The Wnt Signaling Pathway Effector TCF7L2 Controls Gut and Brain Proglucagon Gene Expression and Glucose Homeostasis

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The type 2 diabetes risk gene TCF7L2 is the effector of the Wnt signaling pathway. We found previously that in gut endocrine L-cell lines, TCF7L2 controls transcription of the proglucagon gene (*gcg*), which encodes the incretin hormone glucagon-like peptide-1 (GLP-1). Whereas peripheral GLP-1 stimulates insulin secretion, brain GLP-1 controls energy homeostasis through yet-to-be defined mechanisms. We aim to determine the metabolic effect of a functional knockdown of TCF7L2 by generating transgenic mice that express dominant-negative TCF7L2 (TCF7L2DN) specifically in gcg-expressing cells. The *gcg*-TCF7L2DN transgenic mice showed reduced gcg expression in their gut and brain, but not in pancreas. Defects in glucose homeostasis were observed in these mice, associated with attenuated plasma insulin levels in response to glucose challenge. The defect in glucose disposal was exacerbated with high-fat diet. Brain Wnt activity and feeding-mediated hypothalamic AMP-activated protein kinase (AMPK) repression in these mice were impaired. Peripheral injection of the cAMP-promoting agent forskolin increased brain β-cat Ser675 phosphorylation and brain gcg expression and restored feeding-mediated hypothalamic AMPK repression. We conclude that TCF7L2 and Wnt signaling control gut and brain gcg expression and glucose homeostasis and speculate that positive cross-talk between Wnt and GLP-1/cAMP signaling is an underlying mechanism for brain GLP-1 in exerting its metabolic functions.
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Extensive genome-wide association studies have revealed that specific single-nucleotide polymorphisms (SNPs) in *TCF7L2* are strongly associated with the susceptibility of type 2 diabetes (1). Because these risk SNPs are located within the intronic regions of *TCF7L2*, great effort has been made to assess whether these SNPs affect *TCF7L2* transcription or its alternative splicing (2–6). The genome-wide association studies finding has also redirected our attention to the role of Wnt signaling and its downstream effectors, including TCF7L2 and β-catenin (β-cat), in controlling hormone–gene expression and glucose disposal (7).

The major effector of Wnt signaling is β-cat/TCF, formed by free β-cat and a member of the TCF family, including TCF7, LEF-1, TCF7L1, and TCF7L2 [TCF-4] (8). *TCF7L2*$^{-/-}$ mice die soon after birth, associated with the lack of proliferative compartments in gut prospective crypt regions (8). Early investigations did not reveal expression or function of TCF7L2 in the mouse pancreas (8–10), in contradiction with recent observations for the potential role of TCF7L2 in pancreatic β cells (11–14). Furthermore, complicated observations were also made of the contribution of Wnt signaling in pancreatic islet development (15,16). For example, Murtaugh et al. (15) found that the loss of β-cat does not significantly perturb islet endocrine cell mass or function, although β-cat is crucial for pancreatic acinar cell lineage specification and differentiation. Furthermore, Krutzfeldt and Stoffel (17) demonstrated that Wnt signaling is not appreciably active in the adult mouse pancreas. In addition, an early transgenic mouse study suggested that although the attenuation of Wnt signaling perturbed pancreatic growth, it did not affect islet cell function (18). A number of other studies, however, have shown that Wnt signaling and TCF7L2 are involved in the function of mouse or human pancreatic β cells (11–14,16,19).

Extensive investigations also have shown that the bipartite transcription factor β-cat/TCF functions as the effector of cAMP-dependent protein kinase A (PKA) signaling, and hence mediates the effect of peptide hormones, including GLP-1, which utilizes cAMP as the second messenger (12,20). Cross-talk between Wnt and other signaling pathways, as well as the pathophysiological significance of the cross-talk, has been recognized in recent years (21–23).

The proglucagon gene (*gcg*) encodes both glucagon and GLP-1, among other peptide hormones; *gcg* is expressed in pancreatic α cells, gut endocrine L cells, and certain brainstem neurons, especially in the nucleus of solitary tract. In pancreatic α cells, *gcg* expression leads to the production of glucagon, whereas in the gut and brain *gcg* expression leads to GLP-1 production. The biological activities of GLP-1 include the stimulation of insulin secretion, inhibition of glucagon secretion, and attenuation of gastric emptying. Brain GLP-1 mediates nutritional and other signals in attenuating food intake and glucose homeostasis (24,25), but the underlying mechanism is elusive. A recent study demonstrated that brain GLP-1 signaling represses AMPK activity, involving PKA and mitogen-activated protein kinase activation (MAPK) (26).

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Previously, we found that the Wnt pathway activator lithium can stimulate *gcg* transcription in the gut endocrine L cells (27). The stimulation of *gcg* transcription in endocrine L cells by lithium or cAMP is at least partially mediated by increasing the binding of β-cat/TCF7L2 to the G2 enhancer element of *gcg* promoter (27–29). The role of Wnt signaling in brain GLP-1 expression and metabolic homeostasis is unknown. Because *TCF7L2*$^{-/-}$ mice die soon after their birth (8), precluding a more thorough investigation of the mature phenotype, we used a specific functional knockdown of TCF7L2 in *gcg*-expressing cells only, aiming to assess the in vivo role of TCF7L2 in gcg expression and the resulting contribution to glucose homeostasis.

RESEARCH DESIGN AND METHODS

Transgenic mice and cell cultures. The *gcg*-TCF7L2DN fusion gene construct was generated by replacing the luciferase (LUC) reporter in the 2.3 kb Glu-LUC (30) with the human dominant-negative TCF7L2 (TCF7L2DN), provided by Eric Fearon (31). Transgenic mice (in FVB background) were housed under controlled temperature and a 12-h light/12-h dark cycle with free access to standard chow diet and water, except when noted. All animal procedures were approved by the animal care committee of the University Health Network. Mouse intestinal gcg-expressing GLUTag and brain gcg-expressing mHypoE-20/2 cell lines have been reported in our previous studies (32,33). Mouse primary neurons were isolated using the method described by Weinstein (34). The method for LUC reporter analysis was described previously (30).

Metabolic studies. Male littermates were used in each of the experiments. Blood glucose levels were monitored using tail-vein blood (35). Plasma total and active GLP-1 levels were determined with enzyme-linked immunosorbent assays kits from Meso Scale Discovery (Gaithersburg, Maryland), whereas plasma glucagon and insulin levels were determined with the RIA kits from Millipore (Billerica, MA) (35). Oral glucose tolerance test, intraperitoneal glucose tolerance test (IPGTT), and insulin tolerance tests were conducted by traditional methods (35,36).

Forskolin injection. Mice were injected with 5 mg/kg forskolin intraperitoneally (IP). Four hours after the injection, animals were killed and indicated organs were taken for Western blotting or RNA extraction. Alternatively, mice were injected with 2 mg/kg forskolin per day for 5 days (1:00 PM each day). After an overnight fast, mice were refed for 15 min (without refeeding as controls). The mice were then killed, with indicated tissue taken for Western blotting.

Immunohistochemistry and β-cell mass analysis. Methods for glucagon, GLP-1, and insulin staining, and β-cell mass analysis, have been described previously (35).

Quantitative RT-PCR analysis. Mouse total RNA from indicated tissues was extracted using the Trizol reagent. Quantitative RT-PCR was conducted as previously described (29). The RT-PCR primers for TCF family members are summarized in Table 1.

Western blotting. Antibodies against protein kinase B (PKB)/Akt, phosphorylated PKB (Ser473), Ser675 β-cat, β-actin, AMPK, pAMPK, and TCF7L2 were obtained from Cell Signaling Technology (Beverly, MA). β-cat antibody was the product of Santa Cruz Biotechnology (Santa Cruz, CA). Methods for tissue protein extraction and Western blotting have been described previously (37).

Statistical analysis. Data are expressed as the mean ± SEM. Statistical significance was determined using unpaired two-tailed Student *t* test or ANOVA.

TABLE 1
Primers used in detecting TCF mRNA expression in the brain and liver by RT-PCR

Primer pair	DNA sequence	PCR product size (bp)
TCF7	F: 5′-AGG TCA GAT GGG TTG GAC TG-3′ R: 5′-AGG GTG CAC ACT GGG TTT AG-3′	412
LEF1	F: 5′-CTC ATC ACC TAC AGC GAC GA-3′ R: 5′-TGA GGC TTC ACG TGC ATT AG-3′	386
TCF7L1	F: 5′-GAG TGC GAA ATC CCC AGT TA-3′ R: 5′-ATG CAT GGC TTC TTG CTC TT-3′	384
TCF7L2	F: 5′-CAG CAA GGT CAG CCT GTG TA-3′ R: 5′-CAC CAC CTT CGC TCT CAT CT-3′	321
β-actin	F: 5′-TCA TGA AGT GTG ACG TTG ACA-3′ R: 5′-CCT AGA AGC ATT TGC GGT G-3′	285

RESULTS

Reduced intestinal gcg and brain gcg expression in *gcg*-hTCF7L2DN transgenic mice. The *Not*I/*Eco*RI fragment containing hTCF7L2DN (31) was inserted into the 2.3-kb *gcg*-LUC plasmid (30), replacing the LUC coding sequence. As shown in Fig. 1*A*, hTCF7L2DN lacks the β-cat interaction domain. It functions as a dominant-negative molecule to block, in theory, any functional TCF7L2 isoforms that use β-cat as the partner. We showed previously that hTCF7L2DN repressed basal *gcg* mRNA expression in the gut GLUTag cells and blocked the stimulatory effect of lithium on *gcg* expression in this cell line (28). This *gcg* promoter construct is known to drive reporter gene expression in vivo in the gut, brain, and pancreatic gcg-expressing cells only (38). This fusion gene was used in generating *gcg*-TCF7L2DN transgenic mice. We obtained eight transgenic founders, which express the transgene hTCF7L2DN in their pancreas, gut, and brain (Supplementary Fig. 1*A*–*C*). The transgene was not detected in other organs in the T3 and T4 founders that we have tested (Supplementary Fig. 1*D* and *E*). We then examined male mice from five founders, showing a 40–78% reduction of gcg mRNA expression in their gut and a 50–78% reduction in their brain (Fig. 1*B* and *C*). The gcg mRNA levels in their pancreas, however, were not reduced (Fig. 1*D*). Figure 1*E* is a representative Northern blot, showing the reduction of gut gcg levels in T4 and T8 transgenic mice. The transgenic mice displayed normal islet architecture and pancreatic α cells (data not shown).

Reduced GLP-1–positive cells in the gut. Figure 1*F* shows a representative gut GLP-1 immunostaining in the T4 mouse, along with a sex-matched and age-matched wild-type littermate. We then quantitatively analyzed GLP-1–positive cells in the entire 5-cm distal ileum region, showing that the transgenic mice had reduced gut GLP-1–positive cell numbers by 58%, compared with the wild-type littermates (Fig. 1*G*). Reduced gut GLP-1–positive cells also were observed for the T8 and T3 mice (Fig. 1*H* and *I*). These observations suggest that TCF7L2 is important for gut *gcg* expression and GLP-1 production in vivo (28).

The hTCF7L2DN mice showed impaired glucose disposal on chow diet, which was further exacerbated with high-fat diet feeding. We then assessed the metabolic profiles of the transgenic mice, mainly using the T3 mice and their littermates. The T3 mice showed a moderate but significant elevation in fed plasma glucose levels (Fig. 2*A*). Feeding led to a significant increase in insulin levels in the wild-type animals, but not in the T3 transgenic mice (Fig. 2*B*). No significant difference in plasma glucagon levels was observed, either with fasting or after feeding (data not shown). We then conducted glucose gavage in the T3 mice and measured plasma GLP-1 levels before and after glucose gavage. As shown in Fig. 2*C*, the GLP-1 levels in littermate controls increased 5 min after glucose gavage, whereas in the T3 transgenic mice no such increase was observed. We also have assessed active GLP-1 levels before and after glucose gavage. Glucose gavage did not increase active GLP-1 levels in either the littermate controls

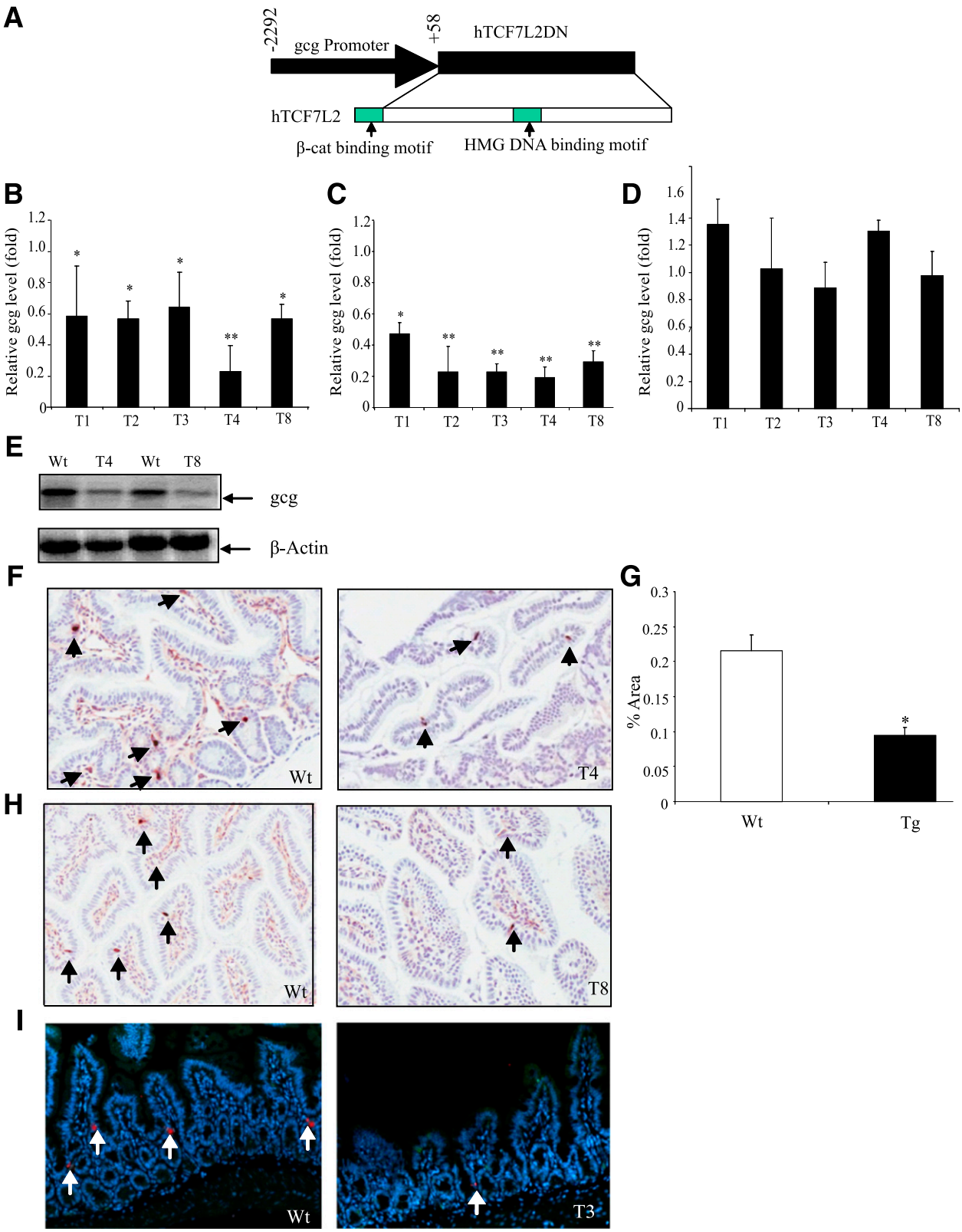

FIG. 1. Reduced intestinal and brain *gcg* expression in *gcg*-hTCF7L2DN transgenic mice. *A*: A schematic representation of the *gcg*-hTCF7L2DN transgene. The lack of the β-cat binding motif makes it function as a dominant-negative molecule (31). Quantitative RT-PCR shows reduced *gcg* levels in the gut (*B*) and brain (*C*), but not in the pancreas (*D*) in five founders of the transgenic mice (T1/Wt, *n* = 3/3; T2/Wt, *n* = 4/4; T3/Wt, *n* = 3/3; T4/Wt, *n* = 3/3; and T8/Wt, *n* = 3/3). This study assessed male mice only. **P* < 0.05; ***P* < 0.01. *E*: A representative Northern blot shows reduced *gcg* mRNA levels in the distal ileum of T4 and T8 mice. *F*: Representative immunostaining shows reduced numbers of GLP-1–producing cells in the distal ileum of T4 mice. *G*: Percentage area of GLP-1–positive staining was calculated with the MacBiophotonics ImageJ program after the Aperio image scan of entire slide containing 5 cm of distal ileum. Representative immunostaining results show reduced GLP-1–positive cells in the distal ileum of T8 (*H*) and T3 (*I*) transgenic mice. Wt, wild type. (A high-quality digital representation of this figure is available in the online issue.)

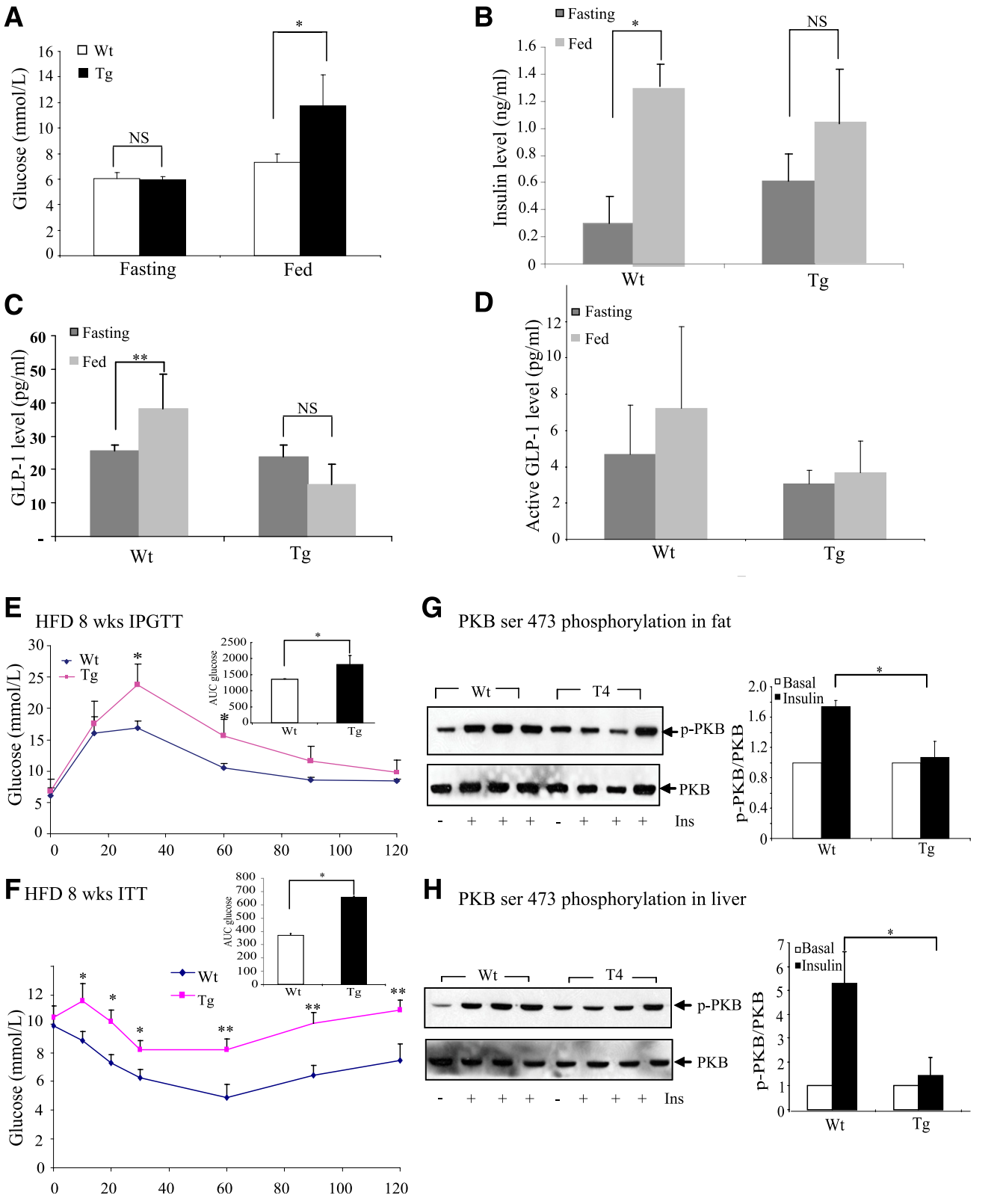

FIG. 2. The *gcg*-hTCF7L2DN transgenic mice show impaired glucose disposal. *A*: T3 and wild-type littermates were fed chow diet for 10 weeks. Blood glucose levels were determined in fasting and after feeding (n = 7 for both types). Similar observations were made for the T4 transgenic mice. *B*: The T3 mice show an attenuated insulin secretion in response to feeding and a trend of elevated fasting plasma insulin levels (n = 4 for both groups of mice, similar results were obtained for T4 transgenic mice and littermate controls). Plasma total (*C*) and active (*D*) GLP-1 levels were determined with the Mesoscale ELISA kits (n = 4 for both the T3 mice and the control group, similar results were obtained for the T4 mice and controls). *E*: A representative IPGTT result for T4 and their wild-type littermates fed with high-fat diet for 8 weeks (n = 4 for each group). *F*: A representative insulin tolerance test result for T4 (n = 5) vs. wild-type littermates (n = 5) 12 weeks after high-fat diet feeding. Western blotting shows impaired responses to IP insulin injection (1 units/kg body weight) in PKB Ser473 phosphorylation in fat tissue (*G*) and liver (*H*) in T4 mice after 12 weeks of high-fat diet feeding. Animals were killed 30 min after IP insulin injection. Representative blots for two independent assessments. *P < 0.05; **P < 0.01. Tg, transgenic; Wt, wild type. (A high-quality color representation of this figure is available in the online issue.)

or the T3 transgenic mice, although a trend of lower active GLP-1 levels was seen in these transgenic mice (Fig. 2*D*).

No statistical difference in glucose disposal was observed in the T3 or T4 transgenic lines after an IPGTT (data not shown). Furthermore, in liver and fat tissue, only a moderate defect in response to IP insulin injection on PKB Ser473 phosphorylation was observed (data not shown).

We then challenged the transgenic mice with high-fat diet. Compared with the wild-type controls, the T4 mice showed a more severely impaired glucose disposal at 8 weeks, as assessed by IPGTT (Fig. 2*E*) or oral glucose tolerance test (Supplementary Fig. 2). Similar IPGTT observations were made on the T3 and T8 mice (Supplementary Fig. 3). After 8 weeks of high-fat diet feeding, the T4 transgenic mice exhibited a marked increase in β-cell mass as a compensatory response (Supplementary Fig. 4). These mice also showed impaired insulin sensitivity, which was assessed by insulin tolerance tests (Fig. 2*F*), as well as PKB Ser473 phosphorylation in response to IP insulin injection in fat tissue and liver (Fig. 2*G* and *H*).

TCF7L2 controls brain gcg expression. Because brain gcg mRNA levels also were significantly reduced in the TCF7L2DN transgenic mice (Fig. 1*C*), we further assessed whether TCF7L2 regulates brain *gcg* expression. First, we found by coimmunostaining that in the brainstem, GLP-1–producing cells express TCF7L2 (Fig. 3*A*). We then detected the expression of TCF7L2 and two other TCF members by RT-PCR in brain tissue (Fig. 3*B*). To verify that TCF7L2 controls brain *gcg* expression, we did a battery of tests in a mouse clonal *gcg*-expressing cell line mHypoE-20/2 (33). As shown in Fig. 3*C*, the hypothalamic neuronal cell line expresses GLP-1, as determined by immunostaining. We have determined previously that in the intestinal GLUTag cell line either forskolin or lithium stimulates *gcg* promoter transcription via enhancing the binding of β-cat/TCF7L2 to the G2 enhancer element (28). Here, we found that in mHypoE-20/2 cells, both forskolin and lithium stimulated the expression of G2S-LUC (Fig. 3*D*), a LUC fusion gene construct that contains the G2 enhancer element (28). In addition, we conducted TCF7L2 knockdown with two mouse-specific small interfering RNA (siRNA) (Supplementary Fig. 5). TCF7L2 knockdown in mHypoE-20/2 cells (Fig. 3*E*) led to reduced gcg mRNA expression, determined by semiquantitative RT-PCR (Fig. 3*F*). Furthermore, we found that in mHypoE-20/2 cells, forskolin treatment for 5 min stimulated β-cat S675 phosphorylation (Fig. 3*G*), an event that is positively associated with increased β-cat/TCF transcriptional activity (37,39). We also have tested the effect of hTCF7L2DN transient transfection in gut GLUTag and brain mHypoE-20/2 cells with the doxycycline inducible system. For this purpose, hTCF7L2 is coexpressed with the mCherry reporter (a red-colored fluorophore). As shown in Fig. 3*H* and *I*, cells expressing hTCF7L2DN did not produce the GLP-1 peptide. These observations suggest that TCF7L2 controls *gcg* expression and GLP-1 production in both gut and brain *gcg*-expressing cells.

Existence of cross-talk between Wnt and GLP-1/cAMP signaling in the brain. Because brain *gcg* expression is also controlled by the Wnt signaling pathway and TCF7L2, we hypothesized that the reduction of brain *gcg* in the TCF7L2DN mice may be partially responsible for the observed defects in glucose disposal. To initiate an examination of this, we used a cDNA microarray to assess the expression of Wnt-related genes in male adult T4 mice and sex-matched and age-matched wild-type littermates. Among 84 Wnt-related genes we have assessed, 26 showed a reduction of at least 1.4-fold, including known Wnt pathway downstream target genes (Supplementary Table 1).

The *gcg*-expressing cells account for a modest proportion of brainstem neurons. How can TCF7L2 knockdown in these cells affect overall brain Wnt activity? We propose that GLP-1, as a downstream target of Wnt signaling, serves as a positive feedback component to stimulate brain neuronal Wnt activity, similar to the action of peripheral GLP-1 on pancreatic β cells, which is mediated by the GLP-1/cAMP/PKA signaling, involving β-cat Ser675 phosphorylation (12). To examine this hypothesis, we injected wild-type FVB mice with forskolin IP. Gut *gcg* mRNA levels were increased nearly four-fold 4 h after forskolin injection (Fig. 4*A*), whereas the levels of Ser133 cAMP-responsive element-binding protein (CREB) in the hypothalamus also were increased significantly (Fig. 4*B*). Furthermore, we found that IP injection of either forskolin or lithium chloride (which mimics Wnt ligand activation) increased *gcg* mRNA level in the brainstem (Fig. 4*C*). These observations indicate that we can use IP forskolin injection to activate both brain cAMP/PKA/CREB signaling and brain *gcg* expression. Brain hypothalamic β-cat Ser675 phosphorylation levels were not substantially increased 4 h after forskolin IP injection (data not shown). We speculate that the in vivo β-cat S675 phosphorylation in response to acute forskolin injection is a temporary event. We then conducted an in vitro assay. Brain hypothalamic neurons obtained from a T4 mouse or an age-matched wild-type littermate were treated with forskolin for 0 to 60 min. As shown in Fig. 4*D*, forskolin substantially increased β-cat S675 phosphorylation in both mice. Furthermore, Exendin-4 treatment also stimulated CREB Ser133 phosphorylation and β-cat Ser675 phosphorylation in brain hypothalamic neurons (Fig. 4*E*).

We also asked whether consecutive forskolin injections could correct brain Wnt activity in these transgenic mice. T4 mice and littermate controls were injected with forskolin IP for 5 consecutive days. The hypothalamic neurons were then collected for analysis of β-cat S675 phosphorylation and Wnt target expression. We found that consecutive forskolin injections elevated Ser675 β-cat levels in the transgenic mice to a level that was comparable with that of the wild-type mice (Fig. 5*A*). This elevation was accompanied by increased expression of c-Myc and cyclin D1, two known downstream targets of the Wnt signaling pathway (Fig. 5*B*). Thus, cAMP/PKA/CREB activation in the brain in response to peripheral forskolin injection at least partially restored the Wnt activity in these transgenic mice.

Forskolin injection restored feeding-mediated hypothalamic AMPK repression. Brain GLP-1 is mainly produced by brainstem neurons, whereas GLP-1 receptors are expressed in regions that include the paraventricular nucleus and arcuate nucleus of the hypothalamus (40). A recent study suggested that brain GLP-1 exerts its anorectic effect via inhibiting AMPK in the brainstem (26). We also have learned that AMPK regulates food intake by responding to hormonal and nutrient signals in the hypothalamus (41). After fasting, active AMPK (pAMPK) level in the hypothalamus is high, whereas refeeding leads to GLP-1 secretion and reduced hypothalamic pAMPK. We compared hypothalamic pAMPK levels in the T4 transgenic mice and the control littermates in both the fasting and refeeding states, which were achieved by starving the mice overnight,

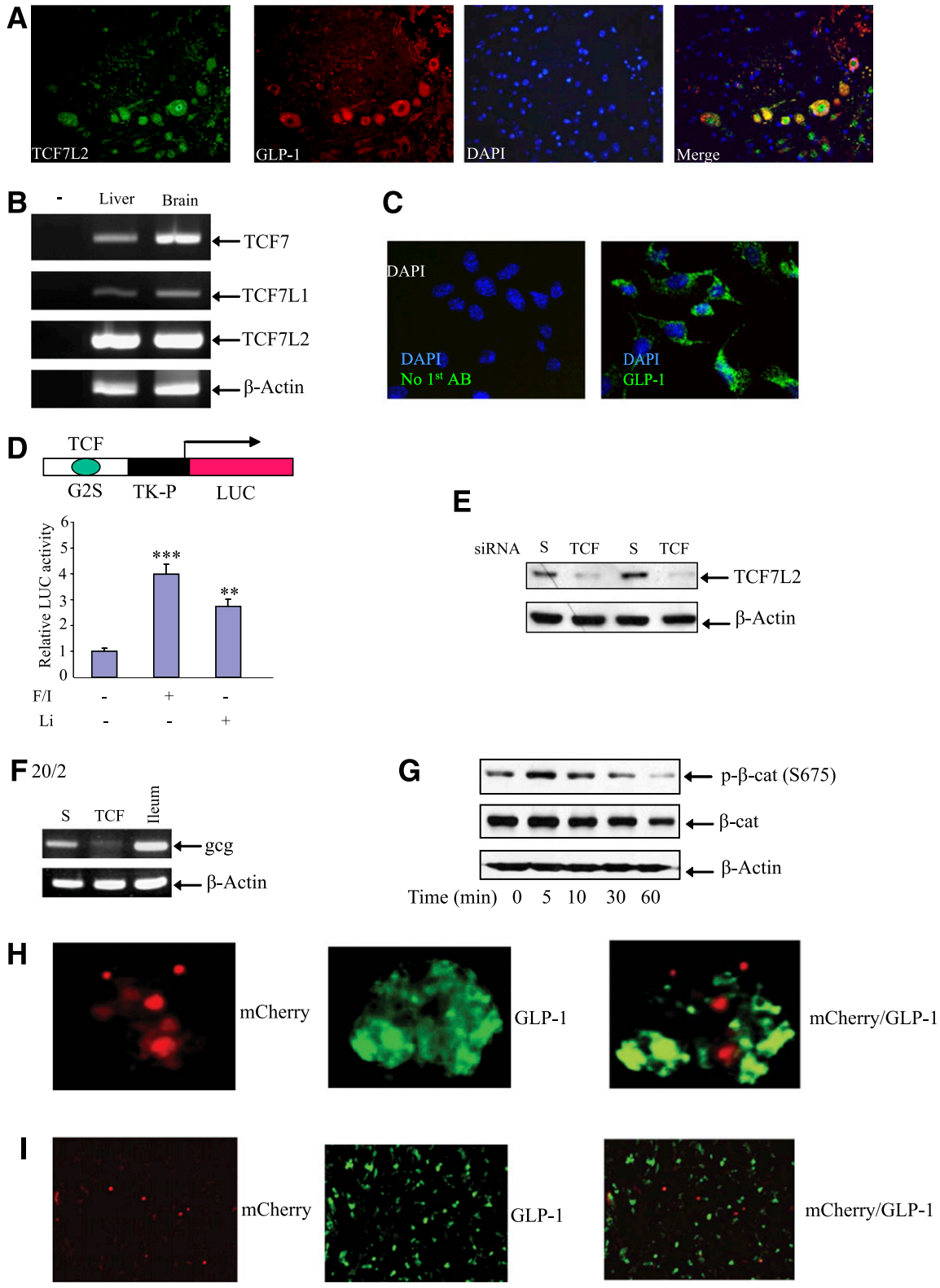

FIG. 3. TCF7L2 controls *gcg* expression in the mouse neuronal cell line mHypoE-20/2. *A*: Immunostaining shows the coexpression of TCF7L2 and GLP-1 in the brainstem of an 8-week-old male FVB mouse. *B*: RT-PCR shows the detection of TCF7, TCF7L1, and TCF7L2 mRNAs in the mouse brain (liver tissue is a control; primer sequences are shown in Table 1). *C*: Immunostaining shows GLP-1 expression in the mouse brain mHypoE-20/2 cell line (20/2). *D*: G2S-LUC expression was stimulated by 4-h lithium (Li; 10 mmol/L) or forskolin and IBMX (F/I; 10 μmol/L each) treatment in mHypoE-20/2 cells. *E*: Western blotting shows that TCF7L2 siRNA (TCF) but not the scrambled siRNA (S) blocked TCF7L2 expression in mHypoE-20/2 cells (nucleotide sequences of the siRNA are shown in Supplementary Fig. 5). *F*: Knockdown of TCF7L2 led to a reduced *gcg* mRNA level (representative RT-PCR result). A mouse distal Ileum (ileum) sample serves as the control for RT-PCR. *G*: Forskolin and IBMX (10 μmol/L each) treatment shows a temporary stimulation of β-cat S675 phosphorylation in the mHypoE-20/2 cell line. Expressing TCF7L2DN (tagged with mCherry, red) blocked GLP-1 production in gut GLUTag (*H*) and brain mHypoE-20/2 (20/2) (*I*) cell lines. The two cells lines were transfected with TCF7L2DN and Tet3G for 24 h, followed by doxycycline (5 ng/mL) treatment for another 24 h. Immunostaining shows that cells express TCF7L2DN (red) do not express GLP-1 (green). ***P* < 0.01; ****P* < 0.001. (A high-quality digital representation of this figure is available in the online issue.)

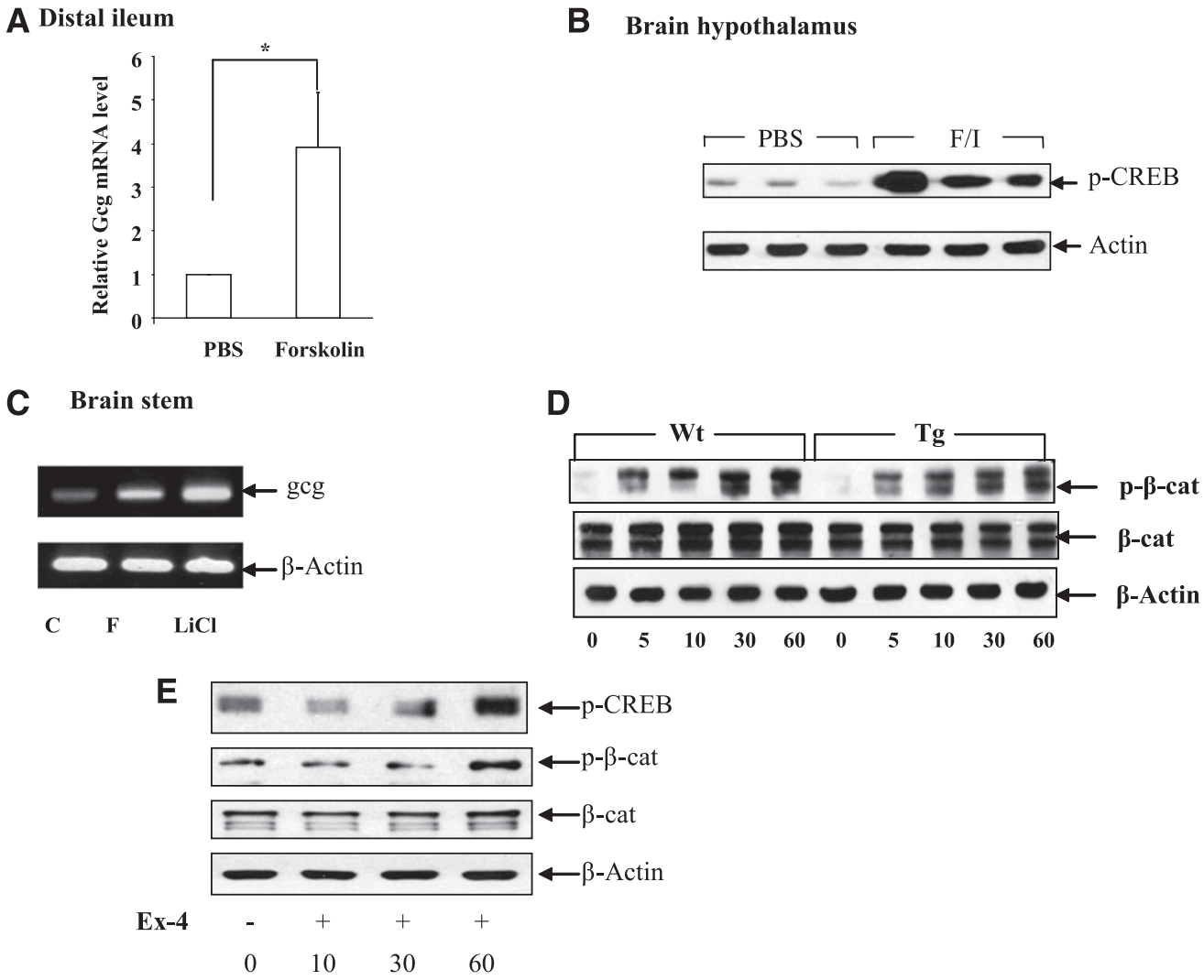

FIG. 4. cAMP elevation increases brain hypothalamic neuron β-cat Ser675 phosphorylation. Peripheral forskolin injection (F/I) (5 mg/kg) increased gut *gcg* mRNA expression (*A*) and CREB phosphorylation (Ser133) in hypothalamic neurons (*B*). *C*: Peripheral forskolin or lithium injection increased brainstem *gcg* mRNA levels (a representative RT-PCR, $n = 3$ for each group). C, control; LiCl, lithium chloride; F, forskolin. *D*: Forskolin stimulated brain β-cat Ser675 phosphorylation. A T4 transgenic mouse and a wild-type littermate (male, at age of 12 weeks) were killed. Brain hypothalamus tissues were taken for making primary cultures. The cells were treated with 10 μmol/L forskolin and 10 μmol/L IBMX for indicated times before being harvested for Western blotting, with indicated antibody. *E*: Exendin-4 (Ex-4; 20 nmol/L) treatment increased CREB and β-cat phosphorylation in the brain hypothalamic neurons. *$P < 0.05$. Tg, transgenic; Wt, wild type.

followed by a 15-min refeeding. The hypothalamus from each of the mice with or without refeeding was isolated, followed by Western blotting for pAMPK. As shown in Fig. 6*A* and *B*, a significant repression of hypothalamic pAMPK levels was observed on refeeding in the wild-type animals, but not in the T4 transgenic mice (Fig. 6*A* and *C*). Consecutive forskolin injections for 5 days, however, restored refeeding-mediated pAMPK repression in the transgenic mice (Fig. 6*A* and *D*). Finally, we tested the direct effect of cAMP signaling on AMPK activity in vitro. As shown in Fig. 6*E*, 1 or 10 μmol/L forskolin substantially repressed AMPK activity in the hypothalamic *gcg*-expressing mHypoE-20/2 cell line.

DISCUSSION

We demonstrated that the functional knockdown of TCF7L2 in *gcg*-expressing cells leads to reduced *gcg* expression in the gut and brain, but not in the pancreas. Although the transgenic mice showed increased fed plasma glucose levels and attenuated insulin secretion in response to feeding, no further abnormality on glucose homeostasis was observed when these mice were fed a chow diet. When the mice were challenged with a high-fat diet, impaired glucose disposal and insulin sensitivity worsened, revealed by IPGTT and insulin tolerance test, associated with a marked increase in β-cell mass for compensation. We suggest that TCF7L2DN expression only attenuated, but did not block, the Wnt signaling pathway, whereas the central and peripheral GLP-1 incretin system were capable of overcoming the moderate attenuation of Wnt signaling without a high-fat–diet challenge.

Soon after the discovery of the strong association between certain TCF7L2 SNPs and the risk of type 2 diabetes (1), Schäfer et al. (42) found that carriers of the type 2 diabetes susceptible TCF7L2 SNPs have impaired GLP-1–induced insulin secretion. Plasma GLP-1 levels in those carriers were not different from the control group. Extensive investigations hence have been focused on the

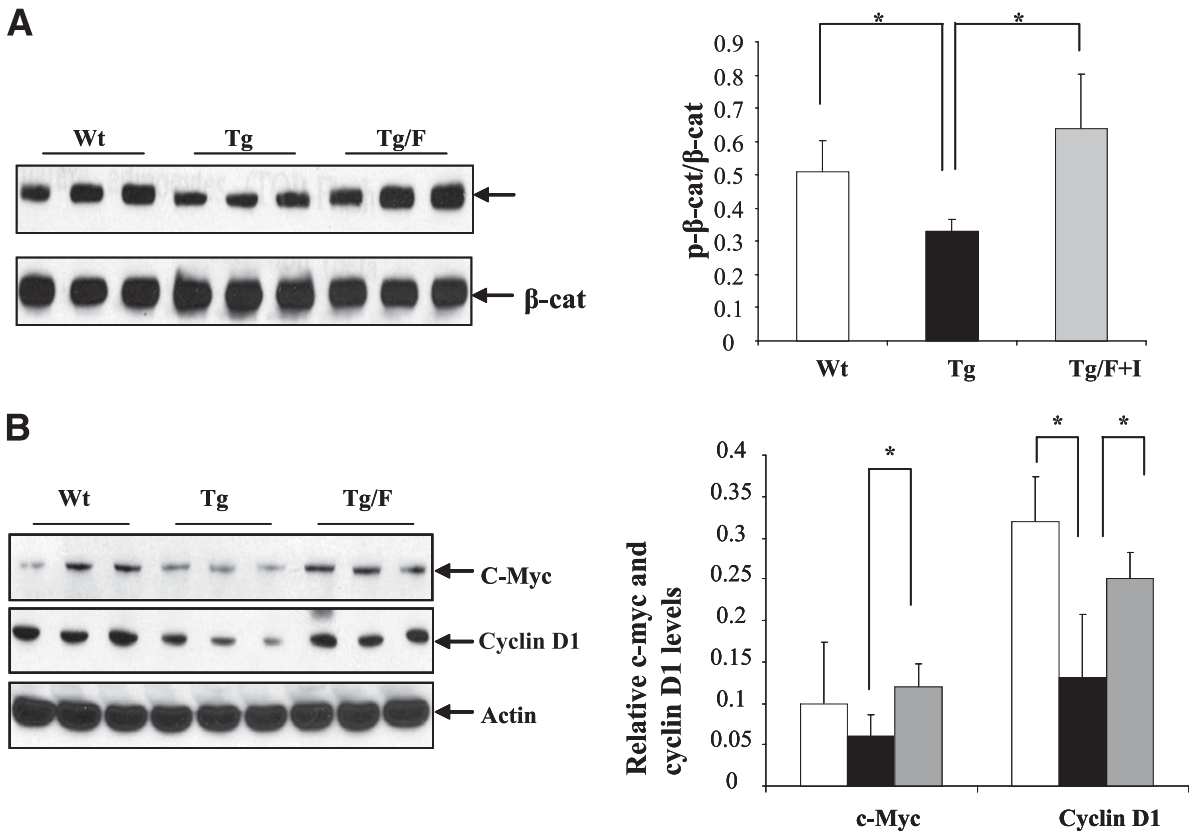

FIG. 5. Consecutive IP forskolin injection in *gcg*-TCF7L2DN mice increased hypothalamic β-cat S675 phosphorylation, associated with increased c-Myc and cyclin D1 levels. T4 transgenic mice were IP injected with forskolin (2 mg/kg, 5 days) for 5 days (at 1:00 PM each day), followed by taking the hypothalamic neurons for Western blotting against Ser675 β-cat (*A*) or c-Myc and cyclin D1 (*B*). *$P < 0.05$.

potential role of TCF7L2 in pancreatic β cells (11–13). It should be pointed out that although GLP-1 stimulates insulin secretion and that exendin-4 or Liraglutide have been used in diabetes treatment, whether plasma GLP-1 levels decline in type 2 diabetic patients is controversial (43,44). Although its level may decline in a portion of diabetic subjects, those individuals are usually not at an early stage of the disease. Plasma GLP-1 levels are not a diagnostic tool for diabetes. In the insulin-resistant MKR mouse model, although nutrient-stimulated GLP-1 secretion was lower than in the control mice, basal plasma GLP-1 levels were higher than levels in the wild-type littermates, making the postprandial plasma GLP-1 levels in the two groups of mice comparable (45). We show here that although the *gcg*-TCF7L2DN transgenic mice lose 40–78% of gut *gcg* mRNA expression and 58% of the gut GLP-1-producing cells, they were still able to release an amount of GLP-1 that is comparable with that of wild-type controls at the basal stage in the absence of glucose gavage. These observations suggest that mammals possess a strong compensatory capacity to maintain plasma GLP-1 at necessary levels. Considering that these transgenic mice did show an attenuated response to glucose on insulin secretion, we cannot eliminate the possibility that current GLP-1 detection methods are not sensitive enough for revealing the subtle differences in the transgenic mice versus the littermate controls. Plasma GLP-1 levels are within the picogram range (5–20 pg/mL), which are much lower compared with that of insulin or glucagon.

As discussed by Krutzfeldt and Stoffel (17), loss of function experiments in mouse models have uncovered conflicting results on the role of Wnt signaling in the pancreas (15,16,18,46). Murtaugh et al. (15) found that the loss of β-cat in transgenic mice did not significantly perturb islet endocrine cell mass or function, although β-cat is essential for pancreatic acinar cell development. Papadopoulou and Edlund (18) used the Pdx1-Cre system to delete β-cat in the pancreas and duodenum. They found that β-cat mutant cells had a competitive disadvantage during development. Although there was a reduction in the endocrine islet numbers during development and the mice had development of pancreatitis perinatally because of the disruption of the epithelial structure of acini, the mice later recovered from the pancreatitis and regenerated normal pancreas and duodenal villi from the wild-type cells that had escaped β-cat deletion (18). These observations do not support a fundamental role of Wnt signaling in pancreatic islets.

Certain seemingly contradictory observations on the role of TCF7L2 in β cells also were made in recent years. For example, Lyssenko et al. (14) found that the risk T-allele in rs7903146 was associated with increased TCF7L2 expression, impaired insulin secretion, incretin effects, and enhanced hepatic glucose production. In addition, they found that TCF7L2 expression correlated inversely with glucose-stimulated insulin release (14). The deleterious effect of TCF7L2 also was reported in a recent study (2), showing that *Tcf7l2*$^{+/-}$ mice displayed enhanced glucose tolerance coupled to significantly lowered insulin levels, whereas transgenic mice harboring multiple Tcf7l2 copies displayed reciprocal phenotypes, including glucose intolerance (2). Shu et al. (11), however, found that TCF7L2

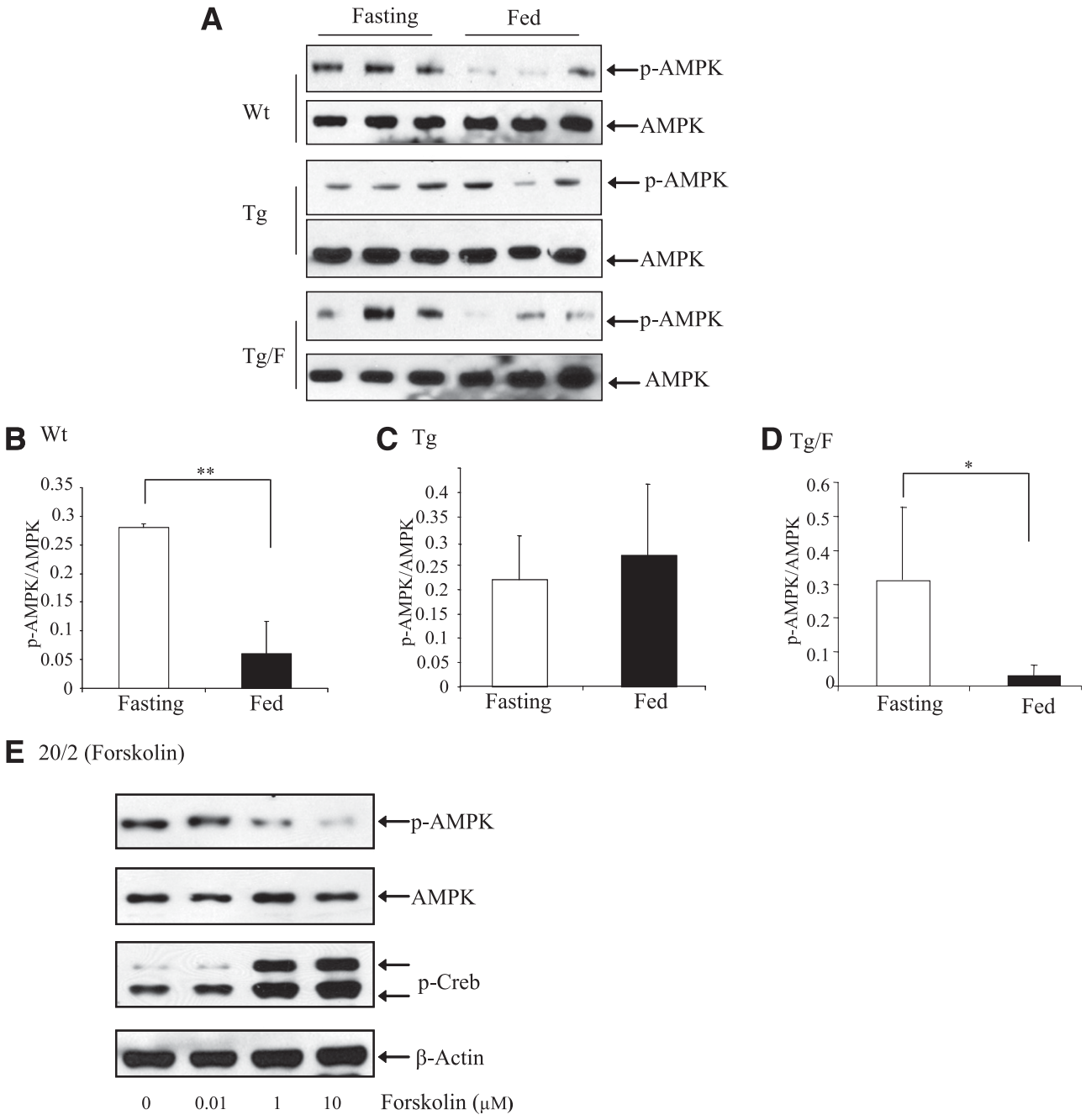

FIG. 6. Consecutive IP forskolin injection in *gcg*-TCF7L2DN mice restored feeding-mediated repression of AMPK. *A*: Refeeding led to inhibited hypothalamic AMPK in the wild-type but not the T4 transgenic mice (top and middle panels), whereas consecutive IP forskolin injection restored this inhibitory effect of refeeding. Representative blots for three mice in each of the three groups. *n* = 6 for each of the three groups. *B–D*: Densitometry analyses of *A*. *E*: Forskolin treatment inhibited AMPK activity in the brain neuronal cell line mHypoE-20/2.

positively regulates β-cell proliferation and glucose-mediated insulin secretion. Furthermore, TCF7L2 overexpression protected islets from glucose and cytokine-induced apoptosis. Obviously, these contradictory observations were made because of the complexity of the Wnt signaling pathway. Because TCF7L2 has multiple alternatively spliced isoforms, it is possible that different isoforms may exert different or even opposite functions (5).

If the risk TCF7L2 SNPs alter the expression of TCF7L2 or the function of Wnt signaling in pancreatic islets, then we would be more interested in the role of Wnt signaling in adult islets rather than in pancreatic development. Utilizing the β-cat/TCF responsive TOPGAL mouse model, Krutzfeldt and Stoffel (17) demonstrated that Wnt signaling is not appreciably active in the adult pancreas. They suggest that abundant expression of the repressive Wnt ligand Wnt-4 is among the mechanisms that determine the lack of appreciable Wnt activity in adult pancreas (17). We show here that *gcg* promoter–directed hTCF7L2DN expression did not alter pancreatic *gcg* mRNA levels or the genesis of pancreatic α cells. Because TCF7L2 is also expressed in many other organs, it is necessary to expand investigations into those organs that are also involved in glucose and metabolic homeostasis.

A recent study by Liu et al. (47) revealed the effect of hepatic β-cat depletion in inhibiting glucose production. In addition, recently, with the method of chromatin-immunoprecipitation combined with massively parallel DNA sequencing (chromatin-immunoprecipitation sequencing), Norton et al. (48) demonstrated that TCF7L2 directly binds to promoters/regulatory elements of a number of genes that are important in regulating hepatic glucose metabolism. They also have conducted an in vitro study showing that TCF7L2 knockdown in a rat hepatic cell line led to increased gluconeogenic gene expression (48).

Here, we verified our previous in vitro findings in vivo, showing that TCF7L2 is important for gut *gcg* expression. Because we cannot determine whether reduced gut gcg expression is fully responsible for the altered glucose homeostasis in these transgenic mice, we assessed the role of TCF7L2 in brain *gcg*-expressing cells. We show here that TCF7L2 is colocalized with GLP-1 in the brainstem, and it controls brain *gcg* expression. More importantly, we revealed the positive cross-talk between Wnt and GLP-1/cAMP signaling in the brain, which is mechanistically achieved via increasing β-cat Ser675 phosphorylation by GLP-1/cAMP signaling. This observation is consistent with the report that peripheral GLP-1 stimulates β-cell proliferation via activating β-cat Ser675 phosphorylation (12). We propose that metabolic defects in *gcg*-TCF7L2DN transgenic mice are partially attributable to the reduction of brain *gcg* expression. To further examine this hypothesis, it is necessary to develop methods to knockdown TCF7L2 in brain *gcg*-expressing cells only.

After the discovery of the role of brain GLP-1 in controlling energy homeostasis (49), enormous efforts have been made to explore the underlying mechanism. Knauf et al. (50) showed that during hyperglycemia, brain GLP-1 inhibited muscle glucose utilization and increased insulin secretion, indicating that central GLP-1 signaling is connected to peripheral insulin signaling and glucose usage. A recent study by Hayes et al. (26) shows that GLP-1 represses brainstem AMPK via activating cAMP and mitogen-activated protein kinase signaling. We found that functional knockdown of TCF7L2 in *gcg*-expressing cells reduced brain *gcg* expression, along with impaired repression of hypothalamic AMPK in response to refeeding, and this defect can be partially restored by cAMP/PKA/CREB activation through peripheral forskolin injection. Based on these observations, we summarize our main findings in Fig. 7. TCF7L2 is among the essential factors for brain GLP-1 production. After a meal, GLP-1 is able to inhibit hypothalamic AMPK, which may be among the mechanisms underlying the anorectic effect of this hormone (26). Furthermore, GLP-1 positively regulates the brain Wnt signaling pathway via stimulating β-cat Ser675 phosphorylation and increasing TCF7L2 levels. How this cross-talk controls peripheral insulin sensitivity and glucose disposal is unknown and needs to be further investigated.

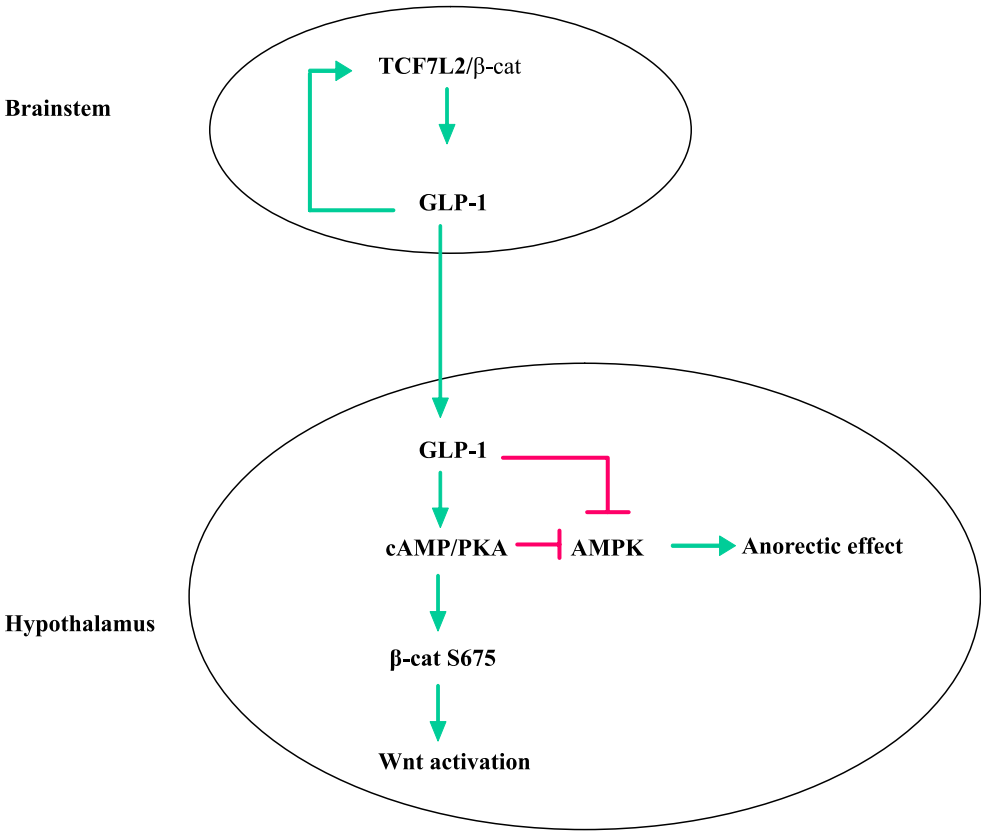

FIG. 7. A diagram shows the existence of positive feedback between the Wnt and GLP-1/cAMP signaling pathways in the brainstem and hypothalamus. In the brainstem, β-cat/TCF7L2 positively regulates gcg expression and the production of GLP-1, which inhibits food intake at least partially by attenuating hypothalamic AMPK activity (26). GLP-1 also stimulates brain Wnt activity via increasing β-cat Ser675 phosphorylation and, possibly, TCF7L2 production. In the brainstem, this leads to increased gcg expression (positive feedback), whereas in hypothalamic neurons this is among the anorectic effects of GLP-1. How the hypothalamic Wnt activation modulates peripheral glucose homeostasis and insulin signaling is currently unknown. (A high-quality color representation of this figure is available in the online issue.)

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W.S., D.W., Y.-T.C., W.I., L.Z., F.X., J.C., X.W., and Q.W. provided research data. D.D.B. and D.M.I. provided research materials. W.S. and T.J. wrote the manuscript. H.Z., D.W., Q.W., D.D.B., D.M.I., and Y.-T.C. edited the manuscript. T.J. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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