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# Glucagon physiology and pathophysiology in the light of new advances

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**Summary.** Recent advances in the understanding of glucagon-insulin relationships at the level of the islets of Langerhans and of hepatic fuel metabolism are reviewed and their impact on our understanding of glucagon physiology and pathophysiology is considered. It now appears that $\alpha$ cells can respond directly to hyperglycaemia in the absence of insulin and $\beta$ cells, but that antecedent hyperglycaemia masks or attenuates this response. Insulin appears to exert ongoing release inhibition upon glucagon secretion, probably via the intra-islet microvascular system that connects $\beta$ cells to $\alpha$ cells. Diabetic hyperglucagonemia in insulin deficient states appears to be secondary to lack of the restraining influence of insulin. The $\alpha$ cell response to glucopenia, by contrast, may be in large part mediated by release of noradrenaline from nerve endings in contact with $\alpha$ cells. Glucagon's action on glucose and ketone production by hepatocytes is mediated by increase in cyclic-AMP-dependent protein kinase. The opposing action of insulin upon glucagon-mediated events probably occurs largely at this level. Consequently, when glucagon secretion or action is blocked, cyclic-AMP-dependent protein kinase activity is low even in the absence of insulin, explaining why marked glucose and ketone production is absent in bihormonal deficiency states.

**Key words:** Glucagon, diabetes, noradrenaline, insulin, fructose-2,6-bisphosphate, cyclic AMP-dependent protein kinase.

Although in certain respects glucagon is the most fully understood of the polypeptide hormones, ironically it has also been the most controversial. However, recently gained insights into insulin-glucagon interactions at both the level of the islets of Langerhans and of hepatic target cells have served to clarify certain of the disputed issues concerning glucagon's role in normal and diabetic fuel metabolism. In this communication controversial questions concerning insulin-glucagon interactions at two controversial sites, the islets of Langerhans and the hepatocytes, are re-examined in the light of information not available at the time of more comprehensive recent reviews [1, 2].

## Insulin-glucagon relationships within the islets of Langerhans

### *Glucose and the α cell*

Regulation of normal fuel homeostasis requires appropriate reactions of $\beta$ and $\alpha$ cells to a spectrum of physiological events. While the various signals that elicit such reactions have been difficult to sort out, clearly the overriding signal to both $\alpha$ and $\beta$ cells is the ambient glucose concentration, changes of which elicit a reciprocal response of insulin and glucagon (Fig. 1). The mechanism by which changes in extracellular glucose concentration influence glucagon secretion has been controversial. It is unclear whether $\alpha$ cells sense and respond directly to glycaemic change or if, as recently proposed [3, 4], insulin, an inhibitor of glucagon secretion [5], mediates their response. According to the latter hypothesis, hyperglycaemia suppresses glucagon by stimulating insulin secretion, while hypoglycaemia stimulates glucagon by suppressing insulin secretion. The idea of insulin mediation of glucagon responses to glycaemic changes was buttressed by the fact that suppression of $\alpha$ cells by glucose had never been demonstrated in the absence of $\beta$ cells and insulin; $\alpha$ cells that lack contact with $\beta$ cells [e.g. those of patients with Type 1 (insulin-dependent) diabetes and of alloxan-diabetic dogs (Fig. 2a) and those in the gastric fundus of dogs or in a glucagonoma] could be suppressed by insulin but not by glucose [6-11]. Recently, however, it was shown

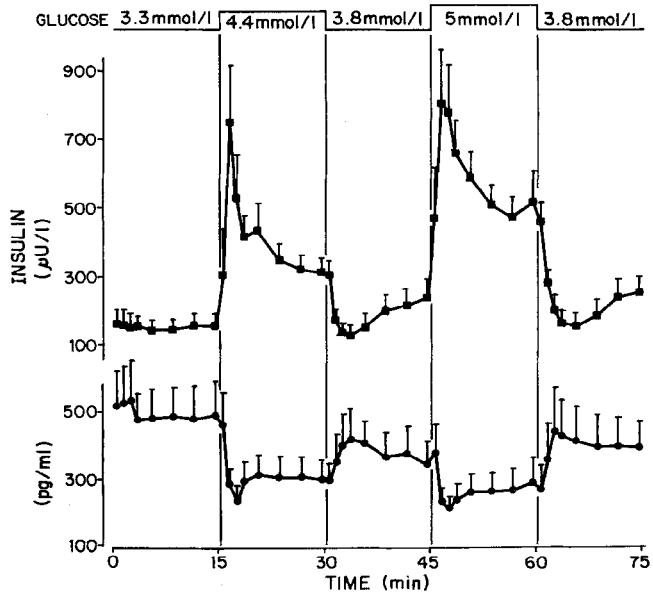


**Fig. 1.** A demonstration of the promt and reciprocal nature of the insulin (■—■) and glucagon (●—●) responses to change in glucose concentration in the isolated perfused dog pancreas. (Unpublished work by K Kawai and RH Unger reproduced from [42])

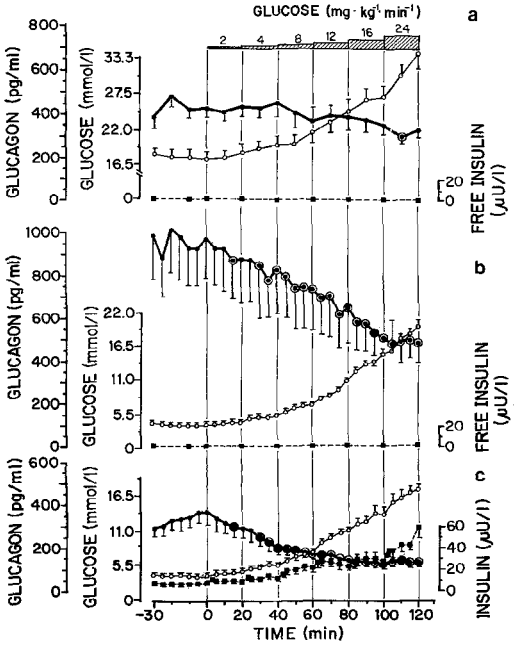


**Fig. 2a–c.** A comparison of the glucagon response (●—●) to progressively increasing rate of glucose infusion in **a** insulin-deprived alloxan-diabetic dogs with hyperglycaemia, **b** the same insulin-deprived alloxan-diabetic dogs pretreated with phloridzin to eliminate hyperglycaemia and **c** non-diabetic phloridzinized dogs. Glucagon levels that differ significantly ($p < 0.05$) from the basline value are circled. Glucose levels are represented by ○—○ and insulin by ■—■. The suppression of glucagon observed in **b** in the absence of insulin or $\beta$ cells is consistent with an intrinsic glucose sensing system for $\alpha$ cells. The fact that glucose-induced suppression of glucagon in B stops when glucagon levels have reached the basal levels of unphloridzinized insulin-deprived diabetic dogs **a** suggests that in the latter group the hyperglycaemia has already achieved maximal glucagon suppression and that the residual hyperglucagonaemia is the consequence of absence of insulin-mediated glucagon suppression (Fig. 3). (Reprinted with permission of Proc Natl Acad Sci USA [12])

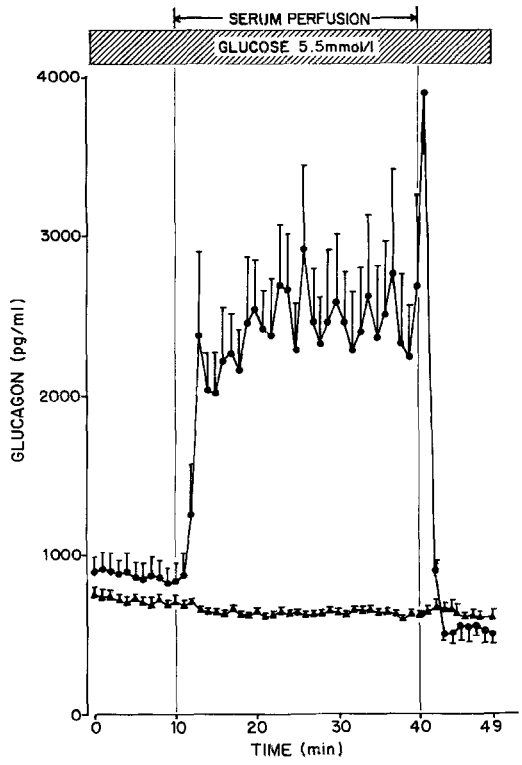


**Fig. 3.** The effect of a potent guinea pig anti-insulin serum (●—●) or nonimmune guinea pig serum (▲—▲) on glucagon levels in the isolated perfused rat pancreas. The antiserum is known to be capable of complete neutralization of up to 20 mU within 1 min under the conditions that pertain in this nonrecirculating perfusion system. The rapidity of the on-off is consistent with neutralization of insulin in the microcirculation of the islets. (Reprinted with permission of the J Clin Invest [14])

that the hyperglucagonaemia of insulin-deprived alloxan-diabetc dogs made normoglycemic by pretreatment with the glucuretic agent, phloridzin, responds normally to small increments in glucose [12] (Fig. 2b). Thus, glucose can suppress pancreatic $\alpha$ cells in the absence of $\beta$ cells and insulin *provided there is no ambient hyperglycaemia*. Hyperglycaemia produces an apparent glucose unresponsiveness of $\alpha$ cells probably by preempting and/or down-regulating the glucose sensing sites that mediate the negative glucagon secretory response to a rise in glucose. It is probable that these cells have responded already to glucose; the hyperglucagonaemia that persists despite the hyperglycaemia is the consequence of insulin deficiency and can be fully reduced to normal by insulin.

### *Insulin and the α cell*

Despite the evidence that insulin is a potent inhibitor of glucagon secretion [3], the regulatory role of this effect upon $\alpha$-cell function has been uncertain. Two recent reports seem to have clarified this point, at least in the rat. Firstly, studies of the microcirculation of the rat islet indicate that blood flows from the $\beta$ cell-rich islet medulla to its $\alpha$ cell-rich cortex before leaving the islet [13]. $\alpha$ cells are thus exposed to the highest insulin concentration in the body. Secondly, single pass perfusion of normal rat pancreas with a potent neutralizing anti-insulin serum results in marked hyperglucagonaemia (Fig. 3) [14]. The rapidity of this effect suggests that it is the consequence of neutralization within the islet microvasculature of insulin en route from $\beta$ cells to $\alpha$ cells and does not involve neutralization of insulin in the islet interstitium. It would seem that insulin within the islet microcirculation acts as a release-inhibiting factor of glucagon. An action of insulin on $\alpha$ cells via interstitial pathways cannot be excluded, but remains to be clearly established.

### *Glucopenia and the α cells*

The mechanism by which glucopenia stimulates glucagon secretion has also been controversial. Simple lack of glucose [15] and/or its metabolites and/or reduction of insulin-mediated $\alpha$-cell suppression [3, 4] in the islet have been favoured explanations. However, recent work in the rat raises the possibility of a novel mechanism. The $\alpha$-adrenergic blocker phentolamine was

found to reduce by about 70% the effect of glucopenia upon glucagon secretion in the isolated perfused pancreas of normal rats [16]. This suggests an adrenergically mediated system within the pancreas that senses and responds to glucopenia without any input from centres in the hypothalmus, which previously were believed to control all sympathetic regulation of islet cell secretion [17]. Preliminary evidence suggests that loss of the glucagon response to glucopenia in the perfused pancreas of streptozotocin-diabetic rats may be, in part, a consequence of damage to this adrenergically mediated system.

To summarize current concepts of $\beta$-$\alpha$ cell relationships, the $\alpha$ cells of the rat are under the constant restraining influence of insulin within the islet microcirculation in the highest concentrations in the body. $\alpha$ cells can sense and respond normally to a rise in glucose concentration in the complete absence of $\beta$ cells and insulin. Glucopenia, on the other hand, stimulates glucagon secretion largely (but not entirely) via an intrapancreatic $\alpha$-adrenergic mechanism that operates independently of the central nervous system.

## Glucagon-insulin relationship in the liver

A host of pathophysiological and clinical observations [18–22] indicate that hepatic over-production of glucose and ketones in the insulin-deficient state cannot occur unless glucagon is present, i.e. that insulin lack produces the catabolic cascade in the liver not directly but via glucagon-mediated mechanisms. This, the so-called "bihormonal abnormality hypothesis" of diabetes mellitus [23], has been perhaps the most controversial of recent glucagon-related issues. Now, however, it can be readily explained at the molecular level.

### *The molecular physiology of glucagon and insulin* (Figs. 4 and 5)

Within seconds after glucagon interacts with its receptor on liver cells, adenylate cyclase is activated and intracellular cyclic AMP concentrations rise. The increase in cytosolic cyclic AMP activates the all-important cyclic AMP-dependent protein kinase which, by phosphorylating certain key enzymes, induces the major hepatic actions of glucagon (stimulation of glycogenolysis, gluconeogenesis and ketogenesis and inhibition of glycogen synthesis, glycolysis and lipogenesis). As depicted in Figure 4, cyclic AMP-dependent protein kinase influences glycogen metabolism by phosphorylating (and thus activating) phosphorylase, the rate-limiting enzyme of glycogenolysis, and by phosphorylating (and thus inactivating) glycogen synthase [24]. Glycogenolysis is thereby increased and glycogenesis inhibited. Insulin opposes this action largely by reducing cyclic AMP-dependent protein kinase activity [25]. (It may also oppose glucagon by increasing the activity of phosphodiesterase, the enzyme that degrades cyclicyclic AMP [26].) In any case, when insulin is not present, the unopposed action of glucagon greatly increases cyclic AMP and cyclic AMP-dependent protein kinase and initiates the catabolic cascade (Fig. 5). But, if glucagon is not present cyclic AMP and cyclic AMP-dependent kinase activity are low and a major site of insulin action on hepatic fuel metabolism is eliminated (Fig. 5). This explains why the excessive hepatic fuel production that characterizes the insulin-deficient state does not occur in the total absence of glucagon.

Increased cyclic AMP-dependent protein kinase activity augments gluconeogenesis and ketone production by the same mechanism, i.e. phosphorylation of key proteins (Fig. 4). The key protein in this case is the bifunctional enzyme that controls the level of fructose-2,6-bisphosphate (F-2,6-$P_2$), the recently discovered regulator of glycolysis and gluconeogenesis [27–29]. When glucagon levels are high relative to insulin and the enzyme is in a phosphorylated state, it acts as a fructose-2,6-bisphosphatase and lowers the levels of F-2,6-$P_2$ within the hepatocyte. This increases gluconeogenesis, thus explaining the increase in hepatic glucose production from non-glucose sources. Low F-2,6-$P_2$ levels also block glycolysis, which reduces the flow of 3-carbon fragments [30], the substrate for fatty acid synthesis (Fig. 4). In addition to substrate depletion, glucagon reduces lipogenesis by inhibiting acetyl-carboxylase activity [31], probably via a phosphorylation mechanism [32]. The reduction in lipogenesis is responsible for the increased ketogenesis. The initial product of fatty synthesis, malonyl-CoA, is the normal inhibitor of carnitine palmitoyl transferase-1 [33], the enzyme that transesterifies fatty acyl-CoA to fatty acyl carnitine, in which form fatty acids can cross into the mitochondria, the site of oxidation to ketones [34]. Depletion of malonyl-CoA by reduction in lipogenesis removes this normal restraint upon ketogenesis [35]. In addition, glucagon increases hepatic carnitine levels via an unknown mechanism [36]. Finally, the lack of insulin increases the availability of non-esterified fatty acids to the liver for oxidation to ketones through diminished insulin-mediated inhibition of lipolysis in adipocytes. As mentioned, insulin reverses all these changes by reducing the activity of cyclic AMP-dependent protein kinase (Fig. 4).

Finally, demonstration of the remarkable potency of glucagon action on the liver explains how over-production of glucose and ketones may occur in insulin-deprived depancreatized patients considered to be deficient in glucagon [37]. Glucagon in concentrations as low as $10^{-13}$ M [38] reportedly reduces intrahepatic F-2,6-$P_2$ levels. This is well below the sensitivity of radioimmunoassays and would account for the fact that seemingly hypoglucagonaemic depancreatized patients developed diabetic ketoacidosis [37]. (However, more recent studies in such patients report glucagon levels to be in the normal or low-normal range [39, 40].)

To summarize, the fact that insulin's major metabolic effect on hepatocytes is to oppose glucagon explains why the presence of glucagon is required for the full metabolic expression of the syndrome of insulin-dependent diabetes mellitus. When glucagon is suppressed or inactivated [41], insulin-dependent diabetes mellitus is,

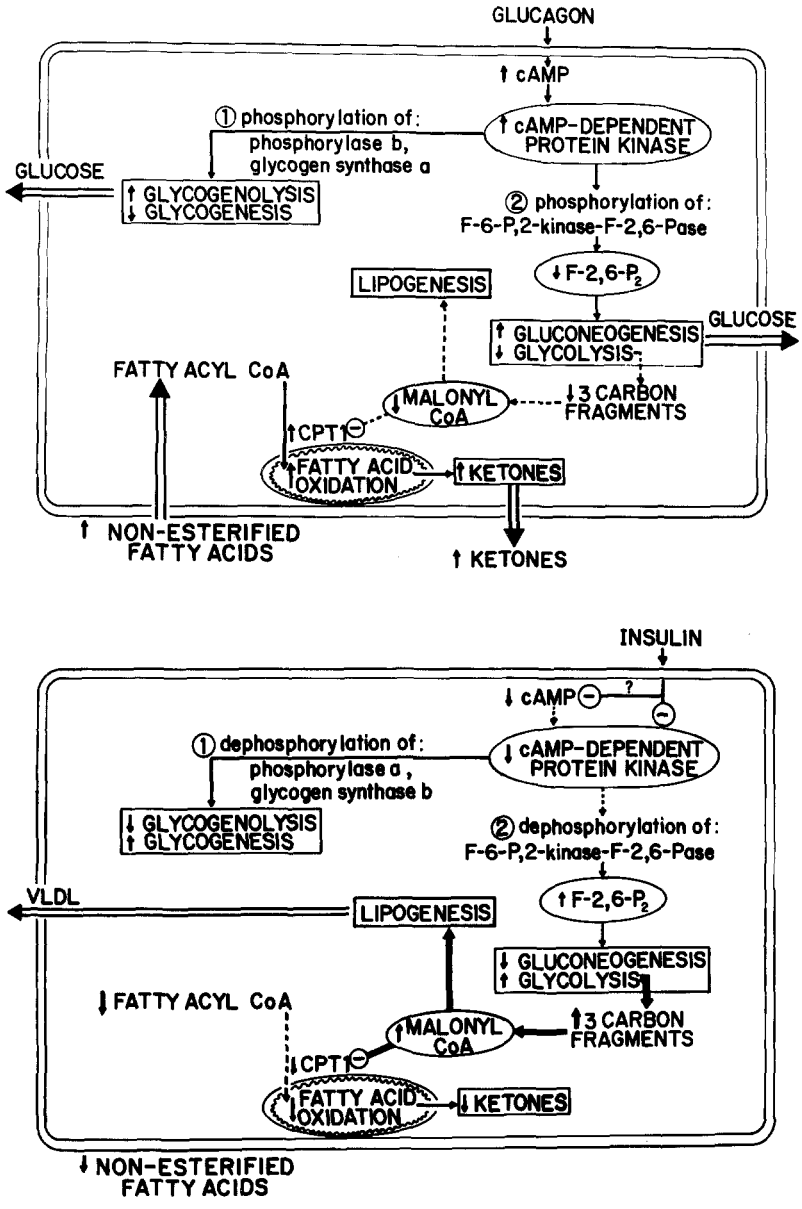


**Fig. 4.** A panoramic perspective of the major sites of glucagon-insulin interactions at the hepatocyte. The upper panel depicts the unopposed effects of glucagon and the lower panel the opposing actions of insulin. CPT = carnitine palmitoyl transferase. (Reprinted with permission from Diabetes Annual)

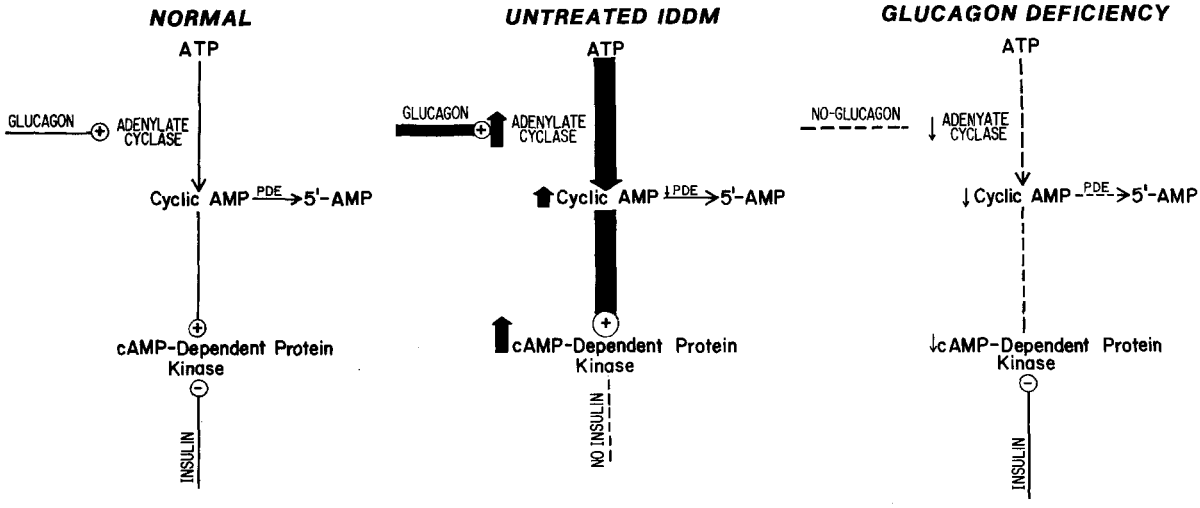


**Fig. 5.** The probable sites of insulin opposition to glucagon action in the hepatocyte. The principal site of opposition appears to be at the level of cyclic AMP-dependent protein kinase, although insulin may also increase phosphodiesterase activity (not shown), thereby lowering cyclic AMP levels. The lefthand panel portrays normal glucagon-insulin interaction at this level; the middle panel glucagon action unopposed by insulin, as would pertain in insulin-deficient states; the righthand panel depicts and explains why insulin's hepatic influence is minimal when glucagon is not present

in a sense, "converted" to a syndrome more compatible with the clinical definition of non-insulin-dependent diabetes [42].

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