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# Problem or solution: The strange story of glucagon

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ABSTRACT

Globally, 13% of the world's adult population is obese, and more than 400 million people suffer from diabetes. These conditions are both associated with significant morbidity, mortality and financial cost. Therefore, finding new pharmacological treatments is an imperative. Relative hyperglucagonaemia is seen in all types of diabetes, and has been implicated in its pathogenesis. Consequently, clinical trials are underway using drugs which block glucagon activity to treat type 2 diabetes. Conversely, exogenous glucagon can increase energy expenditure. Therefore, researchers are designing peptides that combine activation of the glucagon receptor with further incretin properties, which will treat obesity while mitigating the hyperglycaemic effects of glucagon. This review will discuss these conflicting physiological properties of glucagon, and the attempts to harness these effects pharmacologically.

## 1. Introduction

Glucagon as a subject of study had an inauspicious start. It was discovered as an impurity in early preparations of insulin, a 'toxic fraction' causing a rise in blood glucose and even death [1,2]. Kimball and Murlin named it glucagon (GLUCose-AGONist) after a series of experiments designed to concentrate and isolate pure insulin found a precipitant which increased blood glucose in depancreatized dogs. However it took a further 25 years before Sutherland and De Duve purified glucagon itself [3].

Glucagon is a 29 amino acid peptide produced by the alpha cells in the pancreas. It is produced by proconvertase 2 processing products of the pre-pro-glucagon gene. Classically hypoglycaemia triggers glucagon release. Hypoglycaemia is sensed within the hypothalamus, particularly the ventromedial hypothalamic nucleus [4,5], and the parasympathetic nervous system relays the signal to the pancreas to cause glucagon release [6–8]. The sympathoadrenal response to hypoglycaemia also stimulates glucagon release [7,9], and intra-islet glucose levels affect glucagon production as well [9]. Glucagon release is inhibited by hyperglycaemia, insulin, GLP-1 and somatostatin [10–12]. Glucagon acts via a specific G-protein coupled receptor, which has wide-spread expression throughout the body, being particularly abundant in the liver, kidney, heart and adipose [13].

The main function of glucagon is to increase blood glucose, through both glycogenolysis and increased gluconeogenesis. It also affects lipid metabolism, breaking down fat through lipolysis and increasing ketone production [14]. Glucagon affects protein metabolism, increasing ureagenesis and causing amino acid uptake into hepatocytes [15–17]. The resultant carbon skeletons can then enter the gluconeogenic pathway. Glucagon therefore acts in multiple ways to maintain fuel supply to all organs in the body (Fig. 1).

## 2. The bihormonal hypothesis

It was recognised in the 1920s that insulin deficiency was the cause of diabetes, and that administration of pancreatic extracts containing insulin could successfully treat the hyperglycaemia [18,19]. In contrast, glucagon appeared to have little significant function in disease, and its clinical use was limited to rare occasions of reversing the effects of insulin [20,21]. Then in 1973, Roger Unger and Lelio Orci proposed the bihormonal-abnormality hypothesis of diabetes, stating that glucagon elevation was as important as insulin deficiency [22]. This rooted glucagon as a central problem in the disease.

Glucagon has been found to be elevated in all forms of diabetes, from alloxan-induced diabetes in dogs, to patient with type 1 and type 2 diabetes; even following pancreatectomy [23–29]. Physiological studies have also demonstrated that glucagon has opposing effects to insulin on carbohydrate, fat and protein metabolism, with insulin causing glycogenesis, lipid formation and having an anabolic effect on muscle [30–37]. The bihormonal-abnormality hypothesis combines these two findings, stating that the relative glucagon excess and insulin deficiency, plus the opposing actions of these two hormones, leads to the hyperglycaemia of diabetes.

The bihormonal hypothesis was supported by studies that showed

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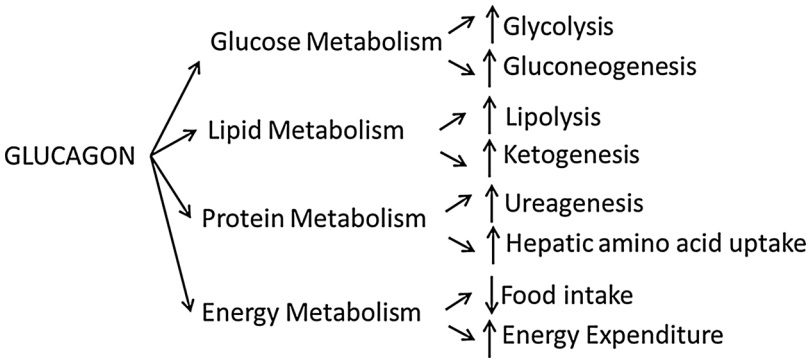


**Fig. 1.** Summary of the metabolic effects of glucagon.

that suppression of glucagon with somatostatin limited the hyperglycaemia seen in patients with type 1 diabetes and alloxan-diabetic dogs [38–40]. Over the following 40 years, further evidence was produce by studies using glucagon-receptor knock-out mice. Compared to wild-type mice, *Gcgr-/-* mice have constitutively lower blood glucose levels, improved glucose homeostasis and a lean phenotype [41]; they are also resistant to the hyperglycaemic and hyperinsulinaemic effects of high-fat diet, and following beta-cell destruction with streptozotocin, have normal glucose levels and improved response to glucose challenges, as well as lower levels of gluconeogenic enzymes, all markers of improved diabetic control [42,43]. Moreover, if the glucagon receptor is restored with adenovirus, the diabetic profile is returned [44]. However, recent studies have shown that while preventing glucagon activity can mitigate the effects of insulin deficiency, this only works if there is some residual insulin signalling left. In absolute insulin deficiency, due to either complete beta-cell destruction or insulin gene knockout, glucagon action blockade does not prevent hyperglycaemia [45–47].

### 2.1. Glucagon receptor antagonists

Nevertheless, the bihormonal hypothesis has made blockade of the glucagon receptor a potential treatment for diabetes. Preclinical studies have supported numerous different approaches Table 1. Glucagon receptor antagonists improve glucose-mediated blood glucose excursions in mice including diabetic models [48–50]. Glucagon receptor antibodies reduce baseline glucose levels and improve glucose tolerance in diabetic rodents and monkeys [51–54]; they also reduce the hepatic expression of gluconeogenic enzyme mRNA [51]. Anti-sense oligonucleotides which reduce expression of the hepatic glucagon receptor also improve glucose levels and improve glucose tolerance in diabetic mice [55]. Even glucagon-neutralizing L-RNA aptamers (Spiegelmers) have been developed, which improve glucose excursions following IPGTTs in diabetic mouse models [56].

The success of these pre-clinical studies has inevitably led to trials in humans of agents which reduce activity at the glucagon receptor. These trials have confirmed that this is an effective approach for treatment of diabetes. Glucagon receptor antagonists improve fasting and post-prandial blood glucose levels, as well as HbA1c [57–61]. Antisense oligonucleotides also improve HbA1C in people with diabetes in phase 2 clinical trials [62–64] while monoclonal antibodies against the glucagon receptor reduce glucagon-induced glucose excursions [65].

**Table 1**
Classes of drugs being developed which target glucagon activity to treat obesity and diabetes.

| Agents developed to block activity at the glucagon receptor to treat diabetes | Glucagon-receptor co-agonists developed to treat obesity |
|---|---|
| Glucagon receptor antagonists [48–50,57–61] | Glucagon/GLP-1 co-agonists [85–90] |
| Antibodies against the glucagon receptor [51–54,65] | Glucagon/GLP-1/GIP tri-agonists [110–112] |
| Antisense oligonucleotides [55,62–64] | T3 coupled to glucagon [113] |
| Glucagon-neutralizing Spiegelmers [56] | |

Each of these classes of glucagon blocking drugs has been associated with significant side effects. Increased hepatic transaminases have been seen with the antisense oligonucleotides [64], glucagon receptor antagonists [57–60,66,67] and humanized monoclonal antibodies [65]. The small molecule glucagon receptor antagonists also increase LDL cholesterol, a highly undesirable side effect given the association of increased cholesterol, type 2 diabetes and cardiovascular disease [60,61,67,68]; and one, LY2409012, causes an increase in hepatic fat fraction [66]. All can exaggerate a fall in blood sugar, with the potential for serious hypoglycaemia, though the number of symptomatic hypoglycaemic episodes actually seen in clinical trials is low [57,58,66,67]. Pre-clinical studies have also shown that glucagon receptor antibodies cause compensatory alpha cell hyperplasia [51,52]. What clinical effect this has long-term has not yet been ascertained, but there is a concern that this hyperplasia may become malignant. These side effects have stymied the development of several GRAs (including MK-3577 and MK-0893), and indeed there are no anti-diabetic agents in current clinical practice which work by blocking glucagon activity. Nevertheless, several agents are still in the developmental pipeline, and the results of further clinical trials are awaited.

### 2.2. Glucagon and obesity

Much research has focused on blocking the action of glucagon to treat diabetes since the articulation of the bi-hormonal abnormality hypothesis. However, there has been recent interest in using glucagon to treat obesity, and subsequently treat type 2 diabetes through weight loss.

The hyperglycaemic effects of glucagon were first noted in the 1920s. It wasn't until the 1950s that glucagon was found to have other metabolic effects. In 1957, Schulman et al. showed that glucagon reduced appetite, and could even cause weight loss in man [69]. Then in 1960, Salter showed through a pair-feeding paradigm that glucagon causes an increase in energy expenditure in rodents [70], an increase that was confirmed by indirect calorimetry [71]. Subsequently, several studies in man have shown that an infusion of glucagon can increase energy expenditure as well as reduce food intake [71–74]. This combination of effects makes glucagon a very attractive anti-obesity treatment, as typically drugs which increase energy expenditure also cause an increase in food intake [75,76], which means there is likely to be no overall loss of weight; and conversely, but equally problematically for an obesity treatment, reducing food intake is usually accompanied by a decrease in energy expenditure, with subsequent limits as to the weight which can be lost [77].

### 2.3. Glucagon/incretin receptor dual agonists

The question remained, however, as to how to harness these effects of glucagon without causing harmful hyperglycaemia. The answer