INSULIN RELEASE

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Insulin is the key hormone controlling rates of glucose removal from the plasma. The interdependence of endocrine system and control of food stuffs and metabolic intermediates has a rather important bearing on the homeostatic regulation of metabolic fuels which involves the generally opposing actions of insulin and glucagon. In healthy individuals, there is a balance between carbohydrate metabolism in the liver and in peripheral tissues which is maintained by a portal-peripheral gradient of Insulin concentration. Further, the basal insulin requirement appears to be closely related to body weight. Hyper-insulinaemia in obese individuals, both in the basal state and after various stimuli of insulin secretion and reduced sensitivity to the effect of endogenous and exogenous insulin, are the quite well known modifications. It is also known that even minor illness may cause glucose intolerance and insulin resistance in normal subject (1) and that severe stress may cause a transient form of overt diabetes-fasting hyperglycaemia (2,3).

In healthy subjects, mild to moderate physiological stress has generally little effect on fasting glucose levels, whereas impaired glucose tolerance commonly occurs (2). It is suggested that increased counter regulatory hormone (epinephrine, glucagon, cortisol) secretion or action may be playing a role in mediating the metabolic response to stress. Recent studies have demonstrated that many metabolic effects like ketoacidosis usually observed in insulin dependent diabetics result from infusion of catecholamines (norepinephrine, epinephrine and dopamine) to levels readily achieved during stress.

Diurnal variations and effects of advancing age on glucose tolerance have been repeatedly reported. In normal subjects, the glycaemic levels after oral glucose were found to be significantly lower in the morning than in the evening. The impairment of oral glucose tolerance later in the day has been attributed to a smaller and delayed insulin response (4,5) and to increased resistance to insulin (6,7). Decline in glucose tolerance and increased insulin secretion with advancing age has also been reported (8,9).

Thus there is considerable evidence to suggest variations in insulin secretion under physiological conditions and stress as also aspects of metabolic profile. In the presentation to follow, some metabolic and endocrinal factors influencing insulin secretion are mentioned with particular emphasis on neural regulation.

Biosynthesis :

Insulin is synthesized in the endocrine pancreatic B cell. Preproinsulin a precursor molecule is produced by DNA/RNA directed synthesis in the rough endoplasmic reticulum of these cells. It is cleaved by microsomal enzymes to proinsulin and then converted to insulin and a smaller connecting peptide, or C peptide chain. Proinsulin transi-

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ently retains one of its connecting terminals between C peptide and the insulin molecules producing two different intermediate molecules. Cleavage of proinsulin occurs either before or after its transport to the Golgi apparatus where it is packed as granules. The mature granules contain insulin and C peptide in equimolar amounts and small quantities of proinsulin (10).

Factors Regulating Insulin Secretion :

The basal rate of secretion of insulin in a 70 kg man has been estimated as 0.5 to 1.0 unit per hour. Ingestion of meals increases the rate of secretion by three to four fold; and the total secretion during a 24 hour period in an adult may be 20 to 40 units.

There are two aspects of insulin secretion that must be examined : (i) Basal secretion which occurs in the absence of exogenous stimuli and is the quantity of insulin secreted in the post absorbtive state. Basal insulin secretion occurs in bursts. It is reduced in fasting state. (ii) Stimulated insulin secretion is that which occurs in response to exogenous stimuli (Fig. 1). This is the response of the β cell to ingested meals. Both insulin and glucagon play an important role in the regulation of carbohydrate, lipid and amino acid metabolism and these metabolic fuels in turn determine the functioning of pancreatic B cells with glucose being the most potent stimulant of Insulin release.
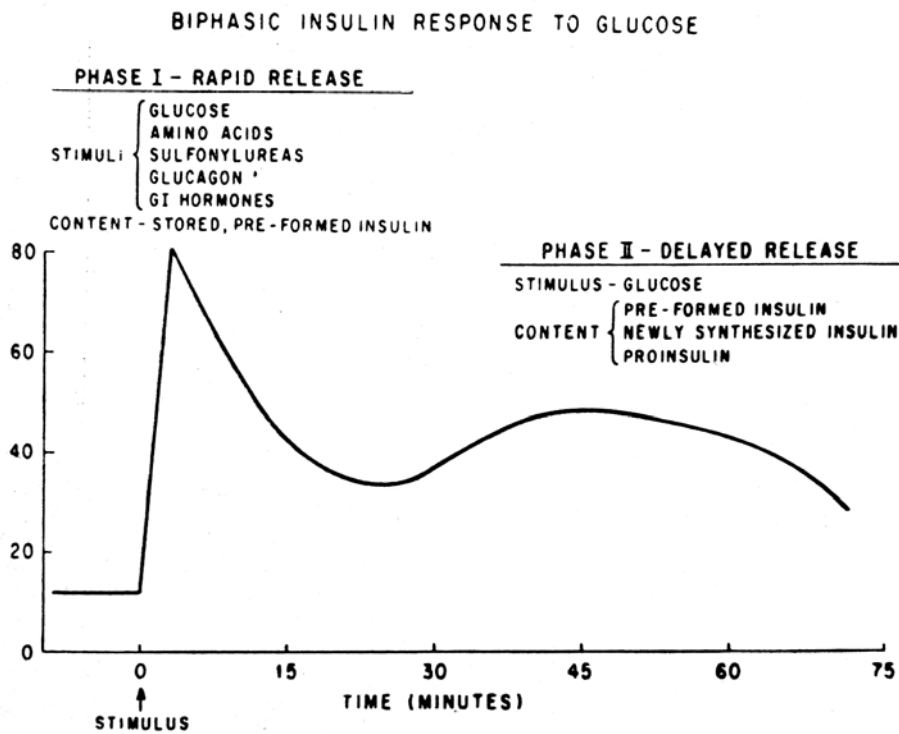
BIPHASIC INSULIN RESPONSE TO GLUCOSE

PHASE I – RAPID RELEASE

STIMULi { GLUCOSE
AMINO ACIDS
SULFONYLUREAS
GLUCAGON '
GI HORMONES

CONTENT - STORED, PRE-FORMED INSULIN

PHASE II – DELAYED RELEASE

STIMULUS - GLUCOSE

CONTENT { PRE-FORMED INSULIN
NEWLY SYNTHESIZED INSULIN
PROINSULIN

TIME (MINUTES)

STIMULUS

Fig. 1 : Biphasic Insulin response showing rapid and delayed release.

Glucose : The exact mechanism of action of glucose on the β cell remains clarified. The perfused rat pancreas has shown a biphasic release of insulin in response to glucose (Fig. 2). When the glucose concentration in the system is increased suddenly, an initial short-lived burst of insulin release occurs (the early phase). If the glucose concentration is maintained at this level, the insulin release gradually falls off and then begins to rise again to a steady level (the late phase). Metabolism of glucose appears essential in stimulating insulin release. Indeed agents such as 2-deoxy-glucose that inhibit the metabolism of glucose interfere with release of insulin. Probably, of more physiological importance is the effect of glucose absorption from the intestine which produces a greater insulin release than would be expected from the rise in glucose alone. This effect is thought to be mediated by gut hormone. There is evidence that glucose may act through a membrance-bound glucose receptor; however, despite extensive research in this area, no such glucose receptor has been identified on the β cell.

It is assumed that the rate of secretion of insulin from β-cell is reflected in the plasma insulin levels which is about 10-59 mu/1 with a half life of about 4 min. Studies with 125-I insulin reveal a direct linear relationship between insulin degradation and plasma insulin concentration over a wide range of steady state insulin levels. These assumptions will not be however correct when the liver is diseased as decreased degradation of insulin takes place under that condition.
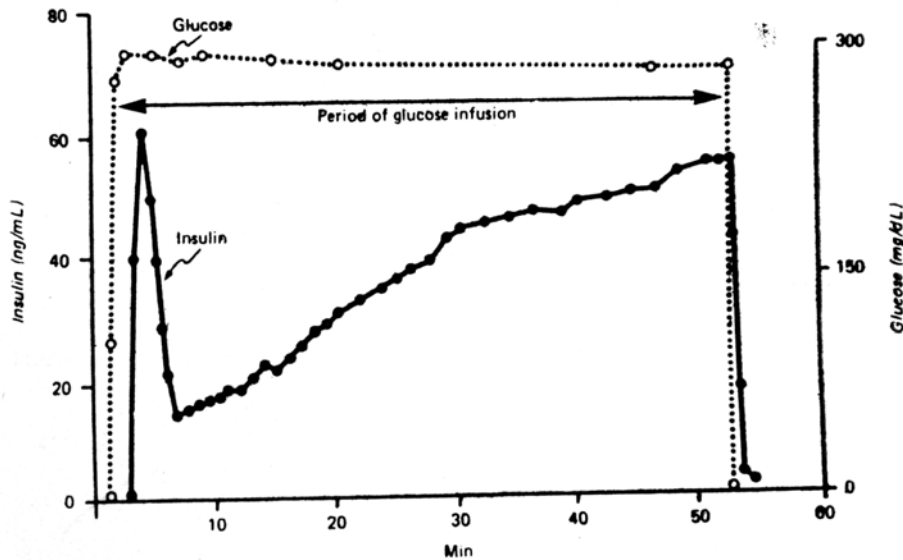

Fig. 2 : Insulin release during constant stimulation with glucose in, invitro perfused pancreas. (From Greenspan and Forsham : *Basic and Clinical Endocrinology*, Lange Medical Publications, Singapore, 1983).

Insulin release has been shown to require calcium. It has been proposed that mature insulin containing granules in the β cell attach linearly to microtubules that contract after exposure to high intracellular calcium, thereby ejecting the granules (Fig. 3). The following effects of glucose on calcium ion movement have been demonstrated :
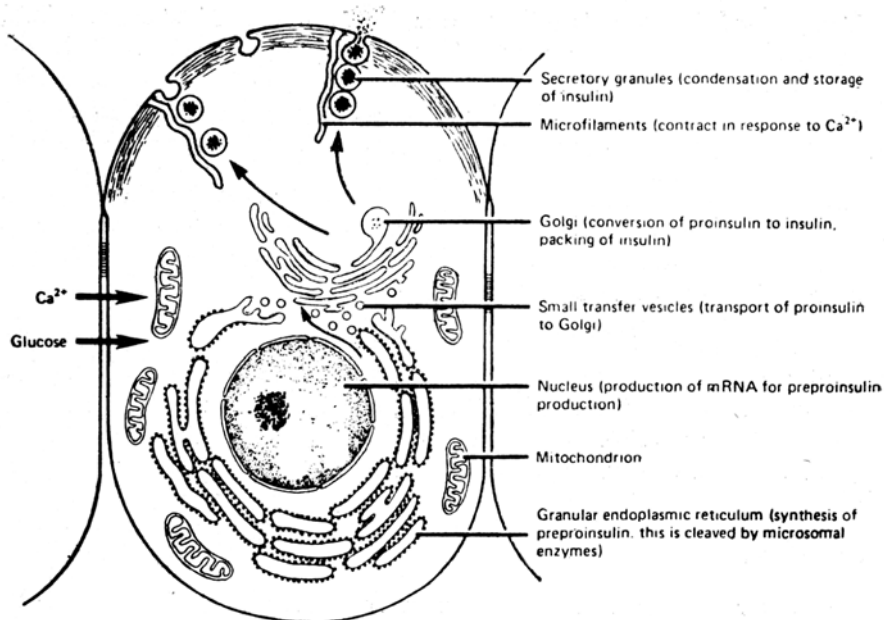
Fig. 3 : Schematic representation of structural components of B cell and the role of Ca²⁺ ions in ejection of insulin containing granules from microtubules. (From Greenspan and Forsham : *Basic and Clinical Endocrinology*, Lange Medical Publications, Singapore, 1983).

i)   Calcium uptake is increased by glucose stimulation of the β cell.

ii)   Calcium efflux from the cell is retarded by some action of glucose.

iii) Mobilization of calcium from mitochondrial compartments occurs secondary to CAMP induction by glucose.

CAMP is another important modulator of insulin release. Glucose has been shown to directly induce CAMP formation. Further more, many non-glucose stimuli to insulin release are known to increase intracellular CAMP. Elevations of CAMP, however, will not stimulate insulin release in the absence of glucose.

Amino Acids-Amino acids stimulate release of both insulin and glucagon. The potency of the different amino-acids in stimulating insulin secretion varies. The most potent are arginine and lysine. Presence of glucose augments insulin response to amino acids, while diminishing those of glucagon. Intravenously administered amino-acids have a direct effect on islet tissue where as a protein meal stimulates insulin release indirectly by causing secretion of G.I. hormones.

Ketone Bodies and Fatty Acids-The effect of free fatty acids, β-hydroxybutyrate and acetoacetate on insulin and glucagon release varies in the different species and their effectiveness in man is limited. It is proposed that these agents may be important during starvation.

Glucagon and Somatostatin-The three major hormones of islet cells i.e. insulin (βcells) glucagon (α-cells) and somatostatin (D-cells) affect each other's synthesis and secretion in a complex, interactive manner. It has been suggested that the local concentration of the hormones within the islets are probably very high and could therefore exercise control locally more effectively than they do after release into the general circulation.

Glucose induced insulin release may be an important factor in decreasing glucagon levels. Conversely, when insulin levels fall, as during fasting. glucagon levels will tend to increase. Glucagon stimulates the release both of somatostatin and of insulin where as somatostatin will tend to decrease insulin release.

Gastrointestinal Peptide Hormones-As already stated the insulin response is much more to orally administered glucose or amino acids than to intravenous infusion. The greater response is attributed to release of the gastro-intestinal tract hormones which reinforce the stimulus of metabolite in boosting the release of insulin. Gastric inhibitory peptide (G.I.P.) is a very potent stimulus. Gastrin secretion and pancreozymin are also effective.

Neural Regulation :

The islets are provided with a rich innervation, consisting of sympathetic postganglionic fibers from the coeliac ganglion and parasympathetic preganglionic fibers from the right vagus. The fibers which are mainly nonmyelinated are vasomotor (sympathetic) and parenchymal (sympathetic and parasympathetic) in their distribution. Fine branches ramify among the islets from perinsular plexus. Fibers frequently make synaptic contact with acinar cells before innervating the islets suggesting a close linkage between the neural control of exocrine and endocrine components of the pancreas. Many fibers end in the islets in company with arterioles.

Three types of nerve terminals have been identified in the islets : adrenergic, chlonergic and peptidergic (11). No selective association with any particular cell type has been found, and sometime more than one type of terminal/s are associated with a single cell type. Some nerve terminals appear to end remote from the surface of the islet cells and neurotransmitter released from such endings into the intercellular spaces of the islets, could perhaps diffuse and affect numerous islet cells. Further, the circulating catecholamines secreting post synaptic sympathetic neurones are located throughout the body including the adrenal medulla. Thus the stress induced, sympathetic stimulation can have direct effects at the tissue level or indirect effects resulting from catecholamines that circulate following their release from sympathetic neurons.

Immunocytochemical techniques have revealed two populations of peptidergic fibers within the islets : one that contains VIP like immunoreactivity and another containing gastrin/CCK like immunoreactivity. It may, therefore be significant that both VIP and gastrin/CCK family of peptides are capable of stimulating the release of both glucagon and insulin under experimental conditions.

Role of Sympathetic Nervous Systems-The catecholamines exert their physiologic effects by binding to receptor molecules on the surface of the target cells. Earlier studies of

sympathetic activation suggested that there were 2 classes of responses designated as inhibitory or excitatory. Subsequent studies revealed that there were 2 types of receptors designated « and ~, based on the relative potencies of a series of adrengergic agonists. β-adrenoceptors stimulation is shown to potentiate the release of insulin where as α-adrenoceptors stimulation is inhibitory. Studies of glucose stimulated insulin response have shown that α-adrenergic stimulation and β-adrenergic blockage decrease insulin responses, while α-adrenergic blockade and β-adrenergic stimulation increase insulin response. The effect of α and β adrenergic blockade has also been studied in 12 hours fast resting human subjects.

These results and the results of glucose-stimulated release suggest that insulin secretion is continually modulated by adrenergic activity in both the basal and stimulated state. It thus appears critical to incorporate the adrenergic nervous system into proposed model of insulin secretion and to fully characterize adrenergic effects upon basal and stimulated insulin secretion in various disease states associated with abnormal insulin responses such as myo-cardial infarction, haemorrhagic shock, sepsis, hypothermia.

Parasympathetic Stimulation-Release of insulin occurs in response to both Vagal stimulation (which is important in the anticipation and disposal of food) and intra-arterial infusions of acetylcholine in isolated perfused pancreas. These effects on perfusion are invariably blocked by atropine indicating that they depend on activation of muscarinic receptors. Effects of blocking by atropine on vagal stimulation however vary with the species. In cat and pig atropine has no effect on release of insulin in response to vagal stimulation. It seems most likely that these responses are mediated by peptidergic transmitter such as V.I.P.

Central Nervous Mechanisms-The C.N.S. was first shown to influence carbohydrate metabolism by Claude-Bernard (12) who demonstrated the glycosuric effect of trau-matizing the floor of the 4th Ventricle. It was after another 100 years or so that the hypothalamic (Ventro-lateral) lesions were shown to produce anorexia and the animals died of starvation even though food was plentiful. Voracious eating and gross obesity was observed after lesions of ventromedial hypothalamic (VMH) nuclei. These two areas have been designated as feeding and satiety centres respectively, and the two opposing mechanisms seem to regulate food intake. The lateral feeding mechanism producing the basic urge to eat and the medial satiety mechanism inhibiting the lateral. The cells of VMH take up radioactive glucose more actively from the blood than do the adjacent parts of the brain. If gold thioglucose is injected in the rat, preferential uptake of glucose by the VMH nuclei leads to cellular degeneration therein owing to the toxic effects of gold which accompanies the glucose (13). As a result rats become hyperphagic and obese.

We had carried on a study (14) way back in 1962-63 to see whether any differences in firing frequency could be detected in neurons in hypothalamic feeding and satiety centers exhibiting spontaneous activity and changes in their activity following I.V. glucose or insulin. The changes in activity of satiety and feeding center neurons were inversly related (Figs. 4, 5).
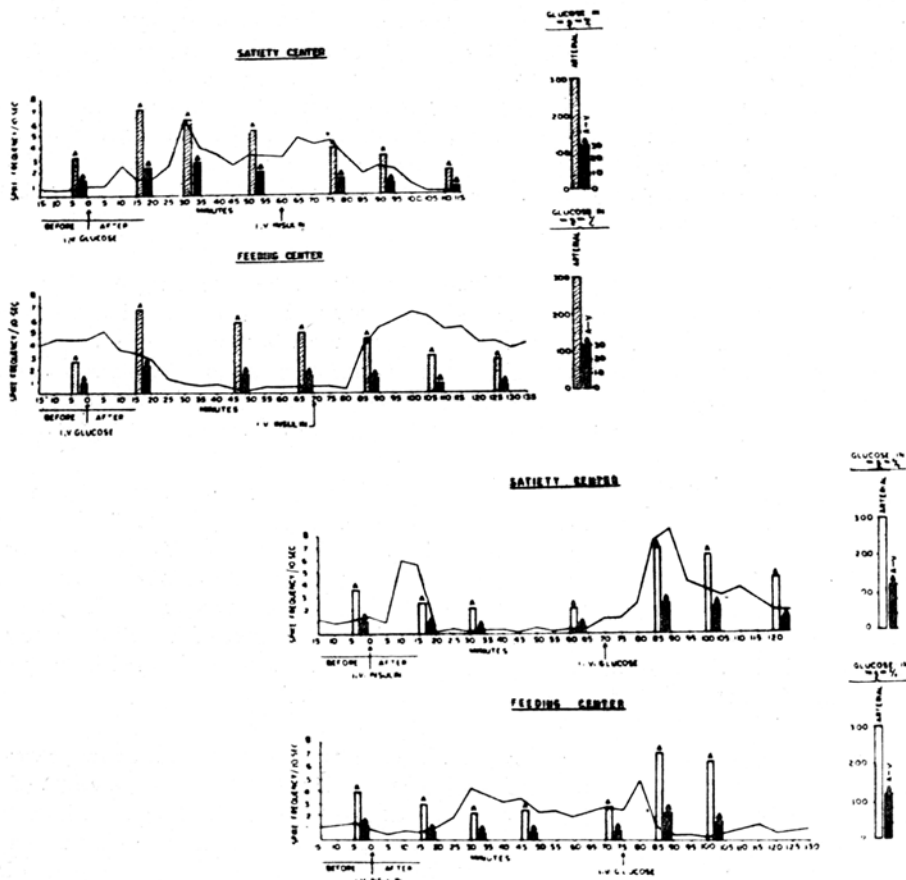
Fig. 4 : Changes in spike frequency of satiety and feeding centres in response to I.V. glucose followed by I.V. insulin (top)a nd I.V. insulin followed by glucose (bottom). (From Anand et al., (1964) Am. J. Physiol., 207 : 1146-1154).

Such a change is suggested to be due to the presence of "glucoreceptors" in the satiety centers. Respiratory studies do suggest an increased utilization of glucose of the satiety region in the fed animals. These central glucoreceptors influence the activity of some of the autonomic efferents has been suggested by Niijima.

I.V. glucose in the anaesthetised rabbit caused an increased in firing rate in nerve filaments of the pancreatic branch of the vagus nerve and a decrease in filaments of the adrenal branch of the splanchnic nerve (15).

Precisely opposite results were observed when blood glucose concentration was reduced after administration of insulin and the same later response was mimicked by 2-deoxyglucose which is believed to interfare in the utilization of glucose by the brain and cause release of adrenal medulla (16).
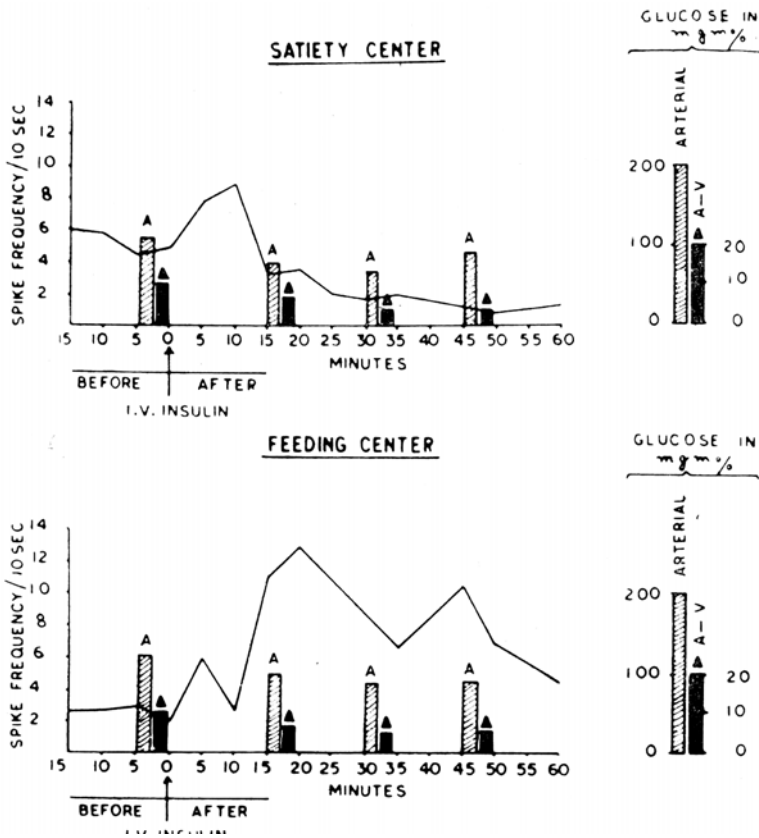
Fig. 5 : Spike frequency response of satiety and feeding centres and A.V. glucose differences before and after I.V. insulin. (From Anand et al., (1964) Am. J. Physiol., 207 : 1146-1154).

Thus in this metabolic homeostasis the autonomic nervous system plays an important part in controlling the activity of the pancreatic islets in terms of bringing about appropriate changes in the output of glucagon and insulin whenever there is an imbalance in external and internal environment.

The parasympathetic influences the islets during ingestion, digestion and absorption of food while sympathetic activity overrides during stress.

The kinetic interactions between the three major secretions of pancreas are complex, not well-understood and yet are very important in assessing the patterns of hormonal and metabolic responses.

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