

## PANCREATIC ALPHA CELL DYSFUNCTION IN DIABETES MELLITUS AND ITS MANAGEMENT STRATEGIES

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### ABSTRACT

#### BACKGROUND

Diabetes mellitus has primarily centered around the insulin deficiency owing to pancreatic beta-cell dysfunction or loss and associated insulin resistance. Recently, numerous findings indicate that defect of glucagon secreting alpha-cell get involved with development and exacerbation of hyperglycaemia in both type 1 and type 2 DM. Aberrant  $\alpha$ -cell responses exhibit both fasting and postprandial hyperglucagonaemia contributes to fasting and postprandial hyperglycaemia caused by inappropriate hepatic glucose production owing to blunted  $\alpha$ -cell suppression. Thus, blockade of glucagon receptor or suppression of glucagon secretion from  $\alpha$ -cell would be novel therapeutic target for control of hyperglycaemia. There have not been remarkable advances in developing new class of drugs, currently glucagon-like peptide-1 and dipeptidyl peptidase-4 inhibitors and amylin agonist are available targeting alpha-cell dysfunction for the treatment of diabetes mellitus.

#### KEYWORDS

Diabetes Mellitus, Glucagon,  $\alpha$ -cell,  $\beta$ -cell, GLP-1, DPP-4 Inhibitors.

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#### BACKGROUND

In 1869, Paul Langerhans, a medical student identified an island of clear cells in the pancreas for which it was named the Islets of Langerhans and it contains alpha cells ( $\alpha$ -cells). In 1907, Micheal Lane discovered that there were two distinct cell type called "alpha" ( $\alpha$ -cell) and "beta cells" ( $\beta$ -cell). In 1923, Charles Kimball, a biochemistry student isolated a substance from the  $\alpha$ -cell and named it "glucagon."<sup>1</sup>

**Islet of Langerhans-** Approximately, one million islets are there distributed throughout a healthy human adult pancreas. Each islet with sizes varying from 100-500  $\mu$ m is made up of 1000-3000 cells.<sup>2</sup> The endocrine cell composition of islets are the beta-cells mainly secrete insulin,  $\alpha$ -cells secrete glucagon,  $\delta$ -cell secrete somatostatin, PP-cells secrete pancreatic polypeptide and Epsilon-cells secrete Acyl-Ghrelin.<sup>3,4</sup> In human islets, the  $\beta$ -cells are about 48-59% and the  $\alpha$ -cells are about 33-46%, that glucagon secretion by  $\alpha$ -cells play a major role in human and one of the main endocrine cell population.<sup>4</sup> These islet cells show a random distribution pattern where the majority of  $\beta$ -cells are in contact with non- $\beta$ -cells suggesting that paracrine interactions among different cell population may be more active and there is intercellular communications.<sup>5</sup> Within the islets,  $\beta$ -cells exert a major paracrine effects,

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through insulin, zinc and/or amylin. Insulin directly suppress glucagon secretion as do amylin and zinc, both co-secreted with insulin.<sup>6</sup>

#### Molecular Biology of Glucagon

Glucagon is a peptide hormone released from pancreatic  $\alpha$ -cells. The proglucagon gene is expressed by pancreatic  $\alpha$ -cells, intestinal L-cells and in some neuronal cells of central nervous system. These cells express different isoforms of the enzyme called prohormone convertase (PC) and therefore differentially process the gene products. In the  $\alpha$ -cells, the predominance of PC2 leads to a major production of glucagon.<sup>7,8</sup> PC 1/3 is predominantly expressed in the intestinal L-cells and cleaves the proglucagon to GLP-1 and GLP-2.

#### Glucagon Receptors

The glucagon receptors are present in multiple tissues including the liver, pancreas, heart, kidney, brain and smooth muscle cells. Thus, it modulates multiple responses in these tissues. In any case, the regulation of glucose homeostasis is the major function of glucagon and its receptors. Glucagon receptor is a 485 amino acid protein. Glucagon binding to this receptors is coupled to GTP-binding heterotrimeric G proteins of the gas type that leads to the activation of adenylate cyclase, cAMP production and PKA. This receptor can also activate the phospholipase/inositol phosphate pathway via Gq proteins resulting in  $Ca^{2+}$  release from intracellular store.<sup>9</sup> Thus, glucagon signaling regulates positively (+ve) or negatively (-ve) of the multiple steps of the hepatic glucose metabolism.

#### Glucagon Control of Glucose Homeostasis

Several lines of defenses protect the organism against hypoglycaemia and its potential damaging effects, especially

in the brain, which depends on a continuous supply of glucose as its principal metabolic fuel. These defenses include decrease insulin release and increased secretion of adrenaline and glucagon. Additionally, glucose sensing neurons in the ventromedial-hypothalamus, further control response to glycaemia changes. Among this regulatory system, glucagon plays a central role in the response to hypoglycaemia and also opposes insulin effects. The main action of glucagon occurs in the liver where the insulin/glucagon ratio control multiple steps of hepatic metabolism. Glucagon stimulates gluconeogenesis and glycogenolysis, which increases hepatic glucose output of 150-250 mg/min. or (2 mg/kg/min.) glucose ensuring an appropriate supply of glucose to body and brain, and at the same time, it decreases glycogenesis and glycolysis. The glucagon receptor in the liver is highly selective for glucagon. Glucagon also stimulates the uptake of amino acids especially involved in gluconeogenesis, such as alanine, glycine and proline and in adipocytes causes lipolysis as well as fatty acid  $\beta$ -oxidation.<sup>10</sup>

### Control of Glucagon Secretion

In health, a rise in blood glucose activates both glucose dependent and independent inhibition of glucagon secretion from the alpha cells, thereby control blood glucose and regulate the storage and utilisation of energy. Direct glucose-dependent pathway for modulating  $\alpha$ -cells-glucagon secretion evident that the  $\alpha$ -cells express ATP regulated potassium channels and glucokinase both require glucose sensing.<sup>11</sup> The glucose-independent pathways for modulating glucagon secretion involves a network of liver, intestine, nervous, muscular and adipose tissue called the "HENAMI" network.<sup>1</sup> The islets of Langerhans are a key part of this network. The  $\beta$ -cells exert a major paracrine effect, which mediate through insulin, zinc and/or amylin. Insulin directly suppresses glucagon secretion as do amylin and zinc, both cosecreted with insulin.<sup>3</sup> Coupling can be found between several human  $\beta$ -cells in clusters within the same islet, but not in the whole  $\beta$ -cells population. This kind of intracellular communications is probably the result of the human islet cytoarchitecture.<sup>5</sup> In addition to nutrients and paracrine signals, islet function is further regulated by sympathetic, parasympathetic and sensory nerves that go deeply into the islets.<sup>12</sup>

### Insulin

The most important paracrine mechanism responsible for inhibiting glucagon release is conducted by insulin acting via several pathways. Insulin act on insulin receptors of the  $\alpha$ -cells and inhibit glucagon release through the activation of phosphatidylinositol-3 kinas (PIK3) and also involved in the modification of K-ATP channels.<sup>13</sup> Insulin also increases K-ATP channel activity inducing an inhibitory effect on glucagon release via membrane hyperpolarisation and suppression of glucagon secretion. In addition to the effect on K-ATP channels of  $\alpha$ -cell, insulin can translocate A-type GABA receptor to the cell membrane, which increases the response by  $\beta$ -cells, favouring membrane hyperpolarisation

and suppression of glucagon secretion.<sup>14</sup> Insulin also inhibit  $\text{Ca}^{2+}$  signals in  $\alpha$ -cells induce by low-glucose concentration, which rely on electrical activity. Therefore, insulin inhibit glucagon release mainly by altering  $\alpha$ -cell membrane potentials.<sup>15</sup>

### Zinc

Insulin is stored within secretory granules of  $\beta$ -cell forming stable hexamers around two atoms of  $\text{Zn}^{2+}$ . After exocytosis, these hexameric crystals are exposed to a change in PH from 5.5 to 7.4 become dissociated and release both atoms of  $\text{Zn}^{2+}$ . The zinc atoms can also work as modulators of the  $\alpha$ -cell function.<sup>16</sup>  $\text{Zn}^{2+}$  can activate K-ATP channels and decreases glucagon secretion.

### Amylin

Islet-Amylin Pancreatic Peptide (IAPP), is an another  $\alpha$ -cell regulator, mainly synthesised in  $\beta$ -cells, co-secreted with insulin (in 100:1 ratio) exocytosis and has an inhibitory effect on glucagon basal concentration as well as after arginine stimulation.<sup>17</sup> Since, amylin also reduces somatostatin and insulin release, it is proposed that endogenous amylin within islet may establish a negative feedback to avoid excessive secretion from  $\alpha$ -,  $\beta$ - and  $\delta$ - cells.

### Somatostatin

Somatostatin is produced and secreted by the  $\delta$ -cells of islets, work as inhibitor of both glucagon and insulin release. Alpha cells express five subtypes of Somatostatin Receptors (SSTRs). SSTR2 is highly expressed in  $\alpha$ -cells, while SSTR1 and SSTR5 are expressed in  $\beta$ -cells. Somatostatin activate  $\text{K}^{+}$  channels in  $\alpha$ -cells and suppressing electrical activity.

### GLP-1

The upper small intestinal L-cells secrete GLP-1 that inhibit glucagon release from the  $\alpha$ -cells and potentiate insulin release from  $\beta$ -cells after food intake<sup>18</sup> and is a potential therapeutic agent for the treatment of diabetes with insulin deficiency as well as hyperglucagonaemia.<sup>19</sup> In isolated rat  $\alpha$ -cells, GLP-1 stimulate glucagon secretion and in  $\beta$ -cell specific knockout mice, the lack of effect of GLP-1 on  $\beta$ -cells unable to inhibit glucagon secretion. Thus, some  $\beta$ -cells presence is necessary for its inhibitory effect on  $\alpha$ -cells.<sup>20</sup> GLP-1 also affect the  $\alpha$ -cell function by interacting with autonomic nervous system.<sup>21</sup>

**GIP-** Intestinal K-cells secrete GIP in response to food intake, which stimulate glucagon release and also protect  $\beta$ -cells from apoptosis<sup>22</sup> in healthy human protect from hypoglycaemia.<sup>23</sup>

**Leptin-** The adipocyte hormone leptin inhibits glucagon release and is associated with satiety.<sup>24</sup>

**IL-6-** Leptin is closely related to IL-6 that is released from muscle tissues in response to vigorous exercise.<sup>25</sup> IL-6 can stimulate  $\alpha$ -cells to secrete low level of GLP-1, which

stimulate  $\beta$ -cells secretion of insulin and has antiapoptotic effect on the  $\beta$ -cells.<sup>26</sup>

### Neural Regulation

The islets of Langerhans is highly innervated by parasympathetic and sympathetic nerves that ensure rapid response to hypoglycaemia and protection from potential brain damage.<sup>12</sup> Some terminals of these nerves store and release classic neurotransmitters as well as several neuropeptides, which stimulate or inhibit glucagon secretion depending on the neural messenger released and they have multiple action.<sup>12</sup> Sympathetic activation also induce adrenaline release from adrenal medulla, which stimulates glucagon secretion. These neural regulation of  $\alpha$ -cell are mainly regulated by glucose sensing neurons of the ventromedial hypothalamus, which response to plasma glucose levels with mechanism very similar to those of  $\beta$ -cells.<sup>27,28</sup>

### Alpha Cell Dysfunction in Type-1 DM

Type 1 DM patients have specific  $\beta$ -cell loss with preservation of  $\alpha$ -cell and other islet cells and some reports suggest mild  $\alpha$ -cell hyperplasia.<sup>29,30</sup> The loss of  $\beta$ -cells in type 1 DM and the resulting loss of insulin mediated suppression of glucagon secretion maybe expected to result in persistently elevated levels of glucagon. In health, falling glucose, triggers glucagon secretion a phenomenon that is often absent in type 1 DM.<sup>31</sup> Furthermore, the greater the loss of  $\beta$ -cell function, the more blunted the glucagon increase with hyperglycaemia. This blunted response increase the risk of hypoglycaemia in type 1 DM treated with insulin. Absolute level of glucagon may not consistently elevated, it is conceivable that glucagon levels are higher than would be expected for the hyperglycaemia associated with type 1 DM. Furthermore,  $\alpha$ -cell responses to both rising and falling levels of glucose appear compromised in type 1 DM and a meal stimulus results in a paradoxical increase in glucagon secretion. The reason for an inappropriate increase in glucagon in type 1 DM are likely to be multifactorial and defect in both glucose-dependent and independent pathways controlling glucagon secretion may contribute to the hyperglycaemia and incretins mediated control of glucagon secretion maybe modulated in type 1 DM.<sup>1</sup> Exploratory trial administrations of GLP-1 analogues in well controlled type 1 DM improve mean fasting glucose and decreases glycaemic excursions suggesting insulin-independent inhibitory effects on  $\alpha$ -cells. Currently, GLP-1 analogues are not licensed for use in type 1 DM. Similarly, DPP-4 inhibitor, sitagliptin and teneligliptin study in type 1 DM patients showed decreased meal stimulated glucagon levels, still unlicensed for type 1 DM and requires large clinical trials to confirm its safety and efficacy for their use in clinical practice.<sup>32</sup>

### Alpha Cell Dysfunction in Type 2 DM

Alpha-cells have a guardian type of role in the islet to maintain the body's capacity to produce insulin<sup>33</sup> and in health the  $\alpha$ - and  $\beta$ -cells in the islet regulate each other

reciprocally and systemic glucose levels are maintained within narrow range. But, the role of alpha cell in diabetes has been neglected for a long time despite suggested as "bi-hormonal" disease with an absolute or relative excess of glucagon, which causes higher rate of hepatic glucose production than utilisation favouring hyperglycaemia.<sup>34,35</sup> The rate of hepatic glucose production has been correlated with the hyperglycaemia in association with hyperglucagonaemia.<sup>36,37</sup>

In type 2 DM, glucagon producing  $\alpha$ -cells in the pancreas remain relatively protected from the toxic environment created by metabolic stress (glucotoxicity and lipotoxicity), while insulin producing  $\beta$ -cells are not protected and die by apoptosis.<sup>38</sup> The impairment of insulin release and insulin resistance is often accompanied by absolute or relative increased levels of glucagon in the fasting and postprandial state.<sup>39</sup> In this situation, insulin is not effective as a negative feedback for hepatic glucose output while glucagon potentiate glucose mobilisation from the liver, thus contributing to hyperglycaemia. There is also lack of suppression of glucagon release in hyperglycaemic conditions contributing further to postprandial hyperglycaemia in both type 1 and type 2 DM.<sup>40</sup> This can be relevant in the context of impairment of insulin secretion or action. Another defect in glucagon secretory response of  $\alpha$ -cell to low glucose concentrations is impaired in type 1 DM and long-lasting type 2 DM increasing risk of episodes of severe hypoglycaemia, especially in patients treated with insulin.<sup>41</sup> The lack of glucagon response to hypoglycaemia has been associated with multiple failure in  $\alpha$ -cell regulation. There are several problem attributed to several defects in  $\alpha$ -cell regulation including defective glucose sensing, loss of  $\beta$ -cells function, insulin resistance or autonomic malfunction. As diabetes is considered as bi-hormonal disorder with progressive  $\beta$ -cell dysfunction as well as number of characteristic  $\alpha$ -cell dysfunction, extreme hyperglycaemia occurs in insulin deficiency state, such as type 1 DM and advanced type 2 DM or ketoacidosis.<sup>37</sup> Fasting hyperglucagonaemia is observed in some that can be 50% greater than nondiabetic subjects. Paradoxical increase glucagon secretion following carbohydrate meal leads to postprandial hyperglycaemia. The response of  $\alpha$ -cell to hyperglycaemia is blunted or vanishing and plasma glucagon remains inappropriately excessive at comparable blood glucose levels. Glucagon production is also elevated by other stimulus such as arginine or protein rich food to greatest extends in type 2 DM than nondiabetic subjects. Fasting blood glucose is closely linked with abnormal elevation of hepatic glucose production accounting for 40-50% of basal hepatic glucose output. A relative increase in ratio of  $\alpha$ - to  $\beta$ -cells in pancreatic islets occurs owing to a decrease in  $\beta$ -cell mass, but the  $\alpha$ -cell mass is similar to that of nondiabetic individuals.<sup>42,43</sup> Interestingly, some type 2 DM patients show apparent  $\alpha$ -cell dysfunction without change of their islet cell composition and failure of adjacent  $\beta$ -cells.<sup>44</sup> There is possibility that diabetic patients have enhanced sensitivity of hepatic glucose production to glucagon.<sup>45</sup> In context of hyperglycaemia and hyperinsulinaemia that the glucagon

levels are relatively high to the ambient glucose level, as the glucagon level might not be increased in absolute term in early phase of type 2 DM.<sup>46</sup> So, the concept of glucagon-insulin ratio has been drawn, the term which depicts the overall islet dysfunction in type 2 DM.<sup>37</sup> After meal ingestion, hepatic glucose production is still remained near fasting level in diabetic subjects<sup>47</sup> and contribute to the postprandial hyperglycaemia and hyperglucagonaemia responsible for as much as 50% of the pathological increase in glucose.<sup>40</sup> There is evidence that  $\beta$ -cell dysfunction could contribute to  $\alpha$ -cell dysfunction.<sup>48</sup> Glucagon secretion is under paracrine control by insulin (the switch-off hypothesis) for the architectural proximity between  $\alpha$ - and  $\beta$ -cells.<sup>49</sup> Beside paracrine regulation mediated by insulin as well as other factors, the  $\alpha$ -cell also have intrinsic control of glucagon secretion. The  $\alpha$ -cells possesses the ability to response to glucose at concentration too low and it involves K-ATP channels similar to those found in  $\beta$ -cells without eliciting insulin or somatostatin secretion.<sup>34</sup> An inverted glucose regulation of glucagon release observed in type 2 DM might be result of a minute increase in  $\alpha$ -cell K-ATP channel activity.<sup>50</sup> Defective counter regulation in advanced type 2 DM with hyperglycaemia occurs and the counter regulatory effect of glucagon to hypoglycaemia is impaired and the degree of  $\alpha$ -cell dysfunction is related with lack of  $\beta$ -cell function in diabetes. Insulin represses glucagon secretion as a pulsatile manner in nondiabetic subjects, but this coordination is disrupted in patients with advanced type 2 DM and it could be potentially contribute to glucagon dysregulation.<sup>51</sup>

### Targeting the Alpha Cell- Therapeutic Potential in Diabetes Mellitus

Absolute or relative excess of glucagon seems to be critical in the development and/or maintenance of hyperglycaemia in diabetes; the strategies targeted to correct this malfunction are suitable for improvement of glucose levels. Since several components of the  $\alpha$ -cell stimulus-secretion coupling are also present in  $\beta$ -cell and  $\delta$ -cells, specific control of glucagon secretion by pharmacological modulation is complex.<sup>36</sup>

#### Preclinical Data

- I). Lack of Glucagon Receptors (Gcgr)- Glucagon receptor knock-out mice have hyperglucagonaemia and  $\alpha$ -cell hyperplasia with improved glucose tolerance and lower fasting and postprandial glucose with normal insulin level and lipidaemia, lower leptin level, normal food intake and energy expenditure.<sup>52,53</sup>
- II). Administration of IV Gcgr antisense oligonucleotide- In various rodent obesity and/or diabetes model significantly reduced blood glucose level, serum and liver triglyceride and improve glucose tolerance and develop hyperglucagonaemia in Zucker diabetic fatty rats and db/db and ob/ob mice.<sup>54,55</sup> There was hyperglucagonaemia and pancreatic hyperplasia in most models.<sup>56</sup>

III). Neutralising antibodies against glucagon- Neutralising monoclonal antibodies against glucagon in nondiabetic rats completely abolished the hyperglycaemia effect of exogenous glucagon administration and postprandial glucose suggesting glucagon antagonism maybe beneficial for diabetes.<sup>57</sup>

IV). Deletion of  $\alpha$ -cells- In transgenic mice by deletion of the  $\alpha$ -cell transcription factor Arx results in complete loss of  $\alpha$ -cell glucagon production and decrease fasting blood glucose indicating pivotal role of glucagon in glucose metabolism.<sup>58</sup>

V). Antagonising the glucagon receptors-

- 1) Peptide-based glucagon receptor antagonist- Several linear cyclic glucagon analogues have been developed to work as glucagon receptor antagonist. Essentially, they impair the ability of glucagon to stimulate adenylate cyclase utility in liver, thus reducing hepatic glucose output and improving plasma glucose levels. The des-His-glucagon antagonist binds preferentially to the hepatic glucagon receptors in vivo and associated with glucose-lowering effect.<sup>59</sup>

- 2) Non-peptide glucagon receptor antagonist-

- i) (N-(3-cyano-6-(1, 1-dimethyl propyl) 4, 5, 6, 7-tetrahydro-1-benzathion-2-y1)-2-ethylbutamide inhibit glucagon-mediated glycogenolysis in primary human hepatocytes and block the increase in glucose level after exogenous glucagon administration in mice.<sup>60</sup>

- ii) Bay 27-9955 is an oral glucagon-receptor antagonist tested in human, decreases glucose levels, but abandoned for clinical development without any rationality.<sup>61</sup> Recently, long-term studies in mice by glucagon antagonist proves its viability.<sup>62</sup> Interestingly, human mutation in Gcgr gene commonly associated with extreme hyperglucagonaemia and marked pancreatic hyperplasia presenting with abdominal mass and did not exhibit disrupted glycaemic control or dyslipidaemia.<sup>63</sup>

- iii) MK-0893- is a glucagon-receptor antagonist in phase II study in 342 type 2 DM patients given once daily in different doses had dose dependent reduction in fasting and postprandial blood glucose and HbA1c. However, there was increased LDL and liver transaminase enzymes dose dependently with increase in body weight and blood pressure lead to discontinuation for its development.<sup>64</sup>

- iv) LY-2409021- In phase I/II study, attenuated increase in both hepatic glucose output and blood glucose up to 84% and 81% respectively and decrease plasma glucose was highly correlated to the reduction in hepatic glucose output.<sup>65</sup> The agent was well tolerated with infrequent hypoglycaemia in higher dose and reversible increased liver transaminase (up to 3 times) and increased bilirubin is still in clinical development.<sup>63</sup>

## Clinically Marketed Drugs Affecting Alpha-Cell and Modulating Glucagon Secretion

### Incretin-Based Therapy

Glucagon-like peptide-1 (GLP-1) is an incretin hormone, a product of proglucagon that has nearly 50% homology to glucagon.<sup>66</sup> GLP-1 binds to a specific receptor that is distinct from, but related to Gcgr. GLP-1 regulates hyperglycaemia through variety of mechanism enhancing glucose dependent insulin secretion from  $\beta$ -cells and reducing the plasma glucagon. Alternatively, indirect control through somatostatin or neural regulation has been postulated. Thus, control of  $\alpha$ -cell secretion is a physiological role of GLP-1 and GLP-1 treatment in type 1 DM was effective to control hyperglycaemia (3-4 mm decrease) coincident with 40-50% decrease in glucose levels. This finding indicate that glucagon suppression is important part of GLP-1 effect on glycaemia control and GLP-1 based treatment in type 2 DM is optimal choice in the context of islet dysfunction with favourable glucose profile and  $\alpha$ - and  $\beta$ -cell function in type 2 DM after 1 week administration of GLP-1 agonist liraglutide.<sup>67</sup> Incretin-based therapy comprise two major class of drugs, namely the GLP-1 receptor agonist and the DPP-4 inhibitors. These agents act by increasing GLP-1 receptor signaling either by administration of exogenous GLP-1 analogues (incretin mimetic) or enhancement of endogenous GLP-1 level by DPP-4 inhibitors. Activation of GLP-1 receptors effectively inhibit glucagon secretion in human together with deceleration of gastric emptying, inhibition of food intake and elevation of insulin secretion.<sup>68,69</sup> Insulin generally thought to be inhibiting glucagon secretion. Local increment in insulin level and other  $\beta$ -cell products might inhibit  $\alpha$ -cell secretion in a paracrine manner. However, the preserved and pronounced inhibitory effect of GLP-1 agonist in type 1 DM without residual  $\beta$ -cell suggest that other mechanisms such as indirect control through somatostatin or through neural regulation have been postulated. Regardless of precise mechanism, the inhibitory effect of GLP-1 on glucagon secretion in vivo is glucose-dependent, only observed at glucose level at or above fasting levels and the inhibitory effect of GLP-1 is lost at glucose level just below normal fasting levels and the normal stimulation of glucagon secretion at hypoglycaemic level is unopposed by GLP-1. Thus, it protect from hypoglycaemia.<sup>70</sup> In contrast, glucagon suppression is progressively enhanced at higher glucose levels.<sup>68</sup>

### GLP-1 Receptor Agonist

In agreement with the effect of native GLP-1 on glucagon secretion, treatment with GLP-1 receptor agonist provides clear-cut reduction in glucagon. The glucose-lowering effect of GLP-1 in the setting of unchanged insulin levels and gastric emptying points to a substantial role of glucagonostatic effect in the treatment modalities. The insulinotropic and glucagonostatic effect were found to contribute equally to the overall glucose-lowering effect of GLP-1 reflecting the equal contribution of decrease endogenous glucose production and increase peripheral disposal to the glucose-lowering effect of GLP-1 in patients

with type 2 DM.<sup>71</sup> Among GLP-1 mimetics, exenatide is a synthetic polypeptide with high resistance to DPP-4 cleavage that decreases glucagon levels in normal and diabetic subjects.<sup>72</sup> Liraglutide is another GLP-1 derivative with long-lasting action can reduce glucagon release after a meal in patients with type 2 DM.<sup>73</sup>

### Dipeptidyl Peptidase -IV Inhibitors (DPP-4 Inhibitors)

DPP-4 is an enzyme that degrades endogenous GLP-1 and by inhibiting DPP-4 by DPP-4 inhibitor potentiates the action of the incretin pathways. In addition to increasing intact levels of GLP-1, also expected to affect glucagon levels through elevated levels of other incretin hormone, i.e. GIP, which has glucagon-releasing effect, however, the glucagonostatic effect of GLP-1 prevails.<sup>74</sup> Beside, the improvement in plasma glucose and HbA1c, trials of DPP-4 inhibition in patients with type 2 DM convincingly demonstrate that glucagon levels are reduced.<sup>75,76</sup> The suppression of glucagon is observed with both acute and chronic treatment. Some studies demonstrated clear dose dependency with regard to restraining postprandial glucagon response and one study reported lowering of fasting glucagon levels dose dependently. Thus, the glucagonostatic effect is consistent finding across the various studies of DPP-4 inhibitors.<sup>63</sup> Recent studies demonstrated that DPP-4 inhibitors enhance  $\alpha$ -cell responsiveness to both the suppression effect of hyperglycaemia and the stimulatory effect of hypoglycaemia. The last effect is due to increased levels of GIP contributing to enhanced glucagon secretion with low risk of hypoglycaemia with DPP-4 inhibitors treatment.<sup>77</sup>

### Amylin and Pramlintide

Amylin is a peptide hormone cosecreted with insulin from  $\beta$ -cells of pancreatic islet, inhibit glucagon secretion stimulated by amino acids, but dose not affect hypoglycaemia-induced glucagon release.<sup>78</sup> Pramlintide is a synthetic analogue of amylin and approved in USA since 2005 for treatment of both type 1 and type 2 DM as an adjunct to insulin in patients who fail to achieve desired blood glucose with optimal meal time insulin therapy, but not approved in European Union. In human, amylin inhibit meal-related glucagon secretion without effect on hypoglycaemic stimulation of glucagon secretion.<sup>79</sup> Since  $\alpha$ -cell response to amino acids is often exaggerated in diabetic patients, amylin or amylinomimetic compounds such as pramlintide are used as an effective alternative for the treatment of postprandial and amino acid induced excess glucagon secretion.<sup>19,80</sup> Amylin also suppress gastrointestinal motility and food intake, which contribute to improved postprandial glycaemia.<sup>79,80</sup> The mechanism of glucagon suppression has been reported to be extrinsic to pancreatic islet; however, others have reported a direct intra-islet inhibitory effect of IAPP on glucagon secretion. Nevertheless, evidences suggest that the pivotal receptors conveying the actions of amylin are situated in the area prostema, a periventricular structure in the hind brain and critical for sensing and integrating peripheral and central

meal-related signals.<sup>80</sup> Several clinical trials of pramlintide in patients with type 1 DM and type 2 DM have demonstrated robust postprandial glucagon suppressive effect in combination with insulin dose-dependent reduction in HbA1c and weight reduction with treatment duration of 3-12 months. However, the relative contribution of the positive effect, i.e. inhibitory effects on glucagon release, gastric motility and appetite are largely unrevealed.<sup>63</sup>

## CONCLUSION

Proper functioning of  $\alpha$ - and  $\beta$ -cells is key to the glycaemic homeostasis. Numerous findings indicate that defects of glucagon secreting  $\alpha$ -cell get involved with development and exacerbation of hyperglycaemia in both type 1 and type 2 DM owing to over secretion of glucagon when it is not needed and poor production when it is needed. Plasma glucagon levels are abnormally high even within normal range and normally not responsive to usual regulation. Studies suggest that normalisation of glucagon secretion could have potential effect on glycaemic control. Since, the specific control of glucagon secretion by pharmacologic modulation is complex and several components of  $\beta$ -cells stimulus secretion coupling are also present in  $\beta$ - and  $\delta$ -cells. Thus, the manipulation of glucagon action by modulating the Glucagon Receptors (Gcgr) signaling seems to be effective alternative. The glucagon receptors antagonist have yet to prove their efficacy and safety and complete glucose-independent antagonism of glucagon is not optimal way to dampen endogenous glucose production. In type 1 DM, drugs that target the incretin pathways such as GLP-1 agonist and DPP-4 inhibitors exert some of their glycaemic effects through a reduction on endogenous glucagon secretion suggesting insulin-independent inhibitory effects on  $\alpha$ -cells and/or insulin sensitising effect. Currently, GLP-1 analogues and DPP-4 inhibitors are not licensed for use in type 1 DM and require large clinical trials to confirm its safety and efficacy. In type 2 DM, convincing evidences from preclinical and clinical humans studies has been demonstrated the applicability of targeting the pancreatic  $\alpha$ -cell and its main secretory product glucagon in the treatment of type 2 DM. The incretin-based therapy, inhibit endogenous glucose production in glucose-dependent manner and improves  $\alpha$ -cell response to hypoglycaemia. The glucagonostatic effect of pramlintide in treating type 2 DM could benefit. Now, the inappropriate glucagon secretion in type 1 DM and type 2 DM seems most favourably addressed by employing incretin-based treatment and have the greatest potential to provide improvement in quality of life and lifespan of diabetic patients.

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