GLUCAGON: ITS SIGNIFICANCE IN HEALTH AND DISEASE

by

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HISTORICAL

In the early 1920's when crude extracts of pancreas were used as the source of insulin for animal experiments, it was noted that a transient rise in blood sugar often occurred before the expected insulin hypoglycaemia. The effect was at first wrongly attributed to adrenaline but soon the presence of a hitherto unknown hyperglycaemic agent was suspected.

Glucagon, the fraction responsible for the hyperglycaemia, was isolated by Collens and Murlin in 1929. However, it was not until the 1950's, following the introduction of new electrophoretic and chromatographic techniques that glucagon was obtained in sufficiently pure form to allow chemical characterization (Staub, Sinn and Behrens, 1953). Porcine glucagon was shown to be a single chain polypeptide of 29 amino acids with molecular weight 3485 (Bromer, Sinn and Behrens, 1957). Subsequently immunofluorescent techniques revealed that the alpha cells of the pancreatic islets were its source (Baum, Simons, Unger and Madison, 1962).

The glucagon molecule shows a striking similarity to the secretin molecule chemically and shares some biological properties with it. Secretin is composed of 27 amino acids. Starting from the N-terminus 14 positions are occupied by the same amino acids. Under certain conditions both hormones can stimulate lipolysis and insulin secretion.

PHYSIOLOGY

Following secretion from the pancreas glucagon is either degraded by proteolytic enzymes in the plasma (Eisentraut, Whissen and Unger, 1968) or metabolised and cleared by the liver (Buchanan et al, 1968) and kidneys (Lefebvre, Luyckx and Niset, 1974). Degraded hormone has no biological activity. At present little is known about the physiological factors controlling rate of breakdown and clearance of the hormone.

Regulation of glucagon secretion by changes in plasma glucose appears to be as sensitive as regulation of insulin secretion by this means. Hypoglycaemia induced by insulin (Ohneda et al, 1969; Gerich et al, 1974c), by a sulphonylurea (Buchanan et al, 1969) or by starvation (Aguilar-Parada et al, 1969) is associated with a rise in glucagon levels while hyperglycaemia causes a fall in the level (Unger et al, 1970). More recently glucagon has been shown to have a role in the maintenance of blood sugar during overnight fasting (Alford et al, 1974; Gerich et al, 1975). In the liver
glucagon stimulates glycogenolysis and gluconeogenesis while inhibiting glycogen synthesis; these changes being associated with a rise in hepatocyte cyclic 3', 5'-adenosine monophosphate (cAMP) level.

Aminoacids stimulate both insulin and glucagon secretion, the rise in glucagon serves to limit the fall in glucose that would occur if insulin alone was secreted after a mainly protein meal (Unger et al, 1969). The aminogenic glucagon response is abolished by hyperglycaemia (Müller et al, 1970) but where there has been prolonged dietary carbohydrate restriction there is a brisk aminogenic glucagon response from an already elevated basal glucagon level (Müller et al, 1971a).

Glucagon can cause lipolysis in rat (Hagen 1961) and avian (Langslow & Hales, 1970) adipose tissue and triglyceride breakdown in perfused rat liver (Penhos et al, 1966). However, it has been difficult to show a lipolytic effect of glucagon at physiological levels in man. Supraphysiological levels have lipolytic and ketogenic effects in the human (Liljenquist et al, 1974). Free fatty acid (FFA) levels and glucagon levels have an inverse relationship in humans (Gerich et al, 1974a) and dogs (Luyckx and Lefebvre, 1969). However, in man should hyperglycaemia coexist with very low FFA levels the effect of glucose on glucagon secretion will predominate (Gerich et al, 1974). Absorption of a fat meal leading to a rise in plasma triglyceride (TG) level causes no change in plasma glucagon level in humans but is associated with a rise in dogs. In dogs, infused TG causes no glucagon rise and it has been postulated that an enteric signal such as pancreozymin may be responsible for the hyperglucagonaemia following a fat meal (Böttger et al, 1973). At pharmacological levels glucagon will lower plasma TG levels (Eaton, 1973) but this effect has not yet been shown at physiological levels of the hormone.

Glucagon at physiological concentrations stimulates insulin release (Ketterer et al, 1967). The insulingenic and hyper glycaemic properties of glucagon are independent, the former probably being important for insulin-dependent glucose metabolism in peripheral tissues during times of starvation or low carbohydrate intake.

Insulin exerts a strong influence over glucagon secretion. High glucagon levels found in experimental insulin deficiency in dogs are promptly lowered by insulin administration (Müller et al, 1971b). Similarly when insulin-deficient isolated pancreatic islets were incubated in high glucose media glucagon secretion was suppressed only after addition of insulin (Buchanan and Mawhinney, 1973).

Although glucagon at pharmacological levels can cause catecholamine release (Sarcione et al, 1963; Sheps and Maher, 1968) this seems not to occur at physiological levels (Broadus et al, 1970). Glucagon secretion rises abruptly under acute stress (Bloom, 1973) and during adrenaline infusion (Gerich, Karam and Forsham, 1973). It is now clear that the autonomic nervous system, especially the parasympathetic is important in mediating the glucagon response to hypoglycaemia (Bloom, Edwards and Vaughan, 1974) and in man following truncal vagotomy this response is very poor (Bloom, Vaughan, Russell, 1974).
Energy consumption rises abruptly with exercise and it is not surprising that levels of glucagon, a fuel mobilizer, rise too. This response can be blocked by propranolol pretreatment (Luyckx and Lefebvre, 1974) and is probably dependent on the sympathetic nervous system.

Food (or its absorption products) and the autonomic nervous system seem to have major roles in the control of glucagon secretion. Secretion can be modified by substances which change the intracellular cAMP level (Marco et al, 1973) or destroy the microtubular apparatus of the alpha cell (Leclercq-Meyer et al, 1974; Edwards, 1973). Potassium imbalance modifies secretion (Kuzuya et al, 1974; Santeusanio et al, 1973). Whereas it has been clearly shown that low calcium levels cause decreased insulin secretion either under experimental conditions (Grodsky and Bennett, 1966) or in man (Laron and Rosenberg, 1970), the role of calcium in glucagon secretion remains controversial (Leclercq-Meyer et al, 1973; Gerich et al, 1974b).

Somatostatin is a tetradecapeptide isolated from the hypothalamus. Recently it has been found in the D cells of pancreatic islets. Somatostatin (growth hormone release inhibiting factor) inhibits the release of both insulin and glucagon.

Other actions of glucagon possibly only of pharmacological importance are augmented urinary excretion of sodium, potassium, calcium, phosphate and magnesium (Birge and Avioli, 1969), positive inotropism on the myocardium (Parmley et al, 1968), inhibition of gastric motility (Necheles et al, 1966) and stimulation of growth hormone release (Mitchell et al, 1969).

**Measurement of glucagon**

Attempts at precise measurement of circulating levels of pancreatic glucagon have met with many technical problems. Circulating levels are low and radioimmunoassay has been the method of choice. One of the important basic needs for a sensitive and precise radioimmunoassay is an antibody with high specificity for the hormone (or antigen) being measured. Experienced workers have found that most antibodies raised to purified glucagon of pancreatic origin will cross-react with a number of polypeptides from the gastrointestinal tract. These peptides are as yet poorly characterized and their relationships to pancreatic glucagon remain obscure. A few antibodies have been found which show little cross-reactivity with these gut peptides.

Measurements of plasma ‘glucagon’ levels with such antibodies have been used to define the physiological circulating levels of glucagon. Such an antibody was first discovered in 1968 (Eisentraut et al, 1968); since then a great deal of work has been done to define glucagon’s role in health and disease. In the resting, non-stressed state in normal individuals the peripheral venous level falls in the range 40–200 pg/ml; the portal venous level is several times greater.
CLINICAL SYNDROMES IN WHICH GLUCAGON IS, OR MAY BE, IMPORTANT

(a) Glucagonoma syndrome

The most convincing evidence that elevated glucagon levels, in the presence of normal insulin secretion, can cause the diabetic syndrome comes from a single case report. Lightman and Bloom (1974) have reported the return of completely normal glucose tolerance in a previously insulin-dependent diabetic following the removal of a glucagonoma.

Glucagonomas are tumours of the pancreatic alpha cells found in patients who have often presented at dermatology clinics with a striking necrolytic, migratory erythematous rash, usually affecting the lower trunk and groin areas. In addition to the rash there is stomatitis, carbohydrate intolerance (usually mild), anaemia and a history of weight loss (Mallinson et al, 1974). Hypoaminoacidaemia is often present as well as the high plasma glucose and the diagnostic high plasma glucagon levels. The high plasma glucagon levels are of aetiological importance in the development of the diabetic state.

(b) Idiopathic diabetes mellitus

Glucagonomas are rare and the role played by glucagon in the aetiology of idiopathic diabetes mellitus is not as fully understood. In the complex biochemical situation occurring in diabetes it may be difficult to decide which abnormalities are primary and possibly of aetiological importance and which occur as a consequence of the diabetic state. It is generally agreed that pathophysiological changes which occur early in the course of a genetic disease are more closely related to the primary disorder than are those which occur later. Since genetic factors are important in the development of diabetes, assessment of persons supposed to be at high risk of developing the disease has always been an area of active investigation. For example, it has been shown that otherwise healthy monozygotic twin sibs of diabetic patients may have decreased and delayed insulin responses to glucose infusion (Cerasi and Luft, 1967). Day and Tattersall (1975) doing a similar study, found that the mean glucagon levels in the unaffected twins tended to be higher than in other healthy control subjects. This would support the concept that in idiopathic diabetes mellitus there may be an inherited abnormality of glucagon secretion or metabolism as well as a primary abnormality of insulin secretion.

In established idiopathic diabetes several abnormalities of glucagon secretion have been reported. There is relative or absolute basal hyper-glucagonaemia (Unger et al, 1970; Müller et al, 1970; Buchanan and McCarroll, 1972; Wise et al, 1973a). Ingested or infused glucose has been reported to cause no suppression or even paradoxical rise in glucagon levels (Müller et al, 1970; Buchanan and McCarroll, 1972).

Although in experimental insulin deficiency the alpha cell unresponsiveness to hyperglycaemia is promptly reversed by administration of insulin (see above) in the human diabetic this unresponsiveness is not fully corrected even by massive doses of insulin (Unger et al, 1972) suggesting that there is some other (perhaps primary) abnormality of the alpha cell in this condition.
Whereas in normal individuals there is a striking rise in glucagon level in response to hypoglycaemia, this response was absent in a group of insulin-dependent diabetics where hypoglycaemia was produced by insulin (Gerich et al, 1973a). This finding again suggests an intrinsic alpha cell abnormality in diabetes. Aminogenic glucagon secretion is exaggerated in diabetics despite hyperglycaemia which in non-diabetics abolishes the aminogenic stimulus (Unger et al, 1970; Müller et al, 1970; Wise et al, 1973a).

For some time it has been known that very high levels of glucagon may occur in diabetic ketoacidosis (Assan et al, 1969; Unger et al, 1970). Recently Gerich and coworkers (1975) have shown that glucagon had aetiological importance in the development of the ketoacidotic state. When they withheld insulin from a group of insulin-dependent diabetics there was a prompt rise in plasma glucagon, glucose and ketone levels. In the same diabetics if glucagon secretion was blocked (by infusion of somatostatin) at the same time as insulin was withheld then plasma glucose rose slowly and ketone levels rose very little over the time period studied (18 hours).

In idiopathic diabetes there is, therefore, some evidence of primary alpha cell abnormalities. In established cases relative or absolute hyperglucagonaemia contributes to the hyperglycaemia and glucagon probably plays a key role in development of diabetic ketoacidosis.

Do glucagon levels change with diabetic management and control? In a large group of mild maturity-onset diabetics treated by dietary methods only, there was very little change in glucagon levels after six months' treatment which resulted in significant improvement both in the glucose tolerance and insulin secretion on glucose challenge. (Care was taken to ensure a similar carbohydrate intake for one week before the initial GTT and the test carried out at six months), (Trimble, 1975).

It has been mentioned above that high glucagon levels associated with ketoacidosis do tend to drop with insulin treatment, however, there is no evidence that the alpha cell responsiveness to glucose is ever fully restored. Unger and coworkers (Unger et al, 1970) found that the glucagon response to arginine was exaggerated and unrelated to duration of diabetes, body weight, or type of diabetic treatment (the diabetic group included juvenile and maturity-onset types and treatment was either by insulin, sulphonylureas or diet alone).

There are contradictory reports in the literature about the effect of sulphonylureas on glucagon secretion. Suppression (Samols et al, 1969), non-suppression (Pek et al, 1972) and stimulation (Harrison and Samols, 1975) have been recorded. It would appear that sulphonylureas may lower glucagon levels if they are already raised by the stimulus of hypoglycaemia. However, it cannot be inferred from the above publications that this is a primary effect on the alpha cell; it may be secondary to the effect of the sulphonylurea-induced insulin secretion on the alpha cell.
(c) Renal and hepatic disease

The kidneys and liver are the important sites of glucagon breakdown and clearance. There is a high incidence of carbohydrate intolerance in renal and liver failure.

Hyperglucagonaemia has been reported in uraemia (Assan, 1972; Bilbrey et al, 1974). Since the hyperglucagonaemia of renal failure may occur early on and in the absence of glucose intolerance (Trimble, McEvoy and Buchanan, unpublished data) it may be of aetiological importance in the development of diabetes in this situation.

High levels of glucagon have also been found in cirrhosis (Marco et al, 1973). However, if liver glycogen stores are poor it is less likely that glucagon plays any role in the development of hyperglycaemia in this situation.

(d) Other endocrine disorders

High glucagon levels occur in Cushing's syndrome (Wise et al, 1973b) and myxoedema but not thyrotoxicosis (Seino et al, 1974). In acromegaly the levels have been reported on as being slightly elevated (Lawrence, 1972) but this could not be confirmed by others (Trimble, 1975).

Adrenaline increases glucagon and decreases insulin secretion, both of these changes probably contribute to the glucose intolerance seen in situations associated with raised catecholamine levels.

The stress reaction may be implicated in the hyperglucagonaemia seen in cases of trauma (Meguid et al, 1972), burns (Wilmore et al, 1974), severe infections (Rocha et al, 1973) and coronary thrombosis (Willerson et al, 1973).

(e) Obesity

Stimulated glucagon output has been reported as decreased (Wise et al, 1973a) or increased (Gossain et al, 1974; Gerich, Langlois, Noacco, 1973) in obesity. At present it is doubtful whether glucagon is important in the carbohydrate intolerance associated with obesity.

(f) Primary endogenous hypertriglyceridaemia

In many patients with primary endogenous hypertriglyceridaemia the glucagon levels are high. Eaton and Schades (1973) have postulated that there is resistance to the triglyceride lowering action of glucagon in this situation.
(g) Disease involving exocrine pancreas

In chronic pancreatitis the glucagon and insulin response to arginine infusion falls into two patterns. In one group of individuals there may be a concomitant decrease in glucagon and insulin secretion while the other group which includes those with severe insulinopenia show relative hyperglucagonaemia (Kalk et al, 1974). In cystic fibrosis Stahl and coworkers (1974) found that there was a tendency for stimulated glucagon output to be decreased by about the same amount as was insulin secretion. However, others have found high, ‘normal’, and low aminogenic glucagon secretion in patients with cystic fibrosis (Redmond, Buchanan and Trimble; in preparation). High levels of glucagon in this series were found in those with most severe pulmonary involvement. Late in the disease extensive pancreatic fibrosis could theoretically limit both insulin and glucagon secretion, earlier on other factors such as stress and bacterial infections may be important.

In acute pancreatitis mild elevations of glucagon have been reported but in the more severe cases glucagon levels have been reported as low (Day et al, 1972).

**Summary**

Although it has been known for a long time that glucagon has many powerful biological activities in the in vitro situation the importance of glucagon has always been overshadowed by that of insulin because of difficulty in proving that its actions are significant at physiological circulating levels in the intact human. The development of (relatively) specific assay systems for its measurement has led to many advances in our understanding of the hormone. If any other single item can be picked as having made an outstanding contribution to our knowledge of the physiological importance of the hormone it has been the experimental use of the hormone somatostatin. Glucagon and insulin often act as a bihormonal unit, there being few situations in which changes in the level of one is not accompanied by changes in the level of the other. Consequently, biological effects noted have been due to the sum of glucagon and insulin activities. By the use of somatostatin the endogenous secretion of both can be blocked and the biological effects of infused glucagon can be observed. Unfortunately, somatostatin has side effects which will probably preclude its further use in humans. However, there is unequivocal evidence that glucagon is a hormone with significant physiological activity and that it is also of importance in many common clinical syndromes.
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(The above represent key references quoted in this paper. The complete references may be obtained from the author, c/o The Metabolic Unit, Royal Victoria Hospital, Belfast BT12 6BA).