

IMPAIRED GLUCOSE SENSING AS INITIATOR OF METABOLIC DYSFUNCTIONS

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INTRODUCTION

The control of blood glucose levels - glucose homeostasis - requires the perfectly integrated function of many organs: liver, muscle, adipose tissue, the endocrine pancreas and specific regions of the brain. Dysfunctions in any of these organs can lead to type 2 diabetes, obesity, and lipid disorders. Type 2 diabetes is characterized by two major physiological dysfunctions. A defect in the secretion of insulin in response to elevations in blood glucose concentrations by the pancreatic islet β cells, and a relative decrease in the action of insulin on its major target tissues: liver, muscle and adipocytes. Over time, these defects aggravate each other so that the secretion of insulin no longer suffice to override the insulin resistance of target tissues and frank hyperglycemia, even in the fasted state, is present. Epidemiological studies have demonstrated that the progression towards diabetes can be initiated by defects in glucose-stimulated insulin secretion (1,2). This points towards a defect in the glucose sensing mechanisms by pancreatic β cells as a possible cause of diabetes.

The prevalence of type 2 diabetes, which is often triggered by obesity, is progressing at a very rapid rate, not only in the developed world but also in developing countries. The worldwide prevalence of diabetes is approximately 5 percent and it is estimated that within the next ten years it will have increased by greater than forty percent to afflict 220 million people (3). Due to the development of complications affecting small and large blood vessels, type 2 diabetes has a severe impact on quality of life and life-expectancy. This disease is so far not optimally treated and much less prevented, due to lack of predictive molecular tools and of the scarcity of potential therapeutic targets.

For many years my work has been focused on studying the molecular pathophysiology of diabetes. A major aspect was to understand the regulation of glucose-stimulated insulin secretion by pancreatic β cells. This work was focused on the role played by specific membrane proteins, referred to as facilitated glucose transporters, which are required to move glucose across the plasma membrane, and the receptors for peptide hormones secreted by gut endocrine cells. These hormones are liberated in the blood after absorption of a meal and strongly potentiate the effect of glucose on insulin secretion. Because these gut hormones can still stimulate insulin secretion in diabetic patients who no longer respond to classical hypoglycemic agents, these receptors are now the target for the development of novel therapeutic agents for the treatment of type 2 diabetes.

In novel studies of glucose sensing by pancreatic β cells, we have generated mice with specific inactivation of the β cell glucose transporter gene. As anticipated, these mice develop a diabetes-like phenotype. Unexpectedly, however, they allowed us to discover the existence of other glucose sensing units, located in different anatomical sites, and which rely on the same molecules as β cells for glucose sensing. These other sensing units control several physiological functions such as food intake, energy expenditure and hormone secretion. Therefore, the pathogenesis of type 2 diabetes may be initiated not only by defect in glucose-stimulated insulin secretion, but also by abnormal control of these other physiological functions.

Here, I will review the different parts of this story and place it in the general context of the pathophysiology of type 2 diabetes.

FACILITATED DIFFUSION GLUCOSE TRANSPORTERS

Glucose is a polar molecule and its transport through biological membranes requires the presence of specific transporter proteins. In 1985, Mueckler et al. (4), were the first to isolate a cDNA for a mammalian glucose transporter. Based on the availability of this initial sequence, four additional hexose carriers were rapidly isolated by

different groups (Figure 1). These transporters, referred to as GLUT1 to GLUT5, are the products of different genes and are functionally distinguished by their affinity for glucose, different hexose specificity, specific tissue and cellular localization and regulated expression in different metabolic states (5,6). GLUT1, GLUT3 and GLUT4, are high affinity glucose transporters (K_m for glucose of 1-5 mM), GLUT5 is mostly a fructose transporter and GLUT2 has a uniquely low affinity for glucose (K_m for glucose ~17 mM) (7). This transporter is expressed selectively in tissues where high glucose fluxes are required such as intestine and kidney where it functions, respectively, in glucose absorption after a meal or in glucose reabsorption. It is also highly expressed in hepatocytes for efficient glucose uptake or release and in pancreatic β cells where glucose needs to be rapidly equilibrated between the extracellular compartment and the cell interior.

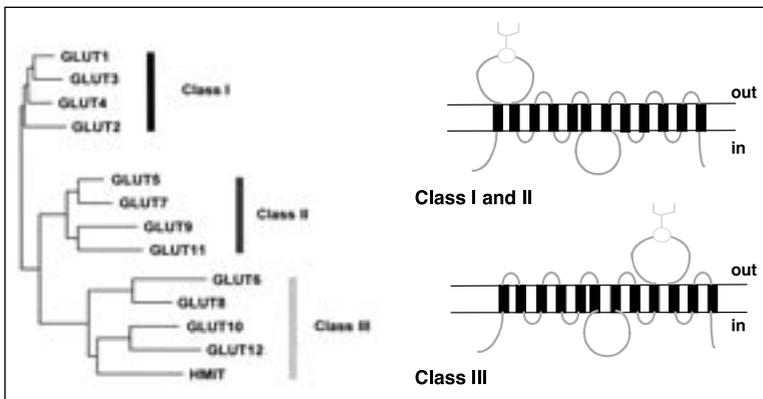


Figure 1:

Dendrogram of sequence similarities between the glucose transporter isoforms. These transporters are categorized in three classes. Class 1 consists in the first identified glucose transporters GLUT1 to GLUT4. Class 2 consists in GLUT5, which is mostly a fructose transporter, and the newly identified sequences, which are highly similar to GLUT5. The function of GLUT7, 9 and 11 is not yet firmly established. The overall structure of the class 1 and 2 transporters is depicted on the right. Class 3 consists of GLUT8, 10, 12 and HMIT. GLUT8 is a glucose transporter mostly expressed in testis and brain; HMIT is a H⁺/myo-inositol transporter present at highest level in brain, both in glial and neuronal cells. The function of GLUT10 and 12 is not yet established.

There is ample experimental evidence that facilitated diffusion glucose transporters play important roles in the control of whole body glucose homeostasis. GLUT4, the transporter which has been most extensively characterized, is present in muscles and adipocytes where it is located in intracellular vesicles. Following insulin stimulation, GLUT4-containing vesicles are translocated to, and fuse with the cell surface (8) thereby increasing the number of cell surface-exposed transporters and the rate of glucose uptake, a rate-limiting step in glucose utilization by these tissues. Over-expressing GLUT4 in adipocytes and/or muscle by transgenesis leads to a constitutive increase in glucose uptake, even in the absence of insulin. This modifies not only the transgenic tissue glucose metabolism but also whole body glucose homeostasis as shown by hypoglycemia in the fasted state and improved glucose tolerance due to more efficient glucose clearance (9-14). One of the major physiological dysfunction of type 2 diabetes, insulin resistance, is caused by inefficient stimulation by insulin of GLUT4 translocation to the plasma membrane.

Heterozygous inactivating mutations of GLUT1 in human induces a defect in the transport of glucose from the blood to the cerebrospinal fluid. This causes hypoglycorrachia, a condition characterized by seizure, delayed development and acquired microcephaly (15). Complete GLUT2 deficiency is at the origin of a very rare syndrome, initially identified by Fanconi and Bickel in Zürich in 1949, characterized by a kidney syndrome, hepatomegaly and impaired plasma glucose homeostasis(16,17). These examples of mutations in glucose transporter isoform very strikingly demonstrate that these proteins play specific and critical roles in whole body glucose regulation.

More recently, this initial glucose transporter family has been extended through bioinformatic screening of gene databases for sequences related to GLUT1 to GLUT5 (18,19). Novel sequences, referred to as GLUT6 to GLUT12 and HMIT have been identified (20) (Figure 1). The function of these newly discovered transporter-like sequences is only partly established. GLUT8 is a glucose transporter (18), and there are indications that GLUT9 (21) and GLUT10 could also transport

glucose. HMIT is a H⁺/myo-inositol symporter expressed at high level in brain, both in glial cells and in neurons (22).

The existence of a now extended family of glucose transporters certainly indicates a far greater complexity in the control of glucose homeostasis than previously anticipated. Detailed analysis of mice with inactivating mutations in each gene will certainly bring new important information on the control of glucose homeostasis.

GLUCOSE-INDUCED INSULIN SECRETION.

Insulin is the only hormone which stimulates glucose uptake and storage in peripheral tissues: liver, muscle and adipose. Its regulated secretion is therefore critical for controlling hyperglycemic excursions but also to avoid excessive secretion which could induce hypoglycemia. The capacity to sense changes in circulating glucose concentration and to precisely adapt the rate of insulin secretion relies on a specific glucose signalling mechanism present within the pancreatic β cells. This signalling pathway requires glucose uptake and catabolism through glycolysis and activation of mitochondrial metabolism. This leads to production of ATP and an increase in the cytoplasmic ATP to ADP ratio which triggers the closure of a KATP channel, depolarization of the plasma membrane and opening of calcium channels. The resulting influx of calcium then stimulates insulin secretion (23-25) (Figure 2). KATP-independent mechanisms, as yet poorly characterized, also participate in the glucose stimulation of insulin secretion (26,27).

Both the KATP channel-dependent and -independent pathways require energy production from glucose catabolism. The rate-limiting step in glucose metabolism in β cells is its phosphorylation by glucokinase. The uptake is only a permissive step allowing free access of glucose to glucokinase (28). Glucokinase activation by glucose displays a sigmoidal dose-response with a K_m for glucose of ~ 6 mM and a maximum rate reached at ~ 20 -30 mM. Glucose phosphorylation represents the rate-controlling step in glucose-stimulated insulin

secretion as indicated by the identity in the dose-dependent increase in glucose phosphorylation, utilization, oxidation and stimulated insulin secretion (23).

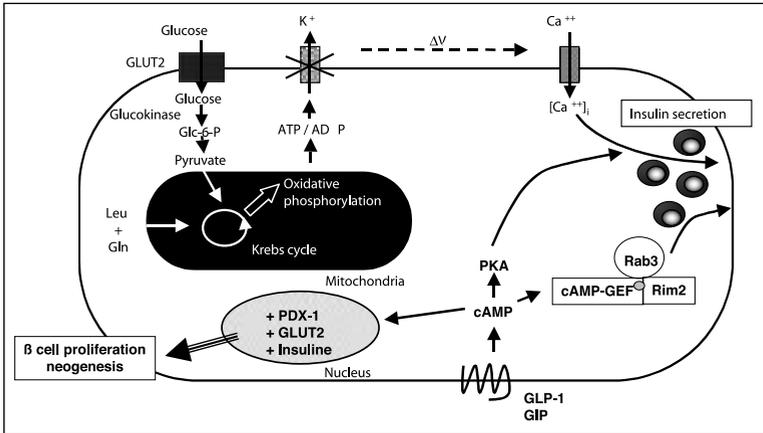


Figure 2:

Glucose and gluco-incretin signalling pathways controlling insulin secretion by pancreatic β cells. Glucose signalling to insulin secretion is initiated by glucose uptake by the glucose transporter GLUT2. Glucose is then phosphorylated by glucokinase, the rate-controlling step in glucose-stimulated insulin secretion. Activation of mitochondrial metabolism by pyruvate leads to production of ATP and an increase in the ATP/ADP cytoplasmic ratio. This leads to closure of the KATP channel, opening of voltage-gated Ca^{++} channels and the increase in cytoplasmic Ca^{++} induces insulin granule exocytosis. This pathway can be potentiated by the action of GLP-1 and GIP, which bind to specific receptors linked to activation of adenylyl cyclase and production of cAMP. Cyclic AMP then activates protein kinase A which phosphorylates a number of substrates, including GLUT2, the KATP channel, the L-type Ca^{++} channel and some as yet uncharacterized proteins involved in the exocytosis of insulin granules. A protein kinase-independent pathway involves activation by cAMP of the complex of cAMP-GEF1/Rim2/Rab3, which also potentiates glucose-stimulated insulin secretion at the exocytosis step. Production of intracellular cAMP, through as yet poorly identified nuclear pathways, also activates β cell proliferation and neogenesis.

GLUCOSE-STIMULATED INSULIN SECRETION AND GLUCAGON-LIKE PEPTIDE-1

Glucose-stimulated insulin secretion is modulated by a number of nervous and hormonal stimulations. In particular, in the postprandial state, secretion is potentiated by hormones originating from intestinal endocrine cells and which are released following nutrient absorption (29). The incretin effect of these hormones has been estimated to be responsible for about half of the postprandial insulin secretion. Today two major incretin hormones have been described: GIP (gastric inhibitory polypeptide or glucose dependent insulinotropic polypeptide) and GLP-1 (glucagon-like polypeptide-1) (30,31). GLP-1 is produced in endocrine intestinal L cells by proteolytic processing of the preproglucagon molecule (32). GLP-1 is the most potent stimulator of glucose-induced insulin secretion characterized so far (33). Interestingly, its stimulatory action requires the presence of glucose at normal or elevated levels and its incretin effect is preserved in type 2 diabetic patients. This peptide is presently developed as a new therapeutic agent for the treatment of diabetes (34,35).

GLP-1 RECEPTOR

The GLP-1 receptor was molecularly characterized by isolating a cDNA clone from a rat pancreatic islet library using an expression cloning strategy (36). The human islet receptor has also been cloned (37) and identical receptor cDNAs were also isolated from rat lung and hypothalamus. This receptor belongs to a subfamily of G-protein-coupled receptors, which includes those for other peptide hormones such as glucagon, GIP and VIP.

The potentiation of glucose-induced insulin secretion by GLP-1 is a very interesting, although not completely understood, example of cross-talk between signalling pathways (Figure 2). The GLP-1 receptor activates adenylyl cyclase and production of cAMP. This can lead to activation of protein kinase A and phosphorylation of proteins participating in glucose signalling pathway. GLUT2, the KATP channel

and the voltage-gated Ca⁺⁺ calcium are all phosphorylated by PKA. The role of these modifications is however not clear as phosphorylation reduces the rate of glucose transport by GLUT2 and increases the opening probability of the KATP channel, both effects being rather inhibitory on glucose-stimulated insulin secretion. This therefore indicates that the key phosphorylation events potentiating the effect of glucose have not yet been identified. It is known however that PKA has a major effect on insulin granule exocytosis (38-40).

Recently, PKA-independent effects have been described which rely on the activation of a cAMP sensor protein, cAMP-GEF, which has a GTP-exchange activity. CAMP-GEF can form complexes with Rim2 and Rab3, a small GTP-binding protein involved in the control of insulin granule exocytosis (41). This PKA-independent effect represents an important part of the GLP-1 effect on insulin secretion (42).

GLP-1 has also a number of additional effects on pancreatic β cells. It stimulates the biosynthesis of insulin at the transcriptional and translational levels (43,44). Recent evidence also suggests that GLP-1, when administrated to rats or mice can stimulate β cell proliferation and increase β cell mass (45,46), an effect also observed when studying diabetic animals. Because of the multiplicity of GLP-1 effects on glucose-stimulated insulin secretion, insulin biosynthesis, β cell proliferation and neogenesis, the signalling pathways activated by this receptor need to be extensively characterized. It is possible that signalling proteins belonging to these pathways become targets to generate novel drugs for the treatment of insulin secretory defects of type 2 diabetes.

IMPAIRED GLUCOSE SENSING IN GLUT2-NULL MICE

Inactivation of the GLUT2 gene in the mouse by homologous recombination led to a diabetic-like phenotype characterized by hyperglycemia, relative hypoinsulinemia and high circulating free fatty acids (47). Unexpectedly, the mice also showed hyperglucagonemia and high plasma β -hydroxybutyric acid levels. As assessed in vitro in

perfusion assays the GLUT2^{-/-} islets had lost the first phase of glucose-stimulated insulin secretion but had a preserved second phase, which was, however, of reduced amplitude as compared to control islets. The defect in stimulated insulin secretion was specific for glucose since a combination of leucine and glutamine, which induce a strong insulin secretory response by directly entering the Krebs cycle to generate coupling factors, induced the same secretory response in control and GLUT2^{-/-} islets.

Importantly, to demonstrate that the secretory defect was only due to the absence of GLUT2, we reexpressed the transporter in mutant islets by transduction of its cDNA using recombinant lentiviruses. Perfusion experiments demonstrated the complete restoration of normal glucose-stimulated insulin secretion following reexpression of GLUT2 (48).

RESCUE OF GLUT2^{-/-} MICE BY TRANSGENIC GLUCOSE TRANSPORTER REEXPRESSION.

The study of the GLUT2^{-/-} islets demonstrated that this transporter was required for normal β cell glucodetection. It was then interesting to evaluate whether this transporter could be involved in other glucose detection units and whether its localization could help identify these sensors. However, the GLUT2^{-/-} mice were not suitable to perform physiological studies since they die between two to three weeks after birth and never reached a body size compatible with in situ metabolic evaluation.

Treatment of the mutant mice by insulin injection or implantation of subcutaneous insulin pellets maintained the mice alive (47). This indicated that the insulin secretory defect was the major cause of death. Transgenic expression of GLUT2, or GLUT1, in the pancreatic β cells of GLUT2^{-/-} mice using the rat insulin promoter (RIP), rescued them from early death and restored the normal glucose-stimulated insulin secretory response (49).

These observations thus indicated that the lack of normal glucose-stimulated insulin secretion was the cause of the death of GLUT2-/- mice, even though amino acid-stimulated insulin secretion was preserved. They also demonstrated that reexpression of GLUT1, which has a lower K_m (~1-3 mM) than GLUT2 (~17 mM) was equally efficient in restoring normal sensing. Thus the absolute flux of glucose at different stimulatory concentrations, rather than the transporter K_m , is key for normal GSIS.

THE HEPATOPORTAL SENSOR

A glucose sensor is located in the hepatoportal vein area (50). It is connected through afferent hepatic branches of the vagus nerve (51) to glucose sensitive neurons in the lateral hypothalamus (52,53) and in the nucleus of the solitary tract (54). Its activation depends on the presence of a positive glucose gradient between the portal and arterial circulation (55,56), which could be established, for instance, after food absorption. This activated sensor induces anorexia (57,58), stimulates hepatic glucose uptake (55,56) and inhibits counterregulation induced by peripheral hypoglycemia (59,60) (see Figure 3).

We recently demonstrated that activation of the hepatoportal sensor by portal glucose infusion (61) induced development of hypoglycemia. This hypoglycemic effect could not be explained by an exaggerated secretion of insulin since insulinemia increased similarly when the mice were perfused through the femoral instead of the portal vein, a condition in which glycemia transiently rises to ~9-10 mM before returning to normoglycemia. Induction of hypoglycemia was correlated with a ~3-fold increase in glucose clearance rate and a preferential increased storage of glucose in brown adipose tissue, heart and soleus muscle.

Although the observed hypoglycemic effect is quite striking, many questions remained unresolved about the cellular nature of this sensor, the way it transmits its signal to peripheral tissues, and the signalling pathway activated at the target tissue level to increase glucose uptake.

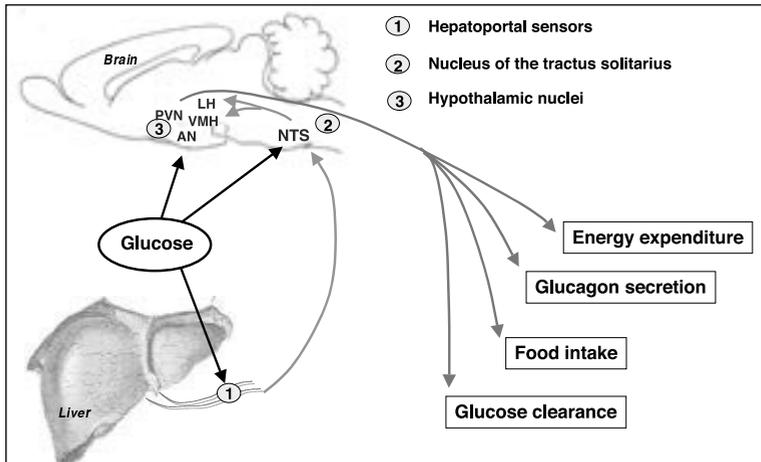


Figure 3

Multiple extrapancreatic, GLUT2-dependent, glucose sensors. 1: The hepatoportalsensor is activated by a glucose gradient established between the portal vein and the periphery. It stimulates afferent branches of the vagus nerve which project on the nucleus of the tractus solitarius (NTS) and the lateral (LH) and ventromedial (VMH) hypothalamus area. This sensor is known to control food intake, counterregulation and to stimulate glucose clearance. GLUT2-dependent sensors are also located in the nucleus of the tractus solitarius (NTS) and different hypothalamic nuclei: LH, VMH, PVN (paraventricular nucleus) AN (arcuate nucleus). The hypothalamic sensors can be directly activated by local changes in glucose concentrations. They may also be mobilized indirectly by hepatoportals or brain stem glucose sensors. Together, through autonomic nervous output, they control food intake, glucagon secretion, glucose clearance and energy expenditure.

SIMILARITY BETWEEN THE HEPATOPORTAL AND PANCREATIC β CELLS SENSORS

The function of the hepatoportalsensor was inhibited by somatostatin, as demonstrated by the absence of hypoglycemic effect when the hormone was co-infused with glucose in the portal vein (61). At the same time, no induction of glucose clearance could be observed and the glycemic profiles were the same as in mice infused with glucose in the femoral vein(62).

The requirement for GLUT2 in the function of the portal sensor was evaluated using GLUT2-null mice rescued by transgenic re-expression of GLUT1 in their β cells. Portal vein glucose infusion in these mice failed to induce hypoglycemia and to increase glucose clearance. These data therefore suggested that the portal sensor required the presence of GLUT2 for its normal function. Alternatively, as GLUT2 is the major liver transporter, it could be possible that the absence of hypoglycemia resulted from the inability of mutant hepatocytes to take up glucose. However, when the portal vein glucose infusion were realized in mutant mice in which GLUT2 was re-expressed in liver by transgenesis (63), hypoglycemia and increased glucose clearance were still not induced. This therefore indicated that GLUT2 was required for the normal function of the portal sensor and that this sensor was distinct from hepatocytes, in agreement with previous published data (50).

The role of GLP-1 on the function of the sensor was evaluated by infusing glucose in the portal vein in the presence of increasing concentrations of GLP-1. This did not change the kinetics and extent of hypoglycemia induction (64). In contrast, co-infusion in the portal vein of glucose with exendin-(9-39), an antagonist of the GLP-1 receptor, not only prevented hypoglycemia development but led to a transient hyperglycemia similar to that seen in mice infused with glucose through the femoral vein. This effect was not observed in portal vein glucose infused mice when exendin-(9-39) was infused in the femoral vein. Also, no induction of hypoglycemia could be induced in GLP-1-R^{-/-} mice infused with glucose in the portal vein. Thus, the presence of the GLP-1 receptor, which is probably constantly activated by the levels of GLP-1 which are directly secreted into the portal vein by the intestinal L cells, is required to maintain the glucose competence of the portal sensor.

Together, these data demonstrate that the hepatportal sensor shares several functional similarities with the pancreatic β cells: requirement for GLUT2 expression; inhibition by somatostatin; requirement for a functional GLP-1 receptor for glucose competence.

PHYSIOLOGICAL ROLE OF THE HEPATOPORTAL SENSOR

The capacity of the hepatoportal sensor to stimulate glucose clearance may be important in the absorptive state when a glucose gradient is established between the portal and peripheral blood. This sensor also stimulates hepatic glucose uptake (56,65,66), inhibits food intake (57,58), and blocks the secretion of counterregulatory hormones (59,60). These actions probably cooperate to minimize postprandial glycemic excursions and regulate termination of food intake. In diabetes, however, hyperglycemia may prevent this gradient to be established and the sensor may not be activated. This would participate in further accentuating the diabetic phenotype.

GLUCAGON SECRETION

Glucagon secretion is an immediate response to hypoglycemia, which stimulates glucose production from liver to ensure preservation of normoglycemia. This counterregulatory response is controlled by glucose sensors whose anatomical locations are not completely identified nor their cellular and molecular make-up. Understanding the mechanisms controlling glucagon secretion is important as defective counterregulation is a major problem limiting the treatment of diabetic patients by insulin injections. Indeed, initial episodes of iatrogenic hypoglycemia lead to increased frequency and severity of subsequent hypoglycemia.

Secretion of glucagon may be controlled at three different levels. Firstly, alpha cells possess a glucose sensor directly controlling their secretory activity (67,68). This is supported by the expression by these cells of molecules essential for gluco-detection in pancreatic β cells such as glucokinase (69) and the KATP channel subunits, SUR1 and Kir6.2 (70). Importantly, however, in contrast to β cells, alpha cells do not express the glucose transporter GLUT2 (71,72).

Secondly, intra-islet insulin may regulate glucagon secretion in particular through its reported inhibitory action on alpha cell secretory activity (73).

Thirdly, glucagon secretion is also under the control of the parasympathetic and sympathetic nervous system and the sympathoadrenal axis (74-76). The control of glucagon secretion by the autonomic nervous system appears to be dominant over the control exerted by the alpha cell sensor itself or by intra-islet insulin levels as revealed, in particular, by the possibility to block hypoglycemia-induced glucagon secretion using ganglionic blockers (74-76).

The autonomic nervous system activity controlling glucagon secretion may be activated directly by hypothalamic centers such as the ventromedial hypothalamus or the lateral hypothalamus. Whether these centers are directly activated by hypo- or hyperglycemia to control counterregulation is still a matter of debate. A major role of the hepatportal sensor in controlling counterregulation has also been proposed (50,59,60), but others favor a preponderant role of hypothalamic nuclei in the normal control of counterregulation (77,78). The respective role of each sensor in stimulating and/or suppressing counterregulation in physiological conditions is however not yet fully elucidated.

Control of glucagon secretion in the absence of GLUT2

In the absence of a functional GLUT2 gene, the glucagon levels are elevated in the fed state. As these mutant mice are normoglycemic and their rate of hepatic glucose production is similar to that of controls, this suggests that glucagon secretion may be abnormally upregulated. The presence of a constant tone of the autonomic nervous system to alpha cells was indeed evidenced by injecting mutant mice with ganglionic blockers. This rapidly led to a correction of the glucagon levels, which returned to those found in the control mice (79). Therefore, in the absence of GLUT2, there is an increased autonomic nervous system tone to the alpha cells which leads to increased glucagon secretion in the fed state.

Glucagon secretion was further evaluated during hypo- and hyperglycemic, hyperinsulinemic clamps. Whereas a hypoglycemia of 2.5 mM induced a robust secretion of glucagon in control mice, no such

stimulation was observed in mice lacking GLUT2 (79). Similarly, hyperglycemia (10 or 20 mM) reduced plasma glucagon levels in control but not in mutant mice. Thus, in the absence of this transporter, the response to both hypo- and hyperglycemia was suppressed. However, when a more profound hypoglycemia was induced by insulin injection (glycemia of ~1 mM) the same strong secretory response was observed in control and mutant mice (79). Therefore, even though control of glucagon secretion by physiologic glycaemic variations was suppressed, a response to deep hypoglycemia was still retained.

As GLUT2 is not normally expressed in alpha cells and since the mutant mice had a normal insulin secretory response to glucose, the above data indicate that regulation of glucagon secretion during physiological variations of blood glucose concentrations is controlled by extrapancreatic, GLUT2-dependent glucose sensors. The response to very low glucose levels appears however to be GLUT2-independent.

CONTROL OF GLUCAGON SECRETION IN THE ABSENCE OF KIR6.2

The KATP-channel is an essential component of the gluco-detection system of pancreatic β cells. Recently, the Kir6.2 subunit of this channel has been genetically inactivated in the mouse (80). The β cells from these mice have impaired Ca^{++} homeostasis and no longer respond to glucose or tolbutamide by the normal insulin secretory response. Secretion of glucagon in response to insulin-induced hypoglycemia was also suppressed (81), as in GLUT2^{-/-} mice. Furthermore, direct intracerebroventricular injection of 2-deoxyglucose failed to activate a glucagon response and electrophysiological measurements showed absence of glucose response of the neurons of the ventromedial hypothalamus. These data therefore further support the role of central glucodetection as a major site of control of glucagon secretion and bring additional evidence for a similarity between the β cell and central glucose sensing mechanisms.

Identity of extrapancreatic glucose sensors controlling glucagon secretion

Where are these glucose sensors located? As mentioned earlier, regulation of the autonomic tone to alpha cells may be controlled by hypothalamic nuclei, in particular the ventromedial and lateral hypothalamus. These brain regions are known to contain two types of glucose regulated neurons: “glucose responsive” neurons, whose firing rate is increased by elevation in extracellular glucose concentrations and “glucose sensitive” neurons which are activated when glucose concentrations decrease (82-85). Glucose sensitive neurons are more abundant in the lateral hypothalamus and glucose responsive neurons in the ventromedial hypothalamus (86). Glucose-modulated neurons are however also found in the arcuate nucleus, the paraventricular hypothalamus and the dorsomedial hypothalamus. Whether these neurons require GLUT2 for their glucose sensitivity is not known although GLUT2 has been reported to be expressed in the arcuate nucleus and the lateral and paraventricular hypothalamus (87,88)

Glucose responsive and glucose sensitive neurons are also present in the brain stem, in particular in the nucleus of the tractus solitarius (NTS) (89-91). This structure plays a key role in integrating internal signals and transmitting them to the hypothalamus. These glucose sensitive neurons are not protected by the blood brain barrier and are directly sensitive to changes in glycemia of small amplitudes (<1 mM), compatible with a major role in sensing glycemetic variations. These neurons are connected to the hypothalamus, in particular to the lateral and ventromedial hypothalamus. GLUT2 has also been detected in neurons of the nucleus of the tractus solitarius (87). Thus, activation by glucose of hypothalamic neurons may involve a direct sensing of glucose, but their activity may also be modulated by glucose secondarily to an activation of NTS neurons.

Hypothalamic GLUT2, insulin secretion and food intake

Hypothalamic GLUT2 may also participate in the control of other physiological functions beside the control of glucagon secretion. This has been demonstrated following intracarotid or intracerebroventricular

injection of GLUT2 antisense oligonucleotides to reduce brain GLUT2 expression. One study by the group of Pénicaud reported that this experimental protocol led to a decrease in nervous control of insulin secretion (92). The other study by the group of Martin demonstrated that intracerebroventricular injection of antisense, but not random oligonucleotides, daily for 13 days reduced food intake, body weight change and reduced food intake following injection of 2-deoxy-glucose (93). Thus GLUT2 may be required in multiple central glucose sensors controlling a diversity of physiological responses.

Concluding remarks

Understanding the pathophysiology of type 2 diabetes and developing new therapies will require to understand at the molecular levels the different dysfunctions which can lead to this disease. It is clear that this is an immensely complicated task since many defects in several different organs can initiate cascades of events leading progressively to a general impairment of glucose homeostasis. It is therefore of critical importance to establish broad-based research projects linking molecular identification of novel genes, to the study of their integrated function in whole animal physiology and pathology. Eventually, these molecular and animal studies should be confronted to the pathophysiological processes in human patients. Here, we have attempted to follow this exploratory path by starting with the molecular analysis of GLUT2, a member of a class of proteins involved in critical aspects of whole body glucose homeostasis. By physiological analysis of mice deficient in the expression of this transporter, we have uncovered a number of physiological functions controlled by this transporter and which give new perspective on the role of glucose sensing not only on glucose but more broadly on energy homeostasis. New physiological studies in human are now warranted to evaluate the role of these glucose sensors in normal physiology but also at the early stage of obesity and diabetes. Finally, with the recent discovery of eight novel glucose transporter isoforms, we can expect that new important information on the regulation of glucose homeostasis will be uncovered which, we hope, will lead to better understanding and novel treatments of type 2 diabetes.

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