated behavioral attraction to sugars; and 6) pharmacological manipulation of the \( K_{ATP} \) channel modulates CPIR magnitude in a predictable manner.

### METHODS

**Animals and housing conditions.** C57BL/6 (B6) mice were purchased from the Jackson Laboratories (Bar Harbor, ME). The gene knockout (KO) mice and their wild-type controls (WT) were generously donated by different individuals and institutions. We obtained the Calhm1 KO mice and their WT controls (56) from Philippe Marambaud (The Feinstein Institute for Medical Research; Manhasset, NY). The SGLT1 KO mice (21) were provided by Hermann Koepsell (University of Würzburg; Würzburg, Germany); we used B6 mice as the WT given that the genetic background of the KO mice is >99% B6. The Sulf1 KO mice and their WT controls (47) were obtained from Robert Margolskee (Monell Chemical Senses Center; Philadelphia, PA). The P2X2+P2X3\(^{\text{Double-/-}}\) KO (P2X-dbl KO) mice (15) were obtained from Affere Pharmaceuticals (San Mateo, CA) and the P2X2+P2X3\(^{\text{Double+/-}}\) WT controls (P2X-dbl WT) from Thomas Finger (University of Colorado; Denver, CO). To minimize experiential effects, each mouse was tested once, unless stated otherwise.

Approximately equal numbers of males and females of each genotype were used in the individual experiments. Samples sizes are provided in the figures or figure legends. The mice were naïve to the taste stimuli before testing. All mice (except the P2X-dbl WT and KO mice) were 7–10 wk old and weighed 21–27 g at the onset of testing. The P2X-dbl WT and KO mice were 20–40 wk old and weighed 21–32 g. We maintained the mice in a vivarium with controlled care and use of laboratory animals.

All animal protocols were approved by the Institutional Animal Care and Use Committee of Columbia University and conducted in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals.

**Taste solutions.** All taste stimuli were dissolved in deionized water (unless indicated otherwise) and presented to mice at room temperature. The type and concentration of taste stimuli used in a given experiment are described below. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO), except SC45647, sucralose, and Polycose. SC45647 was donated by Grant E. Dubois (Almendra, Roswell, GA); sucralose was donated by Tate & Lyle (Dayton, OH); and Polycose (Ross Laboratories; Columbus, OH) was purchased from amazon.com. According to the manufacturer, Polycose is a corn starch hydrolysate, which contains (by weight) 2% glucose, 7% maltose, 55% maltotriosaccharides, and 36% maltopentosaccharides.

**Training and testing in gustometer.** Brief-access lick tests and oral stimulation procedures were conducted in a gustometer (Davis MS160-Mouse; DiLog Instruments, Tallahassee, FL). Before taste tests in the gustometer, each mouse was subjected to three training sessions with water. This served to familiarize each mouse with the gustometer and train it to obtain water from the sipper tube. Each training session began when the mouse took its first lick and lasted 30 min. On training day 1, the mouse could drink freely from a single sipper tube throughout the session. On training days 2 and 3, the mouse could only drink from a sipper tube during sequential 5-s trials. To motivate licking for the water, mice were deprived of water for 22.5 h before each testing session. Afterward, each mouse was returned to its home cage and given 1 h of ad libitum access to water and food; then, it was deprived of water for another 22.5 h but had libitum access to food. Once training was complete, the mouse was given at least one recovery day.

**Water- and food-restriction or deprivation protocols.** To motivate licking during the brief-access taste tests in experiments 2 and 7, we used a food- and water-restriction protocol. This involved placing each mouse in a new cage with fresh bedding 23.5 h before each test session and limiting its rations to a 1-g chow pellet (F0173, Bio-Serv) and 2 ml of water. This deprivation schedule causes B6 mice to exhibit concentration-dependent increases in lick rate for preferred taste stimuli (18).

To motivate licking by the B6 and KO mice during the CPIR tests in experiments 1, 3, 4, 5, 6, and 8, we deprived the mice of water for 23-h before testing. Furthermore, to limit the quantity of food in their stomach, we food deprived the mice for 6 h before testing.

**Oral stimulation procedure for eliciting CPIR.** For each mouse, we obtained a baseline blood sample between 2:00 and 4:00 PM. Immediately afterward, we put the mouse in the gustometer and permitted it a maximum of 3 min to take its requisite number of licks from the taste stimulus (see below). Most mice completed licking within 60 s. Once they did so, a shutter blocked access to the drinking spout and the mouse was transferred to a cage (without food and water) for blood sampling. If a mouse did not take the requisite number of licks within 3 min, then it was removed from the experiment. We collected additional blood samples at specific time points (see below).

**Tail blood sampling.** In experiments 3, 4, 5, 6, and 8, we snipped the distal 1 mm of the tail, obtained a single drop of blood from the severed tail vein, and then determined blood glucose concentration with a handheld glucometer (OneTouch Ultra; Milpitas, CA). For repeated samples, we wiped the tail tip with a moist towel to reinitiate blood flow. For the plasma insulin measurements, we obtained blood from the same tail but had to gently stroke the tail lengthwise to obtain the necessary volume of blood (30 \( \mu l \)) for each time sample.

In experiment 1, we collected blood samples from the submandibular vein, using a technique described elsewhere (20). In brief, we used a lancet to sever the submandibular vein. This technique permitted us to collect >30 \( \mu l \) of blood rapidly (i.e., within 5 s), and thus obtain blood samples at precise time points after the mouse initiated licking. To validate the use of blood samples from different body regions, we compared blood samples taken concurrently from the tail and the submandibular vein of 12 mice. All mice had been maintained on ad lib food and water before testing. Despite large differences in blood glucose concentration across mice (109–212 mg/dl), there was no significant difference in glucose concentration between the tail and submandibular vein samples [mean difference = 4.1 mg/dl; paired \( t \) value = 0.69, degrees of freedom (df) = 11, \( P > 0.53 \)].

The blood samples for the insulin assays were collected in 30-\( \mu l \) EDTA-coated capillary tubes (Innovative Medical Technologies; Shawnee Mission, KS). They were initially stored in ice and were centrifuged later for 3 min at 5,000 rpm. The decanted plasma was stored at –80°C until analysis with the Ultra-Sensitive Mouse Insulin ELISA (Crystal Chem; Downers Grove, IL).
Experiment 1: time course of change in plasma insulin and blood glucose concentration. Here, we asked how long it took for plasma insulin and blood glucose concentrations to increase once a mouse initiated licking for a 1 M glucose solution. Previous studies in rats reported that plasma insulin concentrations rose more quickly than did blood glucose concentrations (6, 49, 53). Each mouse took a weight-specific number of licks (i.e., 4.3 licks/g mouse), as in our previous study (19). They usually completed these licks within 60 s.

For each mouse, we collected two blood samples and measured the concentration of plasma insulin and blood glucose in each. The baseline blood sample was taken from the tail. Immediately after taking this sample, we placed the mouse in the gustometer and permitted it to complete its weight-specific number of licks (4.3/g mouse) for 1 M glucose. The second blood sample was taken from the submandibular vein 1, 2, 3, 4, or 5 min after the mouse initiated licking. To be included in the experiment, the mouse had to complete its requisite number of licks before we took the second blood sample.

We calculated the difference in plasma insulin (or blood glucose) concentration between the first and second blood samples, separately for each mouse. We refer to these differences as the Δ plasma insulin or Δ blood glucose concentration. To determine how many minutes elapsed before the Δ plasma insulin and blood glucose concentrations rose significantly above baseline, we used a one-sample t-test, separately for each time point. In this and all subsequent statistical comparisons, we set the α level at 0.05.

Experiment 2: determination of isoacceptable concentrations of taste stimuli. Here, we sought to identify isoacceptable concentrations of 10 taste stimuli. To this end, we measured licking response of B6 mice to a range of concentrations of glucose (0.03, 0.1, 0.2, 0.3, 0.6, and 1.0 M); sucrose (0.03, 0.1, 0.2, 0.3, 0.6, and 1.0 M); fructose (0.03, 0.1, 0.2, 0.3, 0.6, and 1.0 M); maltose (0.03, 0.1, 0.2, 0.3, 0.6, and 1.0 M); saccharin (0.3, 1, 3, 10, and 30 mM); sucralose (0.3, 1, 3, 10, and 30 mM); SC4567 (0.03, 0.1, 0.3, 1, and 10 mM); Ace K (0.003, 0.03, 0.3, 3, and 30 mM); Polycose (1, 2, 4, 8, 16, and 32%); and α-methyl-D-glucopyranoside (MDG) (0.03, 0.1, 0.3, and 1.0 M), a nonmetabolizable sugar analog that is transported by SGLT1 (21). All of these chemicals have been reported to bind to the T1r2+T1r3 receptor (12), except the latter two. MDG has an attractive taste to B6 mice (29, 46) but not to T1r3 KO mice, indicating that it binds to the T1r2+T1r3 receptor (J. Glendinning, unpublished data). Polycose (a maltodextrin) has an attractive nonsweet taste to rodents, which is mediated by a non-T1r2+T1r3 receptor (42, 62, 72).

Each mouse was subjected to a brief-access taste test (18) with all concentrations of a given tastant. A trial began when the mouse took its first lick from the sipper tube and ended 5 s later when the shutter closed. After a 7.5-s pause, the mouse was presented a different concentration of the same taste stimulus. In this manner, the mouse could initiate up to 144 trials across the 30-min test session. We treated the different concentrations of each taste stimulus as a block and randomized (without replacement) their order of presentation within the block so that each concentration was presented once before the next block began.

Next, we calculated a standardized lick ratio (SLR), separately for each mouse and taste stimulus, using a procedure described elsewhere (18). In brief, we determined each mouse’s local lick rate (expressed as licks/s) during the first training session with water. First, we determined the mean interlick interval (ILI) in milliseconds, based on the population of ILIs <200 ms, and then took the reciprocal of this mean. Next, we multiplied the local lick rate by a scaling factor of 5, resulting in an estimate of the maximal number of licks that the mouse could generate if it licked continuously across the 5-s trial. Finally, we calculated the SLR by dividing average number of licks per trial for a taste stimulus (e.g., 1 M glucose) by the maximal potential lick rate per trial. An SLR approaching 0.0 indicates that the taste stimulus elicited only sporadic licking, whereas one approaching 1.0 indicates that it elicited nearly continuous licking across even 5-s trials.

We defined the isoacceptable concentration of each taste stimulus as the lowest concentration that elicited a mean SLR > 0.70. While this approach did not necessarily identify concentrations of each stimulus that were exactly matched for sweetness intensity or palatability, it did permit us to identify a concentration of each taste stimulus that was highly attractive to the B6 mice.

Experiment 3: do all of the taste stimuli elicit CPIR in B6 mice? The mice took a body weight-specific number of licks for each taste stimulus solution (i.e., 4.3 licks/g mouse). We took tail blood samples at baseline (i.e., 0 min) and then 5, 15, 30, and 60 min after the mouse initiated licking. We tested for time-dependent changes in plasma insulin and blood glucose levels with repeated-measures ANOVA (and Dunnett’s post hoc test) separately for each taste stimulus. We also calculated Δ plasma insulin (i.e., the change in plasma insulin between time 0 and 5 min after initiating licking) as a measure of CPIR magnitude. We analyzed CPIR magnitude in two ways. We used a one-sample t-test to determine which taste stimuli elicited a CPIR; i.e., a Δ plasma insulin concentration that was significantly greater than zero. For those taste stimuli that elicited a CPIR, we compared CPIR magnitudes with one-way ANOVA and Tukey-type multiple comparison test.

Experiment 4: does acarbose prevent sucrose, maltose, and Polycose from eliciting CPIR? Because amylase and α-glucosidases are present in the oral cavity (54), it is likely that they liberated glucose from the glucose-containing carbohydrates that were tested in experiment 3 (i.e., sucrose, maltose, and Polycose). If so, then it is possible that this liberated glucose (and not the larger carbohydrate molecules) elicited CPIR. To test this hypothesis, we had the mice take a weight-specific number of licks (4.3/g mouse) for the isoacceptable concentration of each carbohydrate, with or without acarbose (Sigma-Aldrich).

We used acarbose because it prevents amylase and α-glucosidases from hydrolyzing carbohydrates and thereby liberating glucose (30, 37, 39). We selected 5 mM acarbose for two reasons. First, it effectively blocks the hydrolytic action of amylase and α-glucosidases in human saliva (34). Second, a prior study demonstrated that a 5.6 mg/kg dose of acarbose prevents the rise in blood glucose that typically follows a 0.5 g/kg intraduodenal infusion of sucrose in rats (37). In this prior study, the ratio of the acarbose dose to the sucrose dose was ~1:1 (i.e., 5/0.5). In the present study, the ratio of the acarbose dose (0.51 mg/kg) to the sucrose dose (0.05 g/kg) was ~10:1. Based on the similarity of these ratios, we predicted that 5 mM acarbose should effectively block hydrolysis of sucrose, maltose, and Polycose in the oral cavity and the small intestine of mice.

We took tail blood samples at baseline (i.e., 0 min) and 5, 15, 30, and 60 min after the mice initiated licking for each taste stimulus, and we measured both plasma insulin and blood glucose concentration. We defined CPIR as described above and compared CPIR across treatment levels (+ or – acarbose), separately for each carbohydrate with an unpaired t-test. We tested for an effect of acarbose treatment on plasma insulin and blood glucose dynamics across time with a mixed-model ANOVA and Sidak’s multiple comparison test. We also compared plasma insulin and blood glucose values at each time point (relative to baseline) with Dunnett’s multiple comparison test, separately for each carbohydrate and treatment level.

Experiment 5: does CPIR increase with intensity and duration of oral glucose stimulation? We tested the prediction that CPIR magnitude would increase with intensity of oral glucose stimulation and duration of stimulation. To vary stimulus intensity, we offered B6 mice different concentrations of glucose (i.e., 0, 0.1, 0.3, 0.6, 1, or 3 M) and allowed them to take a mass-specific number of licks for each glucose solution (4.3 licks/g mouse). To vary stimulation duration, we allowed B6 mice to take 0, 50, 100, or 200 licks for a 1 M glucose solution. In each case, we collected plasma insulin both at baseline and 5 min after initiating licking.

We used one-way ANOVA (and a Tukey-type multiple comparison) to determine whether CPIR increased with glucose concentration.
or number of licks. In addition, we used a one-sample t-test to determine whether the CPIR was significantly >0, separately for each glucose concentration and lick number.

**Experiment 6: which taste signaling proteins are necessary for CPIR?** Previously, we established that T1r3 KO mice exhibit normal CPIR following oral stimulation with 1 or 2.8 M glucose. This established that the T1r3 subunit of T1r2+T1r3 receptor does not contribute to CPIR. Here, we examined the necessity of additional taste signaling proteins to CPIR. In each case, we compared mice with a global gene deletion to their appropriate WT control. The KO mice lacked the voltage-gated ATP-release channel (Calhm1), the P2X2+P2X3 receptor for ATP, the sulfonilurea receptor 1 (Sur1), or the Na+/dependent glucose transporter 1 (SLGT1).

All mice, except the SLGT1 KO and WT controls, were maintained on the normal chow diet. The SLGT1 KO mice were maintained on a low-carbohydrate diet from weaning (percent kcal from carbohydrate = 2.2%, from fat = 39.3% and from protein = 58.5%; Envigo, Indianapolis, IN), owing to compromised glucose transport in the small intestine (46). We maintained the B6 WT mice on the same low-carbohydrate diet for 4 wk before testing.

The mice took a weight-specific number of licks (4.3 licks/g mouse) for 2.8 M glucose. We used this high glucose concentration to minimize the risk of a type 2 error. We were concerned that if the KO mice had reduced taste sensitivity to glucose, then we may fail to observe a CPIR simply because the 1 M concentration (which was used in many of the other experiments) might produce a weak response. We obtained tail blood samples at baseline (i.e., 0 min) and 5, 15, 30, and 60 min after the mouse initiated licking for the glucose solution. At each time point, we collected plasma insulin and blood glucose samples. We tested for time-dependent changes in plasma insulin and blood glucose with a mixed-model ANOVA, separately for each type of mouse. Genotype (i.e., KO vs. WT) was a between factor and time a within factor. We also calculated Δ plasma insulin (i.e., the change in plasma insulin concentration between time 0 and 5 min after initiating licking) as a measure of CPIR magnitude. We compared each KO and its associated WT group with a t-test.

**Experiment 7: is Sur1 necessary for normal taste-mediated licking for sugars?** To determine the necessity of the K<sub>ATP</sub> pathway in taste cells for taste-mediated behavioral attraction to sugars, we compared licking responses of Sur1 KO and WT mice to a range of concentrations of glucose (0.03, 0.1, 0.3, 0.6, and 1.0 M) and sucrose (0.03, 0.1, 0.2, 0.3, 0.6, and 1.0 M). We selected these sugars because they elicited the strongest CPIR. Each mouse was tested with both sugars. To control for order effects, we counterbalanced the testing sequence of each sugar across mice. We employed the same gustometer training and testing procedures as described above. We used a mixed-model ANOVA to compare SLR across genotypes, separately for each sugar. We treated genotype as a within factor and concentration as a between factor.

**Experiment 8: does pharmacological manipulation of K<sub>ATP</sub> channel alter CPIR?** For the glyburide test, the experimental solution contained 150 μM glyburide, 0.33% DMSO, and 0.5 M glucose, whereas the control solution contained 0.33% DMSO and 0.5 M glucose. We used a relatively low glucose concentration (i.e., 0.5 M) to minimize the chances of a ceiling effect compromising our results. For the diazoxide test, the experimental solution contained 250 μM diazoxide, 0.66% DMSO, and 1.0 M glucose, whereas the control solution contained 0.66% DMSO and 1.0 M glucose. We used a relatively high glucose concentration (1.0 M) to minimize the chances of a floor effect compromising our results. We selected the indicated concentrations of glyburide and diazoxide based on pilot studies and the literature (23).

After training the mice to lick in the gustometer, we subjected them to CPIR testing. We used different mice in the glyburide and diazoxide tests. For a given test, we ran each mouse through two trials, each on separate days. In one trial, the mouse received the experimental solution; in the other trial, it received the control solution. We counterbalanced the order in which the experimental and control solutions were tested (i.e., day 1 or 2) across mice. During each trial, we collected plasma insulin at baseline (0 min) and 5 min after the mice initiated licking for the experimental or control solution. All mice had to complete 200 licks within a 3-min period to be included. We compared plasma insulin responses to licking for the control versus experimental solutions with paired t-tests, separately for the glyburide and diazoxide tests.

**RESULTS**

**Experiment 1: time course of change in plasma insulin and blood glucose concentration.** Licking for 1.0 M glucose caused a marked increase in both plasma insulin and blood glucose, but over different time frames (Fig. 1). Whereas there was a trend for the Δ insulin concentration to rise above zero within 2 min (one sample t-value = 2.42, df = 6, P = 0.052), it did not do so significantly until 3 min had elapsed (one sample t-value = 6.87, df = 7, P < 0.0003). In contrast, the Δ blood glucose concentration did not rise significantly above zero until 4 min had elapsed (one sample t-value = 4.77, df = 6, P < 0.004). Given that there was virtually no change in blood glucose concentration during the initial 3 min of the test, it follows that the early rise in plasma insulin could not have been elicited by a change in blood glucose concentration. Thus we infer that the early rise in plasma insulin reflects a cephalic-phase response.

**Experiment 2: determination of isoacceptable concentrations of tastants.** To compare the different artificial sweeteners and carbohydrates in terms of their ability to elicit CPIR, we determined isoacceptable concentrations of each (Fig. 2). We found that the SLRs increased significantly with concentration for each taste stimulus in all RM ANOVAs, P < 0.001. Whereas SLR rose monotonically with concentration for most stimuli, that for SC45647 did so until 3 mM but then decreased...

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Fig. 1. Plasma insulin (A) concentration increased more rapidly than did plasma glucose (B) concentration after mice initiated licking for 1.0 M glucose (experiment 1). Each mouse took a weight-specific number of licks (4.3 licks/g mouse). We represent scores from individual mice as a circle, and the mean score as a horizontal line. We use closed circles to indicate the time points when the mean change in plasma insulin (or blood glucose) was significantly greater than zero (P < 0.004; one-sample t-test), and open circles to indicate the time points when it was not significantly greater than zero (P > 0.05).

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That elicited by maltose and Polycose, but the CPIR elicited by glucose was significantly larger than that elicited by sucrose did not differ from that elicited by the other three carbohydrates according to an ANOVA (F3,32 = 6.7, P < 0.002) and Tukey-type multiple comparison.

We also examined the extent to which ingestion of isoacceptable concentrations of each taste stimulus increased plasma insulin over the 60-min sampling period (Fig. 4). Only four of the taste stimuli (glucose, sucrose, maltose, and Polycose) caused a significant increase in plasma insulin concentration over time (Table 1). Glucose and sucrose elicited substantially larger and more protracted elevations in plasma insulin than did maltose and Polycose.

Five taste stimuli (i.e., glucose, sucrose, maltose, Polycose, and fructose) caused blood glucose to rise significantly (Table 1) above baseline at the 5-, 15-, and 30-min time points (Fig. 3). Fructose produced the smallest increase in blood glucose. Polycose caused the largest and most sustained increase in blood glucose, remaining significantly above baseline 60 min after initiating licking. This stems in part from the fact that the 30% Polycose solution (once digested) yielded more glucose molecules than the other carbohydrate solutions.
Maltose was lower at 15 min than that in control mice. It follows that the elevation in blood glucose in acarbose-treated mice was significant (Table 2, Fig. 6). The interaction reflects the fact that acarbose completely inhibited the ability of the amylases and α-glucosidases to hydrolyze Polycose. As a result of acarbose treatment, it rose significantly less than in control mice. Indeed, it appears that acarbose strongly inhibited (but did not eliminate) the ability of the amylases and α-glucosidases to hydrolyze maltose. Polycose from eliciting CPIR?

We found that acarbose treatment blocked CPIR in response to sucrose, Polycose, and to a lesser extent, maltose (Fig. 5). That acarbose prevented digestion of sucrose and Polycose (and maltose to a lesser extent) is demonstrated by the plasma glucose data (see below). The absence of CPIR following acarbose treatment reveals that free glucose is necessary for eliciting CPIR.

We determined whether acarbose altered plasma insulin over the 60-min sampling period. For sucrose, there was a significant main effect of acarbose and a significant interaction of acarbose × time on both plasma insulin and blood glucose (Table 2, Fig. 6). The absence of any rise in blood glucose over the 60-min sampling period indicates that the acarbose treatment not only attenuated the plasma insulin spike at 15 min (compared with the control mice) but also prevented plasma insulin from rising significantly above baseline over the entire 60-min sampling period.

For Polycose, there was a significant main effect of acarbose and significant interaction of acarbose × time on blood glucose (Table 2, Fig. 6). Even though blood glucose rose significantly above baseline (at 5, 15, and 30 min) in acarbose-treated mice, it rose significantly less than in control mice. Indeed, it appears that acarbose strongly inhibited (but did not eliminate) the ability of the amylases and α-glucosidases to hydrolyze Polycose. As a result of acarbose treatment, plasma insulin did not rise above baseline across the entire 60-min sampling period.

Experiment 5: does CPIR increase with intensity and duration of oral glucose stimulation? We found that licking glucose elicited a concentration-dependent increase in CPIR magnitude, peaking at 1 M glucose ($F_{4,58} = 11.7, P < 0.0001$) (Fig. 7A). While 0.3 and 0.6 M glucose elicited CPIR, the magnitude was significantly less than that for 1 M glucose. Figure 7B
Table 1. Did licking for different taste stimuli alter plasma insulin and blood glucose dynamics in B6 mice (experiment 3)?

<table>
<thead>
<tr>
<th>Taste Stimulus</th>
<th>Blood Measurement</th>
<th>F Ratio</th>
<th>df</th>
<th>P Value</th>
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</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>Plasma insulin</td>
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<td>4,32</td>
<td>0.0001</td>
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<td></td>
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<td>4,32</td>
<td>&lt;0.0001</td>
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<tr>
<td>Sucrose</td>
<td>Plasma insulin</td>
<td>10.8</td>
<td>4,20</td>
<td>&lt;0.0004</td>
</tr>
<tr>
<td></td>
<td>Blood glucose</td>
<td>25.7</td>
<td>4,20</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Maltose</td>
<td>Plasma insulin</td>
<td>13.8</td>
<td>4,28</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Blood glucose</td>
<td>23.3</td>
<td>4,28</td>
<td>&lt;0.0001</td>
</tr>
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<td>Polycose</td>
<td>Plasma insulin</td>
<td>5.0</td>
<td>4,28</td>
<td>&lt;0.004</td>
</tr>
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<td>Blood glucose</td>
<td>68.6</td>
<td>4,28</td>
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<td>Fructose</td>
<td>Plasma insulin</td>
<td>2.0</td>
<td>4,20</td>
<td>0.17</td>
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<tr>
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<td>Blood glucose</td>
<td>17.9</td>
<td>4,20</td>
<td>&lt;0.001</td>
</tr>
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<td>MDG</td>
<td>Plasma insulin</td>
<td>1.2</td>
<td>4,24</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>Blood glucose</td>
<td>0.7</td>
<td>4,28</td>
<td>0.51</td>
</tr>
<tr>
<td>Ace K</td>
<td>Plasma insulin</td>
<td>0.3</td>
<td>4,28</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>Blood glucose</td>
<td>1.7</td>
<td>4,28</td>
<td>0.23</td>
</tr>
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<td>Plasma insulin</td>
<td>0.8</td>
<td>4,28</td>
<td>0.54</td>
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<td>Plasma insulin</td>
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<td>4,28</td>
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<tr>
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<td>4,28</td>
<td>0.91</td>
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<tr>
<td></td>
<td>Blood glucose</td>
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<td>4,28</td>
<td>0.14</td>
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We used repeated-measure ANOVA to test for time-dependent changes in plasma insulin or blood glucose concentration, separately for each taste stimulus. The actual results are in Fig. 4, df, degrees of freedom.

shows that CPIR magnitude also increased monotonically with number of licks taken for 1 M glucose ($F_{3,34} = 4.7, P < 0.008$). These results establish that CPIR is a quantitative trait, whose magnitude varies as a function of the intensity and duration of glucose stimulation.

Experiment 6: which signaling proteins are necessary for CPIR? We previously demonstrated that an intact Tlr2+Tlr3 receptor is not necessary for CPIR in mice. Here, we tested the necessity of other peripheral taste signaling proteins (Calhm1, P2X3+P2X3, SGLT1, and Sur1) to CPIR with the use of mice with global gene deletions. We asked whether plasma insulin increased within 5 min of initiating licking for 2.8 M glucose.

There was no difference in CPIR magnitude among Calhm1 KO, P2X-dbKO, or SGLT1 KO mice and their respective WT controls (Fig. 8). In contrast, CPIR magnitude in Sur1 KO mice was blocked relative to Sur1 WT mice (unpaired $t$-value = 2.84, df = 21, $P < 0.01$). These results indicate that CPIR is mediated by the KATP channel (of which Sur1 is a key component) and does not require Calhm1, P2X2+P2X3, or SGLT1.

We also examined the impact of knocking out Calhm1, P2X2+P2X3, SGLT1, and Sur1 on blood glucose and plasma insulin over the 60-min period immediately after ingestion. For Calhm1, there were no significant differences between the KO and WT mice in plasma insulin or blood glucose dynamics (Table 3, Fig. 9). This indicates that Calhm1 is not necessary for normal plasma insulin and blood glucose responses to ingested glucose.

For P2X2+P2X3, there were no significant differences in plasma insulin dynamics between the KO and WT mice, but there was a significant interaction of genotype × time on blood glucose concentration (Table 3, Fig. 9). This interaction reflects two effects of deleting the P2X2+P2X3 receptor. First, blood glucose concentration rose significantly above baseline in the WT but not in the KO mice at the 5-min sampling period. Second, blood glucose concentration was significantly higher in the KO than in the WT mice at the 30-min sampling period, indicating impaired glucose tolerance in the KO mice. Accordingly, these results indicate that P2X2+P2X3 is necessary for normal glucose tolerance but not for normal plasma insulin responses to ingested glucose.

For SGLT1, there was no significant difference between the KO mice and WT controls in plasma insulin dynamics, although plasma insulin concentration was low in both types of
mice (Table 3, Fig. 9). There was a significant main effect of genotype and a significant interaction of genotype/time on blood glucose dynamics. This reflects the fact that blood glucose concentration barely rose above baseline in the KO mice \((F_{4,36} = 3.3, P = 0.03)\); it exceeded baseline only 60 min after initiating licking (Dunnett’s multiple comparison test, \(P = 0.05\)). This shows that SGLT1 is necessary for absorption of glucose but not for eliciting a plasma insulin response to ingested glucose.

For Sur1, there were significant differences between the KO and WT mice in both plasma insulin and blood glucose dynamics (Table 3, Fig. 9). Most importantly, the interaction of genotype/time was significant for plasma insulin, reflecting the absence of an insulin spike during the 5-, 30-, and 60-min sampling periods in Sur1 KO mice. This latter result helps explain why the Sur1 KO mice exhibited such poor glucose tolerance. Thus, Sur1 is necessary for normal plasma insulin and blood glucose responses to ingested glucose.

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![Fig. 6. Acarbose attenuated the plasma insulin (top row) and blood glucose (bottom row) response (means ± SE) to licking for sucrose, maltose, and Polycose (experiment 4). Within each panel, we compared (at each time-point) the plasma insulin or blood glucose concentrations following oral stimulation with the carbohydrate (Carb) solution vs. its corresponding carbohydrate + 5 mM acarbose (Carb + acarbose) solution, using Sidak’s multiple comparisons test (#\(P < 0.05\)). We also compared the plasma insulin or blood glucose concentration at baseline (B) with that at each successive time-point, separately for each treatment group, using Dunnett’s multiple comparison test (*\(P < 0.05\)). For each experimental solution, we tested the following number of mice: sucrose \((n = 6)\), sucrose + acarbose \((n = 9)\), maltose \((n = 8)\), maltose + acarbose \((n = 9)\), Polycose \((n = 8)\), and Polycose + acarbose \((n = 9)\).](http://www.ajpregu.org)

![Fig. 7. CPIR magnitude increased with glucose concentration (A) (i.e., sweetness intensity) and number of licks (B) (i.e., duration of stimulation) (experiment 5). We defined CPIR as a significant increase in plasma insulin concentration, relative to baseline, within 5 min of initiating licking for the taste stimulus (i.e., Δ plasma insulin concentration), based on a one-sample t-test (\(P < 0.05\)). For each treatment level, we represent the scores from each mouse as a circle, and the mean score as a horizontal line. We distinguish treatment levels that either did or did not elicit a CPIR with closed and open circles, respectively. We varied stimulation intensity by allowing B6 mice to take a weight-specific number of licks (4.3 licks/g mouse) for different concentrations of glucose. We varied stimulus duration by allowing mice to take different numbers of licks for 1 M glucose. We compared the mean CPIR values within A or B, using Tukey’s multiple comparison test. Means that differ significantly from one another lack a shared letter (i.e., a, b, or c) above them (\(P < 0.05\)).](http://www.ajpregu.org)
We also made four observations that, when considered together, provide support for the hypothesis that blood glucose levels have limited impact on plasma insulin levels during the 5 min immediately after a glucose drink. First, plasma insulin levels were essentially flat across the 3-, 4-, and 5-min sampling periods, despite a large increase in blood glucose during the 4- and 5-min sampling periods (Fig. 1). Second, the B6 mice experienced a significant rise in blood glucose within 5 min of initiating licking for fructose, but no associated rise in plasma insulin (Fig. 4). Third, the B6 mice exhibited large and roughly equivalent elevations in blood glucose within 5 min of initiating licking for glucose and Polycose (93 and 81.5 mg/dl, respectively). Nevertheless, the rise in plasma insulin elicited by glucose was nearly five times larger than that elicited by Polycose (Fig. 4). Fourth, the SGLT1 KO mice exhibited a small but significant increase in plasma insulin within 5 min of initiating licking for glucose, but no associated change in blood glucose (Fig. 9). The lack of a change in blood glucose in the SGLT1 KO mice reflects the fact that SGLT1 is the primary mechanism for transporting glucose across the intestinal epithelium (21).

If CPIR represents an adaptive feedforward mechanism for helping control blood glucose homeostasis, then one would expect that its magnitude would increase with the intensity and duration of glucose stimulation. In support of this hypothesis, we found that CPIR magnitude increased as a function of both the concentration of glucose and the number of licks taken for 1 M glucose in B6 mice. These results are consistent with a prior study of humans, which reported that CPIR magnitude increases with glucose concentration (67).

Sucrose, maltose, and Polycose also elicited CPIR. However, given the presence of amylases in saliva and α-glucosi-

**Table 3. Did genetic deletion of Calhm1, P2X2+P2X3, SGLT1, or Sur1 alter plasma insulin or blood glucose response to oral stimulation with glucose (experiment 6)?**

<table>
<thead>
<tr>
<th>Signaling protein</th>
<th>Blood Measurement</th>
<th>Source of Variation</th>
<th>F Value</th>
<th>df</th>
<th>P Value</th>
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<tbody>
<tr>
<td>Calhm1</td>
<td>Plasma insulin</td>
<td>Time</td>
<td>15.0</td>
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<td>&lt;0.001</td>
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<tr>
<td></td>
<td></td>
<td>Genotype</td>
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<td>1,13</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Interaction</td>
<td>0.8</td>
<td>4,52</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>Blood glucose</td>
<td>Time</td>
<td>25.7</td>
<td>4,52</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Genotype</td>
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<td>1,13</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Interaction</td>
<td>0.4</td>
<td>4,52</td>
<td>0.82</td>
</tr>
<tr>
<td>SGLT1</td>
<td>Plasma insulin</td>
<td>Time</td>
<td>12.4</td>
<td>4,52</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>1,13</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Interaction</td>
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<td>4,52</td>
<td>0.72</td>
</tr>
<tr>
<td>Sur1</td>
<td>Plasma insulin</td>
<td>Time</td>
<td>32.9</td>
<td>4,52</td>
<td>&lt;0.001</td>
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<td>1,13</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>Blood glucose</td>
<td>Time</td>
<td>10.1</td>
<td>4,72</td>
<td>&lt;0.0001</td>
</tr>
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<td></td>
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<td>1,18</td>
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<td></td>
<td>Interaction</td>
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<td>4,72</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>Blood glucose</td>
<td>Time</td>
<td>25.3</td>
<td>4,72</td>
<td>&lt;0.001</td>
</tr>
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<td></td>
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<td>Genotype</td>
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<td>4,72</td>
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<td></td>
<td></td>
<td>Interaction</td>
<td>4.8</td>
<td>4,84</td>
<td>&lt;0.0002</td>
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<td>Time</td>
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<td>1,21</td>
<td>0.001</td>
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</table>

Actual data are presented in Fig. 9. We performed mixed-model ANOVAs, separately for each transduction protein and blood measurement. In each case, time was a within factor and genotype a between factor.
Crose, maltose, and Polycose are hydrolyzed in the oral cavity. In sum, our acarbose results indicate that su-
interfered with the detection of glucose by taste cells in the
nerve to glucose (54), it is improbable that acarbose
inhibitors did not diminish responses of the chorda tympani
in taste cells. Likewise, given that other classes of glucosidase
lining the small intestine (30, 37), it is unlikely to have done so
consistent with prior studies in humans (30) and rabbits (27).

The fact that acarbose completely blocked hydrolysis of su-
crose and only partially blocked hydrolysis of maltose is
consistent with prior studies in humans (30) and rabbits (27).

What taste signaling pathway mediates CPIR in mice? CPIR
does not appear to be mediated by T1r2+T1r3 and its down-
stream signaling proteins. This inference is based on two lines
of evidence. First, neither the T1r3 subunit of T1r2+T1r3 (19)
or downstream components of the T1r2+T1r3 taste signaling
pathway (i.e., Calhm1 and the P2X2+P2X3 receptor) are
necessary for CPIR. Second, even though fructose and the
artificial sweeteners all bind to T1r2+T1r3 (12), they
nevertheless failed to elicit CPIR at highly acceptable concen-
trations.

We tested two predictions of the hypothesis that CPIR is
mediated by SGLT1. Given that MDG binds to SGLT1 (41),
we predicted that MDG would elicit CPIR. This did not occur.
We also predicted that genetic deletion of SGLT1 would
eliminate the glucose-induced CPIR. Contrary to prediction,
glucose elicited a CPIR in the SGLT1 KO mice that was equal
in magnitude to that in the WT mice. Thus, the glucose-
elicted CPIR is not mediated by SGLT1. It should be noted,
that the glucose liberated from these carbohydrates triggers
CPIR in mice.

Fig. 9. Knocking out Calhm1, the P2X2+P2X3 receptor, SGLT1, or Sur1 differentially impacted plasma insulin and blood glucose dynamics (experiment 6). All B6 mice took a mass-specific number of licks (4.3 licks/g mouse) for 2.8 M glucose. We show changes in plasma insulin (top row) and blood glucose (bottom row) concentrations (means ± SE), relative to baseline (B). In each panel, we compare responses of WT and KO mice. In instances where the time × genotype interaction was significant within a panel (based on results in Table 3), we compared genotypes at each time period with Sidak’s multiple comparison test (#P < 0.05). We also compared the plasma insulin or blood glucose concentration at baseline (B) with that at each successive time-point, separately for each treatment
group, using Dunnett’s multiple comparison test (*P < 0.05). We tested the following number of mice: Calhm1 WT (n = 8) and KO (n = 7); P2X-dbl WT (n = 8)
and KO (n = 7); SGLT1 WT (n = 10) and KO (n = 10); and Sur1 WT (n = 12) and KO (n = 11).
attributed to the low (2%) carbohydrate diet given to these mice; e.g., compare the CPIR displayed by B6 mice fed the normal (i.e., 57% carb) chow diet (Fig. 3) with that displayed by SGLT1 WT mice fed the low-carbohydrate (2% carb) diet (Fig. 8). The low-carbohydrate diet may have downregulated the CPIR signaling pathway in taste cells, diminished insulin production in pancreatic β-cells (66), or enhanced insulin clearance. It is notable that this low-carbohydrate diet does not reduce the preference of SGLT1 KO or B6 WT mice for saccharin (relative to B6 mice fed a normal high-carbohydrate diet) (46).

We obtained four convergent lines of support for the hypothesis that the KATP channel is a novel taste-signaling pathway for detecting glucose and eliciting CPIR. First, glucose was both sufficient and necessary for eliciting CPIR. Second, fructose and MDG, which do not activate the KATP channel on their own (33, 70), did not elicit CPIR. Third, Sur1 (a critical component of the KATP channel) was necessary for eliciting CPIR. Fourth, oral stimulation with drugs that either increase or decrease KATP channel activity produced corresponding changes in CPIR magnitude (experiment 8). Each test involved two sessions: one was conducted with a control solution and the other with an experimental solution. A: in the glyburide test, the control solution was 0.5 M glucose (Glu) and the experimental solution was 0.5 M glucose + 0.15 mM glyburide (GB). B: in the diazoxide test, the control solution was 1 M glucose and the experimental solution was 1 M glucose + 0.25 mM diazoxide (DZ). Within a given panel, we represent the results from each mouse as two points connected by a line. The Δ plasma insulin concentration reflects the change in plasma insulin concentration between measurements obtained at baseline and 5 min after a mouse initiated licking for the experimental or control solution. Within each panel, we compare the Δ plasma insulin concentration elicited by the control vs. experimental solutions (paired t-test, *P < 0.03).

Additional studies are needed to determine how activation of the KATP signaling pathway in taste cells triggers insulin release from pancreatic β-cells. One hypothesis involves a neural mechanism; e.g., activated taste cells stimulate ascending gustatory pathways, which in turn stimulate preganglionic parasympathetic neurons within the DMNV, resulting in the release of acetylcholine onto pancreatic β-cells and secretion of insulin (6, 17). There are several lines of support for this neural mechanism in the rat (7, 8, 50). A second hypothesis involves a humoral mechanism; e.g., activated taste cells release a signaling molecule, which is conveyed directly to pancreatic β-cells via the blood. GLP-1 has been hypothesized to act in this manner based on the observation that it is released from murine taste cells in response to oral stimulation with glucose, sucrose, or saccharin (32, 55). Two findings contradict this hypothesis, however. First, even though GLP-1 enhances glucose-stimulated insulin release from β-cells, it does not stimulate insulin release on its own (43). Second, Takai et al. (55) found that oral stimulation with saccharin causes GLP-1 release from murine taste cells. If this taste cell-derived GLP-1 mediates CPIR, then oral stimulation with saccharin should have triggered CPIR in the present study. This was not the case.

Artificial sweeteners and CPIR. None of the artificial sweeteners elicited CPIR in the B6 mice, despite the fact that we used highly acceptable concentrations of each. For other species, there are contradictory reports about whether artificial sweeteners elicit CPIR. In rats, six studies reported that artificial sweeteners elicit CPIR (6, 44, 59, 60, 65), whereas one reported that they do not (25). In humans, one study reported that artificial sweeteners elicited CPIR (31), whereas four reported that they do not (1, 13, 26, 58). These contradictory findings may stem in part from the fact that the studies did not all use the same concentrations of each artificial sweetener. For example, among the studies that tested saccharin in humans, the one that observed CPIR used a 10 mM concentration (31), whereas the ones that failed to do so used concentrations ≤ 3 mM (26, 58). It is also possible that the CPIR elicited by 10 mM saccharin (31) represents a conditioned response based on prior experience with saccharin and ingested sugars (e.g., see Ref. 64).

We can offer one explanation for how artificial sweeteners could trigger CPIR rats and humans, but not mice. Here, we found that incapacitation of the KATP pathway abolishes CPIR but has no apparent impact on taste-mediated licking for sugars. In contrast, we reported previously that incapacitation of the T1r2+T1r3 pathway abolishes taste-mediated licking for sugars but has no apparent impact on CPIR (19). Taken
together, these observations indicate that despite occurring within the same taste cell (68), the K_Atp and T1r2+T1r3 signaling pathways function (at least partially) independently of one another and generate distinct physiological responses in mice. If we assume that the same basic configuration exists in the taste cells of rats and humans, then it is possible that there is more crosstalk between the two signaling pathways in these species. Accordingly, when artificial sweeteners activate the T1r2+T1r3 pathway in rats and humans, they could also activate downstream components of the K_Atp pathway and thereby elicit CPIR.

Perspectives and Significance

Several studies have postulated the existence of a T1r2+T1r3-independent signaling pathway for tasting carbohydrates (16, 45, 62, 72), but this study is the first to implicate a specific pathway; namely, the K_Atp signaling pathway. This T1r2+T1r3-independent signaling pathway does not appear to play a role in motivating intake of carbohydrates. Instead, it helps animals determine the concentration of glucose or glucose-containing carbohydrates in food and generate a CPIR of appropriate magnitude. Notably, the physiological impact of activating the K_Atp signaling pathway may not be limited to insulin release. There is evidence, for instance, that oral stimulation with glucose alters celiac artery blood flow and gastric myoelectrical activity in humans (14). Given that type 2 diabetes now afflicts nearly 10% of Americans (11), it is critical to gain a more complete understanding of the mechanisms that regulate insulin section and glucose tolerance. Despite clear evidence that oral stimulation with food-related chemical stimuli triggers CPIR and dramatically improves glucose tolerance in humans (10, 57), rats (52) and mice (19), most studies of glucose tolerance in rodents (19), most studies of glucose tolerance in rodents incorporate oral stimulation and administer nutrients either intra- and mice (19), most studies of glucose tolerance in rodents bypass oral stimulation and administer nutrients either intra-gastrically or intravenously (2, 5, 9, 38). We recommend that future studies of glucose tolerance in rodents incorporate oral stimulation into the experimental design.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS


REFERENCES

A motifs of SUR1 in K-ATP channel activation by Mg-ADP and diazoxysodium-glucose cotransporter 1 ameliorates hyperglycemia by mediating the GLUTag cell line.


