Perspectives in Diabetes

Glucose Sensing in Pancreatic β-Cells

A Model for the Study of Other Glucose-Regulated Cells in Gut, Pancreas, and Hypothalamus

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Nutrient homeostasis is known to be regulated by pancreatic islet tissue. The function of islet β-cells is controlled by a glucose sensor that operates at physiological glucose concentrations and acts in synergy with signals that integrate messages originating from hypothalamic neurons and endocrine cells in gut and pancreas. Evidence exists that the extrapancreatic cells producing and secreting these (neuro)endocrine signals also exhibit a glucose sensor and an ability to integrate nutrient and (neuro)hormonal messages. Similarities in these cellular and molecular pathways provide a basis for a network of coordinated functions between distant cell groups, which is necessary for an appropriate control of nutrient homeostasis. The glucose sensor seems to be a fundamental component of these control mechanisms. Its molecular characterization is most advanced in pancreatic β-cells, with important roles for glucokinase and mitochondrial oxidative fluxes in the regulation of ATP-sensitive K^+ channels. Other glucose-sensitive cells in the endocrine pancreas, hypothalamus, and gut were found to share some of these molecular characteristics. We propose that similar metabolic signaling pathways influence the function of pancreatic α-cells, hypothalamic neurons, and gastrointestinal endocrine and neural cells.
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Blood glucose levels are tightly controlled by regulation of insulin release from pancreatic β-cells. This homeostatic function depends on glucose uptake in β-cells and the subsequent signaling pathways that influence the rate of exocytosis. The underlying molecular mechanisms have been outlined in previous reviews (1–4); they contain metabolic steps that are responsible for intercellular differences in glucose sensitivity (5). The glucose signals for insulin release act in synergy with messengers originating from the binding of glucagon or the incretin hormones glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) to their cognate receptors (6). This synergistic action in β-cells may act in concert with glucose regulation of glucagon and GIP-releasing cells. The effects of glucose on the release of nutrient-regulating hormones may also require a balance with its influences on hypothalamic control of food intake and, hence, the size of the ingested nutrient load (7). In this article, we propose that glucose homeostasis is maintained by similar—but not necessarily identical—glucose sensors distributed in endocrine cells of the pancreas and gut and in various hypothalamic neurons that are implicated in feeding behavior. This concept will be explored by reviewing a number of molecular similarities in these diverse cell types. If physiologically operative, understanding this network of glucose-sensing cells may be crucial in preventing development of type 2 diabetes and obesity. In this way, further molecular analysis of the diverse glucose-sensing pathways may indicate the sites of dysregulation in diabetic and obese subjects.

Glucose sensor in pancreatic β-cells. Glucose regulation of insulin release is mediated by metabolic signals (1–4). The putative pathway between uptake of glucose and exocytosis of secretory vesicles (Fig. 1) has emerged from numerous converging observations in rodent islet cells (1–4, 8–11). Briefly, pancreatic β-cells express GLUT2 glucose transporters, which permit rapid glucose uptake regardless of the extracellular sugar concentration (8). At low glucose levels (<2.5 mmol/l), little substrate is phosphorylated in β-cells (9), probably because of low expression in these cells of high-affinity hexokinase isoforms (hexokinase I, hexokinase II or hexokinase III [HK]). The measurement of relatively high HK levels in isolated islets is most likely attributed to its presence in nonendocrine cells that contaminate these preparations (9). Unlike β-cells, most other cell types express HK at sufficiently high levels to keep intracellular glucose-6-phosphate (G6P) concentrations rather constant, and thus guarantee basal ATP production for maintaining cellular functions even at low extracellular glucose concentrations (12,13). In these cells, the rate of glucose transport and/or G6P consumption are the main forces for metabolic flux, keeping the energy charge of the cells constant and independent of the extracellular glucose levels (12–14). In pancreatic β-cells,

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α-MSH, α-melanocyte–stimulating hormone; AgRP, Agouti-related protein; ARC, arcuate hypothalamic nucleus; CART, cocaine and amphetamine–regulated transcript; G6P, glucose-6 phosphate; GIP, glucose-dependent insulinotropic peptide; GK, glucokinase; GLP-1, glucagon-like peptide 1; GLP-1R, GLP-1 receptor; HK, hexokinase I, hexokinase II, or hexokinase III enzyme activity; K_{ATP}, ATP-sensitive potassium channel; LHA, lateral hypothalamic area; MC3-R, melanocortin-receptor type 3; MC4-R, melanocortin-receptor type 4; MCH, melanin-concentrating hormone; NPY, neuropeptide Y; PACAP, pituitary adenylate cyclase activating polypeptide; POMC, pro-opiomelanocortin; PVN, paraventricular nucleus; RT-PCR, reverse transcriptase–polymerase chain reaction; SUR1, sulfonylurea receptor 1; VIP, vasoactive intestinal polypeptide; VMH, ventromedial hypothalamus; VMN, ventromedial nucleus.

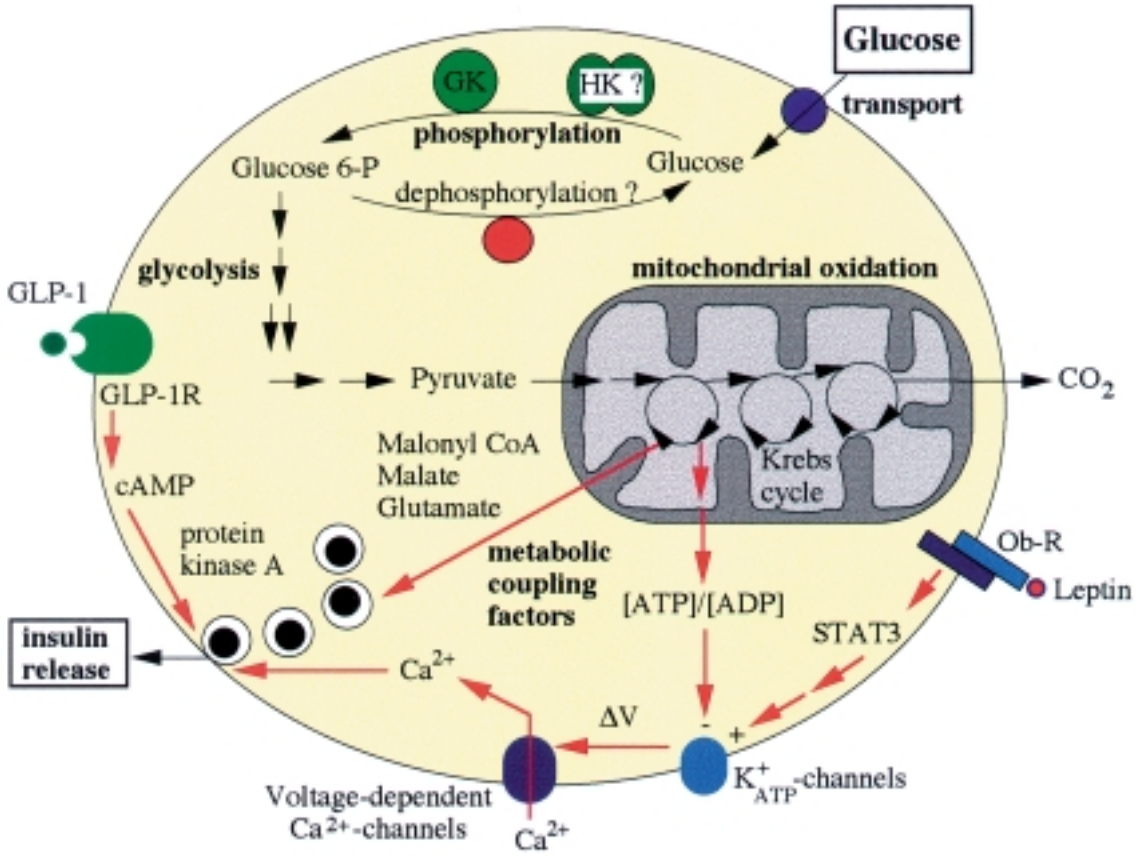

FIG.1. Metabolic hypothesis of glucose-stimulated insulin release from β-cells. In mammalian β-cells, the rate of glucose phosphorylation, catalyzed via glucokinase, is the bottleneck for further metabolic flux. Because of the kinetic properties of GK, this rate is proportional to the extracellular glucose concentration. It is unknown if other hexokinases and G6P dephosphorylation can influence this rate-limiting step. Mitochondrial uptake and metabolism of pyruvate—both carboxylation and decarboxylation—allows production of various messengers for exocytosis, such as the ATP/ADP ratio and the efflux of mitochondrial metabolic intermediates. Glucose signaling pathways are amplified via production of cAMP, which is stimulated by activated GLP-1Rs, GIP, and glucagon (not shown). This effect is further modulated by the phospholipase C pathway (not shown) and the signaling induced by insulin receptors (not shown) and leptin-receptors (Ob-R). Recent data indicate that part of the effect of cAMP on exocytosis in β-cells is mediated via a protein kinase A–independent pathway involving the GDP/GTP-exchanging protein cAMP-GEFII (117). ΔV, membrane depolarization.

however, the absence or very low expression of HK results in a low glycolytic flux and low ATP/ADP levels at glucose levels <2.5 mmol/l (10,11), levels essential for maintaining a low basal rate of insulin release. At glucose levels >2.5 mmol/l, β-cells phosphorylate glucose via high-affinity glucokinase (GK) (hexokinase IV), which explains the observed increase in the ATP/ADP ratio of 5–10 mmol/l (11). As was reviewed previously (1), the expression of GK in β-cells has important metabolic consequences. The fact that GK is not inhibited by G6P forms the basis of a proportional change in the cytoplasmic G6P concentration when extracellular glucose concentrations increase. Moreover, the sigmoidal relationship between GK reaction velocity and glucose concentration ($S_{0.5}$ 8 mmol/l) renders β-cells maximally sensitive to changes in extracellular glucose around the physiological plasma level. Differential usage of two alternative GK gene promoters (15) keeps enzyme expression levels much lower in pancreatic β-cells than in liver parenchymal cells and makes GK activity in β-cells the rate-limiting step for further glucose metabolism (1). On the contrary, abundant GK expression in the liver helps the rapid uptake and disposal of glucose from the portal vein during the postprandial state. To achieve the latter function in nonfasting conditions, the hepatocyte-specific promoter is strongly activated by insulin, whereas it is repressed by glucagon during fasting (16,17). In β-cells, the GK upstream promoter region is not really influenced by acute changes in the nutritional state (16). This difference cannot be attributed to the absence or presence of membrane receptors, because rat (18) and human (19) β-cells express glucagon-receptors and insulin-receptor signaling has been demonstrated in mice (20). Although it is difficult to exclude that β-cell GK gene transcription is regulated to some extent via local islet hormone levels, we consider it unlikely that GK gene transcription in β-cells is regulated by glucagon or insulin. Moreover, the glucagon-induced increase in β-cell cAMP formation synergizes rather than antagonizes with the glucose signals for insulin secretion (21), which is not compatible with glucagon repression of the GK gene. Direct effects of glucose are considered more likely (22,23) and could explain the loss of glucose-responsiveness in β-cells that have been exposed for prolonged periods to low glucose concentrations. The low glucokinase level in β-cells compared with liver parenchymal cells is probably the consequence of a weak promoter. This possibility may be functionally important: on the one hand, small changes in glucokinase were shown to affect the glucose sensitivity of

the cells (24); on the other hand, larger increases in GK expression severely disturbed the glucose-sensing properties by a process comprising G6P accumulation, ATP depletion, and cell death (25). It is conceivable that glucose activates GK molecules posttranslationally, as observed in hepatocytes (26). Liver glucokinase is indeed inhibited by a regulatory protein (26) that anchors the enzyme in the nucleus causing enzymatic inhibition during the postabsorptive or fasted state (27,28). When plasma glucose rises after a meal, nuclear GK is released into an active pool in the cytosol (26,27). It is still unclear whether glucose can cause GK redistribution in β-cells. Glucose-induced translocation of a GK immunoreactive protein from a peri-Golgi region to the cytosol has been described (29), but the significance of this observation is not yet explored. Coexistence of diffusible and nondiffusable GK may result from partial GK binding to subcellular organelles, involving a protein that is different from the liver-regulatory protein (23). Whatever the underlying mechanisms, a rise of plasma glucose in the physiological range (i.e., between 3 and 10 mmol/l) is known to cause a proportional flux of glucose phosphorylation via GK in the active subcellular pool. This leads to a proportional increase in overall glycolytic flux and mitochondrial glucose oxidation. The crucial role of GK as the "gatekeeper" for metabolic signaling in β-cells (1) is further illustrated by three observations. First, β-cells with lower glucokinase expression exhibit a lower glucose-induced insulin release than those with a higher expression (30). Second, reduction of GK expression or functional enzyme activity in humans is associated with an inadequate insulin-secretory response to glucose in patients with maturity-onset diabetes of the young (31). Third, targeted disruption of the GK gene in β-cells results in disturbed glucose-induced insulin release in mice (32,33).

Unlike most other mammalian cell-types, pancreatic β-cells express low lactate dehydrogenase levels (10,34) and high pyruvate carboxylase activity (10). These two properties can explain why virtually all pyruvate that is formed from G6P enters the Krebs cycle (10) and why β-cells produce little lactate (34,35). Tight coupling between glycolysis and mitochondrial oxidation has been considered to be crucial for more distal steps in the signal generation in β-cells (2,36). The first and best characterized signal proceeds via ATP-sensitive K^+ (K_{ATP}) channels (37). An acute rise in the extracellular glucose concentration from 1 to 10 mmol/l glucose induces a dose-dependent increase in the ATP/ADP ratio (11). This shift in adenine nucleotide pools is thought to stimulate insulin release via closure of K_{ATP} channels (37). Intracellular ATP is indeed a negative allosteric regulator of the Kir6.2 pore-forming unit of these channels (38), whereas the concomitant decrease in ADP further contributes to this effect via the associated regulatory subunit, sulfonylurea receptor 1 (SUR1) (39). The membrane depolarization that occurs upon closure of the K_{ATP} channels leads to the opening of L-type voltage-dependent calcium channels and induction of exocytosis (40). The second glucose-induced signaling pathway proceeds independently of K_{ATP} channels (41), but the exact nature of the produced signal(s) is still unclear (42). It has been proposed that increased influx of glucose carbon into the Krebs cycle (anaplerosis) leads to accelerated production of intermediates that leave the mitochondria and accumulate into the cytosol to stimulate insulin release (2). Examples of such intermediates could be malate (10,43), citrate (10,44), and glutamate (45). Malate efflux from the mitochondria has been proposed to activate the malate/pyruvate shuttle and to promote electron transfer from cytosolic NADH to NADPH (43). Citrate is the precursor of malonyl CoA, the key metabolite for the production of acyl CoA esters (44). Mitochondrial glutamate efflux was reported to cause glutamate uptake by the secretory vesicles, an ATP- and proton-dependent step that may induce exocytosis (45).

In rat β-cells, the glucose metabolic signaling pathway interacts synergistically with cAMP for the stimulation of insulin release (21). Although the exact mechanism of this synergism is not yet known, a direct accelerating effect of cAMP on the rate of glucose metabolism is unlikely; addition of glucagon to purified rat β-cells does not increase the rate of glucose oxidation in these cells (46). Synergism at the level of target proteins involved in the exocytosis of secretory granules has been proposed (47). The initiating signals of the cAMP pathway appear to be well conserved. In rat and human β-cells, glucagon and the incretin hormones GLP-1 and GIP increase cAMP production, and expression of the corresponding receptors has been demonstrated (19,48). The physiological relevance of the incretin effect is illustrated in the model of targeted disruption of the GLP-1 receptor gene in mice (49). However, this gene defect does not cause a severe loss of glucose-induced insulin release (50) probably because compensation develops through GIP secretion (51) and GIP action (51,52).

It can be concluded that normal rates of glucose-induced insulin release are maintained by at least two key mechanisms: acceleration of the metabolic flux, which is controlled at the level of glucose phosphorylation by the β-cell isoform of glucokinase, and cAMP production, which is stimulated by glucagon, GLP-1, and GIP.

Glucose sensors in hypothalamic neurons and in neurons of the enteric nervous system. Not only islet β-cells, but also neurons in various hypothalamic nuclei (53) and (possibly) in the gut (54), possess the capacity to detect variation in glucose concentrations between 3 and 10 mmol/l. Like pancreatic β-cells, glucose-sensing hypothalamic neurons appear strongly implicated in nutrient homeostasis, as they control both feeding behavior and whole-body adiposity (7) and, on the other hand, influence on pancreatic hormone release via autonomous islet innervation (55). These latter effects are relevant both to prevent hypoglycemia and to dampen the surge in plasma glucose after a meal. Glucose-responsive myenteric neurons may be relevant for the glucose-induced release of the incretin hormones GLP-1 and GIP, which in turn influence insulin release and nutrient homeostasis (6,56). Because so little is known about these cells (54), the main focus of this paragraph is on hypothalamic neurons. Glucose-responsive neurons can be distinguished from most other neurons in electrophysiological recordings (53), because the latter exhibit high basal firing rates that can only be suppressed by incubating at glucose concentrations <1 mmol/l. Glucose-responsive neurons are particularly abundant in certain regions of the hypothalamus, as evidenced by a localized increase in Fos-like immunoreactivity after injection of glucose into the carotid artery (57). It can be hypothesized that the glucose-activation of these neurons results from metabolic signaling. We examined this possibility by analyzing similarities to glucose sensing in pancreatic β-cells.

The metabolic hypothesis for the glucose-induced activation of glucose-responsive neurons has been supported by

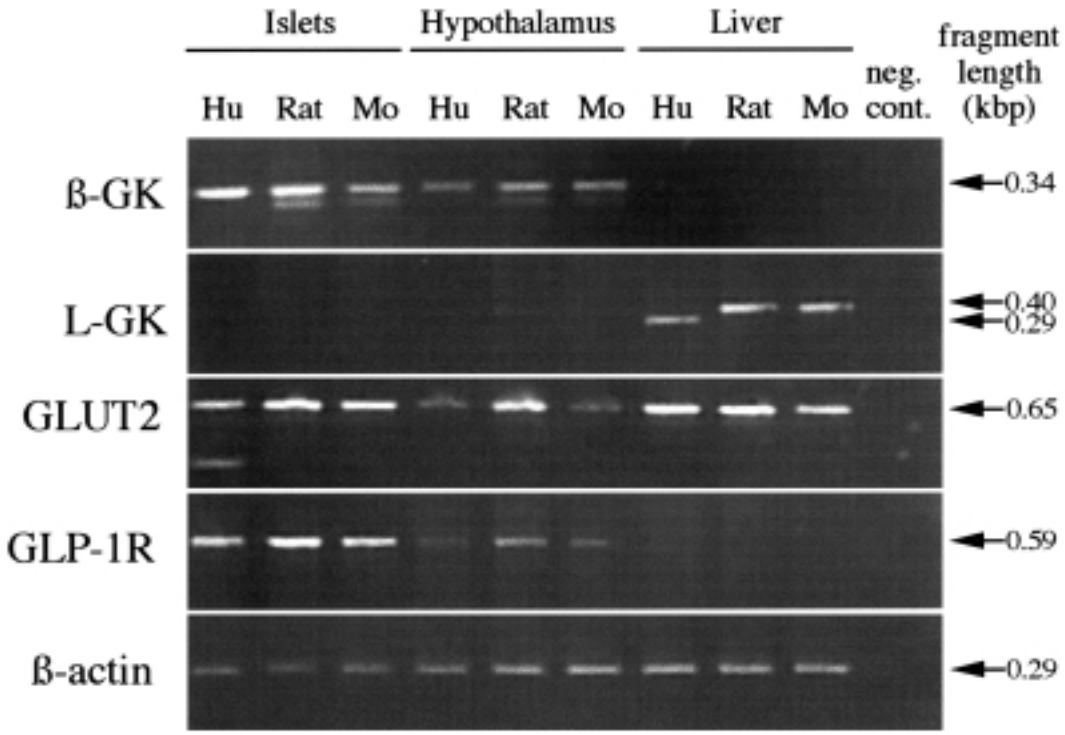

FIG.2. Expression of transcripts involved in glucose sensing in mammalian hypothalamic cells and islet cells. RT-PCR analysis was performed using primer sets allowing species-specific cDNA amplification. Amplification of β-actin cDNA served as a positive control for each tested tissue, and RT-PCR in samples without added cellular RNA served as a negative control (neg. cont.) for each run. The experiment shown is representative of three independent experiments. β-GK, β-cell isoform of glucokinase; Hu, human; L-GK, liver isoform of glucokinase; Mo, mouse.

electrophysiological measurements in rat brain slices (58). Blockers of glucose transport (phloridzin) or glycolysis (glucosamine, 2-deoxyglucose, or iodoacetic acid) abrogated glucose-induced electrical activity while other nutrients, such as mannose, galactose, glyceraldehyde, glycerol, and lactate, mimicked the effects of glucose (58). Like pancreatic β-cells, glucose-responsive neurons might exhibit enhanced glucose uptake and phosphorylation, thus accelerating metabolic flux when exposed to higher glucose levels. The proteins mediating these effects in a glucose concentration–dependent fashion may be similar to those in β-cells. Three lines of evidence support the possibility that glucokinase plays a similar key role in the glucose-sensing phenotype of these cells. The first indication comes from the microscopic localization of glucokinase gene expression in hypothalamic nuclei that contain glucose-responsive cells (59–61). Notably, glucokinase mRNA signals in rat hypothalamus were highest in the ventromedial hypothalamus (VMH) and arcuate (ARC) nuclei (60), which are implicated in the control of feeding behavior and energy expenditure (7). The second element is the observed coexpression in hypothalamic neurons of glucokinase and GLUT2 (61), another protein that is considered to be important for the glucose sensor in β-cells (4,8). A third argument in favor of a similar flux-determining role of GK in glucose-sensing neurons and β-cells is illustrated by how the GK gene is transcribed in neurons. As explained before, β-cells and hepatocytes use different promoter regions of the glucokinase transcription unit (15). Reverse transcriptase–polymerase chain reaction (RT-PCR) analysis (60) (Fig. 2) suggests that the β-cell glucokinase transcript (but not liver-type transcript) is formed in the rat hypothalamus. We noticed that the selective use of the β-cell glucokinase promoter by hypothalamic cells is evolutionary well preserved among mammals, because it was found in RNA extracts from mice, rats, and humans (Fig. 2). As in β-cells, this evolutionary conservation may be functionally relevant. Because rat hypothalamus contains transcripts encoding the liver-type GK-regulatory protein (62), it is conceivable that β-cells and hypothalamus also exhibit differences in their GK regulation. It still remains to be investigated whether GK in hypothalamic neurons can translocate between different intracellular compartments according to the nutritional state.

The similarity between glucose-induced signaling in β-cells and in hypothalamic neurons is also supported by the effects of the sulfonylurea tolbutamide on cellular electrical activity. In neurons (58), as well as in β-cells (63), the sulfonylurea bypasses the signal of an increased ATP/ADP ratio by closing K_{ATP} channels, resulting in membrane depolarization (37). However, contrary to that observed in β-cells (63) and myenteric neurons (54), diazoxide, a substance that opens K_{ATP} channels in β-cells via the SUR1 subunits (37), blocks the effects of glucose only in a minority of glucose-responsive VMH neurons. This difference between VMH neurons and pancreatic β-cells is still unexplained. One possible explanation concerns splice variation in transcripts encoding the SUR1 subunit (64): in β-cells, full-length transcripts were detected, whereas hypothalamic glucose-responsive neurons were found to produce a SUR1-splice variant from which the diazoxide-binding domain is deleted. The participation of K_{ATP} channels in glucose-sensing neurons was further examined by identifying cells that coexpress mRNA that encode GK and the pore-forming unit Kir6.2 of the K_{ATP} channel. Cells positive for

both transcripts were noticed in both the rat hypothalamus (60) and myenteric neurons (54). In the hypothalamus, the Kir6.2/GK-expressing cells were particularly abundant in the ARC nucleus, the main site of neuropeptide Y (NPY) and pro-opiomelanocortin (POMC) production and a pivotal site of signal integration with respect to nutrient homeostasis (7). Accordingly, ARC neurons often coexpress NPY transcripts and GK transcripts (60). More detailed quantification of GK^+/NPY^+ and GK^-/NPY^+ neurons is needed to understand the physiological role of GK in feeding behavior and regulation of body adiposity. It may also be interesting to quantify the number and localization of neurons that coexpress transcripts encoding GK, POMC, and subunits of the K_{ATP} channel.

Despite these known similarities to the β-cell, the glucose sensor in hypothalamic neurons is still poorly understood. The extent to which these cells express HK is still not known. It has been recently reported that low-affinity glucose phosphorylation accounts for 60–85% of total glucose phosphorylation in hypothalamic extracts (62). However, immunocytochemical (60) and electrophysiological studies (53,58) indicate that only a minority of hypothalamic neurons is glucose responsive, suggesting that the observed low-affinity glucose phosphorylation in whole hypothalamic extracts (62) originates mainly, if not solely, from other cells. This situation would then be comparable with that in the pancreas and the liver, in which (respectively) β-cells (9) and parenchymal cells (65) express GK, and surrounding pancreatic exocrine cells or hepatic sinusoidal cells express HK. This particular state could be altered in conditions of stress or injury, during which induction of low-affinity hexokinases accompanies the loss of physiological glucose regulation of β-cells (66) or hepatocytes (67). Another question to be examined is whether the β-cell isoform of GK functions as the flux-controlling step of glucose metabolism in glucose-sensing neurons. If this is indeed the case, then small changes in neuronal GK expression level should lead to functional changes in these cells. Analysis of hypothalami isolated from mice with tissue-specific inactivation of one of the two alleles of the GK gene (33) could help answer this question. Furthermore, it is unknown whether the GK-expressing neurons can be distinguished from GK^-/HK^+ cells by their glucose-dependent ATP/ADP ratios, as is the case in pancreatic β-cells (11). This would imply a very low ATP/ADP ratio at 0–3 mmol/l glucose and concentration-dependent stimulation >3 mmol/l substrate. This response requires regulation of the cellular energy charge that is fundamentally different from that in other neurons in which the constant and high-energy charge is maintained by a continuous and high rate of glucose usage. Given that these metabolites fail to stimulate β-cells, further studies should be undertaken on the effects of glycerol and lactate on electrical activity of VMH neurons (58). One possible explanation for this apparent discrepancy may reside in the presence (neurons) or absence (β-cells) of the corresponding nutrient transporters and fuel-consuming enzymes. In fact, a recent study on INS-1 cells (68) has demonstrated lactate-responsive insulin secretion after gene transfer for both MCT (encoding a lactate transporter) and LDH-A (encoding a lactate dehydrogenase). Because of the important differences between glucose and lactate as signals for whole-body nutrient homeostasis, a detailed analysis of hypothalamic neurons that are positive for MCT/GK-mRNA or LDH/GK-mRNA should then be considered. It should also be investigated whether glucose-responsive neurons utilize glucose aerobically, like pancreatic β-cells (10), and, if not, whether lactate production by a particular subset of glucose-responsive neurons influences the activity of surrounding GK^- cells. If, on the other hand, glycolysis is essentially aerobic, as it is in β-cells, the influence of glucose on fluxes of mitochondrial metabolism should be analyzed. In this context, glucose-sensing neurons may exhibit a high expression of pyruvate carboxylase and glucose-induced export of Krebs cycle metabolites, such as citrate and malate, into the cytosol. Subsequently, the recent suggestion that malonyl CoA participates in the hypothalamic control of food intake (69) is in agreement with the existence of an anaplerotic/malonyl CoA pathway (2) in glucose-sensing neurons.

Our present understanding is limited by the difficulty in investigating metabolic signal transduction in well-characterized subsets of hypothalamic neurons. Albeit the glucose response of GLUT2/GK-expressing neurons is, nevertheless, concentration dependent between 3 and 10 mmol/l substrate, this requires a combination of cellular and molecular studies on isolated hypothalamic cells. According to recent data, GK activity in hypothalamic neurons is linked to the process of glucose sensing. This similarity to the pancreatic β-cells (Fig. 1) may serve as a guide for further studies into the mechanisms involved in nutrient activation of hypothalamic neurons.

Glucose inhibition of pancreatic α-cells and hypothalamic neurons. Some endocrine cells and hypothalamic neurons are inhibited by increasing the extracellular glucose concentration from 3 to 10 mmol/l. Examples are the pancreatic glucagon-secreting α-cells (70) and the so-called glucose-sensitive neurons that are particularly abundant (71) in the lateral hypothalamic area (LHA). Relatively little is known about the mechanism underlying this type of glucose inhibition. For example, whether part of this inhibitory effect is achieved indirectly through the release of an inhibitory substance from neighboring cells that are glucose-responsive is not known. For islet α-cells, somatostatin-14 might be such an inhibitory substance (72), originating from neighboring δ-cells known to be glucose dependent (73). Two other possibilities may be *1*) the direct inhibition of α-cells by insulin (74,75), GABA (76), or other secretory products of the β-cell and *2*) glucose suppression of the sympathetic islet nerves that directly activate islet glucagon release through norepinephrine and galanin (77). Likewise, the suppressive effect of glucose on the firing rate of glucose-sensitive neurons might be mediated through neighboring glucose-stimulated neurons that produce inhibitory neurotransmitters or neuropeptides. There is, however, evidence for a direct glucose-sensing device in rat islet α-cells. These cells express the β-cell form of glucokinase, both at the RNA and protein level, and the expressed protein seems responsible for high-affinity glucose phosphorylation (78). The presence of the β-cell GK isoform may be essential for α-cell function. First, it allows a concentration-dependent glucose-inhibition within the physiological range. Second, it may be associated with low expression of the enzyme, resulting in cellular rates of glucose phosphorylation that are comparable to those of pancreatic β-cells (10). In addition to GK, islet α-cells may share other molecules of the metabolic signaling pathway with β-cells. In situ hybridization experiments indicate that islet α-cells contain the subunits Kir6.2 (79) and SUR1 (80) of the K_{ATP} channels. The role of these proteins in glucose sensing by α-cells is not clear, however, because

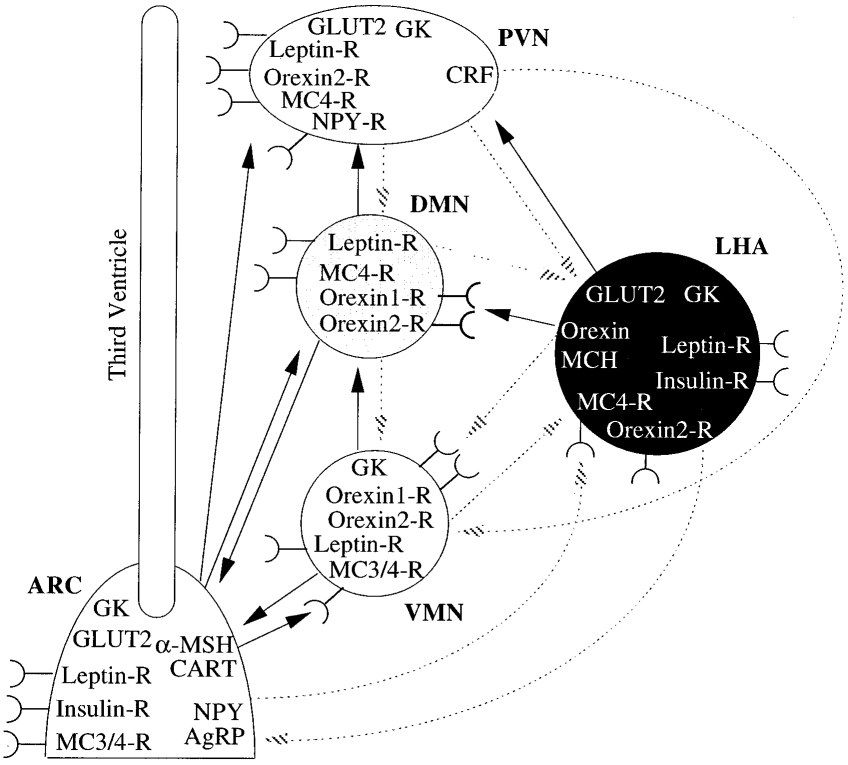

FIG.3. Hypothalamic network of glucose-sensing cells. Gene products involved in the process of glucose sensing in glucose-stimulated (white) and glucose-inhibited (black) neurons are indicated. The putative interconnecting synaptic contacts between hypothalamic nuclei (major, solid arrows; minor, hatched arrows) are also indicated. Gray shading was used for dorsomedial nucleus (DMN) neurons from which the glucose responsiveness is unknown.

raising the glucose concentration from 1 to 10 mmol/l did not change the ATP/ADP ratio in fluorescence-activated cell sorter–purified α-cells (11), which suggests that glucose inhibition is not directly mediated via an opening of K_{ATP} channels. In purified rat α-cells, transition from basal to elevated glucose elicits a decrease (81) in the cytosolic Ca^{2+} concentration, suggesting that glucose closes Ca^{2+} channels or enhances the rate of removal of Ca^{2+} from the cytosol. At present, it is unclear how this occurs in molecular terms. One possibility is that at low extracellular glucose concentrations the plasma membrane of α-cells depolarizes by inactivating a pool of Na^+/K^+-ATPases, analogous to the process in certain LHA neurons (82). Recent work on a glucagon-secreting cell line supports this idea (83). In addition to these differences, it seems likely that glucose-stimulated and -inhibited cells can be distinguished at the level of several other points of glucose metabolism. Examples are more anaerobic glycolysis and less anaplerosis from glucose carbon in α- versus β-cells (10).

In summary, little is known about the molecular mechanism of the inhibition on the activity of α-cells and hypothalamic neurons by glucose. Glucokinase may be involved, but the signaling pathway shows several differences when compared with glucose-activated β-cells.

Network of hypothalamic and endocrine glucose-sensing cells. As was recently reviewed (7) and as was pioneered by experiments by Coleman (84) and Jeanrenaud (85), nutrient homeostasis is controlled by a powerful and complex network of neurons and endocrine cells. At risk of oversimplification, a scheme can be proposed in which glucose-responsive and glucose-sensitive endocrine cells and neurons communicate with each other and regulate the nutritional state of the organism via their secretory products (Figs. 3 and 4). We have omitted in this scheme the position of δ-cells, which are localized adjacent to glucose-sensing cells in pancreas, gut, and hypothalamus (86) and may influence the latter by local somatostatin release. We will also only briefly mention the role of adipocytes, the main body storage site of triglycerides, because these cells lack a glucose sensor. This does not deny the possibility that adipocytes and β-cells may—under certain circumstances—communicate with each other via leptin and insulin (87).

Within the hypothalamus, an important role can be attributed to the ARC nucleus, which contains two important populations of cells with antagonistic effects on food intake (7). The first consists of appetite-stimulating cells that express two orexigenic peptides: NPY, an agonist of NPY receptors, and Agouti-related protein (AgRP), the antagonist of the melanocortin receptors (types 3 [MC3-R] and 4 [MC4-R]). These cells project to different hypothalamic nuclei, such as the ventromedial nucleus (VMN), the paraventricular nucleus (PVN), and the LHA (Fig. 3), which in turn are linked to centers regulating appetite and energy expenditure (7,88). It was mentioned earlier that some of these cells express both GK and GLUT2 and, thus, may be capable of directly monitoring nutrient homeostasis via measurement of plasma glucose levels. A second population of ARC neurons produces the anorectic peptide α-MSH, an end product of POMC processing (89), and the cocaine/amphetamine-regulated transcript (CART) peptide (90). The importance of GLUT2 and GK in

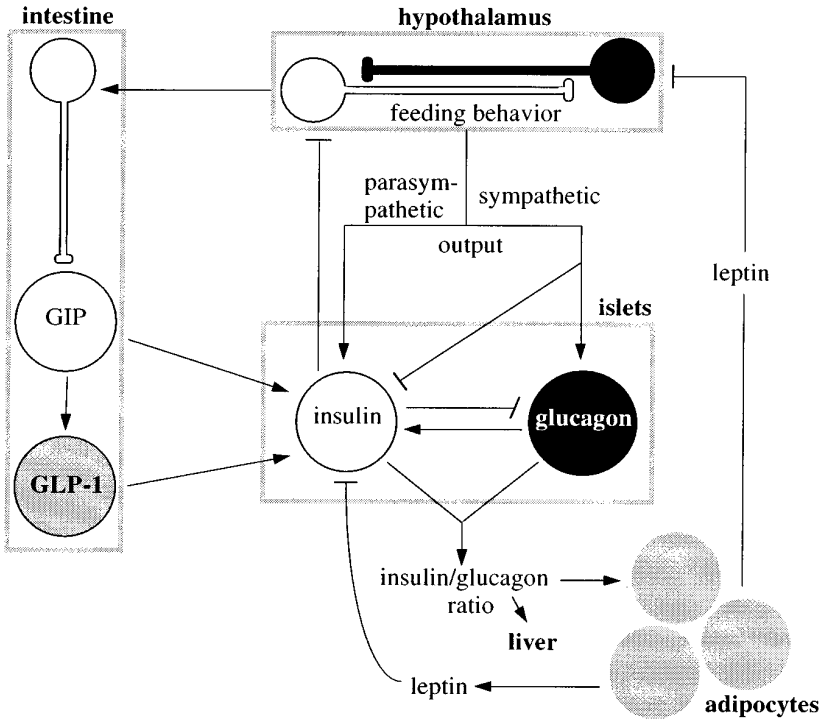

FIG.4. Neuroendocrine network of glucose-sensing cells. Simplified scheme of the putative neuroendocrine network comprising islet α- and β-cells, intestinal K- and L-cells, and neurons in hypothalamus and myenteric plexus. Glucose-stimulated cells (white) and glucose-inhibited cells (black) communicate with cells that are not directly regulated by glucose (gray). Arrowheads indicate (neuro)endocrine stimulatory signaling, whereas lines with blunt ends (⊣) symbolize (neuro)endocrine inhibition. For the sake of simplicity, somatostatin-producing δ-cells in pancreatic islets, in hypothalamus, and in gut mucosa have been ignored, but it is known that release of this inhibitory peptide is nutrient-dependent (73,80). Interaction of hypothalamus and pancreatic islets with the leptin-secreting adipocytes is indicated.

these cells is presently not known. We suggest that the glucose sensors in these hypothalamic cells may act in synergy with hormonal signals, as they were found to do in pancreatic β-cells. The glucose-responsive neurons may be sensitive to leptin, the adipocyte hormone known to determine body adiposity via two ways, namely through inhibition of NPY/AgRP cells and stimulation of POMC/CART cells (7,91). Interestingly, both NPY/AgRP-secreting and POMC/CART-secreting neurons are also under control of plasma insulin, which reinforces the dual effects of leptin. Therefore, the ARC neurons seem specialized in the integration of messages coming directly from extracellular nutrients and indirectly from hormones that signal the nutritional state, just as β-cells integrate such signals for the regulation of insulin release (21). Remarkably, the glucose-responsiveness of pancreatic β-cells can be modulated by activation of insulin receptors (20) and leptin receptors (87).

When blood glucose falls to hypoglycemic levels, acute adaptations in the network are required to preserve brain function from minute to minute. The precise mechanisms of this response, which is of utmost importance for survival, as is regulation of body adiposity, are not yet fully elucidated. It seems likely, however, that glucose-sensitive neurons in the LHA are involved in the prevention of hypoglycemia, because LHA is responsible for increased sympathetic activity when blood glucose falls (92). Although it was shown that GLUT2 and GK are present in some LHA neurons (61), the question whether the expression of these glucose-sensor proteins is associated with the production of appetite-stimulating neuropeptides, such as orexin A (93) or melanin-concentrating hormone (MCH) (94), is still unanswered. It is conceivable that certain appetite- and arousal-stimulating LHA neurons are directly stimulated when extracellular glucose falls below a certain threshold.

The endocrine part of this network involves the pancreatic α- and β-cells, which are known to adjust their hormone release according to the prevailing nutrient and (neuro)hormonal signals (Fig. 4). The direct effects of glucose on α- and β-cells have already been discussed. The amplitude of glucose-induced insulin release is highly dependent on the action of the gastrointestinal hormones GLP-1 and GIP (6,56). Of potential interest is the possibility that the release of GLP-1 and GIP might be regulated by glucokinase-expressing endocrine cells (59) and glucose-responsive neurons in the myenteric plexus (54). It has been reported that the release of GIP by intestinal K-cells is directly controlled by glucose (95), which further extends the integrating properties of the enteroinsular axis (Fig. 4). However, it should be considered that the nutrient control of K-cells differs form that of β-cells: the regulation of K-cells occurs via carbohydrate, fat, and protein concentrations in the gut lumen rather than via changes in plasma glucose levels (96,97). On the other hand, and in parallel with the regulation of insulin release from β-cells (21,72), is the potentiation of nutrient-induced GIP release by agents that increase cAMP and suppression of this effect by somatostatin (95).

Release of insulin and glucagon are both dependent on the islet-brain axis (Fig. 4). Multiple interactions have been previously described between hypothalamic and islet glucose-sensing cells. By means of a retrograde labeling technique (98), it has been possible to localize those cells controlling

activity of parasympathetic and sympathetic pancreatic nerves (most cells reside in nuclei of the hypothalamus and brain stem [99]). It is conceivable that such cells are responsive to changes in the glucose concentration, either through their glucose sensor or, indirectly, by their communication with neighboring glucose-responsive cells. This possibility implies that measurement of blood glucose by hypothalamic neurons orchestrates the autonomous nerve activity within the endocrine pancreas, a concept in accord with the knowledge that islet autonomous nerve activity is dependent on the nutritional state of the organism (55). The neural effectors are potent regulators of insulin and glucagon release, modulating the effect of the glucose-sensor in α- and β-cells. When plasma glucose decreases, as it does between meals or during more prolonged periods of fasting, increased sympathetic output via the splanchnic nerves is observed (92). The increased sympathetic activity of islet nerves directly activates glucagon release and inhibits insulin release (55). The main effects are probably mediated via the neurotransmitter norepinephrine (100) and the neuropeptide galanin (101), but the neurotransmitters and receptors involved in this process may differ among individual mammalian species. In rat purified islet cells, the inverse effects of norepinephrine are mediated by, respectively, α_2-receptors on β-cells and β-receptors on α-cells (102). These direct neural effects on islet α- and β-cells are potentiated by at least three different factors. First, as we have outlined above, glucose is a potent activator of insulin release and a direct inhibitor of glucagon release. Second, a concomitant rise in plasma adrenaline concentrations, as a result of stimulation of the adrenal medulla, may contribute to the suppression of insulin release (103). Third, activation of sympathetic nerves directly affects blood flow through islet capillaries (104), but the influence of these changes on the rate of hormone secretion is not known.

An inverse situation occurs during a meal or in the postprandial state, when elevated plasma insulin/glucagon ratios are required for the net peripheral uptake of glucose, triglycerides and amino acids, and subsequent nutrient storage. Glucose-induced insulin release from islet β-cells is potentiated by the parasympathetic activity of islet autonomous nerve fibers (55). This effect acts in concert with increased levels of the glucoincretin hormones GLP-1 and GIP. It proceeds via release of the neurotransmitter acetylcholine and the two homologous neuropeptides, pituitary adenylate cyclase activating polypeptide (PACAP) and vasoactive intestinal polypeptide (VIP). In mice, the cholinergic effects are mediated by M3 muscarinic receptors on β-cells (105), resulting in activation of phospholipase C and production of inositol phosphates. PACAP stimulates insulin release at picomolar concentrations, whereas VIP stimulation of insulin release requires nanomolar quantities (106). The effects of both peptides are mediated by specific PACAP receptors on islet cells (107) and proceed via increased cyclic AMP production, elevation of cytosolic calcium, and activation of phosphatidylinositol-3 kinases (108). It is not known whether these stimulatory effects occur in the same β-cells as those stimulated by glucoincretin hormones or glucagon. Furthermore, the relative importance of peptidergic insulinotropic effects of PACAP and VIP versus the cholinergic stimulation of insulin release also needs to be defined in more detail. Further studies should consider the possibility of species differences. For example, VIP has been found to potentiate acetylcholine-induced pancreatic polypeptide release in ruminants (109), whereas the peptide primarily stimulates glucagon release in dogs (110).

In summary, various glucose-sensing neurons and endocrine cells communicate in a complex network to maintain nutrient homeostasis. The glucoincretin hormones and the vagal nerve support glucose-induced insulin secretion during or just after meals, whereas the splanchnic nerve activity reinforces the effects of low glucose on the pancreas, both at the level of suppression of insulin release and stimulation of glucagon release.

Extrapolation to diseases. In this article, we have collected evidence for the existence of a network of glucose-sensing endocrine cells and neurons that use similar biochemical pathways to translate changes in extracellular glucose concentration into finely tuned states of cellular activity. Because most studies have been performed on animal cells, the validity of this model for human physiology is still to be assessed. Similarities have already been reported in signaling molecules (11,111), but quantitative differences were also noticed (111). The question should also be addressed whether genetic or environmental factors can directly alter the communication between glucose-sensing cells and thus cause diabetes or obesity. Rare mutations that cause diabetes and/or obesity have been described. Examples include mutations in the genes encoding leptin (112), glucokinase (31), and a growing number of transcription factors that regulate insulin and glucose-sensor protein expression levels (113). Identification of gene defects responsible for more common forms of obesity and diabetes is needed. This may be achieved by large-scale genome analysis. Gene disruption studies in mice might help explore disease mechanisms (32,33,49,114). For instance, these studies may clarify possible mechanisms of redundancy or compensation. In the case of the GLP-1 receptor knockout (49), compensation has been noticed at the level of GIP secretion and signaling (51,52); in the NPY knockout (114), compensation was found at the level of hypothalamic AgRP signaling (115). Finally, numerous molecular and cellular studies have focused on relatively few proteins that were chosen as candidate elements of the glucose sensor. The expression of such proteins in all glucose-sensing cells is an argument in favor of their physiological relevance in glucose sensing. A more systematic approach will be needed, however, to fully understand the molecular basis of a glucose-induced cellular response. Large-scale analysis of the glucose-induced transcriptome (116) or proteome of glucose-activated cells will certainly facilitate this approach.

In summary, certain hypothalamic neurons and pancreatic endocrine cells exhibit a striking similarity in functional organization, both at the level of glucose recognition and at the level of cellular activation. Examples are the expression of the β-cell isoform of glucokinase, GLUT2, and the modulation of functional responses by peptides secreted by a (neuro)endocrine network. Glucokinase plays a fundamental role as a signal generator, not only in β-cells, but in a myriad of glucose-sensing neuroendocrine cells scattered throughout the mammalian hypothalamus, gut, and pancreas. This idea may help to understand complex metabolic disturbances observed in diabetes and obesity and to identify new targets for pharmacological intervention.

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