EFFECT OF ADRENERGIC BLOCKERS ON GLUCAGON LEVEL DURING INSULIN INDUCED HYPOGLYCAEMIA IN NORMAL SUBJECTS

THESIS

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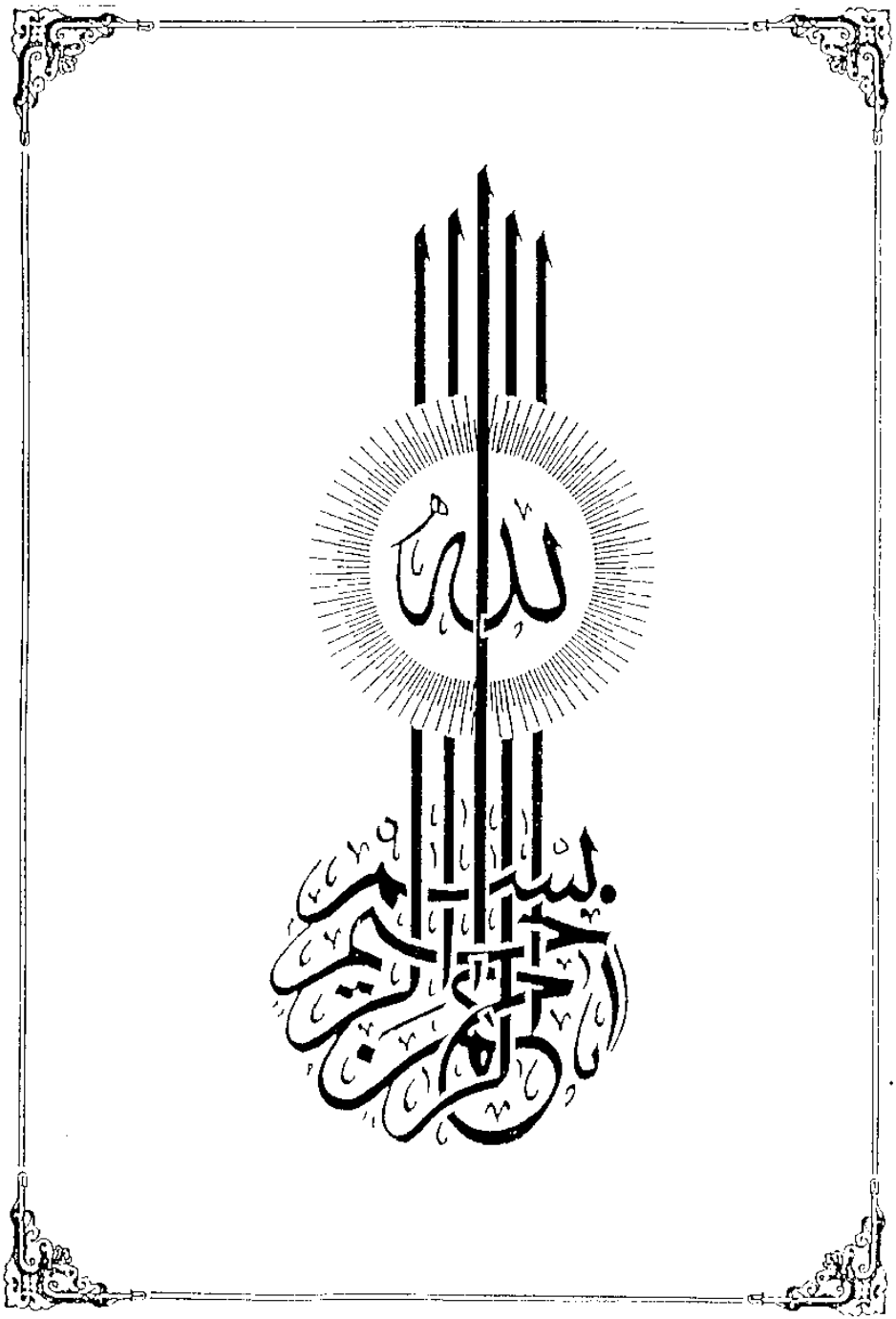

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AIM OF THE WORK

Both catecholamines and glucagon were reported to be increased in isulin-induced hypoglycaemia. Furthermore, hyperglucagonemia has been reported to occur during certain stressful conditions e.g. bacterial infection, trauma, burns, diabetic ketoacidosis, shock and myocardial infarction, i.e. conditions known to be associated with enhanced catecholamine release (Walter et al, 1974).

Whether glucagon increment is evoked by the enhanced catecholamine release or as a response to hypoglycaemia per se is yet unclear.

So the aim of this work is to clarify the relationship between adrenergic nervous system and glucagon secretion.

If hyperglucagonemia-resulting from insulin-induced hypoglycaemia-declines after alpha and beta adrenergic blockade, then it is most probably adrenergic dependent and if it does not decline and persists, then it is most probably adrenergic independent and may be due to hypoglycaemia per se or any other unknown mechanism.

Review of Literature

PANCREATIC ISLETS OF LANGERHANS

To understand the pancreatic secretory mechanisms, few anatomical features must be considered.

Both the exocrine and endocrine portions of the pancreas develop as dorsal and ventral buds from the cells of the duodenal and hepatic diverticulum and the primitive foregut. The islets of Langerhans are those responsible for the endocrinal function of the pancreas. These are collections of ovoid cells 75 X 175 U scattered throughout the pancreas, although they are more plentiful in the tail than in the body and head. They comprise 1-2 % of the weight of the pancreas. In humans, there are 1-2 million islets,each has a copious blood supply and blood from the islets drain into the portal vein (like that from G.I.T. but unlike that from any other endocrine organ).

The cells in the islets can be divided into types on the basis of their morphology, granulation, and staining properties.

There are 4 distinct cell types in human pancreas A, B,D, and F cells.

A-cells constitute 20 % of the granulated cells, secrete glucagon and stain red with modified Mallory aniline blue stain. B-cells comprise > 50 % of granulated cells, secrete insulin and stain bluish purple with Mallory stain. D-cells (1-8 %) secrete somatostatin, gastrin and secretin. F-cells (< 2 %) secrete pancreatic polypeptide. Islets in the tail are relatively rich in A-cells while those in the head are rich in F-cells (Ganong, 1981 and Porte and Halter, 1981).

A and B-cells are close to each other separated only by pericapillary spaces. Glucagon from A-cells passes into pericapillary spaces to B cells to promote secretion of insulin (Keele and Neil, 1974).

A-cells are polyhydral in shape, present in groups, contain large acidophilic secretory granules. These granules are symmetrically rounded, electron dense and surrounded by a limiting membrane. Prominant Golgi complex and abundant endoplasmic reticum are found in A-cells. Synthesis of glucagon takes place in endoplasmic reticulum, transported to Golgi complex where it is packed for export as granules (Porte and Halter, 1981).

A-cells granules show less species variations than the B-cells. This is because of the great similarity of the primary amino-acid sequence among species. However,

bovine and porcine glucagon provokes the formation of antiglucagon antibodies in the rabbit. These antibodies have been used to measure glucagon in humans (Ganong, 1981).

GLUCAGON HORMONE

Historical View :

Early preparation of insulin, including the crude acid-alcohol extracts of Banting and Best produced a transient hyperglycaemia that preceeded the expected fall in blood glucose concentration. Since some preparations, particularly the crystalline insulin prepared by Abel were devoid of hyperglycaemic effect, it was apparent that transient hyperglycaemia was not a property of insulin but rather of some contaminant. In 1923, the hyperglycaemia--producing substance was separated from insulin by Kimbale and Murlin and named glucagon. Later workers referred to it as the hyperglycaemic-glycogenolytic factor (HGF.), since many years were to pass before the hormonal nature of glucagon was established. The availability of antibodies to glucagon made possible an immunofluoresceent examination of pancreas and confirmed that glucagon is localized in A-cells (Mountcastle, 1980).

Chemistry and Biosynthesis :

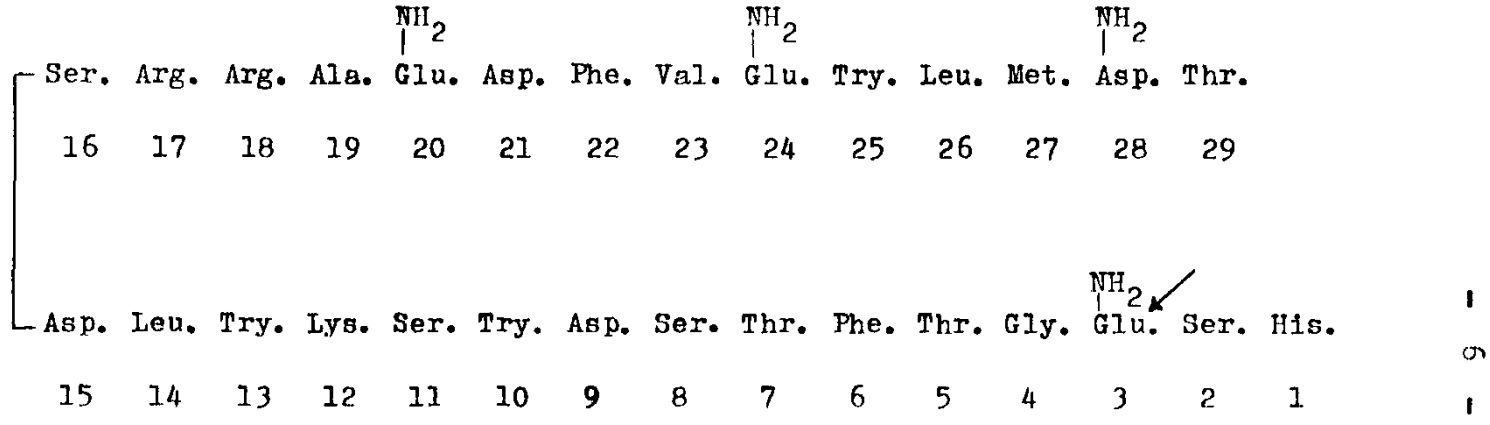
Glucagon is a linear polypeptide with a molecular weight of 3485, containing 15 different amino-acids with a total of 29 amino-acids residue arranged in a straight chain (Thomson et al, 1972).

It contains-in contrast to insulin-no cystine, proline or isoleucine. Furthermore it can be crystallized in the absence of zinc or other metals (Harper, 1979 and Porte and Halter, 1981).

Glucagon is relatively insoluble in water, however mild acidic or basic conditions increase its solubility (Porte and Halter, 1981). There is some evidence for the formation of glucagon from a larger polypeptide precursor "proglucagon" in the A-cells of pancreas (Ganong, 1981).

Glucagon is cleaved by carboxy peptidase, trypsine, leucine amino-peptidase, dipeptidyl amino-peptidase 1 and chemotrypsin. Since none of degradation products of glucagon retains hyperglycaemic activity, the integrity of structure of molecule seems to be required for physiological activity (Porte and Halter, 1981).

Glucagon is thought to circulate in the blood without binding to carrier proteins (Mountcastle, 1980).



Amino acid sequence of human glucagon, showing the site of cleavage by dipeptidyl aminopeptidase I.

(After Port and Halter, 1981)

Valvered et al, (1975) and Kuku et al, (1976) identified four types of plasma immunoreactive glucagon (IRG). Three correspond to IRG components present in glucagon secreting tissues :

1. IRG 40.000 : big plasma glucagon, probably not biologically active-constitute 53 % of total.
2. IRG 9.000 : corresponding to proglucagon, not present in normal fasting plasma.
3. IRG 3.485 : corresponds to standard glucagon and constitute 46 % of total.
4. IRG 2.000 .

A significant amount of glucagon comes from the A-cells in the stomach and duodenum. Consequently, the plasma glucagon level does not fall to zero after pancreatectomy, indeed it may be even elevated. This gut hormone, also called enteroglucagon or glucagon-like immuno-reactive factor, is immunologically similar but not identical to the pancreatic A-cell hormone. Furthermore it is less active in stimulating adenylate cyclase and therefore cannot duplicate many of the actions of the pancreatic hormone. Its physiological role is unknown, however Ghareeb and Ghalioungui (1978) reported that the enteroglucagon mobilizes liver glycogen and prevents the uptake and storage of glucose by the liver during glucose absorption. This is beneficial, since post-prandial insulin release by causing hypoglycaemia

may otherwise adversely affect the cerebral cortex, this is prevented by glucagon which protects the brain from hypoglycaemia.

Glucagon-like immunoreactive factor can be differentiated from pancreatic glucagon by radio-immunoassay (Samols et al, 1966 and Harper, 1979).

Another gut molecule with glucagon-like immuno reactivity had been discovered and named glycentin (Porte and Halter, 1981). It has a larger molecular weight >3500 and shown to contain the structure of glucagon with a C-terminal extension of 1500 daltons. Glycentin is found in the small intestine in cells similar to but not identical with A-cells of pancreas and stomach. It is known to circulate in plasma and its concentration is increased by oral nutrients including glucose but its regulation is different from that of pancreatic or stomach glucagon (Porte and Halter, 1981).

Kuku (1976) found that normal fasting level of plasma glucagon is 113 ± 9 pg/ml. Ghareeb and Ghalioungui(1978) recorded normal level to be 110 pg/ml. The concentration of true glucagon in hepatic portal blood ranges from 300 to 5.000 pg/ml depending on environmental conditions. This difference between peripheral and portal blood reflects not only its greater dilution in peripheral blood but also the

fact that a considerable amount of glucagon is destroyed during its passage through the liver (Mountcastle, 1980).

The Half-Life and Clearance :

Glucagon has a half life of only 3-4 minutes. The principal site of its clearance from the blood is the liver which degrades it enzymatically and also excretes about 0.5 mg into the bile each day (Mountcastle, 1980). This was confirmed by Ganong (1981) who found raised levels of peripheral blood glucagon produced by excitatory stimuli in patients with cirrhosis (Ganong, 1981). The kidney appears to be the major site for glucagon removal. Recent studies suggest that proglucagon is disproportionately increased in patients with chronic renal failure (Kuku et al, 1976). This work was confirmed later on by Freinkel, (1977) who explained hyperglucagonemia of renal insufficiency, and found that impaired conversion of proglucagon to glucagon may contribute to high IRG levels.

Insulin/Glucagon Molar Ratio (I/G) :

Insulin is glycogenic, antigluconeogenic and antilipolytic, thus favouring storage of absorped nutrients i.e. "a hormone of energy storage". Glucagon, on the other hand is glycogenolytic, gluconeogenic and lipolytic, it mobilizes energy stores, so it is a "hormone of energy release"

(Mackrell and Sokal, 1969). So the blood level of both hormones must be considered in any given situation. On molecular basis, glucagon is many times more powerful than insulin which , in part, may explain insulin resistance in diabetic patients (Unger, 1971). So the molar ratio is much more accurate. It could be readily calculated from the blood level of the hormones as determined by immunoassays, and their molecular weights.

The I/G ratio varies inversely with the needs for endogenous glucose production, being lowest in total starvation and highest during loading with exogenous carbohydrate (Unger et al, 1970). The ratio after overnight fast is approximately 3. It may rise as high as 70 after large carbohydrate meal (Muller et al, 1970) and drops to 0.4 after 48 hours of total starvation (Aguilar-Parada et al, 1969). The I/G ratio is taken as a measure for gluconeogenesis as explained by Unger (1971), increased I/G ratio would promote storage of all types of nutrients, endogenous glucose and free fatty acids production would be inhibited, protein biosynthesis would be favoured. On the other hand a decline in I/G ratio would favour mobilization of stored nutrients :

1. Increased hepatic glucose production from glycogen and from available amino-acids at expense of protein synthesis.