Pancreatic α-cell dysfunction in diabetes

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Abstract

A major, yet poorly understood, feature of type 2 diabetes is the excessive hepatic glucose production and the corresponding insulin resistance leading to fasting hyperglycaemia. The tremendous amount of work done to provide the physiological and molecular mechanisms explaining this impairment has led to the emergence of several consensual hypotheses. Among these, is the increased daily and unregulated plasma glucagon concentration in type 2 diabetic patients. Therefore, studies aiming to understand the physiological regulation of glucagon secretion and the corresponding impairment during diabetes are directly relevant to the treatment of type 2 diabetes. Glucagon secretion by α-cells is an immediate response to glucopenia. Abnormal secretion of glucagon and other counterregulatory hormones is a hallmark of type 1 and type 2 diabetes and a major limitation to the use of strong hypoglycaemia agents. A few molecular mechanisms of glucose detection triggering counterregulation and in particular inducing glucagon secretion or suppressing it during hyperglycaemic episodes, have been identified. Such mechanisms are related to those of the insulin secreted β-cell. The glucose transporter GLUT2 and the K-ATP dependent channel, as well as regulatory mechanisms, involved the central nervous system and the gut-brain hormone GLP-1. Over the last years, glucoincretins have provided promising results for the normalization of plasma glucagon concentration of type 2 diabetic patients, which could partly explain the therapeutic benefits of incretin-related therapy. The underlined mechanisms of GLP-1 regulated glucagon secretion are most likely related to the action of the hormone on the activation of the portal and brain glucose sensors. Certainly, strategies aiming to restore glucose-regulated glucagon secretion are important milestones for the treatment of diabetic patient and the prevention of iatrogenic hypoglycaemia.

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Résumé

Dysfonction de la cellule α-pancréatique au cours du diabète

L’une des caractéristiques majeures, bien qu’encore mal connue, du diabète de type 2, est l’excès de production hépatique de glucose et l’insulinorésistance qui lui est liée, conduisant à une hyperglycémie à jeun. Le nombre considérable de travaux effectués pour mettre en évidence les mécanismes physiologiques et moléculaires expliquant cet excès de production a conduit à l’émergence de plusieurs hypothèses consensuelles. Parmi celles-ci, figure l’augmentation journalière et non régulée de la concentration plasmatique de glucose au cours du diabète de type 2. En conséquence, les études ayant pour but de comprendre la régulation physiologique de la sécrétion de glucagon et les altérations au cours du diabète sont d’un intérêt direct pour le traitement du diabète de type 2. La sécrétion de glucagon par les cellules α-pancréatiques est une réponse immédiate à la glucopénie. La sécrétion anormale de glucagon et d’autres hormones contre-régulatoires est une caractéristique des diabétiques de type 1 et de type 2, et une limitation majeure à l’utilisation d’agents hypoglycémiants puissants. Quelques mécanismes moléculaires de la détection du glucose, déclenchant les phénomènes contre-régulateurs, et en particulier provoquant la sécrétion de glucagon, ou la supprimant, durant les épisodes hyperglycémiques, ont été identifiés. Ces mécanismes sont liés à ceux qui régulent la sécrétion d’insuline par les cellules β-pancréatiques. Le transporteur du glucose GLUT2 et les canaux K-ATP dépendants, tout comme les mécanismes de régulation, impliquent le système nerveux central et l’hormone cérébro-intestinale GLP-1. Ces dernières années, les gluco-incretines ont montré des résultats prometteurs pour la normalisation des concentrations plasmatiques de glucagon chez les patients ayant un diabète de type 2, ce qui peut expliquer en partie, le bénéfice thérapeutique observé avec les traitements agissant sur les hormones incrétines. Les mécanismes sous-jacents du GLP-1 régulant la sécrétion de glucagon sont vraisemblablement liés à l’action de cette hormone sur l’activation de capteurs de glucose au niveau du système porte et du cerveau. Il est vraisemblable que les

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stratégies thérapeutiques visant à restaurer la sécrétion de glucagon régulée par le glucose représentent une étape importante pour le traitement des patients diabétiques et pour la prévention des hyposglycémies iatrogènes.

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Keywords: Diabetes; Glucagon; hypoglycaemia; Glucose sensor.

Mots clés : Diabète ; Glucagon ; Hypoglycémie ; Senseur de glucose.

1. Introduction

The undercovered mechanisms of glucagon secretion and action are not yet established and are actually mostly controversial. The discrepancies reside essentially on:

1) The glucose detection system, i.e. the direct and indirect pathway regulating the function of the α-cell;

2) The glucagon targeted tissue and the corresponding functions involved in the regulation of blood glucose during type 2 diabetes.

We will here provide molecular evidences to discuss the relevance of -cell impairment during type 2 diabetes. The impaired action of glucagon and their deleterious effect on glycaemia are beyond the scope of this review.

Glucagon secretion by α-cells is an immediate response to glucopenia. Since hypoglycaemia is a major acute complication of diabetic patients, essentially type 1 diabetes, contributing to its morbidity and mortality, it is of a major importance to determine the molecular mechanisms responsible for hypoglycaemia-induced glucagon secretion. On the other hand, hyperglycaemia is also an important contributor to diabetic complications as defined by the so-called glucotoxicity. This involves that excessive blood glucose concentration impairs the cell function by the mean of the production of reactive oxygen species or the production of advanced glycation-end products [1]. Therefore, it is important to understand the molecular and regulatory mechanisms that mediate the normal glucagon response to hypoglycaemia and hyperglycaemia and to determine how these mechanisms are impaired in diabetic patients.

2. Hypotheses for the regulation of glucagon secretion

Glucagon is a small peptide hormone; its secretion is stimulated by hypoglycaemia and inhibited by hyperglycaemia. The control of glucagon secretion is multifactorial. Roughly, three hypotheses can be proposed for the control of glucagon secretion.

- First, the direct hypothesis implies that variation in plasma glucose concentration is directly detected by the α-cells and regulates glucagon secretion [2,3]. In support to this hypothesis was that infused-rat pancreas regulates glucagon secretion in response to stepwise increment of glucose plateaus [4-6]. Other more recent evidences show that a single α-cell secretes glucagon by a mechanism that mirrors the secretory mechanism of β-cells [7]. Pancreatic α-cells are electrically excitable and generate spontaneous Na+ and Ca2+ dependent action potentials [8, 9]. Glucagon release is Ca2+ dependent through a mechanism in tight relationship with N-type Ca2+ channels at low glucose concentration [10]. Furthermore, evidences showed that α-cells are equipped with ATP-sensitive K+ channels of the same type as those constituting the resting conductance in β-cells [11,12]. Along the line of these hypotheses it has been recently shown that proteins involved in protein-protein interaction systems of exocytosis like SNAREs are also implied in the regulation of glucagon secretion together with the α-gated K+ and Ca2+ channels. These proteins and the SNARE associated proteins Munc-13-1, Munc-18a, caveolins, syntaxin 1A, SNAP-25, VAMP-2 are expressed in α-cells and targeted to lipid raft domains on α-cell plasma membranes. The biochemical disruption of lipid raft domain prevents inhibition of α-type gated K+ currents and enhancement of glucagon secretion from α-cells [13].

However, these results were confounded by the complexity of the pancreas. This involves the presence of the insulin secreting β-cells and of the pancreatic nervous system [14]. The tight interrelationship between pancreatic cells (α – β – δ, and others) could explain the discrepancy about the direct glucose sensitiveness of the α-cells. Other reports that lowering the glucose levels, to which isolates α-cells are exposed, failed to stimulate glucagon secretion [15].

- The second hypothesis would be that a local inhibition of insulin secretion during hypoglycaemia would uncover glucagon secretion [16]. This mechanism requires that blood within the islet flows from the β-cells to the α-cells, thereby exposing them to high concentration so intra-islet insulin [17-19]. This hypothesis also involves that more general β-cell released product could regulate glucagon secretion as β-cell insulin receptor are only very modestly expressed in a cells, although this hypothesis is also controversial [20]. Thus, other β-cell products would directly inhibit glucagon secretion. Very recent evidences show the role of the glucose-dependent regulation of gamma-aminobutyric acid (GABA) receptor mouse pancreatic α-cells [20]. Added to isolated mouse islets in the presence of 0.5 mmol/l glucose, GABA inhibited glucagon release to a similar extent as 10 mmol/l glucose (55%), and the selective GABA(A) receptor GABA(A)R antagonist SR95531 substantially reversed the inhibition of glucagon release by high
glucose. This effect could be related to the regulation of the GABA receptor expression. Recent hypothesis proposes that zinc atoms suppress glucagon secretion via their ability to open α-cell K<sub>ATP</sub> channels. Since insulin binds zinc and zinc is co-secreted with insulin it makes sense that this ion would be one of the insulin-released related products regulating insulin secretion. This was studied in streptozotocin-diabetic Wistar rats. The authors observed that switching off intrapancreatic artery insulin infusions in vivo during hyperglycaemia greatly improved glucagon secretion however, switching off zinc-free insulin infusions had no effect. Therefore, zinc atoms, not the insulin molecule itself, provide the switch-off signal from the α-cell to the α-cell to initiate glucagon secretion during hyperglycaemia [21].

In conclusion, a tonic inhibition by insulin secretion and β-cell products of glucagon secretion would be removed when insulin secretion is impaired as in type 1 diabetic patients. This is however, not true in type 2 diabetic patients characterized by hyperinsulinemia and hyperglucagonae mia. Therefore, other non insulin dependent signals must be suggested for the normal and impaired glucagon secretion. Another biochemical evidence is that glucagon secretion is reduced in vivo when the glycaemic level increased from 3 to 5 mM which do not yet stimulate insulin secretion [22].

- A third potential mechanism for the control of glucagon secretion is related to the central nervous system and the autonomic output to the α-cells. Pancreatic islets are richly innervated to enable autonomic regulation of endocrine cell hormone secretion. The most extensively studied are the sympathetic (adrenergic) and parasympathetic (cholinergic) nerves, which can project deeply into the islet, but other types of sensory neurons have also been detected, including GABAergic nerve bodies [23]. The sympathetic nervous system is activated by hyperglycaemia or exercise stress, causing the release of norepinephrine and other neurotransmitters as well as neuropeptides into the islet [24]. These agents trigger glucagon secretion from α-cells. Norepinephrine and epinephrine inhibit secretion from β- and α-cells. There is some evidence that hyperglycaemia will suppress sympathetic neuronal activity, reducing islet glucagon output [25]. The islets are also richly supplied with parasympathetic fibers originating from intrapancreatic, cholinergic ganglia [26,27]. At least four different neurotransmitters of the parasympathetic system (acetylcholine, vasoactive intestinal peptide - VIP, pituitary adenyl cyclase-activating polypeptide, and gastrin-releasing polypeptide) can stimulate glucagon secretion from islet α-cells although their relative contribution to enhancing glucagon release in vivo remains to be elucidated [24]. Furthermore, circulating neurohormones, like neuropeptide Y (NPY), somatostatin, adrenaline, vasopressin, oxytocin, irrigate the pancreas and could regulate glucagon secretion. It is also noteworthy that a large diversity of products is released by the pancreatic nerve ends. Catecholamines, NPY, VIP, acetylcholine, among others, could regulate glucagon secretion [25,28].

A noteworthy important regulator and, of therapeutic relevance, is the gut-brain hormone glucagon-like peptide-1 (GLP-1). It is an incretin released from the L cells in the small intestine after meal ingestion. One of the primary actions of GLP-1 is the augmentation of glucose-induced insulin secretion, directly by activation of GLP-1 receptors expressed on α-cells and indirectly via the autonomic nervous system [29]. The glucose dependency of the hormonal mechanism and potency of GLP-1 in type 2 diabetic patients has led the peptide to a successful therapeutic future. However, to date, the precise mechanism which account for the antidiabetic effect of the peptide are still a matter of debate since the hormone has multiple physiological targets. Evidence suggests that GLP-1 may also have suppressive effects on glucagon release from α-cells [3]. Any inhibitory effect of GLP-1 on glucagon release may involve direct (via GLP-1 receptor expression in α-cells) and/or indirect mechanisms. An indirect action could be mediated by the stimulatory effect of GLP-1 on neighbouring β- or α-cells, causing intra-islet paracrine inhibition of glucagon release. Clinical studies have demonstrated that patients with type 1 diabetes have decreased plasma glucagon levels in response to intravenous application of GLP-1 after an overnight fast [30]. Long-term (5 days) administration of GLP-1 to type 1 diabetic patients caused a small reduction in postprandial plasma glucagon levels [31]. However, in such in vivo studies, it is difficult to address the direct role of GLP-1 on α-cells for the control of glucagon secretion. So far, no clear data related to the expression of the GLP-1 receptor at the membrane of α-cells exists which shortened the interpretation of the above data. Conversely, biochemical evidences have showed that a direct GLP-1 application to α-cells did not alter glucagon secretion [32] or caused an increase in cAMP levels, in contrast to glucose-dependent inhibitory peptide (GIP), another glucocincretin. Although, such data are also controversial since GLP-1 receptor expression was detected by immunocytochemistry in a subpopulation (20%) of glucagon-positive cells in dispersed rat islets and conversely, some biochemical evidences showed that GLP-1 caused an increase in the rate of exocytosis in single rat α-cells [33, 34]. This chapter denotes the discrepancies and the lack of clarity in the data, which does not allow a clear conclusion.

Another yet unexplored possibility is that GLP-1 inhibition of glucagon secretion is mediated by local neural networks. GLP-1 is rapidly degraded in the circulatory system. Indeed, reports indicate that probably a substantial component of the potentiating effect of GLP-1 on glucose-induced insulin secretion is mediated by the autonomic nervous system in mice [35, 36] possibly via GLP-1 activation of the vagal hepatic nerves [37-39]. Beside the autocrine/paracrine factors which control glucagon secretion in response to glucose, the central nervous system is a major player. The above debate delineates the ambiguities related to the direct α-cells, intra-islet, or pancreatic glucose sen-
singing for the control of glucagon secretion. A more clear-cut regulatory mechanism is based on brain glucose sensing and glucagon secretion.

3. Glucagon secretion during diabetes

Along the last 30 years, convincing evidences have shown that in addition to the relative hypoinsulinaemia, hyperglucagonaemia was detected in all type of diabetic patients. It is still unclear whether hyperglucagonaemia results from an α-cell dysfunction or from an upstream impaired regulatory system. As discussed above, the role of the central and autonomic nervous systems is a key for the regulation of pancreatic islet secretions, suggesting that a neural dysregulation would be tightly linked to hyperglucagonaemia. In type 1 diabetic patients, hypoglycaemia is a major issue. Recurrent hypoglycaemia is also a factor preventing hypoglycaemia-induced glucagon secretion [40]. The mechanism related to this observation could be due to the deleterious effect of hypoglycaemia itself, inducing a failure of the central and autonomic nervous systems. The other hypothesis would be that hyperglycaemia per se would desensitize the α-cells and the corresponding nervous regulatory system for the control of glucose-regulated glucagon secretion. In any case, the loss of glucagon secretion in diabetic patients seems to be glucose specific since glucagon secretion remains normal in response to arginine [4,41].

Thus, the secretory capacity of the α-cell is normal, therefore, the glucagon cell is either unresponsive to the stimuli specifically associated to hypoglycaemia or these stimuli are reduced during diabetes. Certainly, the restoration of normal blood glucose profiles improves hypoglycaemia induced-glucagon secretion [42]. Whether, the improved glycaemic levels directly restores β-cell glucose detection is unclear, as β-cell secretory capacity and autonomic responses to hypoglycaemia are also restored. Upstream regulatory mechanisms are most likely the target of the deleterious hyperglycaemic state. In type 1 and type 2 diabetic patients, it has been several times proposed that a progressive impairment of the autonomic responses to hypoglycaemia may contribute to the impairment and eventual loss of the α-cell response to hypoglycaemia. This hypothesis was supported by the fact that diabetic patients have a delayed epinephrine response to hypoglycaemia [43]. Consequently, such impaired catecholamine release could influence glucagon secretion. Similarly, one could incriminate the upstream mechanisms leading to catecholamine secretion. Such mechanisms are related to the threshold of glucose concentration which needs to be reached to trigger either catecholamine or glucagon secretion. Epinephrine is released at a lower glycaemia level than that required for glucagon secretion. However, the origin of catecholamines, i.e. nerve terminal ends or adrenal glands, is also important. The direct stimulation of glucagon secretion by α-cell in response to noradrenaline released by the nerve terminal ends could occur at a higher glycaemic level than that required for adrenaline release. Therefore, due to the extent of the autonomic dysfunction occurring during the natural history of diabetes, several progressive steps of α-cell impairment could be defined where a lack of glucose sensing for the direct stimulation of α-cell secretion could be occurring at the early onset of diabetes. Therefore, subtle subclinical forms of neuropathy may be present early in the natural course of diabetes and account for the α-cell glucose unresponsiveness.

In type 2 diabetes, hyperglucagonaemia has also been characterized. Its contribution to the hyperglycaemic syndrome could be due to two mechanisms. First, hyperglucagonaemia could explain part of the excessive hepatic glucose production rate in the fasting state; second, its impaired regulated secretion could contribute to the deleterious effect of iatrogenic hypoglycaemia following excessive sulfonylurea dosage for example. It is noteworthy that the role of hyperglucagonaemia in type 2 diabetes is still a matter of debate as the literature reflects numerous controversies. Indeed, glucagon concentration has been found to be elevated in numerous reports [44-48], while normal in others [49,50]. The admitted data is that glucagon concentration is inappropriate with regards to the ambient hyperglycaemia. This could mainly be due to an impaired secretion, or to impaired glucose detection, as debated above. The molecular mechanism for this lack of glucose sensing remains to be fully determined in human, and remains unclear in animal models since the latter are seldomly appropriate for the study of glucagon. Data from the literature demonstrate that the suppression of glucagon after the carbohydrate stimulus, apparent in healthy volunteers, does not occur in patients with type 2 diabetes [51]. This was accompanied, in the diabetic subjects, by a significant blunting of the plasma insulin rise, indicative of impaired glucose sensing in the β-cell. We hence, could extrapolate that the α-cell was also impaired. Furthermore, in the diabetic subjects, plasma glucagon remained at, or above, the preprandial concentration, despite the marked fasting and postprandial hyperglycaemia. This impaired regulation of glucagon secretion in type 2 diabetes was further demonstrated by the fact that there is an exaggerated stimulation of glucagon secretion by aminoacids or a high protein meal, particularly in the context of hyperglycaemia [52]. Importantly, the glycaemia required for half-maximal suppression of arginine-stimulated glucagon release is elevated in diabetic subjects, which further suggest that the sensitivity of the α-cell to the suppressive effects of glucose is decreased [53].

The role of hyperglucagonaemia in type 2 diabetes could be summarized by the effect of the hormone of the overt stimulation of glucose production by the liver in fasting state. The increase in hepatic glucose production seen in patients with overt type 2 diabetes primarily reflects increased gluconeogenesis [48,54]. This is associated with an impaired insulin-mediated suppression of glucose production [55]. These data have been further supported by others, which showed that somatostatin reduced circulating glucagon con-
centration and lowered fasted glycaemia and hepatic glucose production [56]. In addition, glucagon repletion following somatostatin infusion further supports the role of glucagon in the control of glycaemia. Although these experiments must be taken with caution since glucagon or somatostatin infusions were not performed into the hepatoportal vein which is the physiological site of production. Therefore, the data obtained might be of a pharmacological importance.

As mentioned above, animal models are not absolutely appropriate with regards to studies on glucagon. Recent evidences have shown that glucagon receptor knockout mice were characterized by surprising data, when the mutant mice were minimally affected. Mice with a targeted disruption of the glucagon receptor gene (GR<sup>-/-</sup>) were found to have a mild, but still significant, lower fasting and fed plasma glucose concentration [57]. The GR<sup>-/-</sup> mice also had improved oral glucose tolerance, but no overt hypoglycaemia was observed. Surprisingly, in the mutant mice, plasma glucagon concentration was markedly elevated to supraphysiological levels under both fed and fasting conditions. Plasma insulin, cholesterol, and triglycerides, were unaltered. Hence, despite a total absence of glucagon receptors, these animals maintained a near-normal glycaemia. It is also very surprising that no hypoglycaemia episode could be detected at birth, when the peak of glucagon secretion occurs.

Despite these data in rodents, in human the potential of reducing glucagon secretion by the mean of GLP-1 or inhibiting glucagon action is considered a major therapeutic strategy in type 2 diabetes. In a first attempt, pharmaceutical companies have proposed functional glucagon receptor antagonists to block the action of the hormone at the level of the receptor [58, 59]. The recent effort has been on non-peptide based antagonist [60]. However, clinical developments of these compounds were not pursued. This could be due to the fact that hepatic glucose production in vivo could not be suppressed. However, it is still an important challenge to determine a useful glucagon receptor antagonist. The genetic deletion of the glucagon receptor gene led to surprising results [61-63]. The mice exhibited α-cell hyperplasia, markedly increased levels of circulating glucagon and GLP-1. Furthermore, the mice were resistant to high-fat diet-induced obesity, but were not hypoglycaemia and had normal lipid profile [61].

4. Conclusion

Despite a tremendous amount of work performed over the last 40 years, no therapeutic strategy have been set up to counteract the excessive glucagon secretion or action in diabetes. The molecular mechanism related to hyperglucagonaemia in diabetic patients are not totally understood but seemed to be related to the impaired glucose detector units. Therapies aiming to restore normal glucose detection and autonomic nervous system should help reducing hyperglucagonaemia. The use of GLP-1 analogs or DPP-IV inhibitors could be key strategies. Their respective potency in restoring functional GLP-1 levels which in turn alleviate the deleterious glucotoxicity, should provide long-term efficacy for the normalization of hyperglucagonaemia.

Conflicts of interest: No potential conflict of interest relevant to this article was reported.

References


