Sweet taste receptor signaling in beta cells mediates fructose-induced potentiation of glucose-stimulated insulin secretion

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Postprandial insulin release is regulated by glucose, but other circulating nutrients may target beta cells and potentiate glucose-stimulated insulin secretion via distinct signaling pathways. We demonstrate that fructose activates sweet taste receptors (TRs) on beta cells and synergizes with glucose to amplify insulin release in human and mouse islets. Genetic ablation of the sweet TR protein T1R2 obliterates fructose-induced insulin release and its potentiating effects on glucose-stimulated insulin secretion in vitro and in vivo. TR signaling in beta cells is triggered, at least in part, in parallel with the glucose metabolic pathway and leads to increases in intracellular calcium that are dependent on the activation of phospholipase C (PLC) and transient receptor potential cation channel, subfamily M, member 5 (TRPM5). Our results unveil a pathway for the regulation of insulin release by postprandial nutrients that involves beta cell sweet TR signaling.

saccharin | G-protein coupled receptor | T1R3 | glucagon-like peptide-1 | MIN6

After a meal, the increase in circulating glucose is the primary stimulus of insulin release from pancreatic beta cells. In turn, circulating insulin facilitates glucose disposal to peripheral tissues, thus restoring plasma glucose to preabsorptive levels and preventing further insulin secretion. Consequently, insulin release must be tightly regulated to ensure adequate postprandial fuel supply to the tissues, while preventing the detrimental effects of hypoglycemia when energy availability is scarce. Although the uptake and metabolism of glucose by the beta cells is indispensable for the stimulation of insulin release, numerous other insulin secretagogues have been identified, suggesting that several independent signaling pathways may synergize with glucose to fine-tune insulin secretion (1). For instance, nutrients such as some amino acids are metabolized by beta cells to stimulate insulin secretion (2), whereas other insulin modulators such as glucagon-like peptide-1 (GLP-1) interact instead with cell-surface G protein-coupled receptors (GPCRs) to activate signaling cascades leading to insulin secretion (3). Interestingly, the list of nonmetabolizable insulin secretagogues extends beyond known neurotransmitters and hormones. For instance, the major dietary monosaccharide fructose, or sugar substitutes such as saccharin, are poorly or not metabolized by beta cells (4–7), yet potentiate insulin secretion in vitro at physiological glucose concentrations (8–12). Therefore, it is conceivable that natural or artificial sweet compounds may be agonists for specific cell-surface receptors on beta cells.

Sweet sensing in the tongue is mediated by the T1R2-T1R3 heterodimer of the T1R family of GPCRs. In taste buds, ligand interaction with taste receptors (TRs) triggers a conserved signaling cascade that induces ATP exocytosis and stimulation of local sensory nerves (13, 14). Notably, TRs and their signaling components play pivotal roles in other sensory organs, such as the airway epithelium of the lungs (15), the enteroendocrine cells of the intestine (16, 17), and possibly in mouse islets, as recently reported (18). Considering that fructose is the sweetest natural sugar, evidenced by its high affinity for sweet TRs (19), it is plausible that postprandial fructose could potentiate insulin release by interacting with beta cell sweet TRs. This possibility is particularly interesting in light of the suggested link between high-fructose consumption and the development of adverse metabolic effects (20). Nevertheless, the pertinent signaling mechanisms regulated by TRs in beta cells and their interplay with known stimulatory pathways have not yet been described. In the present study, we show that sweet TRs are expressed in mouse and human islets, and that the T1R2 subunit is essential for potentiating insulin release in response to the dietary sugar fructose in islets and in vivo. The initial steps of the signaling pathway we describe can be triggered independently of glucose availability and metabolism, but the later steps converge with the canonical glucose metabolic pathway contributing to calcium flux and potentiation of insulin release.

Results

Ablation of T1R2 Sweet TR Eliminates Fructose-Induced Increase in Intracellular Calcium and Insulin Release in Mouse Beta Cells. Several nutrients, including fructose, can induce insulin release, but their efficacy is tightly dependent on the presence of glucose. This suggests that glucose uptake and metabolism is essential for providing the initiating stimulus for insulin release, whereas nutrients such as other sugars, amino acids, and lipids may enhance the effects of glucose through converging signaling pathways (1). Therefore, we tested whether the presence of physiological glucose concentration was necessary for fructose-induced insulin release in isolated mouse islets. Addition of 10.0 mM fructose to 8.3 mM glucose augmented insulin release compared with glucose alone (Fig. 1*A*), but these effects were absent when glucose concentration was 3.0 mM. These data show that, similar to other nutrients, fructose requires the presence of stimulatory glucose concentrations to induce insulin release in vitro and implies that the physiological role of fructose is to enhance the effects of glucose.

An increase in intracellular calcium ($Ca^{2+}{}_i$) is required for the stimulation of insulin release in beta cells. Using Fura-2 cell imaging in dispersed mouse beta cells, we tested whether fructose mediates its effects on insulin release by enhancing $Ca^{2+}{}_i$ responses. Addition of fructose induced a sustained increase in $Ca^{2+}{}_i$ of single WT beta cells, which exhibited different sensitivity thresholds to the sweetener. Traces of the most prevalent single-cell patterns are shown (Fig. 1*B*), accounting for approximately 80% of the cell population imaged. Similar to insulin

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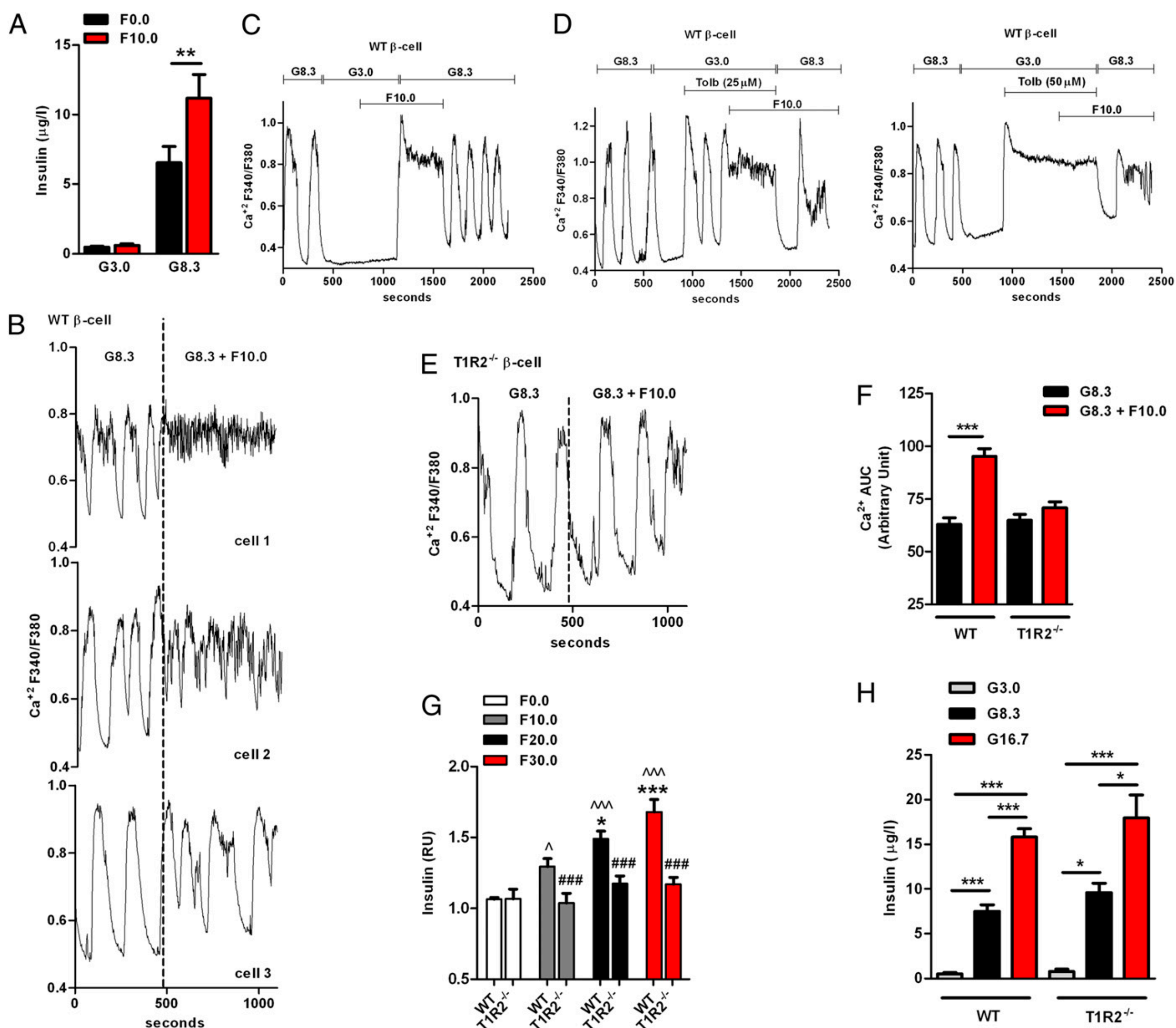

Fig. 1. Ablation of sweet TR protein, T1R2, obliterates fructose-induced calcium responses and insulin release in mouse islets. (*A*) Static insulin release at different glucose ("G"; in mM) concentrations with or without fructose ("F"; in mM) in WT islets (quadruplicate batches of 10 islets from $n = 8$ mice). (**$P < 0.01$, paired Student *t* test.) (*B–E*) Representative single-cell traces of calcium responses in dispersed primary beta cells from WT and T1R2$^{-/-}$ mice treated with glucose ("G"; in mM) and combinations of fructose ("F"; in mM), and tolbutamide as shown. (*F*) Area under the curve (AUC) calculated by using 10 to 20 single-cell calcium traces per mouse islet isolation ($n = 6$ per genotype). (***$P < 0.001$, ANOVA with Tukey posttest.) (*G*) Static insulin release in response to increasing concentrations of fructose ("F"; in mM) in the presence of glucose (8.3 mM) in islets from WT and T1R2$^{-/-}$ mice ($n = 6$ per group). Data are expressed as relative units (RU) of fold insulin change from baseline (8.3 mM glucose; set at value 1) using paired experiments. Baseline secretion at 8.3 mM glucose was similar in WT and T1R2$^{-/-}$ islets (5.4 ± 0.4 μg/L and 5.9 ± 0.6 μg/L, respectively). *$P < 0.05$ and ***$P < 0.001$ vs. F10 WT; ^$P < 0.05$ and ^^^$P < 0.001$ vs. F0 WT; $^{###}P < 0.001$ vs. corresponding WT; two-way ANOVA with Bonferroni posttest. (*H*) Static GSIS at various glucose ("G"; in mM) levels in isolated islets from WT and T1R2$^{-/-}$ mice ($n = 6$ per genotype).

release, the presence of a physiological glucose concentration was also essential for fructose-induced Ca^{2+}_i responses, as these effects were obliterated at substimulatory glucose levels (3.0 mM; Fig. 1*C*). This suggests that a depolarization threshold, provided by adequate glucose, is essential for fructose-mediated effects. To further test this hypothesis, we used tolbutamide, an established inhibitor of ATP-sensitive K^+ (K^+_{ATP}) channels, which can cause beta cell depolarization and calcium influx independent of glucose levels. At 3.0 mM glucose, a moderate concentration of tolbutamide (25 μM) induced submaximal Ca^{2+}_i responses, which were further amplified by the addition of fructose (Fig. 1*D*, *Left*). However, fructose had no additive effects at a tolbutamide concentration (50 μM) that induced maximized Ca^{2+}_i responses (Fig. 1*D*, *Right*) (21). As hypothesized, these observations suggest that the closure of K_{ATP} channels by glucose is necessary to reach the depolarizing threshold, and fructose signaling positively modulates global membrane depolarization, thus enhancing calcium influx and insulin release (Fig. 1 *A–C*).

Considering the documented negligible oxidation of fructose in islets (5, 10), we next hypothesized that the effects of fructose on insulin release may be mediated by cell-surface sweet TRs on beta cells. The three members of the T1R family of TRs form heterodimers to confer umami (T1R1-T1R3) or sweet (T1R2–T1R3) sensing (14). Heterodimerization is necessary for taste

signaling and perception, so ablation of *T1R1* or *T1R2* is adequate to obliterate umami or sweet taste respectively, whereas ablation of *T1R3* eliminates both taste responses (22, 23). Notably, mouse islets and MIN6 beta cells express sweet TRs (Fig. S1 *A* and *B*) (18). Because sweet taste response is inevitably dependent on the presence of both T1R2 and T1R3, we used mice lacking the *T1R2* receptor gene ($T1R2^{-/-}$), which therefore are deprived of sweet taste signaling, to assess the direct role of sweet TRs in the regulation of insulin release. In contrast to WT beta cells (Fig. 1 *A* and *B*), addition of fructose to $T1R2^{-/-}$ beta cells had no significant effects on $Ca^{2+}{}_i$ (Fig. 1 *E* and *F*). In agreement with the $Ca^{2+}{}_i$ responses, fructose also failed to induce insulin secretion in isolated $T1R2^{-/-}$ islets (Fig. 1*G*), even at supraphysiological concentrations, highlighting the essential role of the T1R2 protein of sweet TRs in the regulation of fructose-induced insulin release in vitro.

Because glucose metabolism is essential for fructose-induced insulin release, we tested whether $T1R2^{-/-}$ islets have altered insulin response to glucose in vitro. No significant differences in glucose-stimulated insulin secretion (GSIS; Fig. 1*H*), insulin content (Fig. S1*C*), or glucose-induced $Ca^{2+}{}_i$ responses (Fig. S1*D*) were noted between WT and $T1R2^{-/-}$ islets, suggesting that the lack of fructose response in $T1R2^{-/-}$ islets is not caused by defects in glucose metabolism. To exclude the possibility of other ectopic effects on beta cells, we assessed the expression of genes involved in gustatory signaling or glucose uptake and metabolism. Compared with WT mice, no significant differences were observed in members of the known gustatory signaling pathway in the tongue, including *phospholipase C* (*PLC*) *β2*, *T1R1*, *T1R3*, or *transient receptor potential cation channel, subfamily M, member 5* (*TRPM5*) (Fig. S1*B*). Similarly, there were no differences in the expression of *glucose transporter 2, glucokinase* (the rate-limiting enzyme in GSIS), or *insulin* (Fig. S1*E*).

Islet Sweet TRs Sense Circulating Fructose and Rapidly Induce Insulin Release in Vivo. Our findings in isolated islets led us to speculate that postprandial fructose could rapidly and directly induce insulin release via beta cell TRs. To address this hypothesis, we used an i.v. bolus of fructose (1.0 g/kg) in catheterized conscious male mice and monitored plasma glucose and insulin. We used i.v. administration of fructose to bypass the gastrointestinal tract, because TRs in enteroendocrine cells can be activated by intestinal nutrients to induce the secretion of GLP-1, a known amplifier of GSIS (16, 17). Consistent with our hypothesis, fructose induced an early transient increase of plasma insulin in WT mice (Fig. 2 *A* and *E*), whereas $T1R2^{-/-}$ mice showed no insulin response (Fig. 2 *B* and *E*). Total plasma GLP-1 was not altered after the i.v. bolus of fructose (6.8 ± 0.9 pmol/L at 0 min vs. 8.3 ± 1.4 pmol/L at 2 min; $n = 7$; $P = 0.38$). Similar to plasma GLP-1, glucose levels also remained unaltered immediately after the injection of fructose (Fig. 2 *C* and *D*), suggesting that the rapid increase in plasma insulin cannot be explained by changes in plasma glucose or GLP-1, but rather can be attributed to the direct effects of fructose on pancreatic islets. We observed, however, a mild increase in plasma glucose over time that is likely attributable to the documented metabolic effects of fructose in the liver or kidneys (24). To test this hypothesis, we injected ^{13}C-labeled fructose and monitored the incorporation of ^{13}C to plasma glucose over time. Indeed, ^{13}C-labeled glucose progressively increased at 10 and 20 min after injection of ^{13}C fructose (Fig. S2*A*), confirming that the delayed increase in plasma glucose is a result of hepatic glucose output derived by fructose metabolism. To further verify that the rapid insulin response is a result of the direct stimulation of beta cell TRs, we used the artificial sweetener saccharin that, similar to fructose, is a potent sweet TR ligand, but is not metabolized by the liver or the pancreas (7, 25, 26). Injection of saccharin (1.0 g/kg) induced a rapid increase in plasma insulin at a time course (2–5 min) comparable to a bolus of fructose (Fig. S2*B*). In contrast to fructose, however, the delayed increase in plasma glucose was absent (Fig. S2*C*). Consistent with the in vivo response, saccharin also induced insulin release in isolated mouse islets at 8.3 mM glucose (Fig. S2*D*). These observations corroborate that, as hypothesized, the rapid increase in plasma insulin in response to fructose (or saccharin) is a result of direct effects on beta cells.

To assess whether global deletion of *T1R2* alters glucose homeostasis, we performed an i.p. glucose tolerance test (IPGTT). Glucose excursions during the IPGTT (Fig. 2*F*), fasting insulin levels (Fig. 2*G*), or body weight (24.13 ± 0.20 g vs. 25.53 ± 0.70 g) were no different between 8- to 10-wk-old WT and $T1R2^{-/-}$ chow-fed male mice. These findings suggest that, at least under these conditions, the absence of insulin response to circulating fructose in $T1R2^{-/-}$ mice cannot be attributed to disturbances in systemic glucose metabolism. Collectively, these results are consistent with our in vitro data and strongly suggest that activation of sweet TRs on beta cell acutely and rapidly induces insulin release in response to circulating fructose.

Postprandial Concentrations of Fructose Potentiate GSIS via Sweet TRs. The ability of fructose to stimulate insulin release within physiological glucose concentrations led us to hypothesize that circulating nutrients, such as fructose, may synergize with glucose to potentiate insulin release. We demonstrated that, at 8.3 mM glucose, addition of 10 mM fructose was sufficient to induce insulin release in vitro. Nevertheless, it is conceivable that, during a mixed meal, both sugars will be proportionally absorbed by the gut, entering the systemic circulation simultaneously (27). Thus, we assessed whether fructose, at levels similar to those found in postprandial circulation, can potentiate GSIS in vitro. For that purpose, isolated mouse islets were incubated at 8.3 mM glucose (i.e., preprandial/baseline conditions) followed by an increase in glucose to 16.7 mM (i.e., postprandial/stimulatory conditions) with or without the presence of low concentrations of fructose (3.0 mM). Increasing glucose from 8.3 to 16.7 mM induced a substantial increase in insulin release both in WT and $T1R2^{-/-}$ islets. However, in WT islets the addition of 3.0 mM fructose further potentiated GSIS (from 8.3 to 16.7 mM), but not in $T1R2^{-/-}$ (Fig. 3*A*). At baseline glucose concentration (8.3 mM), insulin release was similar between WT and $T1R2^{-/-}$ islets (4.05 ± 0.50 μg/L vs. 4.07 ± 0.44 μg/L) and the presence of 3.0 mM fructose had no additional effect on secretion, suggesting that fructose potentiation is glucose-dependent.

To assess the potentiating effects of fructose on GSIS in vivo, we administered glucose (0.5 g/kg) alone or with a low amount of fructose (0.3 g/kg) in conscious catheterized mice. Infusion of fructose at this rate has been shown to elicit a steady-state plasma fructose concentration lower than 2.0 mM (28), which is comparable to the concentrations reported in postprandial circulation (29, 30) and in our in vitro experiments. A bolus injection of fructose (0.3 g/kg) did not alter plasma glucose or insulin, suggesting that a moderate increase in circulating fructose alone is not sufficient to stimulate insulin release (Fig. 3*B*, gray trace). This contrasts the insulinotropic effect observed in response to a larger (1.0 g/kg) bolus of fructose (Fig. 2*A*). As expected, i.v. administration of glucose or glucose with fructose increased plasma glucose levels comparably (Fig. 3*C*); however, whereas injection of glucose induced an anticipated increase in plasma insulin (Fig. 3*B*, black trace), the addition of fructose caused a significant potentiation (Fig. 3*B*, red trace). To evaluate the physiological significance of insulin potentiation, we compared plasma glucose slopes after the injection (time 2–20 min). The rate of glucose disappearance after an i.v. bolus of glucose plus fructose was significantly higher than that of glucose alone, consistent with the plasma insulin responses (8.1 ± 0.34 mg/dL/min vs. 11.4 ± 0.54 mg/dL/min; $n = 8$; $P < 0.0001$). In $T1R2^{-/-}$ mice, insulin response to a bolus of glucose or fructose alone was

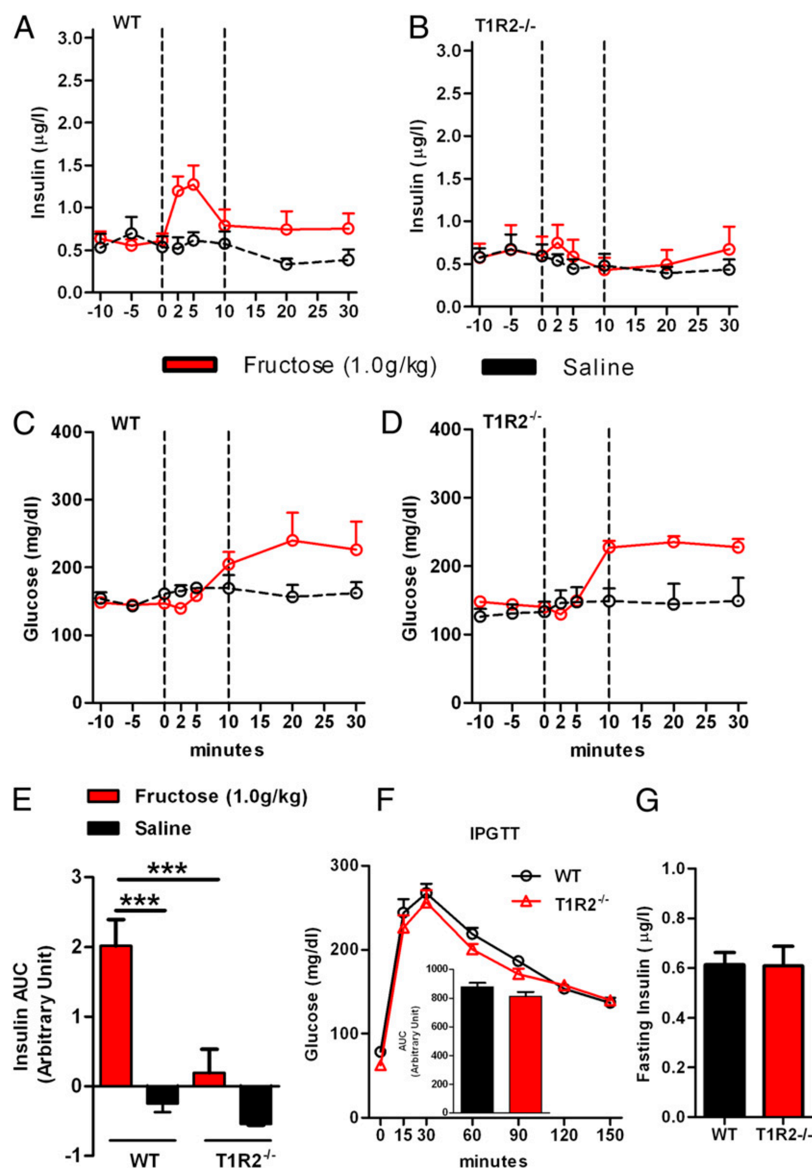

Fig. 2. A bolus of fructose induces rapid insulin release in vivo mediated by islet sweet TRs. (*A* and *B*) Plasma insulin in response to a bolus of fructose (1.0 g/kg) or saline solution injected at time 0 in WT and $T1R2^{-/-}$ mice (n = 8 per treatment). (*C* and *D*) Plasma glucose in response to a bolus of fructose or saline solution in WT and $T1R2^{-/-}$ mice. (*E*) Area under the curve (AUC) calculated between 0 and 10 min (vertical lines). (***$P < 0.001$, ANOVA with Tukey posttest.) (*F*) Blood glucose levels during IPGTT in age-matched WT and $T1R2^{-/-}$ male mice (n = 8 per treatment; *Materials and Methods*). *Inset*: Area under the curve (AUC). (*G*) Plasma insulin levels after 5 h fasting in WT and $T1R2^{-/-}$ mice (n = 12 per genotype).

similar to that in WT mice; however, addition of fructose did not induce any potentiation (Fig. 3 *D* and *E*). These results support our in vitro findings and unveil an important physiological regulatory role for circulating fructose in potentiating GSIS in vivo through the sweet TRs.

PLC Activation Is Necessary for TR-Mediated Insulin Release in Beta Cells. To better elucidate TR signaling in beta cells, we used MIN6 cells. Similarly as in primary mouse beta cells, fructose (10 mM) induced an increase in $Ca^{2+}{}_i$ in MIN6 cells (Fig. S3*A*). TRs belong to class-C GPCRs that stimulate the activation of PLCβ2, leading to IP_3 generation and subsequent ER Ca^{2+} release (14). PLCβ2 is expressed in MIN6 beta cells and mouse islets (Fig. S1 *A* and *B*). Inhibition of PLC with U73122 abolished the fructose-induced stimulation of $Ca^{2+}{}_i$ in primary beta cells (Fig. 4*A*) and MIN6 beta cells (Fig. S3*B*), and diminished insulin release in response to the sweeteners in isolated islets (Fig. 4*B*).

To gain further insight into how the TR-mediated and the glucose metabolic signaling pathways interact to promote insulin release, we investigated the time course of PLC activation and its effects on downstream signaling by using a cellular biosensor that has the pleckstrin homology (PH) domain of PLCδ1 fused to GFP (PH-PLCδ-GFP) (31). We used total internal reflection fluorescence (TIRF) microscopy (32) to assay the translocation of PH-PLCδ-GFP between the plasma membrane and the cytoplasm (i.e., PLC activity). PLC translocation from the plasma membrane to the cytoplasm rapidly increased upon fructose stimulation (a decrease in fluorescence intensity within 20 s) followed by subsequent PLC recruitment to the plasma membrane (Fig. 4*C*, left axis, vertical line). The activation of PLC (Fig. 4*C*, black trace) immediately preceded the onset of $Ca^{2+}{}_i$ response (Fig. 4*C*, right axis, red trace), suggesting that, similar to the tongue, TR agonists in beta cells directly induce PLC signaling. A similar pattern of PLC activation was observed after

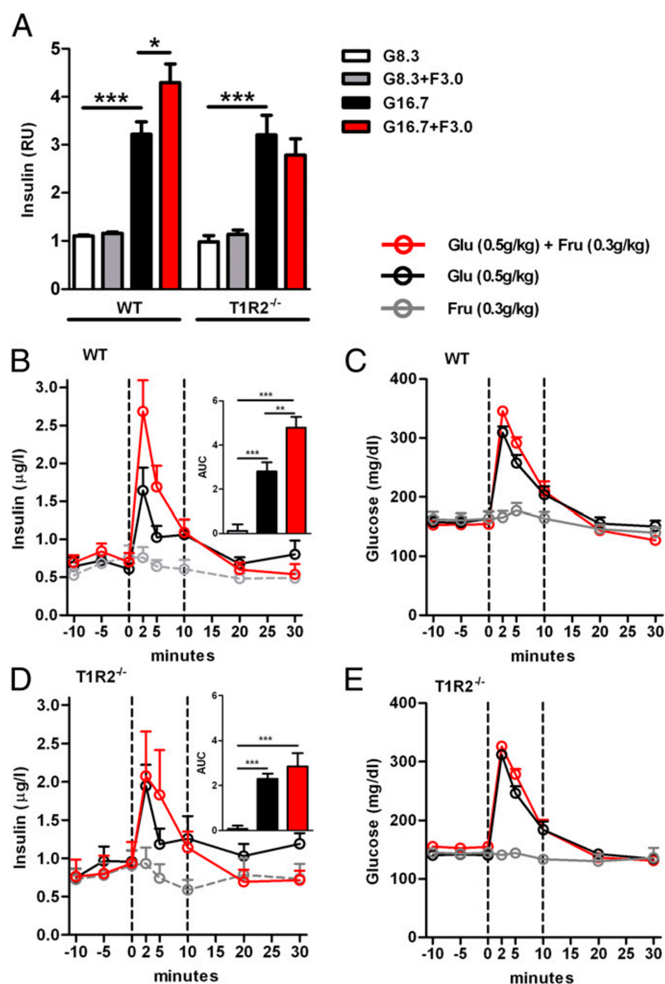

Fig. 3. Fructose potentiates GSIS dependent on sweet TRs. (*A*) Static insulin release of WT and T1R2$^{-/-}$ islets (quadruplicate batches of 10 islets from six mice per genotype) incubated at 8.3 mM glucose (WT, 4.05 ± 0.50 μg/L; T1R2$^{-/-}$, 4.07 ± 0.44 μg/L) and then transferred to media with glucose ("G") and fructose ("F") as shown. Data are expressed as relative units (RU) of insulin fold change from baseline (8.3 mM glucose; set at value 1) using paired experiments. (**P* < 0.05 and ****P* < 0.001, ANOVA with Tukey posttest.) (*B* and *D*) Plasma insulin in response to a bolus of glucose (glu; 0.5 g/kg) and/or fructose (fru; 0.3 g/kg; injected at time 0) in WT and T1R2$^{-/-}$ mice (n = 8 per treatment). *Inset*: Area under the curve (AUC) calculated between 0 and 10 min (vertical lines). (***P* < 0.01 and ****P* < 0.001, ANOVA with Tukey posttest.) (*C* and *E*) Plasma glucose in response to a bolus of glucose and/or fructose (injected at time 0) in WT and T1R2$^{-/-}$ mice.

treatment with carbachol, a known inducer of the PLC-IP_3 pathway in beta cells (33), whereas inhibition of PLC activity abolished its translocation between the plasma membrane and the cytoplasm, confirming the specificity of the assay (Fig. S3*C*).

Given that TR agonist stimulation of $Ca^{2+}{}_i$ response and insulin release is dependent on glucose availability (Fig. 1 *A* and *C*), we tested whether similar glucose conditions were essential for PLC activation. Fructose-induced PLC activation was similar at substimulatory glucose (3.0 mM) levels (Fig. 4*D*) compared with normal glucose levels (8.3 mM; Fig. 4*C*, left axis), suggesting that PLC activation occurred independently of glucose availability and therefore does not account for the absence of the TR-mediated insulin response at low glucose levels (Fig. 1*A*). Because voltage-dependent calcium channel (VDCC) activation and calcium influx are required for GSIS, we used nifedipine, a known inhibitor of VDCCs, to test whether the TR agonist potentiation of GSIS also depends on the activation of extracellular calcium entry. As expected, pretreatment with nifedipine (Fig. S3*D*) or removal of extracellular calcium (Fig. S3*E*, Ca^{2+}-free) diminished influx of extracellular Ca^{2+} in response to TR agonists in MIN6 cells. To demonstrate that PLC activation precedes the activation of VDCCs and calcium influx, we analyzed changes in PLC activity under similar conditions. Neither the inhibition of VDCCs with nifedipine (Fig. 4*E*) nor the absence of extracellular calcium (Fig. 4*F*, Ca^{2+}-free) affected the activation of PLC in response to fructose stimulation, suggesting that PLC activation is upstream of calcium influx. Collectively, these data suggest that TR signaling involves the direct activation of PLC. This early step of TR signaling is initiated independently of the glucose metabolic pathway, suggesting that the two are converging to amplify known downstream steps required for insulin secretion, such as membrane depolarization and VDCC-dependent calcium influx.

Cation Channel TRPM5 Mediates Effects of TR Signaling in Beta Cells. The calcium-activated cation channel TRPM5 plays a central role in mediating TR signaling in the tongue through IP_3-mediated release of ER Ca^{2+}, contributing to subsequent cell membrane depolarization and influx of extracellular Ca^{2+} required for the vesicular release of ATP (34). It was recently shown in beta cells that TRPM5 also affects the frequency of glucose-induced calcium oscillations and GSIS in vitro, suggesting a critical role for cation channels in glucose homeostasis (35). Therefore, we hypothesized that TRPM5 activation is the likely downstream convergence point between TR- and glucose-induced insulin secretion in beta cells. According to this, glucose closes K_{ATP} channels thus withdrawing their antagonizing effects on membrane depolarization, whereas TR-mediated TRPM5 activation induces Na^+ influx that further depolarizes the membrane. To address the direct role of TRPM5, we monitored $Ca^{2+}{}_i$ in dispersed beta cells isolated from *TRPM5* KO mice (*TRPM5*$^{-/-}$). Beta cells deficient in TRPM5 failed to induce a sustain increase in $Ca^{2+}{}_i$ in the presence of fructose compared with WT controls (Fig. 5 *A*–*C*). Consequently, fructose-induced insulin release was also diminished (Fig. 5*D*). These findings suggest that TRPM5 is required for TR signaling in beta cells and that its activation is the likely step that contributes to the global membrane depolarization and induction of calcium influx.

TRs Are Expressed in Human Islets and Mediate Potentiating Effects of Fructose on Insulin Secretion. Interestingly, sweet TRs are also present in human islets (Fig. 6*A*). To address the potential role of TR signaling in human physiology, we used islets from individual donors, which were first tested to ensure normal GSIS (Fig. 6*B*). In view of the heterogeneity of absolute insulin release of individual donor preparations, we expressed insulin data relative to baseline/unstimulated conditions. Similar to mouse islets, fructose and saccharin stimulated insulin release in the presence of a physiologically relevant concentration of glucose (Fig. 6*C*). Consequently, we tested whether a low concentration of fructose, similar to those found postprandially, were adequate to potentiate GSIS in human islets. When 3.0 mM fructose was included with 11.0 mM glucose, it potentiated GSIS, whereas 3.0 mM fructose at 5.5 mM glucose had no effect (Fig. 6*D*). To assess the direct role of TR-mediated insulin release in human islets we used lactisole (Endeavor), a characterized allosteric inhibitor specific to the human T1R3 (36). In humans and rodents, T1R2 and T1R3 interact to function as a heteromeric receptor. Therefore, inhibition or elimination of T1R3 is equivalent in preventing sweet taste responses to the inhibition or elimination of T1R2. Indeed, *T1R3* KO mice (*T1R3*$^{-/-}$) lack sweet taste perception (14) and, as expected, islets from *T1R3*$^{-/-}$ did not respond to fructose-induced insulin release in vitro (Fig. S4). Consistent with the functional role of T1R3 in TR signaling,

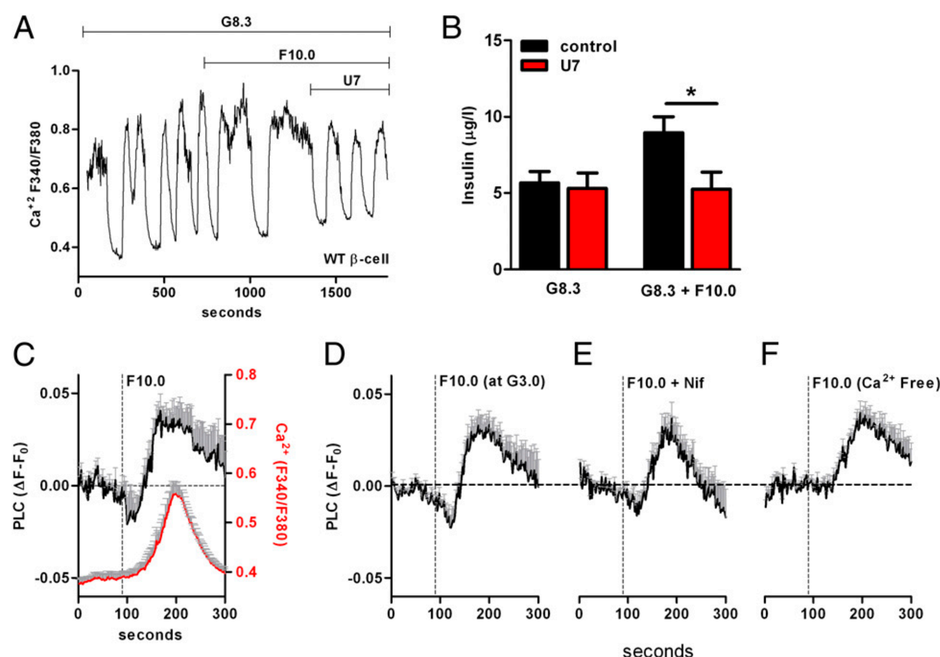

Fig. 4. PLC activation is essential for calcium and insulin responses in beta cell TR signaling. (*A*) Representative single-cell trace of calcium responses in dispersed primary beta cells from WT mice treated with glucose ("G"; in mM) and combinations of fructose ("F"; in mM) and U73122 (U7; 2.0 μM) as shown. (*B*) Static insulin release in response to glucose ("G"; in mM) or fructose ("F"; in mM) with or without U73122 (U7; 5.0 μM) in WT islets (quadruplicate batches of 10 islets from six mice). (*$P < 0.05$, Student *t* test.) (*C*) Average of traces (n = 4–6 cells per trace, total of six independent experiments) showing relative change in PLC activity (left axis) or intracellular calcium (right axis) from baseline (set at 0) in MIN6 beta cells treated (vertical line) with fructose (10.0 mM) in the presence of glucose (8.3 mM). (*D–F*) Average of traces (n = 4–6 cells per trace, total of six independent experiments) showing relative change in PLC activity from baseline (set at 0) in MIN6 beta cells treated (vertical line) with fructose ("F"; 10.0 mM) in the presence of glucose ("G"; 3.0 mM or 8.3 mM), nifedipine (Nif; 1.0 μM), or no extracellular calcium (Ca^{2+} free). Experiments were performed at the presence of 8.3 mM glucose unless otherwise stated.

its inhibition with lactisole abolished fructose-induced insulin release (Fig. 6*E*) and eliminated the potentiating effects on GSIS (Fig. 6*F*), confirming the significance of TR signaling in human islet physiology.

Discussion

Whereas gut absorption of glucose and other nutrients can be sporadic depending on food availability, glucose delivery must be continuous at the cellular level to meet energy demands. Consequently, after a meal, several distinct pathways operate simultaneously in beta cells to provide sufficient regulation of insulin release, thus ensuring immediate energy distribution to the tissues for storage or consumption. Here we describe a pathway for the regulation of postprandial insulin release involving cell-surface GPCRs that are activated by circulating sweet nutrients. Specifically, we show that the dietary monosaccharide fructose potentiates GSIS mediated by sweet TRs expressed on beta cells.

The ability of fructose to induce insulin secretion has been the subject of conflicting reports for several years. Nevertheless, these discrepancies are partly resolved considering that the insulinotropic effects of fructose (8, 10, 37) disappear when physiological levels of glucose are absent (38, 39). Our data illustrate that the required TR-mediated increase in $Ca^{2+}{}_i$ is eliminated at subphysiological glucose levels. This observation not only explains the absence of insulin response shown under these conditions, but also suggests that adequate glucose concentrations may be vital to maintain the necessary membrane depolarization threshold that can be amplified by TR signaling to induce further calcium influx. Therefore, fructose is likely to play only a potentiating role in insulin release that is dependent on glucose levels. This should not be surprising, as a typical mixed diet involves the simultaneous absorption of both glucose and fructose because sucrose is the most common dietary disaccharide (27). Thus, the obligatory role of glucose for the fructose-induced insulin secretion suggests a synergistic interaction of the two sugars in modulating postprandial insulin release (40–42). Consistent with this hypothesis, we showed that low physiological concentrations of fructose were adequate to potentiate GSIS in mouse and human islets, and in vivo.

Our data obtained with the use of isolated islets and the observation that both i.v. fructose and saccharin can induce identical time-dependent increases in plasma insulin, without concomitant changes in plasma glucose, suggest that the insulinotropic effects of fructose are mediated by a mechanism directly targeting beta cells. Unlike other nutrients, however, fructose is neither significantly metabolized by the beta cells, nor does it interfere with beta cell glucose metabolism (4, 6, 43). For example, at equimolar concentrations, the utilization of fructose by the beta cells is less than 10% than that of glucose (5, 10), suggesting that our key experimental fructose concentrations (3–10 mM) had negligible metabolic effects. Therefore, the rapidity of fructose-induced insulin response together with the absence of known metabolic effects in beta cells supports the presence of a receptor-mediated mechanism. TR signaling requires the formation of the T1R2-T1R3 heterodimer. Consequently, genetic ablation of *T1R2* alone is sufficient to obliterate sweet taste sensing in the tongue (23). As a result, T1R2 is a bona fide candidate for mediating the effects of fructose in beta cells. Indeed, lack of T1R2 prevented the dose–response effect seen in WT islets, supporting the notion that, even at high concentrations, sweet TRs account for the vast majority of fructose-mediated effects on beta cells, with little or no contribution of alternative mechanisms.

The explicit involvement of T1R2 in the regulation of insulin secretion in vitro was further supported by in vivo experiments showing that the potentiation of insulin release, induced by the

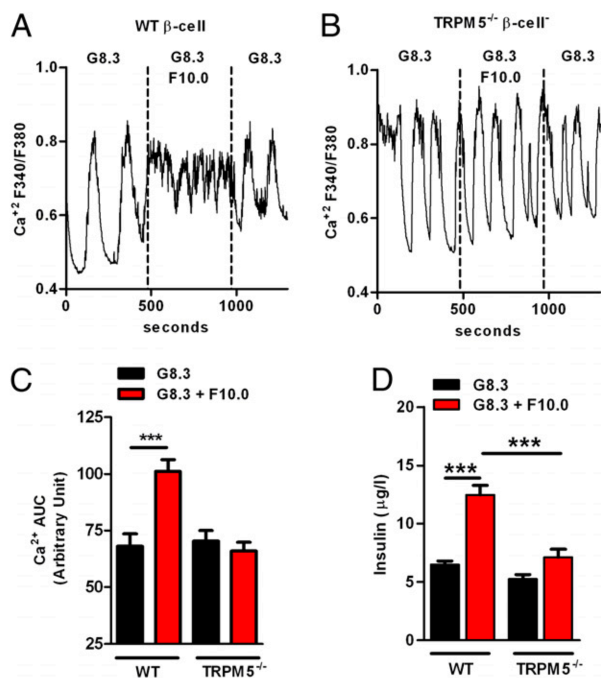

Fig. 5. Ablation of TRPM5 abolishes the effects of TR signaling in mouse islets. (*A* and *B*) Representative traces of calcium response in WT and TRPM5$^{-/-}$ primary beta cells treated (vertical line) with fructose ("F"; 10.0 mM) in the presence of 8.3 mM glucose (quadruplicate batches of 10 islets from six mice per genotype). (*C*) Area under the curve (AUC) calculated using 10 to 20 single-cell calcium traces from six mice per treatment. (***$P < 0.001$, ANOVA with Tukey posttest.) (*D*) Static insulin release in response to fructose ("F"; 10 mM) in the presence of 8.3 mM glucose in WT and *TRPM5*$^{-/-}$ islets ($n = 6$ per treatment). (***$P < 0.001$, ANOVA with Tukey posttest.)

addition of fructose to an i.v. bolus of glucose, disappeared in *T1R2*$^{-/-}$ mice. Surprisingly, WT and *T1R2*$^{-/-}$ mice had similar glucose excursions after an IPGTT and similar GSIS responses in vitro, despite glucose being a de facto sweet TR ligand. Considering, however, that glucose is a low-affinity ligand compared with fructose (19, 44) and that multiple substrate binding sites have been reported for sweet TRs (45), it is plausible that the TR-mediated effects of glucose may be masked by its metabolic effects, or that the two sugars must cooperatively bind to activate the T1R2-T1R3 heterodimer. Although these hypotheses require further investigation, our core data show that TR signaling in beta cells is an auxiliary amplifying module for insulin release, unlikely to induce a strong metabolic phenotype in *T1R2*$^{-/-}$ mice under these circumstances. Nevertheless, it is reasonable to speculate that, under conditions of metabolic stress, such as obesity, high-fat diet, or aging, an undisclosed pathophysiological role of TR signaling in beta cells may emerge.

Our data demonstrate the potential role of TR signaling in rodent islets, but because of the heterogeneous taste perception among species (23, 44, 46), it is not apparent whether these findings are relevant to human physiology. Although there is no known genetic model in which to assess the functionality of sweet TRs in human beta cells, the pharmacological inhibitor lactisole interacts specifically with the transmembrane domain of human, but not mouse, T1R3 and eliminates sweet taste perception (36, 47). Inhibition of human T1R3 with lactisole obliterated fructose-induced insulin release and the potentiating effects of fructose on GSIS, confirming the importance of TR signaling in human islets and revealing potential molecular targets for pharmacological intervention. The contribution of TR-mediated regulation of insulin release in human physiology and its interaction with other known insulinotropic mechanisms requires further investigation.

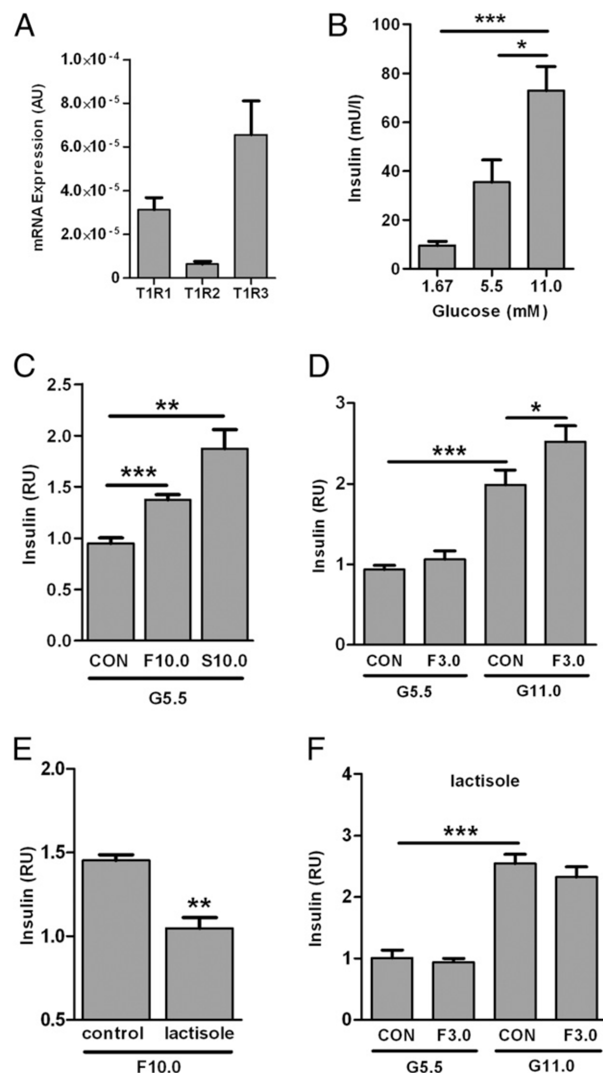

Fig. 6. Fructose-mediated potentiation of GSIS in human islets is abolished by pharmacological inhibition of sweet TRs. (*A*) *T1R* gene expression in human islets ($n = 5$ donors) measured by quantitative real-time RT-PCR. Arbitrary units (AU) shown normalized to 18s rRNA. (*B*) Insulin release in human islet preparations in the presence of glucose ($n = 8$ donors). (*C*) Static insulin release in response to fructose ("F"; in mM) or saccharin ("S"; in mM) at the presence of 5.5 mM glucose in human islets ($n = 6$ donors). Data are expressed as relative units (RU) of fold insulin change from baseline (5.5 mM glucose; set at value 1) using paired experiments. (**$P < 0.01$ and ***$P < 0.001$, Student *t* test.) (*D* and *F*) Static GSIS in human islets ($n = 6$ donors per condition) incubated at 5.5 mM glucose and then transferred to media with glucose ("G"; 5.5 mM or 11.0 mM) and fructose ("F"; 3.0 mM) as shown, with or without lactisole (2.0 mM). Data are expressed as relative units (RU) of fold insulin change from baseline (5.5 mM glucose; set at value 1) using paired experiments. (*$P < 0.05$ and ***$P < 0.001$, ANOVA with Tukey posttest.) (*E*) Static insulin release in response to fructose ("F"; in mM) with or without lactisole (2.0 mM) in the presence of 5.5 mM glucose ($n = 6$ donors). Data are expressed as fold insulin change relative to release at 5.5 mM glucose (set at value 1) using paired experiments. (**$P < 0.01$, Student *t* test.)

Insulin secretagogues trigger diverse signaling pathways that eventually induce membrane depolarization and calcium influx required for insulin exocytosis. In a similar way, TRs in the tongue signal via a conserved pathway in which PLC-IP_3 is activated inducing ER calcium release. Calcium activates TRPM5 channels, allowing sodium flux and membrane depolarization,

which causes calcium influx and ATP exocytosis (48). Our data suggest that the insulinotropic effects of fructose in beta cells necessitate a sustain increase in Ca^{2+}_i, which is absent in *T1R2*$^{-/-}$ beta cells. Therefore, key components of the canonical TR signaling may also be essential for TR-mediated insulin exocytosis in beta cells. However, beta cells secrete insulin by integrating signaling cues that emanate from several independent mechanisms. Because the metabolism of glucose is the unequivocal principal mechanism for insulin release, we attempted to elucidate the functional interaction between the two pathways.

Notably, PLC inhibition blocked TR signaling without altering steady-state insulin release at 8.3 mM glucose, indicating that TR activation amplifies GSIS via a signaling cascade that operates, at least in part, in parallel and independent to the known glucose metabolic pathway. In fact, by using TIRF microscopy, we demonstrated that TR-mediated activation of PLC was rapid, preceded the increase in Ca^{2+}_i, and was induced independently of glucose availability or VDCC activation. It was recently reported that sweeteners induce a delayed increase in cAMP, which correlated with insulin release in beta cells, but it is unclear whether these effects are direct (18). Although our data do not exclude the possibility that TR signaling may induce an increase in second messengers such as cAMP (49), the rapidity of PLC activation shown with TIRF microscopy indicates that such effects may be secondary. On the contrary, TRPM5 is a calcium-activated cation channel that is likely activated after PLC-IP_3–induced mobilization of ER calcium, contributing to membrane depolarization (34, 48). We demonstrated that TRPM5 is required for TR signaling in beta cells, but it has been recently reported that ablation of TRPM5 also disturbs GSIS and systemic glucose homeostasis (35). Thus, TRPM5 seems to be a common denominator linking TR signaling with the canonical glucose-stimulated pathway of insulin release.

The mechanism by which fructose stimulates insulin release in the presence of glucose was unknown for decades. Here we show that fructose is a natural ligand for functional sweet TRs expressed on mouse and human beta cells. Pancreatic TRs sense circulating fructose and activate a distinct signaling pathway that potentiates GSIS. Our data, together with previous reports showing that sweet TRs in the intestinal epithelium stimulate dietary glucose absorption and regulate GLP-1 secretion (16, 50, 51), suggest a TR-dependent intestinopancreatic axis that participates in the regulation of postprandial insulin release by absorbed sugars. Although in vivo human studies have failed to show an effect of consumed artificial sweeteners on insulin or blood glucose levels (52, 53), an oral solution of sucrose (disaccharide of glucose and fructose) can potentiate insulin release compared with equimolar glucose alone (54), and can cause a sustained increase in insulin levels starting short after ingestion (55). These findings are consistent with our in vivo potentiation of insulin release by fructose. Taken together, it is intriguing to speculate that dietary fructose, typically consumed in the form of sucrose or high-fructose corn syrup, might target the TR machinery in the intestine and, soon after, the pancreas to establish a preparatory and effecter mechanism, respectively, for the regulation of insulin release and glucose delivery to the tissues. Such a scenario requires further investigation because it may be part of the possible link between the adverse effects of high fructose consumption and the pathogenesis of metabolic diseases (20).

Materials and Methods

Additional details of experimental procedures can be found in *SI Materials and Methods*.

Reagents and Animals. All chemicals and cell culture reagents were purchased from Sigma-Aldrich or Invitrogen unless otherwise specified. Mice with the homozygous deletion for the *T1R2*, *T1R3*, or *TRPM5* gene (provided by C. S. Zuker, Columbia University, New York, NY) were bred and genotyped in house for all experiments (23, 48) All animals were maintained in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, and all experiments were performed with approved protocols from the institutional animal care and use committee of the Sanford–Burnham Medical Research Institute at Lake Nona.

Static Insulin Secretion. The day of the experiment, cultured human and mouse islets (*SI Materials and Methods*) were equilibrated in Krebs–Ringer Hepes buffer (KRH; 119 mM NaCl, 4.74 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 1.2 mM KH_2PO_4, 10 mM Hepes, 0.5% insulin-free BSA, pH 7.4) with glucose (mouse, 8.3 mM; human, 5.5 mM, unless otherwise stated). Then, islets were hand-picked and transferred to custom-made wells with glucose only for 30 min (i.e., baseline). Islets were then transferred to new wells and challenged for an additional 30 min as shown in figures (i.e., stimulated). The supernatant from both incubations was collected, and static insulin secretion was measured by using mouse insulin ELISA (Mercodia). Islet insulin secretion was expressed as absolute values (in μg/L), or as fold change of stimulated/baseline insulin secretion by using paired experiments. For each experimental condition, we independently measured insulin from four separate wells (10 islets each), which were then averaged to represent insulin values for $n = 1$. All insulin secretion data are averages of six to eight independent mouse islet isolations as shown. If necessary, solutions were osmotically balanced by using appropriate concentrations of dextran that has no affinity for sweet TRs. All islet incubations were at 37 °C without CO_2 in 100 μL of KRH buffer.

Plasma Insulin and GLP-1 Measurements. Plasma insulin was measured by using ultrasensitive mouse insulin ELISA (Mercodia). Plasma GLP-1 was measured by using mouse/rat total GLP-1 assay kit (Meso Scale Discovery) after collection of samples in tubes containing DPP-IV inhibitor (DPP4, Millipore, 10 μl/1ml whole blood).

Calcium Imaging. MIN6 beta cells (56) were plated at a density of 1×10^6 cells per 35-mm glass-bottom dish (MatTek) the day before the experiment. Islets were trypsinized and dispersed in MatTek dishes precoated with poly-L-lysine the day before experimentation. Dispersed islets were transfected (Lipofectamine 2000; Invitrogen) at the time of plating with a construct expressing the far red fluorescent protein Katushka (Evrogen) under the rat insulin promoter II to exclusively identify and image beta cells. Cells were loaded with 3 μM Fura-2 acetoxymethyl ester in KRH buffer containing 8.3 mM glucose for 20 min and then washed twice with KRH and incubated for an additional 30 min at 37 °C without CO_2. Dishes were placed into a heated chamber mounted on the stage of an inverted fluorescence microscope (Eclipse TiE with perfect focus and DG-5 Xenon excitation; Nikon) and perifused with KRH plus 8.3 mM glucose (unless otherwise stated) at a rate of 1.5 mL/min. Baseline was established for at least 6 min before stimulation, as shown in the figures. Fura-2 dual excitation images were captured through a Nikon S Fluor 20× objective (NA 0.75) with a Photometrics QuantEM 16-bit EMCCD camera using 340 nm and 380 nm excitation filters and a 470- to 550-nm emission filter. Data were acquired and analyzed using Nikon Elements software. The fluorescence intensities of several single primary beta cells or 20 to 40 MIN6 cells per dish/condition were background subtracted and expressed as ratio of excitation 340/380 nm.

TIRF Microscopy. MIN6 beta cells were transfected 24 h before the experiment with a $PH_{PLC\delta 1}$-GFP construct in which the PIP_2-binding pleckstrin homology domain of PLC-δ was fused to the GFP (provided by A. Tengholm, Uppsala University, Uppsala, Sweden) by using Lipofectamine 2000 reagent. Growth medium was removed and replaced with KRH buffer containing 8.3 mM glucose. Similar procedures to those listed earlier (*Calcium Imaging*) followed with the same microscope platform. Cell-surface associated $PH_{PLC\delta 1}$-GFP was excited with an evanescent wave created by a 488-nm argon laser line, and GFP emission was visualized using a 500- to 550-nm band-pass emission filter. Images were captured by using an Apo TIRF 60× oil immersion objective (NA 1.49; Nikon) using a Photometrics CoolSNAP HQ2 14-bit CCD camera. Data were acquired and analyzed using Nikon Elements software. The fluorescence intensities (F) of four to six cells were background (F_0)-subtracted and normalized to average base-line values (i.e., $\Delta F - F_0$).

In Vivo Experiments. All in vivo experiments were performed with 8- to 10-wk-old male mice on regular chow (no. 2016; Harlan-Teklad). Catheters were surgically implanted into the left common carotid artery and right jugular vein as described (57), except that anesthesia was induced (2%) and maintained (1–2%) with isoflurane. The arterial catheter was used to obtain blood samples in conscious, unrestrained mice. The venous catheter was used for infusions and bolus injections. After a 5-d recovery, mice were

morning-fasted for 5 h to achieve glycemic levels of approximately 160 mg/dL (9.0 mM), which corresponds with the glucose concentration (8.3 mM) used in the majority of the in vitro experiments. Three blood samples were obtained via the arterial catheter at times −10, −5, and 0 min before the end of the fast for baseline glucose and insulin measurements. At the end of the fast (time 0 min) a single bolus of fructose, ^{13}C-labeled fructose (U-$^{13}C6$ D-fructose; Cambridge Isotope Labs), or saccharin (all at 1.0 g/kg body weight) was administered via the venous catheter as explained in the figure legends. Blood samples were taken at time 2, 5, 10, 20, and 30 min for glucose and insulin measurements. This was followed by a 20-min washout period (time 30–50 min) during which a blood glucose measurement was taken at time 40 min. Three blood samples were acquired at times 50, 55, and 60 min for new baseline glucose and insulin measurements. A second bolus of saline control (100 μL) was given at time 60 min. Blood samples were taken at times 62, 65, 70, 80, and 90 min for glucose and insulin measurements. Saline-washed erythrocytes were infused throughout the study to prevent a decrease of more than 5% in hematocrit. The in vivo fructose potentiation experiments were carried out as described earlier by using a bolus of glucose (0.5 g/kg body weight), fructose (0.3 g/kg body weight), or a combination of the two. Blood glucose was measured using ACCU-CHEK Aviva (Roche), and no cross-reactivity with fructose was observed.

Statistical Analysis. All results are presented as mean ± SEM. The level of significance was set at $P < 0.05$. Statistical significance was calculated as shown in the figure legends.

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1. Torres N, Noriega L, Tovar AR (2009) Nutrient modulation of insulin secretion. *Vitam Horm* 80:217–244.
2. Liu Z, Jeppesen PB, Gregersen S, Chen X, Hermansen K (2008) Dose- and glucose-dependent effects of amino acids on insulin secretion from isolated mouse islets and clonal INS-1E beta-cells. *Rev Diabet Stud* 5:232–244.
3. Ahrén B (2009) Islet G protein-coupled receptors as potential targets for treatment of type 2 diabetes. *Nat Rev Drug Discov* 8:369–385.
4. Ashcroft SJ, Hedeskov CJ, Randle PJ (1970) Glucose metabolism in mouse pancreatic islets. *Biochem J* 118:143–154.
5. Capito K, Hedeskov CJ, Landt J, Thams P (1984) Pancreatic islet metabolism and redox state during stimulation of insulin secretion with glucose and fructose. *Acta Diabetol Lat* 21:365–374.
6. Giroix MH, et al. (1999) Metabolic and secretory interactions between D-glucose and D-fructose in islets from GK rats. *Endocrinology* 140:5556–5565.
7. Byard JL, Goldberg L (1973) The metabolism of saccharin in laboratory animals. *Food Cosmet Toxicol* 11:391–402.
8. Grant AM, Christie MR, Ashcroft SJ (1980) Insulin release from human pancreatic islets in vitro. *Diabetologia* 19:114–117.
9. Sener A, Malaisse WJ (1988) Hexose metabolism in pancreatic islets. Metabolic and secretory responses to D-fructose. *Arch Biochem Biophys* 261:16–26.
10. Zawalich WS, Rognstad R, Pagliara AS, Matschinsky FM (1977) A comparison of the utilization rates and hormone-releasing actions of glucose, mannose, and fructose in isolated pancreatic islets. *J Biol Chem* 252:8519–8523.
11. Bailey CJ, Day C, Knapper JM, Turner SL, Flatt PR (1997) Antihyperglycaemic effect of saccharin in diabetic ob/ob mice. *Br J Pharmacol* 120:74–78.
12. Malaisse WJ, Vanonderbergen A, Louchami K, Jijakli H, Malaisse-Lagae F (1998) Effects of artificial sweeteners on insulin release and cationic fluxes in rat pancreatic islets. *Cell Signal* 10:727–733.
13. Bachmanov AA, Beauchamp GK (2007) Taste receptor genes. *Annu Rev Nutr* 27:389–414.
14. Chandrashekar J, Hoon MA, Ryba NJ, Zuker CS (2006) The receptors and cells for mammalian taste. *Nature* 444:288–294.
15. Shah AS, Ben-Shahar Y, Moninger TO, Kline JN, Welsh MJ (2009) Motile cilia of human airway epithelia are chemosensory. *Science* 325:1131–1134.
16. Jang HJ, et al. (2007) Gut-expressed gustducin and taste receptors regulate secretion of glucagon-like peptide-1. *Proc Natl Acad Sci USA* 104:15069–15074.
17. Jeon TI, Zhu B, Larson JL, Osborne TF (2008) SREBP-2 regulates gut peptide secretion through intestinal bitter taste receptor signaling in mice. *J Clin Invest* 118:3693–3700.
18. Nakagawa Y, et al. (2009) Sweet taste receptor expressed in pancreatic beta-cells activates the calcium and cyclic AMP signaling systems and stimulates insulin secretion. *PLoS ONE* 4:e5106.
19. Bantle JP (2006) Is fructose the optimal low glycemic index sweetener? *Nestle Nutr Workshop Ser Clin Perform Programme* 11:83–91.
20. Tappy L, Lê KA (2010) Metabolic effects of fructose and the worldwide increase in obesity. *Physiol Rev* 90:23–46.
21. Jonkers FC, Guiot Y, Rahier J, Henquin JC (2001) Tolbutamide stimulation of pancreatic beta-cells involves both cell recruitment and increase in the individual Ca(2+) response. *Br J Pharmacol* 133:575–585.
22. Damak S, et al. (2003) Detection of sweet and umami taste in the absence of taste receptor T1r3. *Science* 301:850–853.
23. Zhao GQ, et al. (2003) The receptors for mammalian sweet and umami taste. *Cell* 115: 255–266.
24. Mayes PA (1993) Intermediary metabolism of fructose. *Am J Clin Nutr* 58(5 suppl): 754S–765S.
25. Sweatman TW, Renwick AG (1980) The tissue distribution and pharmacokinetics of saccharin in the rat. *Toxicol Appl Pharmacol* 55:18–31.
26. Byard JL, McChesney EW, Golberg L, Coulston F (1974) Excretion and metabolism of saccharin in man. II. Studies with 14-C-labelled and unlabelled saccharin. *Food Cosmet Toxicol* 12:175–184.
27. Bizeau ME, Pagliassotti MJ (2005) Hepatic adaptations to sucrose and fructose. *Metabolism* 54:1189–1201.
28. Sestoft L (1985) An evaluation of biochemical aspects of intravenous fructose, sorbitol and xylitol administration in man. *Acta Anaesthesiol Scand Suppl* 82:19–29.
29. Macdonald I, Turner LJ (1968) Serum-fructose levels after sucrose or its constituent monosaccharides. *Lancet* 1:841–843.
30. Topping DL, Mayes PA (1971) The concentration of fructose, glucose and lactate in the splanchnic blood vessels of rats absorbing fructose. *Nutr Metab* 13:331–338.
31. Thore S, Wuttke A, Tengholm A (2007) Rapid turnover of phosphatidylinositol-4,5-bisphosphate in insulin-secreting cells mediated by Ca2+ and the ATP-to-ADP ratio. *Diabetes* 56:818–826.
32. Axelrod D, Thompson NL, Burghardt TP (1983) Total internal inflection fluorescent microscopy. *J Microsc* 129:19–28.
33. Thore S, Dyachok O, Gylfe E, Tengholm A (2005) Feedback activation of phospholipase C via intracellular mobilization and store-operated influx of Ca2+ in insulin-secreting beta-cells. *J Cell Sci* 118:4463–4471.
34. Zhang Z, Zhao Z, Margolskee R, Liman E (2007) The transduction channel TRPM5 is gated by intracellular calcium in taste cells. *J Neurosci* 27:5777–5786.
35. Colsoul B, et al. (2010) Loss of high-frequency glucose-induced Ca2+ oscillations in pancreatic islets correlates with impaired glucose tolerance in Trpm5-/- mice. *Proc Natl Acad Sci USA* 107:5208–5213.
36. Jiang P, et al. (2005) Lactisole interacts with the transmembrane domains of human T1R3 to inhibit sweet taste. *J Biol Chem* 280:15238–15246.
37. Ashcroft SJ, Bassett JM, Randle PJ (1972) Insulin secretion mechanisms. *Diabetes* 21 (Suppl 2):538–544.
38. Lacy PE, Young DA, Fink CJ (1968) Studies on insulin secretion in vitro from isolated islets of the rat pancreas. *Endocrinology* 83:1155–1161.
39. Matschinsky FM, Landgraf R, Ellerman J, Kotler-Brajtburg J (1972) Glucoreceptor mechanisms in islets of Langerhans. *Diabetes* 21(2 suppl)555–569.
40. Curry DL, Curry KP, Gomez M (1972) Fructose potentiation of insulin secretion. *Endocrinology* 91:1493–1498.
41. Dunnigan MG, Ford JA (1975) The insulin response to intravenous fructose in relation to blood glucose levels. *J Clin Endocrinol Metab* 40:629–635.
42. Lawrence JR, et al. (1980) The insulin response to intravenous fructose in maturity-onset diabetes mellitus and in normal subjects. *Diabetes* 29:736–741.
43. Miwa I, Taniguchi S (2002) Acceleration by fructose of the ATP-sensitive K(+) channel-independent pathway of glucose-induced insulin secretion. *Horm Metab Res* 34:450–454.
44. Nelson G, et al. (2001) Mammalian sweet taste receptors. *Cell* 106:381–390.
45. Vigues S, Dotson CD, Munger SD (2009) The receptor basis of sweet taste in mammals. *Results Probl Cell Differ* 47:187–202.
46. Li X, et al. (2002) Human receptors for sweet and umami taste. *Proc Natl Acad Sci USA* 99:4692–4696.
47. Galindo-Cuspinera V, Winnig M, Bufe B, Meyerhof W, Breslin PA (2006) A TAS1R receptor-based explanation of sweet 'water-taste'. *Nature* 441:354–357.
48. Zhang Y, et al. (2003) Coding of sweet, bitter, and umami tastes: Different receptor cells sharing similar signaling pathways. *Cell* 112:293–301.
49. Margolskee RF (2002) Molecular mechanisms of bitter and sweet taste transduction. *J Biol Chem* 277:1–4.
50. Mace OJ, Affleck J, Patel N, Kellett GL (2007) Sweet taste receptors in rat small intestine stimulate glucose absorption through apical GLUT2. *J Physiol* 582:379–392.
51. Margolskee RF, et al. (2007) T1R3 and gustducin in gut sense sugars to regulate expression of Na+-glucose cotransporter 1. *Proc Natl Acad Sci USA* 104:15075–15080.
52. Anton SD, et al. (2010) Effects of stevia, aspartame, and sucrose on food intake, satiety, and postprandial glucose and insulin levels. *Appetite* 55:37–43.
53. Bellisle F, Drewnowski A (2007) Intense sweeteners, energy intake and the control of body weight. *Eur J Clin Nutr* 61:691–700.
54. Crapo PA, Reaven G, Olefsky J (1976) Plasma glucose and insulin responses to orally administered simple and complex carbohydrates. *Diabetes* 25:741–747.
55. Ma J, et al. (2009) Effect of the artificial sweetener, sucralose, on gastric emptying and incretin hormone release in healthy subjects. *Am J Physiol Gastrointest Liver Physiol* 296:G735–G739.
56. Miyazaki J-I, et al. (1990) Establishment of a pancreatic beta cell line that retains glucose-inducible insulin secretion: Special reference to expression of glucose transporter isoforms. *Endocrinology* 127:126–132.
57. Ayala JE, Bracy DP, McGuinness OP, Wasserman DH (2006) Considerations in the design of hyperinsulinemic-euglycemic clamps in the conscious mouse. *Diabetes* 55:390–397.