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Amino acids and the changing face of the α-cell

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ABSTRACT

Glucagon has long been defined by its glucogenic action and as a result α-cells have been characterised based largely on their interaction with glucose. Recent findings have challenged this preconception, bringing to the fore the significant role glucagon plays in amino acid breakdown and underlining the importance of amino acids in glucagon secretion. The challenge that remains is defining the mechanism that underlie these effects - understanding which amino acids are most important, how they act on the α-cell and how their actions integrate with other fuels such as glucose and fatty acids. This review will describe the current relationship between amino acids and glucagon and how we can use this knowledge to redefine the α-cell.

1. Glucagon and the glucose-centric paradigm

Glucagon was discovered in 1923 as a glucogenic factor in pancreatic extracts [1]. Since then it has been established as the principle counterregulatory hormone, acting in opposition to insulin to increase blood glucose levels and prevent hypoglycaemia. It does so by increasing hepatic glucose output, primarily by stimulating glycogenolysis [2]. Glucagon is produced and secreted by α-cells, the second most abundant cell type of the pancreatic islets behind the insulin-producing β-cells. Like insulin secretion, glucagon secretion is glucose-dependent, following a bell-shaped relationship - increasing progressively at glucose concentrations <5 mM glucose and also increasing slightly at concentrations >15 mM [3]. In diabetes, glucagon secretion becomes dysregulated with inappropriately high glucagon levels at high glucose, which compound the effects of insufficient insulin action driving diabetes pathogenesis [4]. Understanding how glucagon secretion is regulated is therefore critical in the fight against diabetes.

Regulation of glucagon has primarily been assigned to glucose, whether directly through an intrinsic effect on the α-cell or indirectly via paracrine signalling from neighbouring β-and δ-cells [5]. Thus, a ‘glucose-centric paradigm’ has emerged whereby glucagon is predominantly viewed in axis with glucose. Recent advances in the field, however, have underlined the versatility of action of glucagon, encompassing control of plasma amino acids and even lipids, bringing into question whether glucose is the master regulator of glucagon secretion.

2. Glucagon – more than meets the eye

A central argument against the ‘glucose-centric paradigm’ is the phenotypes of glucagon excess and deficiency. In these models, effects on glucose homeostasis are minimal, yet effects on protein metabolism are stark. Glucagon producing tumours (glucagonomas) generally cause mild hyperglycaemia (80 %), despite glucagon levels being increased 100–1000 fold and when glucagon signalling is blocked using a glucagon antibody or in GCGKO mice, fasting and postprandial blood glucose are only mildly decreased with no effect on hypoglycaemia incidence [6]. Contrastingly, glucagonomas result in a 45 % fall in amino acid levels [7], which manifests as a skin rash known as necrolytic migratory erythema due to insufficient skin turnover, a condition that, notably, can be resolved by amino acid infusion [7–9]. Meanwhile pancreatectomy results in hyperaminoacidemia, which is reversed by glucagon infusion [10].

These observations have led to the suggestion that a negative feedback loop exists between α-cells and the liver, which is driven by changes in amino acids. In the liver-α-cell axis, amino acids stimulate glucagon secretion, which then stimulate amino acid breakdown via ureagenesis in the liver. Several studies from different groups have demonstrated the existence of this axis [11–14], showing that the hyperaminoacidemia caused by glucagon receptor antagonism drives α-cell hyperplasia and hyperglucagonaemia. It should be noted that these studies were predominantly performed in murine models and that α-cell hyperplasia has not be observed in humans. However, human

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islets transplanted into immunodeficient mice do exhibit α-cell proliferation upon glucagon receptor blockade [11,13].

3. Amino acids and α-cell – the current picture

Intrinsic regulation of glucagon secretion by glucose predominates at concentrations below 5 mM where β- and δ-cells are generally inactive [3]. The current intrinsic models rely on increased ATP derived from glucose oxidation, which then inhibits glucagon secretion by closing K<sub>ATP</sub> channels in the plasma membrane [15] or SERCA pump activation [16]. The K<sub>ATP</sub> model of glucagon secretion relies on K<sub>ATP</sub> channel activity being minimal even at low glucose resulting in depolarization that opens voltage-sensitive Na<sup>-</sup> channels and at the peak of the action potential P/Q-type Ca<sup>2+</sup> channels. As glucose increases K<sub>ATP</sub> channel activity is fully inhibited causing stronger depolarization that inactivates Na<sup>-</sup> channels leading to a drop in action potential height that means P/Q-type Ca<sup>2+</sup> channels do not open and glucagon secretion is ablated [15]. The SERCA model states that when glucose is low, SERCA pump activity is low meaning the ER releases Ca<sup>2+</sup> activating a store-operated current at the plasma membrane, leading to an increase in [Ca<sup>2+</sup>]<sub>cyt</sub>, that stimulates glucagon secretion. When glucose levels increase, ATP drives SERCA pump activity, the store-operated current dissipates and glucagon secretion is inhibited [16]. However, given the need for ATP to maintain control of cytosolic Ca<sup>2+</sup> and vesicular priming in exocytosis [18,19], it is counterintuitive that α-cells would have lower ATP levels at low glucose, when the cell is most active. In addition, there is evidence that glucose oxidation is lower in α-cells compared to β-cells indicating that it may not be the primary fuel source [20–22]. Current understanding of the α-cell may be limited as in most functional studies glucose is the only substrate being used. This means the input of other metabolites such as fatty acids and amino acids, which may be the main fuel sources used by α-cells, are omitted, meaning experiments do not reflect the true physiological conditions. Switching focus to include alternative substrates such as amino acids and fatty acids together with glucose could be the key to finally resolving the regulation of glucagon secretion.

Amino acid-induced glucagon secretion is not new. A 1968 study in dogs was the first to show administration of amino acids augmented glucagon secretion [23]. Follow-up studies then measured the effect of individual amino acids in dogs [24], and sheep [25] showing most amino acids stimulate glucagon secretion and that they have synergistic effects when added together [26]. The mechanisms underlying these effects however are poorly understood and amino acid-induced glucagon secretion has been widely viewed as secondary to glucose regulation [27]. Some of the difficulties may be due to the sheer diversity of amino acids, with differences in size, polarity and hydrophobicity, as well as different transport-mechanisms and cell surface receptors. Thus defining a general amino acid effect is difficult. Some efforts have however been made to define how individual amino acids may affect α-cells.

Amino acids can feasibly affect glucagon secretion in four different ways – through metabolic, electrogenic, receptor-mediated and mitogenic effects. All amino acids are potentially capable of exerting metabolic effects as they feed into the anaplerotic pathways of the TCA cycle [28]. However, extensive detail of amino acid metabolism in α-cells is lacking. Electrogenic effects rely on amino acid transport inducing depolarization and can occur due to transport of cationic amino acids, this may be the case with arginine, which induces membrane depolarization in mouse α-cells upon cell entry [29] and also induces Ca<sup>2+</sup> influx [30]. Transport of neutral amino acids via ion-coupled transporters could also induce depolarization (see Fig. 1). Amino acids can also bind to cell surface ionotropic and metabotropic amino acid receptors and there is evidence for this occurring in α-cells. Glycine was identified as the most potent glucagonotropic amino acid in human islets, eliciting its effects via opening of the glycine receptor, an ionotropic receptor permeable to Cl<sup>-</sup>, leading to an increase in cytosolic Ca<sup>2+</sup> [31]. Whilst in human, monkey and mouse islets, glumate was shown to be an autocrine signal secreted by α-cells that acted via ionotropic glutamate receptors, inducing depolarization that increases cytosolic Ca<sup>2+</sup> and stimulates glucagon secretion [32].

However, when considering electrogenic effects whether via amino acid transport or ionotropic receptors, one should note that the effect of depolarization in α-cells is unclear. In the K<sub>ATP</sub> model, glucose-induced depolarization inhibits glucagon secretion [15]. In support of this,
depolarization with sulfonylureas has generally been shown to inhibit glucagon secretion [15,33–35] with some exceptions [30], while KCl-induced depolarization appears to be dose-dependent with 15 mM KCl inhibiting and 40 mM KCl stimulating glucagon secretion [36]. These discrepancies may occur due to paracrine signalling from β-cells, which when activated by sulfonylureas or KCl may inhibit glucagon secretion directly or via activation of δ-cells [37,38]. The dose-dependence of KCl stimulation is therefore likely due to the direct effect of 40 mM KCl on α-cells being able to override any paracrine effect, exemplified by 15 mM KCl stimulating glucagon secretion when insulin secretion was ablated [36]. Furthermore, when insulin secretion is blocked depolarization evoked by sulfonylureas increases glucagon secretion [36], the same is also seen in somatostatin KO mice [35,39], indicating that any inhibitory effect may be mediated via the δ-cell. Notably however, these experiments were performed in cultured mouse islets. In freshly isolated mouse islets, tolbutamide was still inhibitory even when somatostatin signalling was blocked; the same was true for inhibition going from 1 mM to 6 mM glucose [15]. The effect of depolarization may be context-dependent. Adrenaline depolarizes mouse alpha cells but increases glucagon secretion via stimulation of L-type Ca^{2+} channel exocytosis [40]. The effect of glutamate is also dependent on L-type Ca^{2+} currents [32]; the same may be true for other amino acids. Therefore, ultimately if amino acids selectively depolarize α-cells via L-type Ca^{2+} channel activation one would expect stimulation of glucagon secretion.

Mitogenic effects can occur as amino acids can target mTOR [41]. This is evident with glutamine, which drives α-cell hyperplasia in an mTOR-dependent manner when glucagon signalling is disrupted [11]. Recently, this has been developed upon with G_max signalling via the calcium-sensing receptor (CaSR), which is amino acid sensitive, shown to act synergistically with mTOR signalling to induce α-cell proliferation in mouse and zebrafish models [42]. The current picture suggests that amino acids that acutely stimulate glucagon secretion do not directly correlate with those that promote α-cell proliferation. Although as a Gαζ-coupled receptor, the CaSR could feasibly increase Ca^{2+} via IP_{3}R Ca^{2+} release and acutely affect glucagon secretion [43].

Although some mechanistic understanding has been attained from these studies, the overall picture is sparse and there is a need to prioritise which amino acids should be studied. It must also be noted that in many of these studies supraphysiological concentrations are used. Arginine, for example, is commonly applied at 10 mM despite plasma concentrations ranging from 41 to 114 µmol/L [44]. This could produce misleading data and it is essential that the dose-dependence of individual amino acids is investigated.

4. Amino acids during fasting

In redefining the α-cell, considering what fuels are available during fasting, when α-cells are active, is critical. Free fatty acids increase upon fasting [45,46], and there is growing evidence that they are oxidised by the α-cell and play a crucial role in maintaining glucagon secretion [47–55]. For amino acids, the picture is less clear. Although muscle release of glucogenic amino acids (alanine, serine, glycine, methionine, tyrosine & lysine) increases in response to 60 h fast in humans, arterial plasma levels drop due to utilisation by the liver [56]. Therefore, any sustained effect on glucagon secretion is unlikely. It may instead be that glucogenic amino acid plasma levels increase transiently upon fasting, stimulate glucagon release from the pancreas, which then acts on the liver, rapidly bringing amino acid levels down. Taking plasma samples at regular intervals upon the initiation of fasting should give better resolution between fasting amino acid levels and glucagon secretion.

Branched-chain amino acids (BCAAs) do increase in response to 60 h fasting [56], and after 3 days starvation [57], coinciding with a peak in glucagon [58]. However, in early studies in dogs they were among the least glucagonotrophic amino acids [24,59]. In vivo studies in humans have contested this with BCAA-rich protein hydrolysates affecting postprandial glucagon the most [60], and valine and isoleucine stimulating glucagon secretion [61]. However, these studies may be confounded by effects on the gut and interconversion of BCAAs into more glucagonotropic amino acids. A direct effect has been observed in mouse islets, when all three BCAAs were applied together glucagon secretion was stimulated via an increase in cytosolic Ca^{2+} [62]. Notably however, supraphysiological concentrations of 40 mM were used.

Studies in the liver have underlined a shared relationship between BCAA and lipid metabolism during fasting, with the kinase Branched chain ketoacid dehydrogenase kinase (BDK) and Protein Phosphatase 1 K, Mitochondrial (PP1MK), which control Branched-chain α-ketoacid dehydrogenase (BCKDH), the rate-limiting enzyme in BCAA catabolism, also inversely regulating ATP citrate lyase, responsible for the first step of De novo lipogenesis. This ultimately means that in the fasted state BCAA and FA oxidation are upregulated while in the fed state glucose oxidation and lipid synthesis are prioritised. BDK:PP1MK thus integrates glucose, lipid and BCAA metabolism [63]. The existence of this axis in the α-cell would provide an elegant model for α-cell metabolism and glucagon secretion in the fasted state with FA oxidation and BCAA catabolism fuelling the TCA cycle. Indeed, the poor effects of BCAAs alone on glucagon secretion may be because they require fatty acid supplementation. BCAAs could also have mitogenic effects, as important regulators of mTOR [64], and there is evidence for leucine being involved in α-cell proliferation [11]. To establish the true effect of BCAAs on glucagon secretion and α-cell proliferation further ex vivo islet studies are required in both rodents and humans using physiological concentrations.

The falling plasma levels of glucagonotropic amino acids and the seemingly weak effects of BCAAs on glucagon secretion, mean it is currently unclear whether the fasting glucagon response is regulated by amino acid levels. They could play a more prominent role under conditions of prolonged starvation (10 days) however. Glycine begins to increase from 10 days onwards [57]. It is consistently one of the most glucagonotropic amino acids so could heavily influence glucagon secretion during starvation [31]. Threonine and serine also increase so warrant further study [57].

5. Regulation of the α-cell by the liver-α-cell axis

Studies have identified a number of key amino acids associated with glucagon secretion. Cysteine, glycine, alanine, arginine and prol ine have been identified as the most potent glucagonotropic amino acids, and acute mediators of the liver-α-cell axis [65,66]. These five amino acids are therefore particularly interesting. As these amino acids do not increase in response to fasting [56,57] (excl. the potential effect of glycine during prolonged starvation). It is thus likely that they affect glucagon secretion due to a postprandial increase or tonically. Based on the current literature, the receptor-mediated effect of glycine appears valid and importantly was tested using physiological concentrations [31]. Further work should establish the current glycine stimulation elicits. Specifically, the direction of Cl− conductance through the glycine receptor in α-cells. Cl− efflux would depolarize the membrane potential whilst influx would repolarize. Ascertain the direction and how this leads to increased cytosolic Ca^{2+} is essential. Arginine, however, must be tested at physiological concentrations as current setups effectively introduce 10 mM of positive charge to the extracellular solution so are not biologically plausible.

Alanine, the primary amino acid substrate for gluconeogenesis in the liver, seems a key determinant of glucagon secretion. It is consistently associated with glucagon action [67,68] with postprandial alanine concentrations among the highest of the amino acids and is capable of increasing Ca^{2+} in α-cells, although the precise mechanism remains undefined [66]. Moreover, in mice alanine has been shown to act in conjunction with the gut hormone GIP to amplify glucagon secretion, orchestrating the optimal response to a mixed meal [66], and may be crucial for proper postprandial glucagon and insulin responses [69].
Mechanistically, a metabolic effect seems unlikely; alanine is metabolized to pyruvate like glucose yet has opposing effects on glucagon secretion. It is transported however via the Na⁺-coupled Slc38a5 in α-cells, which has been strongly linked to α-cell hyperplasia induced by glucagon receptor inhibition in mice [11,13]. This transporter is actually electroneutral as it is in antiport with H⁺ at a 1:1 stoichiometry [70] but could feasibly generate inward currents via uncoupled movement of ions [13] as has been observed in members of the same family [71,72]. In humans, Slc38a4 appears to be the prominent isoform, displaying heightened expression in α-cells after glucagon receptor inhibition [73]. Notably, it transports Na⁺ without exchange of other ions so could feasibly generate inward currents [70]. Alanine-induced glucagon secretion is lower in Slc38a5⁻/⁻ mice [13]. Furthermore, cysteine, the most potent amino acid in the perfusion study, inducing a 5.6-fold increase in glucagon secretion [65] is also transported by Slc38a4 and Slc38a5 [70]. Other amino acid transport mechanisms should not be ruled out however. Transport via Slc38a5 may only be important under conditions of glucagon resistance and during development, as expression in adult α-cells is low [11]. Lastly, proline has not been extensively studied but notably can be transported by the Na⁺-coupled Slc38a2 along with alanine and cysteine [70], which is expressed in α-cells [74,75].

6. Reframing α-cell dysfunction in diabetes

Hyperglucagonaemia and loss of the glucagon response to hypoglycaemia are features of both type 1 and type 2 diabetes [76–78]. Hyperglucagonaemia exacerbates hyperglycaemia [79], working in conjunction with impaired insulin signalling to drive blood glucose levels up, while hypoglycaemia is the main barrier for effective treatment of diabetes with insulin, with the loss of the glucagon response a significant factor in its development [80]. Therefore, understanding their aetiology is crucial for the effective management of diabetes. Currently, glucagon dysregulation in diabetes is linked to loss of...
paracrine suppression by neighbouring β-cell [81,82] and δ-cells [83, 84], as well as intrinsic dysregulation due to SGLT-driven acidification from hyperglycaemia [85]. However, with the shift towards amino acid and fatty acid regulation of the α-cell, a reframing of how diabetes may drive glucagon dysregulation is required (see Fig. 2).

Notably, BCAAs are elevated in type 2 diabetes and contribute to insulin resistance [86]. Plasma levels of BCAA increase due to obesity driving alterations in enzymes involved in BCAA oxidation in the liver [87,88] and adipose tissue [88–90]. Dysregulation of BCAA metabolism is also evident in the α-cell with BCAA application shown to drive hyperglucagonaemia in several diabetic mouse models, effects that can be reversed by stimulating BCAA oxidation with the compound BT2 [62].

Research into the liver α-cell axis has also shed some light on the causes of glucagon secretion abnormalities. Fasting hyperglucagonaemia is not observed in lean type 2 diabetic patients and occurs in obese patients with normal glucose tolerance. This means it is a feature of obesity not type 2 diabetes [91]. This has led researchers to surmise that obesity-driven steatosis is the driver of fasting hyperglucagonaemia. It has subsequently been found that in patients with non-alcoholic fatty liver disease (NAFLD), the liver-α-cell axis is disrupted with steatosis causing hepatic glucagon resistance [92,93], and crucially that this occurs independently of type 2 diabetes [68]. It should be noted that inappropriate glucagon responses to a glucose load are a feature of type 2 diabetes and do not occur independently in NAFLD [91]. Liver zonation means hepatocytes that perform glycogenolysis or ureagenesis are different and it appears that steatosis only affects the ureagenic hepatocytes [94]. This means that amino acid levels are driven up in NAFLD; the increased amino acids stimulate glucagon secretion, which then acts on the glycogenolytic hepatocytes to drive glucose levels up.

However, given the glucogenic role of several amino acids, the focus on ureagenesis and separation of amino acids from glucose homeostasis may be amiss. The acute effect of glucagon on hepatic glucose output is exclusively due to glycogenolysis, with glucagon not capable of liberating gluconeogenic substrates from the muscle or adipose tissue [2]. However, if gluconeogenic substrates are available, e.g. during fasting [95], the slower acting effects of glucagon on gluconeogenic gene expression will result in increased hepatic glucose output. Gluconeogenesis and ureagenesis are not spatially separated in the liver [94], indicating that further work is needed to fully understand glucagon resistance in metabolic disease. Indeed, steatosis in rats actually results in decreased hepatic glucose output [96], while in humans there is no difference in endogenous glucose production between obese subjects with hepatic steatosis and lean subjects [92].

The relationship between amino acids and glucagon also has implications for the development of glucagon receptor antagonists (GRAs) to treat diabetes. Upon blockade of the glucagon receptor, amino acids will increase, inducing α-cell hyperplasia, and increasing the risk for glucagonomas. Mice treated with glucagon receptor antibodies did not develop glucagonomas but due to the severity of the condition patients treated with GRAs should be monitored [97].

7. Characterising amino acids’ effect on α-cell function

Experimental design is vital for the proper characterisation of amino acid-induced glucagon secretion. In vitro studies provide flexibility with the option for gene knockdown and measurement of various cell parameters. However, they are limited by the availability of good α-cell cell lines. The most commonly used are Alpha T1C–6 cells but they show poor correlation to mature α-cells and are difficult to culture. In vivo studies may be useful for determining the effect of dietary amino acids but are confounded by the interconversion of amino acids meaning a non-gluconeotropic amino acid can feasibly be converted into a gluconeotropic amino acid and vice versa [98]. Assessing the effect of individual amino acids is therefore challenging. For these reasons, ex vivo studies on isolated islets or pancreas perfusion studies present the best option for characterising the effects of individual amino acids. α-Cell function is maintained, paracrine signalling is intact and islet studies allow the assessment of various cellular parameters (e.g. Ca2+, cAMP and membrane potential) enabling true mechanistic insight.

Amino acids have been shown to potentiate insulin secretion in the presence of high glucose concentrations [99]. An effect on δ-cells can also not be ruled out. Thus to get the full picture of amino acid-stimulated glucagon secretion, paracrine effects must be considered - especially postprandially or at >5 mM glucose. Experimental setups where paracrine contacts are maintained should be prioritised.

It is also known that amino acids act synergistically. This could feasibly occur due to the interaction between different pathways activated by different amino acids. For instance, the metabolic effect of one amino acid may be potentiated by heightened intracellular [Ca2+]i, stimulating Ca2+-dependent mitochondrial enzymes [100] or synergy between CAMP and Ca2+ signalling, as observed with the dual action of alanine and GIP on glucagon secretion [66]. Assessing secretion and cellular parameters (e.g. Ca2+, cAMP) in response to individual gluconeotropic amino acids and then in combination, one amino acid at a time, should provide a better understanding of the mechanisms underlying the synergy.

8. Conclusion

The emergence of the liver α-cell axis has reenergised the glucagon field, providing fresh impetus for how the enigmatic α-cell may function. However, unpicking how amino acids stimulate glucagon secretion remains a significant challenge. To address this, assessing the dose-dependence of individual amino acids on glucagon secretion will be a good place to start. This will ensure that previously documented effects are not simply due to supraphysiological artefacts. Then, a thorough characterisation of individual effects of amino acids on cellular parameters such as cAMP, membrane potential, Ca2+ or ATP should follow. This will reveal the underlying mechanisms whether they be metabolic, electrochemical or receptor-mediated. Amino acid effects do not occur in isolation however, glucose and fatty acid concentrations must be carefully considered, as it is most likely the integration of the three macronutrients and their cellular pathways that determines α-cell function. This will shine a light on the true nature of glucagon secretion, away from the glucose-centric paradigm of the past. In doing so, glucagon dysregulation in diabetes can be fully understood and resolved.

Declaration of Competing Interest

None.

Data Availability

No data was used for the research described in the article.

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