“Neural Orchestration of Metabolism and Islet function”
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The International Group on Insulin Secretion – IGIS – was established in the late 1990s by a group of academic researchers to boost interest in islet biology and insulin secretion and to promote the dissemination of front-line research results to a wider medical public.

As a company with a long-standing interest in promoting research in diabetes, Servier provided IGIS with a long-term grant. Thanks to this support, a series of yearly closed symposia was initiated, each focusing on a central theme related to islet function in relation to type 2 diabetes. Attended by senior scientists and younger researchers, these symposia were developed into high-level meetings with an emphasis on extensive interaction.

The XVth Servier-IGIS Symposium, held on the theme “Neural Orchestration of Metabolism and Islet Function,” was another successful meeting where leading experts were able to interact and share their views of the subjects discussed at the meeting.

With a view to sharing the latest developments with scientists and clinicians working in the field of diabetes, the present Digest summarizes a range of topics covered at the symposium.
Claude Bernard first demonstrated the involvement of the brain in the control of glucose homeostasis, and this later led to the concept and subsequent discovery of glucose-sensing mechanisms. The brain is now considered as a center of integration of incoming nutritional, hormonal, and neuronal signals, translating this information into appropriate signals out to the periphery to control energy balance and food intake.

I. General points on the central control of energy balance and food intake

1. Integrative centers

Control of energy balance and food intake involves many brain areas and neurotransmitters, in which the cortical and limbic areas, hypothalamus, and brainstem play a central role. The hypothalamus receives information on internal and behavioral states as well as on the cognitive and nutritional aspects of food. The brain stem is considered as a central processor of peripheral information further relayed to other brain areas, particularly the hypothalamus. Both areas harbor a specific access to blood flow (the median eminence in the hypothalamus and the area postrema in the brainstem) via fenestrated capillaries, allowing detection of hormonal and nutrient signals at higher concentration than in other areas of the brain.

The hypothalamus

The hypothalamus can be divided into four principle zones (Figure 1). The periventricular zone, including the arcuate (ARC) nucleus, is mainly involved in the detection of signals from the circulation and in the organization and control of endocrine responses. The medial zone is primarily composed of large nuclei, such as the dorsomedial (DMN) and ventromedial (VMN) nuclei, which receive various sensory inputs, interconnected heavily with the rest of the hypothalamus, and which are involved in the organization
of adaptive behaviors. The lateral zone (LHA) has an extensive intra- and extra-hypothalamic communication system and could be viewed as the interface between more medial hypothalamic areas with cortical/limbic areas on the one hand and the somatic and autonomic motor systems on the other. The paraventricular nucleus (PVN) can be considered as a microcosm within the hypothalamus, because of its connection with all three effector systems (endocrine, autonomic, and behavioral).1

Within hypothalamic nuclei, the ARC nucleus has an integrator role including connections to the lateral zone, brain stem, and cortical and limbic systems.2,3 The ARC nucleus is in a position to receive an impressive array of information on energy balance. Information arrives concerning the status of long-term energy stores (via leptin from adipose tissue), intermediate available fuels (via hormones such as insulin and ghrelin and vagal afferents from the gastrointestinal tract and liver), and immediate available fuels (via local nutrient sensing). Additional interoceptive information about energy balance and other homeostatic needs can reach the ARC neurons via the abundant intrahypothalamic connections. Neural inputs from the various cortical areas and limbic structures are likely to carry information relating to emotional aspects of particular foods and to other impeding needs and behaviors. In turn, ARC neurons have easy access to endocrine effectors in the medial hypothalamus and pituitary, to cognitive reward and emotion-related areas of the forebrain, and to motor and autonomic areas of the brain stem and the spinal cord, either directly or via connections in the LHA.

In the ARC nucleus, two main populations of neurons are involved in feeding behavior (Figure 1). These populations constitute the first-order neurons of the melanocortinergic system. Neurons co-expressing proopiomelanocortin (POMC) and cocaine-amphetamine-related transcript (CART) rapidly respond to nutritional information by inducing anorexigenic signals. POMC is cleaved into melanocyte-stimulating hormones (MSH), which exert anorectic stimuli by binding to melanocortin receptors (MC3 and MC4R on second-order neurons). Conversely, neurons co-expressing neuropeptide Y (NPY) and agouti-related protein (AgRP) induce orexigenic signals. NPY/AgRP neurons have an opposite effect to POMC/CART neurons through the antagonism of AgRP on MC3 and MC4R. NPY can also directly control the activity of POMC/CART neurons.
via its binding to its Y1 receptor. POMC/CART and NPY/AgRP neurons express several nutrient and hormonal receptors, including insulin, leptin, and glucocorticoids.4

**The brain stem and the parabrachial nucleus**

The brain stem, harboring major visceral sensory and motor output pathways, is an integration center with the nucleus of the solitary tract (NTS) and the parabrachial nucleus (PBN) receiving most attention. The dorsal vagal complex is composed of the NTS, the area postrema and the dorsal motor nucleus of the vagus (dmnX). Nutrients and gastrointestinal hormones have direct access to the NTS through receptors expressed in the area postrema and via numerous projections from the area postrema to the NTS. Apart from the ARC nucleus, the NTS is the only brain area expressing POMC neurons, and the NTS and dmnX nuclei exhibit the highest level of MC4R within the brain.5 The dorsal vagal complex contains leptin receptors along with receptors and enzymatic machinery required for the detection of nutrients. Moreover, the NTS is connected to the brain areas involved in the regulation of food intake and energy balance (cortical/limbic areas and hypothalamic nuclei). Although there are no direct projections from the NTS to the cortex, there are rich polysynaptic projections via the PBN, thalamus and amygdala. Moreover, the cortex receives information from the NTS via brain stem arousal systems such as the locus coeruleus and raphe nuclei.1

The PBN is localized in the dorsal pons and is considered to integrate sensory information via reciprocal projections to various brainstem, diencephalic and forebrain areas. Concerning the regulation of energy balance and food intake, the PBN receives sensory inputs from the NTS in the medial and lateral part, which in turn project to various hypothalamic nuclei such as PVN, ARC, VMN, and LHA.3 The descending projections from the PBN are directed toward the lateral NTS and the spinal cord. Based on the strategic location and anatomical connection of the NTS and PBN, both structures are considered as part of the central processor circuit regulating energy balance and food intake.1

**2. The autonomic nervous system and pancreatic function**

The autonomic and enteric nervous systems and the pituitary-endocrine axis significantly modulate metabolic processing of food as well as the partition and oxidation of metabolites. They are thus considered as part of the output system controlling energy balance and food intake. The efferent autonomic nervous system is composed of parasympathetic and sympathetic outflows. The parasympathetic system is composed of vagal nerves and the sympathetic system is mainly composed of the splanchnic nerve.1
The autonomic nervous system plays a crucial role in pancreatic function. The parasympathetic pathway potentiates insulin secretion induced by hyperglycemia. This potentiation is mediated by acetylcholine, which binds to the muscarinic receptor m3AchR on β-cells, but might also involve PACAP, VIP, and GRP (gastrin). Parasympathetic activity can also be modulated by mild hypoglycemia to trigger the release of glucagon.7

The sympathetic system affects both α- and β-cell function. Whereas norepinephrine stimulates glucagon secretion through binding to β2 adrenergic receptors in α-cells, it inhibits insulin secretion through activation of the α2 receptors in β-cells. Sympathetic activity is an important response to hypoglycemia, which is activated at deeper hypoglycemic levels than the parasympathetic pathway.8

Moreover, the parasympathetic system is involved in the induction of β-cell proliferation in adults and in the postnatal period, whereas the sympathetic pathway has a role in the architecture and maturation of the islet during development.9

II. Mechanisms of direct detection of nutrients and hormones by the brain

1. Glucose sensing

The brain plays a pivotal role in controlling metabolic homeostasis, including the control of blood glucose. Brain glucose sensing is a key determinant of the counter-regulatory responses to hypoglycemia. Both the hypothalamus and the brain stem, areas where the blood-brain barrier is limited, play a crucial role in the response to hypoglycemia. Two populations of glucose-sensitive neurons, either activated (GE) or inhibited (GI) by glucose, have been identified in an increasing number of brain areas. The PVN and ARC nuclei of the hypothalamus, like the NTS and area postrema of the brain stem, possess both GE and GI neurons. GI neurons are particularly present in the LH nucleus and GE in the VMN. The close metabolic coupling between neurons and surrounding glial cells might also be a mechanism involved in glucose sensing. In that case, neurons would be responding to lactate produced by the catabolism of glucose in glial cells rather than to glucose per se.

Glucose sensing depends on mechanisms involving either glucose detection or glucose metabolism. Glucose detection by taste receptors or the sodium-coupled glucose co-transporter SGLT3 triggers electrogenic signals as in intestinal cells. Binding of glucose to SGLT3 promotes Na+ entry and subsequent membrane depolarization (Figure 3A).10 Taste receptor activation leads to the release of Ca2+ from the endoplasmic reticulum where the Ca2+ activates the cation channel TRPM5 (transient receptor potential member 5) leading to membrane depolarization (Figure 3B).11 (Mark Evans, Bernard Thorens, Lectures).

Glucose-sensing mechanisms based on cell metabolism are close to those used by pancreatic β-cells. In β-cells, the low-affinity transporter Glut2 and glucokinase (GK) play a key role in glucose sensing. In the canonical model, glucose catabolism induces the closure of ATP-gated potassium (KATP) channels, allowing membrane depolarization and entry of Ca2+, which triggers insulin release (Figure 3C).12

In the brain, Glut2 has a low level of expression but is dispersed in many regions with sometimes long projections to neighboring structures. In the hypothalamus, Glut2
is necessary for the regulation of the melanocortin pathway by glucose, but Glut2 protein is present in neither POMC nor NPY neurons of the ARC nucleus. However, these neurons are in close contact with numerous Glut2-positive nerve terminals, whose cell bodies have not yet been identified (Bernard Thorens, Lecture). GK also has a low level of expression in the brain, but is expressed in 20% to 30% of POMC-GE, NPY-GI, and orexin-GI neurons. Ex-vivo or in-vivo inhibition of GK in the VMN, either with inhibitors or shRNA, largely abolishes GE and GI glucose-sensing activities, whereas GK activation potentiates both GE and GI responses. Outside the hypothalamus, GK-dependent glucose sensing was also identified in the medial amygdala nucleus connected to basomedial hypothalamus. These neurons seem to exert a control loop to increase or decrease counter-regulatory responses to hypoglycemia. One-third of GK-expressing cells are glial cells, demonstrating the involvement of direct and indirect sensing mechanisms (Mark Evans, Lecture).

Metabolism-dependent glucose sensing also involves AMPK, which is widely expressed in the GE and GI neurons of the brain, and KATP channels, more particularly in the VMN. Glucose sensing in orexin GI neurons of the LHA involves K⁺ leak channels, but independently of cell metabolism. The response of GI neurons of the VMN depends on metabolism via GK and AMPK, but activates the closure of chloride channels, possibly the CFTR. These examples illustrate the diversity and the complexity of glucose-sensing systems in the brain (Figure 4) (Bernard Thorens, Lecture).

In the NTS, Glut2 is expressed in a small population of GI neurons. In these cells, glucose sensing involves metabolic processes dependent on GK, AMPK, and K⁺ leak channels.

Figure 3. Models of glucose-sensing mechanisms. A. Glucose sensing by SGLT3 based on studies in enterochromaffin cells. Glucose binding to the sodium-coupled glucose co-transporter (SGLT3) induces the entry of Na⁺, which leads to membrane depolarization and Ca²⁺ entry, which triggers the release of serotonin (5-HT). B. Glucose sensing by taste receptors. Glucose binding to taste receptors (T1R3/T1R2) activates the G-coupled protein gustducin and then the phospholipase C β2 (PLCβ2). This activation leads to the release of IP3, which induces the release of Ca⁺ from the endoplasmic reticulum. Ca²⁺ then activates the transient receptor potential member 5 (TRPM5) leading to membrane depolarization. C. Canonical model of glucose sensing in β-cells. Glucose catabolism through glucokinase (GK) and glycolysis produce ATP. The increase in ATP level induces the closure of ATP-gated potassium (KATP) channels, allowing membrane depolarization and entry of Ca²⁺, which triggers insulin release.
Glut2 neurons of the NTS are GABAergic and some project to the DMN of the vagus. Specific activation of these neurons induces an increase in vagal nerve firing followed by an increase in glucagon secretion (Bernard Thorens, Lecture).

There is no compelling evidence for an effect of brain GK on appetite and energy balance. However, inhibition of GK in the third ventricle stimulates feeding responses to glucoprivation via the activation of NPY and orexigenic cells. As mentioned above, brain Glut2 is involved in the stimulation of food intake induced by glucose via an indirect regulation of the melanocortin pathway. Interestingly, glucose sensing in the hypothalamus controls the early phase of insulin release by a mechanism depending on GK (Mark Evans, Bernard Thorens, Lectures).

2. Fatty acid sensing

Fatty acid–sensitive neurons are present in the brain, especially in the hypothalamus, and participate in the control of energy homeostasis. Infusion of fatty acids in the hypothalamus decreases food intake, sympathetic activity, and hepatic glucose production and is associated with exaggerated glucose-stimulated insulin secretion. The brain can detect free fatty acids crossing the blood-brain barrier, but also free fatty acids delivered locally from the hydrolysis of triglycerides. Inactivation of lipoprotein lipase in the hypothalamus induces an increase in body weight through a ceramide-dependent pathway.

As with glucose sensing, fatty acid sensing involves pathways dependent on and independent of cellular metabolism. Neurons of the VMN express fatty acid transporters and the main enzymes involved in fatty acid metabolism (such as long-chain acyl-CoA synthase-ACS, carnitine palmitoyltransferase-CPT1, and uncoupling protein 2-UCP2). Fatty acid catabolism is associated with an increase in the acyl-CoA intracellular pool, which is considered as the “final” satiety signal rather than fatty acids themselves. Fatty acids are indeed detected via the production of ATP through β-oxidation.

The central inhibition of CPT-1 (rate-limiting step in mitochondrial β-oxidation) mimics the effect of fatty acids on energy homeostasis. Malonyl-CoA is a potent inhibitor of CPT-1 and is produced by fatty acids via β-oxidation or by glucose via glycolysis. An increase in malonyl-CoA and acyl-CoA levels is associated with reduced food intake, suggesting that malonyl-CoA may be the metabolic sensor of energy levels in the hypothalamus. Since a
selected population of fatty acid–sensitive neurons also sense glucose, the responses to fatty acids is dependent upon the metabolic state of the animal. In addition, since so much fatty acid oxidation takes place in the astrocytes, the latter probably contribute to brain fatty acid sensing. Astrocytes might have their primary effects by the production of ketone bodies, which are further utilized by neurons to alter their fatty acid and glucose sensing (Figure 5).

The hypothalamus contains two populations of fatty acid–sensitive neurons: one excised by oleic acid and another inhibited by oleic acid. Neurons of the ARC nucleus detect fatty acids mainly by mechanisms dependent on cell metabolism. The excitatory effect of oleic acid is due to the closure of chloride channels leading to membrane depolarization and increased action potential frequency. The inhibitory effect of oleic acid involves KATP channels. The β-oxidation of oleic acid induces an increase in POMC and a decrease in NPY expression in the ARC nucleus.

In VMN neurons, cell metabolism accounts for a relatively small percentage of fatty acid sensing. Fatty acid sensing is almost completely abolished by inhibition of the fatty acid transporter CD36. In most fatty acid–sensitive neurons, CD36 may act primarily as a receptor for long-chain fatty acid–activating store-operated calcium channels to alter membrane potential (Figure 5). Reduction of CD36 in VMN neurons decreases expression of AgRP and POMC. Finally, some fatty acids, such as palmitic acid, alter cell signaling molecules by altering their function (covalent attachment) or their location in the membrane.

3. Brain glucose sensing and glucose effectiveness

The regulation of hepatic glucose uptake by insulin through an indirect mechanism involving the brain is still a source of controversy. However, several findings point to a role of the brain in the decrease of hepatic glucose uptake by insulin. For example, the inactivation of the insulin receptor in the hypothalamus by antisense oligonucleotides impairs the suppression of HGP by insulin. The mechanism involves K\textsubscript{ATP} channels, which are targeted by the IRS-Pi3K pathway in some neurons and which induce the activation of efferent vagal fibers. In the DVC, some neurons also respond
to insulin, but the mechanism involves the ERK pathway. Moreover, in streptozotocin-induced diabetic mice, the capacity of insulin to decrease blood glucose levels is attenuated by a central PI3K inhibitor. The collective data suggest that both the direct and the indirect pathways are sufficient to control HGP and that when one does not function properly, the other can compensate. Insulin might regulate HGP indirectly via a FoxO1-independent mechanism, which is blocked by excessive FoxO1 signaling (Michael Schwartz, Lecture).

Secretion of FGF15/19 by enterocytes is induced by bile acids via FXR signaling. FGF19 improves glucose tolerance in diet-induced obesity mice, while inactivation of FGF19 impairs glucose tolerance. FGF19 signaling might depend on FGFR1, which is expressed in the brown and white adipose tissue and in the mediobasal hypothalamic areas. Infusion of FGF19 in the brain of diet-induced obese mice or ob/ob mice improves glucose tolerance. The systemic effect of FGF19 is blunted by 50% with the infusion of an FGFR antagonist in the brain, demonstrating the key role of the central nervous system. The beneficial effect of FGF19 depends on an increase in glucose effectiveness, or the ability of glucose to stimulate its own disposal independently of insulin. Glucose effectiveness contributes at least as much as insulin to normal glucose tolerance and is altered in type 2 diabetes and in ob/ob mice.

III. Gastrointestinal and vagal detection of nutrients

Food intake is regulated by hormonal and physical sensation signals from the gastrointestinal tract. Food ingestion notably induces gastric distension, changes in gut motility, and release of several gastrointestinal hormones, such as ghrelin, cholecystokinin (CCK), peptide YY
t_{3-36} (PYY\_3-36), and glucagon-like peptide-1 (GLP-1). The enteric neural system plays a role in the transmission of the physical sensation from the gastrointestinal tract to the brain, but also in the central and systemic effects of these hormones. Disruption of afferent vagal-brain communication markedly suppresses the control of food intake mediated by gastrointestinal hormones. Recent data demonstrate a crucial role of vagal-brain communications in the transmission of regulatory signals produced by specific nutrients.

Glutamate and aspartate are amino acid neurotransmitters that have a unique taste, known as the “umami” taste. Glutamate is first detected during mastication in the mouth, and this oral stimulus is transmitted to the NTS through the autonomic facial, glossopharyngeal, and vagal nerves. This signal is part of the cephalic phase of digestion preparing the body to eat. Second, glutamate sensing takes place in the stomach and is transmitted to the brain by vagal afferent fibers. Functional magnetic resonance imaging demonstrates that detection of glutamate in the stomach leads to a specific activation of brain areas regulating learning, emotion, and memory (hippocampus and amygdala), and of areas controlling thermogenesis (DMN of the hypothalamus and medial pre-optic area). Unlike glucose, glutamate sensing in the stomach does not activate brain centers involved in reward and addiction. Brain activation by glutamate is abolished by subdiaphragmatic total vagotomy. Since glutamate is completely metabolized by the intestine, glutamate sensing is restricted to the gastrointestinal tract. Glutamate is detected by its binding to the metabotropic glutamate receptor type 1 in mucus cells. Mucus cells expressing NO synthase release NO on their basal sides inducing the release of serotonin from enterochromaffin cells and the further activation of the vagal nerve. This activation of the autonomic nervous system by glutamate sensing induces
digestive secretions to improve food digestion and increase nutrient absorption. A role of glutamate sensing in the regulation of energy homeostasis has recently been demonstrated. Rats fed a high-fat diet containing 1% glutamate were resistant to the development of obesity and harbored lower fat deposits, with reduced circulating leptin levels. Functional magnetic resonance imaging suggests that glutamate sensing likely induces thermogenesis and this additional energy expenditure prevents fat deposition and obesity (Figure 6). (Kunio Torii, Lecture)

Vagal brain communications are also crucial for the maintenance of essential amino acid homeostasis. The central nervous system is able to identify the sensory characteristics of a food deficient in an essential amino acid such as lysine. Following the detection of lysine deficiency, the brain develops a specific behavior resulting in motivation to eat other foods that potentially contain lysine. This detection is linked to the induction of the sensitivity of the afferent vagal hepatic branches to lysine. The presence of lysine in the stomach induces the activation of the hippocampus and the hypothalamus, more particularly the LHA. Lysine deficiency results in an increase in the magnitude of this activation and in the activation of other brain areas. Notably, lysine deficiency results in the appearance of lysine-sensitive neurons in the nucleus accumbens. The nucleus accumbens is connected to the VMA, which is involved in the regulation of the motivation to eat. This mechanism could be linked to the development of the motivational behavior induced by a lysine-deficient diet. Moreover, neural plasticity of specific neurons of the LHA allows the CNS to associate environmental (sound), sensory (smell, taste), and digestive (visceral information) characteristics with lysine-deficient food. This plasticity is induced by the activation of activin A in the olfactory bulb (smell), LHA (feeding), the median eminence of the hypothalamus, and the NTS (tastes and visceral information). Interestingly, the neurons of the LHA that become sensitive to lysine were previously activated by glutamate (Kunio Torii, Lecture).

The anatomic location of the portal vein makes this area highly suitable for nutrient sensing. An increase in portal glucose levels induces a decrease in food intake, changes in food preference, and an improvement in hepatic insulin sensitivity. Portal glucose sensing is also involved in the detection of slow
developing hypoglycemia. Portal glucose sensing depends on afferent vagal nerve stimulation, where glucose is detected in the wall of the portal vein and induces a signal transmitted to the brainstem by both vagal and spinal afferents. A high glucose level in the portal vein seems to be detected by Glut2, whereas low glucose is detected via its binding to SGLT3 (Gilles Mithieux, Lecture).

Sensors present in the portal vein are also involved in the inhibition of food intake induced by a protein-enriched diet. Oligopeptides derived from the digestion of dietary protein are delivered to the portal blood. These peptides have an antagonistic effect on the µ-opioid receptors present in the portal nervous system and activate brain regions receiving inputs from both the vagal afferents (DVC) and the spinal afferents (PBN), and the hypothalamic nuclei regulating food intake (PVN). This in turn induces the expression of gluconeogenic genes (glucose-6-phosphatase and phosphoenol-pyruvate carboxykinase) in the intestine and the further release of glucose in the portal vein. This glucose is then detected by the glucose sensor SGLT3 and induces a decrease in food intake. In summary, proteins act indirectly on food intake by inducing intestinal gluconeogenesis and its sensing by the portal glucose sensor (Figure 7A and C). Proteins reduce food intake by a mechanism independent of the melanocortinergic pathway, as paradoxically, a protein-enriched diet induces increased expression of orexigenic AgRP and decreased expression of the anorexigenic POMC protein (Gilles Mithieux, Lecture).

Dietary fiber improves insulin sensitivity and glucose tolerance in lean and obese subjects. In the distal gut, soluble dietary fiber is fermented by the microbiota into short-chain fatty acids (acetate, propionate, and butyrate). Propionate and butyrate

Figure 7. Nutrient control of energy homeostasis via intestinal gluconeogenesis and portal glucose sensing (with the permission of G. Mithieux). Panel A: The digestion of dietary protein (1) results in the release of peptides in the portal vein (2). Peptides bind to and antagonize MORs present in the peri-portal afferents (3), leading to activation of brain targets through both the vagal and the spinal pathway (4). A reflex arc promotes the induction of the expression of gluconeogenesis genes in the intestine (panel 1C). Panel B: After a meal rich in dietary fiber (1), the latter is fermented by the microbiota, producing propionate and butyrate (2). Butyrate directly activates the expression of gluconeogenic genes via a cAMP-driven mechanism (panel 1C). Propionate released in the portal vein (3) binds as an agonist to FFAR3 (4), leading to activation of brain targets through both the vagal and the spinal pathway (5). A reflex arc drives the induction of gluconeogenic gene expression in the gut (panel 1C). Panel C: In addition to their indirect effect on gluconeogenic genes, amino acids deriving from dietary protein or propionate deriving from dietary fiber can serve as glucose precursors of intestinal gluconeogenesis. Intestinal gluconeogenesis (5) promotes glucose release in the portal vein (6). Glucose binds to SGLT3 as an agonist (7), which sends a second message to the brain to decrease hepatic glucose production and initiate various metabolic benefits, all processes driven by the brain. (Gilles Mithieux, Lecture)
both stimulate intestinal glucose release by inducing the expression of gluconeogenic enzymes in the intestine and in the colon. In addition, propionate is used as a substrate for glucose production. Butyrate induces gene expression by increasing cAMP levels in enterocytes, which is known to induce the expression of gluconeogenic genes. As oligopeptides, propionate induces gluconeogenic gene expression indirectly via a portal-brain neural circuit initiated by agonist binding to the fatty acid receptor FFAR3. The causal role of intestinal gluconeogenesis in the beneficial effect of protein or fiber has been confirmed by the use of intestinal gluconeogenesis-deficient mice. Moreover, using these mice confirms that even if the microbiota is important for the conversion of fiber into short-chain fatty acids, the changes in microbiota composition per se have no role in the beneficial effect of dietary fiber.

These recent data highlight the key role of the portal glucose signaling in nutrient sensing by the brain. Nutrient sensing by the portal nervous system induces a reflex arc with the brain, thus inducing intestinal gluconeogenesis. Portal glucose sensing might thus be related to a prolonged effect of hunger sensation, namely a "satiety" phenomenon, rather than to a "satiation" phenomenon.

IV. Control of β-cell function by the brain

Autonomic activation, particularly sympathetic neural activation, contributes to the glucagon response to hypoglycemia. The autonomic nervous system accounts for 75 to 90% of the glucagon response to marked hypoglycemia and there is sequential recruitment of each arm of the autonomic nervous system as hypoglycemia deepens. In addition, sensory inputs involved in the cephalic phase of insulin secretion also induce the parasympathetic system, thus inducing anticipatory responses that prepare the body for efficient utilization of glucose.

1. Early loss of islet sympathetic nerves

The glucagon response to insulin-induced hypoglycemia is severely impaired in type 1 diabetic patients. Data show that this defect is not due to impairment of the adrenal medullary or parasympathetic branch. However, type 1 diabetic patients exhibit a 93% loss of islet sympathetic nerves. This early sympathetic islet neuropathy is not due to diabetic neuropathy induced by hyperglycemia since it develops before diabetes in animal models of type 1 diabetes. Type 1 diabetes is characterized by invasive insulitis and by a progressive loss of β-cells. The release of sympathetic neurotrophins such as NGF by β-cells could regulate sympathetic innervation. However, specific experiments demonstrate that early sympathetic islet neuropathy is neither linked to decreased secretion of nerve growth factor by β-cells in response to islet destruction, nor to lymphatic infiltration of islets. Sympathetic neurons express the neurotrophin receptor p75 (p75NTR), and agonists of p75NTR induce a rapid sympathetic denervation due to localized pruning of axonal segments, which leaves both the parent axon and the neuronal cell body intact. Inactivation of p75NTR in a type 1 diabetes mouse model prevents early sympathetic islet neuropathy, but does not affect lymphocyte infiltration, or the onset and magnitude of diabetic hyperglycemia. An increase in BDNF within the infiltrated islet likely activates the p75NTR causing the loss of islet sympathetic nerves. However, BDNF-releasing cell types within the islet and the stimulus triggering this release have yet to be identified.
2. The autonomic nervous system and pulsatile secretion of insulin

Pulsatile secretion of pancreatic hormones is physiologically important and compromised in diabetes. This secretory pattern is controlled by neurotransmitters released from islet cells, which shape the pulse in auto-paracrine feedback loops. Blood insulin and glucagon levels oscillate in opposite phase, independently of central neural influences, and the loss of this phase relationship has been associated with prediabetes. Glucose, neurotransmitters, and signaling molecules produced by the islet control pulsatile insulin release. In β-cells, Ca\(^{2+}\) oscillations induced by glucose metabolism might depend on oscillatory activity of rate-limiting glycolytic enzymes and/or mitochondrial metabolism. On one hand, an increase in ATP production drives influx of Ca\(^{2+}\), which temporarily interrupts the increase of ATP, while on the other, processes extruding Ca\(^{2+}\) from the cytoplasm increase energy consumption. Thus, mutual ATP-Ca\(^{2+}\) interaction may generate intracellular calcium oscillation underlying pulsatile insulin secretion.

In addition to this internal β-cell oscillator, autocrine feedback mechanisms are important for shaping the pulses. Positive feedback loops may help to initiate secretory pulses whereas negative feedback loops could be involved in their termination. The neurotransmitters and signaling molecules, which are released by β-cells in parallel to insulin, participate in the feedback regulation of intracellular calcium oscillations. Insulin controls Ca\(^{2+}\) levels via binding to its receptor. Negative feedback regulation by insulin may involve autocrine signaling: PI3K-dependent formation of phosphatidylinositol(3,4,5)-triphosphate in the plasma membrane is able to activate KATP channels, which then leads to reduction in intracellular Ca\(^{2+}\) levels. ATP stimulates insulin release by its interaction with ionotropic P2X receptors, whose cation permeability adds to voltage-dependent Ca\(^{2+}\) influx. ATP also interacts with G-protein-coupled receptor P2Y, which leads to increased intracellular Ca\(^{2+}\) levels via the activation of different phospholipases. In mice, ATP also has an inhibitory effect on insulin secretion via the mitochondrial Ca\(^{2+}\) release channel. Pancreatic islets contain high concentrations of GABA released in parallel with insulin. GABA has a hypothetical role in pulsatile insulin release by promoting the early depolarization and/or the termination of insulin pulses. Zn\(^{2+}\) co-released with insulin might exert negative feedback on β-cells by inducing a decrease in cAMP formation. A loss-of-function mutation in the zinc transporter ZnT8 protects against the development of type 2 diabetes, indicating the physiological importance of Zn\(^{2+}\) in β-cell autocrine regulation. Both serotonin and dopamine inhibit insulin secretion and may therefore participate in negative feedback of insulin release. However, serotonin and dopamine seem to be released from β-cells rather than from nerve terminals.

Intracellular calcium oscillations are synchronized by depolarization spreading in clusters of β-cells and within islets via gap junctions and additional humoral and neural factors. At the level of the organ, pulsatile release of insulin is synchronized by the autonomic nervous system. ATP can synchronize glucose-induced calcium oscillations by the regulation of PKC activity via P2Y receptors. Neural signals such as NO, CO (from nonadrenergic, noncholinergic neurons), and acetylcholine (from sympathetic neurons) promote synchronization of calcium oscillation (Figure 8). As for insulin secretion, Ca\(^{2+}\) is believed to be the main trigger of glucagon release. In α-cells, Ca\(^{2+}\) oscillations are induced in parallel to the stimulation of glucagon secretion by low concentrations of glucose. Control of glucagon secretion depends on cell metabolism, but a consensus is lacking on how this metabolism controls Ca\(^{2+}\) influx. Several mechanisms have been proposed, but the most convincing one suggests that glucose metabolism induces Ca\(^{2+}\) sequestration in the endoplasmic reticulum.
lum via the activation of the SERCA pump. This triggers the entry of Ca^{2+} in the cells and the stimulation of glucagon secretion. The most obvious positive feedback loop is glucagon, which amplifies its own secretion by raising cAMP. Synchronization of Ca^{2+} oscillations is not performed by gap junctions, which are lacking in α-cells. Ca^{2+} oscillations are not synchronous in the presence of low glucose levels. The synchronization of pulsatile glucagon release and Ca^{2+} oscillations are controlled by paracrine signals from β-cells (insulin, Zn^{2+}, ATP, and GABA). These regulations do not control glucagon secretion when blood glucose is low, but might take place at high glucose concentrations when secretion of insulin and glucagon is pulsatile and in opposite phase. Somatostatin is a potent inhibitor of glucagon release and might thus also contribute to the regulation of pulsatile secretion of glucagon under hyperglycemic conditions. It is unclear if neural signaling has direct effects on the synchronization of glucagon pulsatility from the pancreas or if it is entirely mediated by paracrine factors secondary to the coordination of insulin pulsatility (Erik Gylfe, Lecture).

3. Cooperation between cAMP signaling and sulfonylurea in insulin secretion

cAMP regulates exocytosis by directly phosphorylating proteins of the exocytotic machinery in secretory cells. Intracellular cAMP signaling involves the protein kinase A (PKA), the cAMP guanine nucleotide exchange factor EPAC2, and cAMP-gated ion channels. In β-cells, few proteins affecting insulin secretion are phosphorylated by PKA. EPAC2, which possesses guanine nucleotide exchange factor activity towards the GTPase Rap, is required in β-cells for the potentiation of insulin secretion by cAMP. Sulfonylureas stimulate insulin release by binding to the SUR1 subunit of the K_{ATP} channel. Inactivation of EPAC2 in mice shows that EPAC2 is necessary for the effect of cAMP and sulfonylureas on insulin secretion. cAMP levels are increased via activation of G-protein–coupled receptors such as GIP or GLP1 released by enterochromaffin cells after a meal, or such as the neurotransmitters of the vagus nerve (PACAP or VIP). This increase in cAMP potentiates the first and second phases of insulin secretion induced by glucose. The binding of norepinephrine and epinephrine (released by sympathetic nerves or adrenal gland) to Gi-coupled α2 receptors induces a decrease in cAMP levels. This decrease is linked to membrane repolarization, which
induces a decrease in Ca$^{2+}$ influx resulting in the inhibition of insulin secretion. Inactivation of the Ca subunit of PKA in β-cells induces the activity of PKA and stimulates the acute phase of insulin secretion. PKA stimulates insulin secretion by phosphorylating proteins, which promotes the interaction between exocytosis-associated proteins. As an example, PKA phosphorylates MyRIP, a scaffolding protein linking PKA to exocytosis-related protein, exocyst complex, and myosins. In mouse pancreatic β-cell lines, EPAC2 activates Rap1 in a cAMP-dependent manner. This activation is required for the first phase of potentiation of insulin secretion. The potentiating effect of incretins is linked to the interaction of EPAC2 with Rim2a, an effector of the small G protein Rab3. Activation of EPAC2 also stimulates a rise in Ca$^{2+}$ levels via the inositol 1,4,5-triphosphate receptor and ryanodine receptor. Monitoring of EPAC2 activation in β-cells demonstrates that many sulfonylureas (except for gliclazide) directly activate EPAC2/Rap1 signaling. β-Cells from EPAC2−/− mice have a lower response to tolbutamide, confirming in vitro data. The activation of EPAC2 by sulfonylurea depends on the cAMP-binding domain of the protein. The number of hydrogen bonds between this cAMP-binding domain and the core of the sulfonylureas determines the binding affinities of the drug with EPAC2. Moreover, binding and activation of EPAC2 by sulfonylurea require the presence of cellular cAMP. The reduced cAMP levels induced by adrenalin block the activating effect of sulfonylurea on EPAC2 and Rap1. Thus, in a situation in which the sympathetic nerve is stimulated, the effect of sulfonylurea on activation of EPAC2 and Rap1 might be diminished (Figure 9).

In humans, the interacting effect between incretin/cAMP signaling and sulfonylurea through EPAC2 has to be taken into account when establishing diabetes therapies (Susumu Seino Lecture).

**4. Autonomic nervous system and β-cell function**

At the level of the endocrine pancreas, the autonomic nervous system influences acute hormone secretion, but also developmental processes required to establish islet structure and α- and β-cell number. These developmental processes are also regulated by glucose through glucose-sensing mechanisms in the CNS. During the weaning period, carbohydrates present in the diet activate the parasympathetic activity through Glut2-dependent glucose sensing and induce β-cell proliferation. This stimulatory effect of glucose is restricted to the early postnatal period, but is critical for the long-term control of β-cell mass and function. The specific inactivation of
Glut2 in neurons induces a defect in the stimulation by the parasympathetic activity of β-cell proliferation at the weaning period in mice. This early defect results in impaired glucose-stimulated insulin secretion leading to progressive impaired glucose intolerance (Bernard Thorens, Lecture).

V. Conclusion

The data summarized above highlight the complexity of nutrient and hormone sensing and the prominent role of the hypothalamus as an integrative center. Subpopulations of neurons, particularly in the hypothalamus, are selectively either activated or inhibited by nutrients. While interaction of nutrients with specific receptors modulates activity of a specific population of neurons, some of the signaling effects of nutrients appear to be dependent on intracellular metabolism, production of ATP, and the subsequent activation of the $K_{ATP}$ channel. As an example, neurons need to integrate signals from glucose and fatty acid metabolism as well as metabolic signals from surrounding cells such as astrocytes. Thus, these neurons are ideally suited to respond differentially under a variety of metabolic conditions. These complex cross-talk mechanisms should provide clues to an understanding of the relationship between brain sensing and metabolic diseases such as type 2 diabetes.

Data presented here also point out the crucial role of gut-brain interaction in nutrient and hormone sensing and regulation of energy homeostasis. Particularly, the portal vein, which receives the primary information of nutrients and gastrointestinal hormones, is a strategic point of indirect metabolic sensing by the brain. Interestingly, nutrient sensing in the portal vein requires the induction of intestinal gluconeogenesis through a reflex arc, allowing the nutrients to extend the duration of their signal to the brain after digestion, during the post-absorptive state.

Finally, the brain translates sensory information to control energy homeostasis and more particularly insulin secretion, via the autonomic nervous system. The modulation of the autonomic nervous system by brain glucose sensing is crucial for the counter-regulatory responses induced by hypoglycemia and for the proliferation of β-cells in the postnatal period. In addition, this system is required for the fine tuning of insulin secretion, including the regulation of insulin secretion pulsatility and the potentiation mechanisms of insulin release.

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Bibliography:

Lectures during IGIS meeting

- Andrea Brand (Cambridge): Nutrient control of neural stem cells
- Mark Evans (Cambridge): Brain glucose sensing
- Erik Gylfe (Uppsala): Neurotransmitters and oscillation of pancreatic hormones
- Tony Lam (Toronto): Role of vagus in hepatic glucose production
- Wolfgang Langhans (Zurich): Enterocyte - vagus interactions in fat sensing
- Christophe Magnan (Paris): Brain FFA sensing
- Gilles Mithieux (Lyon): Metabolic effects of portal vein sensing
- Susumu Seino (Kobe): Cooperation between cAMP signalling and sulfonylureas in insulin secretion
- Michael Schwartz (Seattle): The brain and metabolic control
- Jay Taborsky (Seattle): Intra-islet neural regulation of islet function
- Tony Tang (Hsinchu): Imaging of the islet neural network
- Bernard Thorens (Lausanne): Neural regulation of islet cell mass and function
- Kunio Torii (Tokyo): Brain amino acid sensing
- Morris White (Boston): IRS signaling balances metabolic homeostasis and neurodegeneration
- Derek Wildman (Detroit): Human cerebral cortical gene expression and glucose metabolism
- Toshihiko Yada (Shimotsuke): Ghrelin signalling in insulin secretion
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