

MECHANISM OF ACTION

1. **Role of c-AMP:** Calcitonin binds to specific calcitonin receptors on the plasma membrane of bone osteoclasts and renal tubular epithelial cells, activates adenylyl cyclase which increase c-AMP level which mediates the cellular effect of the hormone. This is the principal mechanism by which calcitonin acts.
2. **Cellular Shift:** It has been suggested that calcitonin may directly affect the relative distribution of bone cells. The hormone both in vitro and in vivo produced a cellular shift, in which the number of osteoclasts decreased.
3. **PH change:** Calcitonin may regulate PH at cellular level producing more alkaline medium which diminishes resorption.

METABOLIC ROLE

Calcitonin acts both on (a) bone (b) Kidneys. Indirectly, the effects on these two organ systems account for.

Hypocalcaemia and hypophosphataemia

a. Action on Bones

- Calcitonin inhibits the resorption of bone by osteoclasts and thereby reduced mobilization of calcium and inorganic PO_4 from bones into the blood.
- It also stimulates influx of phosphates in bone
- There is decrease in activities of lysosomal hydrolases, pyrophosphatases and alkaline phosphates in bones.
- Decrease in collagen metabolism and decreased excretion of urinary OH-proline
- Whether or not calcitonin promotes bone formation is uncertain and controversial. But it has been established that the hormone in addition to causing a decrease in number of osteoclasts, it increase osteoblast cells, which are thought to be involved in bone laying.

b. Action on kidneys

- The hormone acts on the distal tubule and ascending limb of loop of Henle and decrease tubular reabsorption of both calcium and inorganic phosphate thus producing calcauria and phosphaturia.
- The hormone inhibits α -1-hydroxylase and inhibits synthesis of 1-25-d-OH-D3 thus decreasing calcium absorption from intestine.
- Both the above effects account for hypocalcaemia.

INSULIN

Insulin is a protein hormone, secreted by β -cells of islets of Langerhans of pancreas. It plays an important role in metabolism causing increased carbohydrate metabolism glycogenolysis/ and glycogen storage, FA synthesis/TG storage and amino acid uptake/ protein synthesis. Thus insulin is an important anabolic hormone which act on variety of tissues. Major target tissues of insulin are the muscles, liver, adipose tissues and heart.

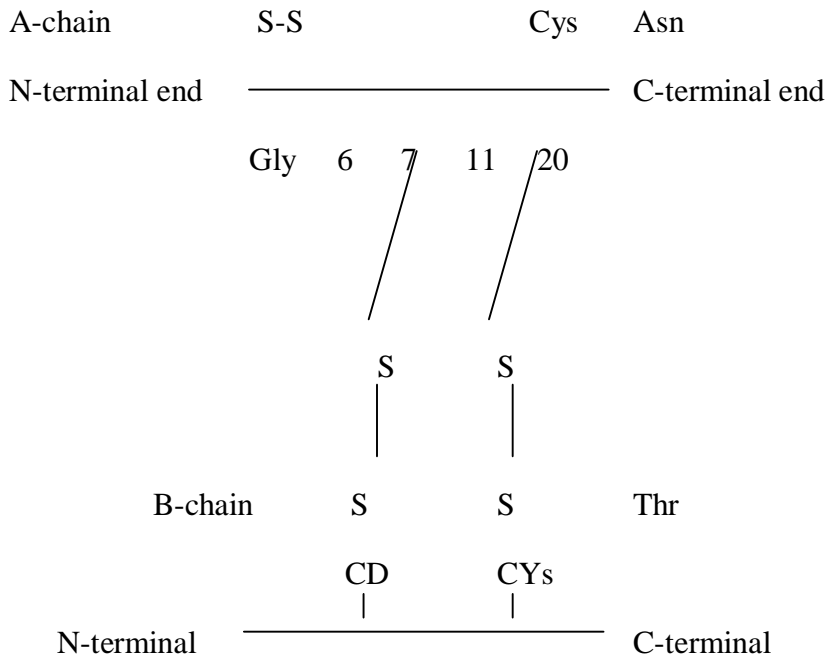
Note: RBC, GI tract epithelial cells and renal tubular epithelial cells are rather generally unresponsive to insulin.

CHEMISTRY

Insulin is a heterodimeric protein; it has been isolated from pancreas and prepared in crystalline form. For crystallization it requires zinc is also a constituent of stored insulin and normal pancreatic tissue is relatively rich in zinc. Insulin molecule is composed of two polypeptide chains, called A-chain and B-Chain, containing total of 51 amino acids. A-chain contains 21 amino acids and B-chain contains 30 amino acids. In A-chain, N-terminal amino acid is phenylalanine and C-terminal is threonine.

DISULFIDE BRIDGES

Both the chains are held together by two s-s-linkage. Cys 7 and Cys 20 of A chain are joined to Cys7 and Cys 19 of B chain respectively. In addition, the A-chain carries an intra-chain s-s linkage between cys 6 and cys 11.



INSULIN FROM OTHER SPECIES

Porcine insulin: Porcine insulin is similar to human insulin. It differs by only terminal amino acid No-30 of B-chain.

- In human: It is threonine
- In porcine: it is alanine in place of threonine. Removal of alanine (de-alaninated) retains the biological activity.

Note: De-alaninated insulin has been used in treatment of diabetes mellitus because of its low antigenicity.

- Human insulin has been produced by recombinant DNA technology.

Important of S-S Bridges

Breaking of the disulfide bonds with alkali or reducing agents inactivate insulin. Digestion of insulin protein with proteolytic enzymes also inactivates the hormone; hence insulin cannot be given orally. Minimum calculated Mwt is 5734. Insulin can exist in different polymeric forms (dimer, trimer etc) depending on pH, temperature and concentration.

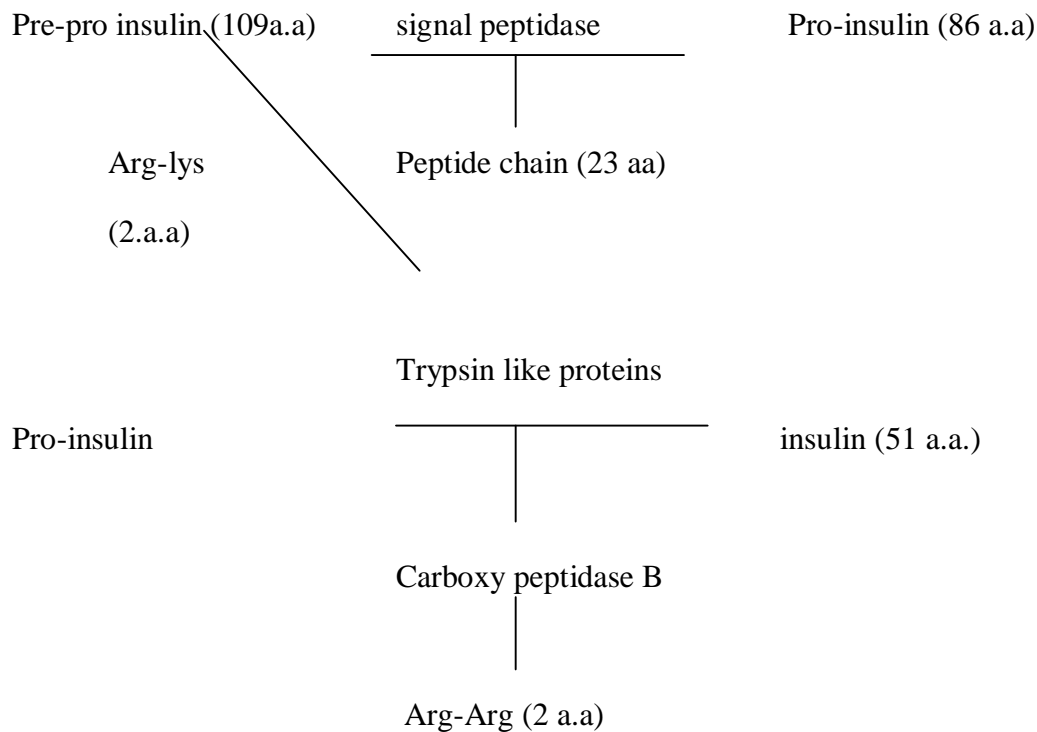
BIOSYNTHESIS OF INSULIN

In biosynthesis of insulin, first “prepro-insulin” is formed which is converted to pro-insulin. The latter is finally converted to insulin.

1. Synthesis of pre-pro insulin: Pre-pro insulin is synthesized in polysomes, attached to the membrane of rough endoplasmic reticulum in β -cell of islet of Langerhans. It is a polypeptide consisting of 109 amino acids, Mwt= 11,500
2. Conversion of pre-pro insulin to proinsulin
 - Pre-proinsulin after synthesis is transferred to lumen of rER cisternae.
 - A peptide chain consisting 23 amino acids in its N-terminal called leader sequence is split by an enzyme called signal peptidase present in the membrane of rER and pro-insulin is formed.
 - Pro-insulin has 86 amino acids Mwt = 9000
3. Conversion of pro-insulin to insulin

Pro-insulin containing small vesicles are detached from ER and fuses with cisternae of Golgi apparatus.

- In the Golgi cisternae, Proinsulin is acted upon by a trypsin-like protease which hydrolyzes the peptide chain at two sites, so that an inactive connective C-peptide is liberated and two active peptide chain are left which forms the A and B chain.
- A carboxypeptidase B like enzymes splits the C-terminal peptide bonds in the two intermediates to release two C-terminal basic amino acids from each of them viz “Arg-63-lys 62” to form ‘A’ chains and “Arg 31-Arg 32” to form ‘B’ chain. C-peptide which is split off has 31 amino acids.
- Condensing vacuoles are pinched off from Golgi cisternae with equimolar amounts of insulin and C-peptide in their lumen. Insulin molecules form dimmers by hydrogen bonding between the peptide groups of phe 24 and tyr 26 residues of their B-chains. Gradually with increasing concentrations, condensing vacuoles change into secretory granules. In them insulin forms crystalloid forms of hexamers with two Zn^{2+} . C-peptides remain in the fluid surrounding the crystalloid granules.



Note: Pro-insulin is comparatively inactive biologically, but it can cross-react with antisera prepared against insulin.

- Plasma pro-insulin is not elevated in human diabetes or in normal after glucose stimulation, but it may be the predominant circulating form in some subjects with islet cell tumours.

CATABOLISM OF INSULIN

Insulin is very rapidly catabolised. Its plasma $t_{1/2}$ is less than 3-5 minutes under normal conditions. Major organs where insulin is catabolised are liver, kidneys, and placenta.

About 50% of insulin is degraded in its single passage through the liver.

Mechanism

Two enzyme systems are involved for degradation of insulin

- Protease: an insulin-specific protease has been found in many tissues with highest concentration in liver and kidneys. The protease is-SH dependent and active at physiological PH.
- Second mechanism is more important. The enzyme is glutathione-insulin transhydrogenase (also called insulianse). This enzyme is found in higher concentration in liver and kidneys. Also present in skeletal muscles and placenta. This brings about reductive cleavage of the insulin molecule. Reduced glutathione (G-SH), acting as a co-enzyme for the transhydrogenase, donates the H-atoms for the reduction and is itself thus converted to oxidized glutathione.
- After insulin is reductively changes, the A-chains and B-chains are further hydrolyzed by proteolysis.

INSULIN RECEPTORS

Insulin acts on target tissue by binding to specific insulin receptors, which are glycoproteins. The human insulin is found on chromosome 19. The insulin receptors are being constantly synthesized and degraded. Their $t_{1/2}$ is 6-12 hrs only. It is synthesized as a single chain polypeptide pro-receptor” in the rER and is rapidly glycosylated in Golgi region. The “pro-receptor” has 1382 amino acids and most 190,000.

The pro-receptor is cleared to form mature “ α ” and “ β ” subunits ($\alpha_2\beta_2$) which is a heterodimer, linked by S-S bonds. Both subunits are extensively glycosylated and removal of sialic acid and galactose decreases insulin binding and insulin action. Insulin receptors are found in target cell membrane, up to 20,000 per cell.

Binding of insulin to the receptor, stimulates its, tyrosine kinase activity. Tyrosine kinase enzyme phosphorylates the phenolic –OH group of tyrosine residues in specific protein including that of a tyrosine in the β -chain of insulin receptor itself to modulate their activities, $ATP + \text{tyrosineprotein} \rightarrow ADP + \text{phosphor-tyrosine protein}$.

Regulation of insulin receptors

High blood insulin level decreases the number of insulin receptors on target cell membrane, probably through internalization of the insulin-receptor complex into the cell and thus decreases the insulin sensitivity of the target tissue.

MECHANISM OF ACTION OF INSULIN

When insulin binds to the specific receptor several events of actions take place.

- A conformational change of the receptor
- The receptor crosslink and form microaggregates
- The receptor complex is internalized and
- One or more signals is generated

But nature of the intracellular signal and intracellular second messenger” remains still uncertain and vague.

Various mechanisms have been proposed.

1. **Role of c-AMP:** It is proposed that insulin promotes the phosphorylation of c-AMP phosphodiesterase. The active phosphodiesterase hydrolyses c-AMP and lowers the c-AMP level in the cells. The consequent fall in activities of c-AMP dependent protein kinase reduce phosphorylation of specific enzymes.
2. **Role of c-GMP:** The insulin receptor binding may activate guanylate cyclase which forms c-GMP. Increased concentration of c-GMP act as second messenger” to activate c-GMP dependent protein kinase. These may phosphorylate some enzymes to modulate their activities
3. **Role of protein phosphatase:** Insulin may act through the protein phosphates I which may dephosphorylate certain key enzymes thereby activating them. Best examples are the key enzyme glycogen synthase and pyruvate dehydrogenase complex. On the other hand inhibits phosphorylase enzyme and triacylglycerol lipase.

4. **Action through tyrosine kinase**” Activity of β -subunit Receptor: The binding of insulin to its receptor enhances tyrosine kinase activity. Tyrosine kinase in turn phosphorylates phenolic-OH group of tyrosine residues of specific proteins leading to changes in enzyme activities.
5. **Role in mRNA translation:** Insulin is known to affect the activity or amount of at least more than 50 proteins in variety of tissue and many of these effects involves covalent modification. A role of insulin in the translation of mRNA has been proposed largely based on studies of ribosomal protein 6S, a component of the 40S ribosomal unit. Such a mechanism accounts for the general effect of insulin on protein synthesis in liver, heart muscle and skeletal muscles.
6. **Role on gene expression (Nuclear action):** insulin also affects the rate of transcription of specific genes, thereby regulates the synthesis of specific m-RNAs and thus changing the rate of synthesis of specific protein coded by them. e.g insulin decreases the transcription of gene involved in synthesis of the enzyme phosphoenol-pyruvate carboxy kinase (PEPCK), the key enzyme for gluconeogenesis. On the other hand insulin induce the synthesis of phosphofructokinase and pyruvate kinase required for glycolysis, by increasing the transcription of these genes.

METABOLIC ROLE OF INSULIN

A. Action on carbohydrate metabolism

Net effect is lowering of blood glucose level and

Increase glycogen store

The above is achieved by several mechanisms.

1. Increase glucose uptake:

- Insulin increases glucose uptake from extracellular fluid by the various tissues viz, muscles, adipose tissue, mammary glands, lens, etc.
- In adipose tissue and other extrahepatic tissues, insulin stimulates translocation of glucose transporters from their intracellular pool in Golgi cisternae to the plasma membrane where they participate as carrier in transportation of D-glucose and D-galactose across the membrane.

- Also in hepatocytes, insulin increases hepatic uptake of glucose (freely permeable to liver cells) it induces the synthesis of the enzyme glucokinase which simultaneously phosphorylates glucose, thereby lower intracellular concentration.
- 2. Increases glycolysis: increase utilization of glucose for providing energy which takes place in muscles, liver and many other tissues. Insulin enhances glycolysis because it induces the synthesis of key enzyme phosphofructokinase and also pyruvate kinase.
- 3. Increase conversion of pyruvate to acetyl CoA: insulin increase aerobic oxidative decarboxylation of pyruvate to acetyl CoA, because it causes dephosphorylation of pyruvate dehydrogenase complex which is thus converted to the form.
- 4. Stimulate glycogenesis: insulin stimulates glycogenesis in the liver and muscles by increasing dephosphorylation of the key and rate limiting enzyme, glycogen synthase, thus converting it to its active form. Insulin stimulates the protein phosphatase-1 directly, which brings about dephosphorylation.
- 5. Decrease Gluconeogenesis: Insulin reduces gluconeogenesis:
 - By repressing the synthesis of the key rate limiting enzyme phosphoenol pyruvate carboxykinase (PEPCK) by decreasing the transcription rate of the gene.
 - Also inhibits allosterically fructose-1,6-bisphosphatase another key enzyme for gluconeogenesis.
 - Insulin dephosphorylates fructose 2,6-bisphosphatase so that it is converted to inactive form, which increases the concentration of fructose 2,6-bisphosphate in the cell, which in turn allosterically inhibit fructose 1,6-bisphosphatase.
- 6. Decrease glycogenolysis: insulin decreases glycogenolysis.
 - By dephosphorylating the key and rate limiting enzyme glycogen phosphorylase thus converting it to inactive form
 - Also represses the enzyme glucose-6-phosphatase.

B. Action on lipid Metabolism

Net effects are lowering of free fatty acid level and increase in triglyceride store.

The above is achieved as follows:

1. Decrease lipolysis: Insulin decreases lipolysis in adipose tissue cells and consequently lower plasma FFA. Lipolysis is reduced due to
 - Insulin activates phosphoprotein phosphatase which dephosphorylates the triacylglycerol lipase and thus converted to inactive form.
 - At the same time, insulin activates phosphodiesterase which degrades c-AMP and prevent phosphorylation and reactivation of TG lipase
2. Increases fatty acid synthesis: Insulin increases the extramitochondrial de novo fatty acid synthesis by making available of more substrate acetyl CoA and also increasing the activity of acetyl CoA carboxylase.

The above is done as follows:

- Insulin promotes dephosphorylation of pyruvate dehydrogenase complex and converts into active form so that more acetyl CoA is available from pyruvate
 - Insulin induces the synthesis of ATP-citrate lyase to increase cleavage of citrate, so that more acetyl-CoA is available in cytosol.
 - Insulin lowers the plasma FFA level, so prevent long chain acyl-CoA from inhibitory acetyl-CoA carboxylase.
 - It induces the synthesis of acetyl-CoA carboxylase and fatty acid synthase, the cytosolic enzymes required for FA synthesis.
 - Insulin activates acetyl-CoA carboxylase by dephosphorylation of the enzyme (Converting to active form).
 - Provides more NADPH for the reductive steps in FA synthesis by stimulating HMP-shunt pathway.
3. Increase synthesis of TG: Insulin enhances TG synthesis in adipose tissues by:
- Providing more α -glycerol-P as glucose uptake and utilization is enhanced in adipocytes.
 - Increased synthesis of FA provides the acyl CoA (FFA pool 1) required for TG synthesis.
 - Insulin also induces the synthesis of lipoprotein lipase. This enzyme hydrolyzes TG of circulating chylomicrons and VLDL and releases FFA (FFA pool 2) which are taken up by the adipocytes and used for TG synthesis.
- 4 Decreases ketogenesis: As plasma FFA level is decreased less is oxidized by β -oxidation and less acetyl-CoA will be available for cholesterol synthesis and ketogenesis.

C. ACTION ON PROTEIN METABOLISM

Net effect is insulin promotes protein synthesis.

This is achieved as follows:

- Insulin increases amino acids uptake by the tissues by enhancing the rate of synthesis of membrane transporters for amino acids.
- Adequate supply of insulin is necessary for protein anabolic effect of growth hormone (permissive effect)
- Insulin increase protein synthesis by providing more amino acids in cells, by affecting gene transcription (nuclear level) by regulating specific m-RNA synthesis and affecting translation at ribosomal level.
- Regulation of ribosomal translation is done by two ways:

- Increase the synthesis of polyamines-required for ribosomal RNA synthesis, by increasing the synthesis of key and rate limiting enzyme ornithine decarboxylase.
 - Secondly, insulin modulates ribosomal activity by causing phosphorylation of 6S ribosome (α component of 40S)
- d. Action on mineral Metabolism

Decrease in concentration of K^+ and inorganic P in blood due to enhanced glycogenesis and phosphorylation of glucose.

e. Actions on growth and cell Duplication

Insulin stimulates growth in vivo and also cell proliferation in vitro. Cultural fibroblasts have been used most frequently in studies of cell proliferation. It has been found that insulin potentiates the ability of fibroblast growth factor (FGF), platelet-derived growth factor (PDGF) and epidermal growth and cell proliferation are seen in many tissues such as liver, mammary glands and adrenals and also in embryogenesis and tissue differentiation. These effects are largely due to stimulation of DNA replication, gene transcription, protein synthesis and modulation of various enzyme activities through phosphorylation dephosphorylation.

GLUCAGON HYPERGLYCAEMIC-GLYCOGENOLYTIC FACTOR

Glucagon is a hormone produced by α -cells of islet of Langerhan of pancreas and is an important hormone involved in

- Rapid mobilization of hepatic glycogen to give glucose by glycogenolysis and
- To a lesser extent FA from adipose tissue.

Thus it acts as a hormone required to mobilize metabolic substrates from storage depots.

CHEMISTRY

Glucagon has been purified and crystallized from pancreatic extracts and also the hormone has been synthesized. It is a polypeptide containing 29 amino acids. There are only 15 different amino acids in the molecule. Amino acid sequence has been determined, histidine is the N-terminal amino acid and threonine is the C-terminal. Mwt is approx 3485.

Unlike insulin

- It does not require zinc or other metals for its crystallization.
- Glucagon contains no cystine, proline, isoleucine but contains tyrosine, methionine and tryptophan

SYNTHESIS

It is synthesized first as a pro-hormone, proglucagon in α -cells. Lysosomal enzyme peptidase like carboxy-peptidase B and trypsin-like peptidase in α -cells hydrolyze pro-glucagon from both its N-terminal end and c-terminal end to yield glucagon and inactive peptides.

ENTERO-GLUCAGON OR GLUCAGON-LIKE IMMUNE REACTIVE FACTOR.

A glucagon-like immunoreactive factor (GLI) has been identified in gastric and duodenal mucosa. GLI is immunologically similar though not identical to the pancreatic hormone. Moreover, it is less active than pancreatic glucagons in stimulating adenyl cyclase and therefore cannot duplicate many of the functions of pancreatic hormone. GLI is stimulated by absorbed glucose causing an apparent elevation of circulating pancreatic glucagons.

Recently, two different molecular fractions have been isolated:

- One having mol.wt =3500, has hyperglycaemic and glycogenolytic activity but far less potent than pancreatic glucagons.
- The other fraction, mol.wt=2000; devoid of the above activity.

Both have insulin releasing activity

MECHANISM OF ACTION

Glucagon binds to specific receptors on the plasma membranes of hepatocytes and adipocytes and activates adenyl cyclase to produce c-AMP in these cells, which is the principal "second messenger" and duplicates the functions of the hormone. C-AMP in turn activates c-AMP dependent protein kinases which further phosphorylate specific enzymes to increase/decrease their activities. C-AMP also induces synthesis of certain specific enzymes like glucose-6-phosphatases by increasing the transcription of their genes.

METABOLIC ROLE

1. Action on carbohydrate metabolism

Net effect of the hormone is to increase the blood sugar level (hyperglycaemia). Hyperglycaemic effect is due to various causes.

- Glycogenolysis: glucagons increases glycogenolysis in liver. In muscles, it cannot bring about glycogenolysis as muscle cell membrane lacks the glucagons specific receptors glucagons also induces the synthesis of glucose-6-phosphatase enzyme.
- By increasing gluconeogenesis in liver: glucagon stimulates the conversion of lactic acid and glucogenic amino acids to form glucose.
- The increased hepatic c-AMP produced after glucagon action has been shown to increase protein kinases that catalyze nuclear histone phosphorylation in liver cell nucleus. This reaction inhibits the repressive effect normally exerted by histones on DNA and allows the initiation of a sequence of events leading to the synthesis off new enzyme proteins involved in gluconeogenesis. Thus, glucagons induces the synthesis of phosphoenol pyruvic carboxykinase, pyruvate carboxylase and fructose - 1-6-biphosphatase enzyme, all key enzymes of gluconeogenesis.
- Also glucagon increases the pool of glucogenic amino acids in liver, so that they can be used for gluconeogenesis. This is achieved by increasing protein breakdown in liver and by reducing hepatic protein synthesis.

2. **On lipid metabolism**

- Lipolysis: In adipose tissue and also possibly in liver, glucagon increases the breakdown of TG to produce FFA and glycerol. FA undergo β -oxidation, increased breakdown may lead to ketone bodies formation and ketosis. Thyroid hormones help in the lipolytic action of glucagon, probably the hormones increases the number of glucagon specific receptors on adipocytes.
- Anti-lipogenic Action: Glucagon reduces F.A. synthesis. This is achieved in 2 ways:
- Increased lipolysis raise the concentration of FFA in blood. Long-chain acylCoA inhibits the rate limiting enzyme acetyl-CoA carboxylases.
- Increased c-AMP level in cells activates c-AMP dependent protein kinase which phosphorylates acetyl-CoA carboxylase. Phosphorylated form of the enzyme is inactive.

3. **On protein metabolism**

- Glucagon reduces protein synthesis by depressing incorporation of amino acids into peptide chains. This may be due to the inactivation of some ribosomal component by a protein kinase whose activity is enhanced by glucagon-induced rise in c-AMP.
- Glucagon also stimulates protein catabolism especially in liver thus increases the hepatic amino acid pool which is utilized for gluconeogenesis. Also increases urinary NPN and urea.

4. Action on Heart: Glucagon exerts a positive inotropic effect on heart without producing increased myocardial irritability. Hence, use of glucagon in treatment of heart disease, viz in cardiac failure and cardiogenic shock.

Advantage over non-epinephrine: glucagon increases the force of contraction, but does not produce any arrhythmias, tachycardia or increase in O_2 consumption.

5. Calorigenic effect: glucagon increases heat production and rise in BMR. The calorigenic action is not due to hyperglycaemia per se but is probably due to increased hepatic deamination of amino acid, with thyroid hormones stimulating the utilization of deaminated residues. The calorigenic action requires the presence of thyroid and adrenocortical hormones and fails to occur in their absence.

6. **On mineral metabolism:**

Potassium: glucagon increases K^+ release from the liver, an action which may be related to its glycogenolytic activity

Calcium: Glucagon can increase the release of calcitonin from the thyroid.

SOMATOSTATIN

The peptide somatostatin (growth hormone release inhibiting factor) was first isolated from the hypothalamus and was implicated as a regulator of growth hormone secretion.

CHEMISTRY

It is a peptide consisting of 14 amino acids. There is an intrachain S-S linkage joining cysteine 3 and cysteine at position 14

Sources: there are three sources

- Hypothalamus
- Pancreas; somatostatin is also secreted by δ -cells of islet of Langerhans of pancreas.

- GI tract: it is also produced by D-Cells of antral mucosa of stomach and also duodenal mucosa.

a. *Hypothalamic somatostatin*

- Acts as a regulator of growth hormone secretion
- It inhibits growth hormone (GH) release
- It may also serve as a neurotransmitter

b. *Pancreatic somatostatin*

- It inhibits both insulin and glucagon secretion and thus may serve as an intraislet regulator of secretion of these hormones. Thus act as intraorgan “synaptic transmitters” or neuromodulators.
- Somatostatin is secreted into the portal vein blood as a result of glucose or amino acid stimulus indicating extra-islet role
- Also directly inhibit secretion of both HCO_3^- and enzymes in pancreatic juice.

c. G.I. somatostatin

- Inhibit the secretions of gastrin, CCK and motilin.
- Also inhibits gastric acid secretion, secretion of Brunner’s glands pancreatic HCO_3^- and enzymes secretions gastric emptying and gall bladder contraction.
Since somatostatin can inhibit a variety of G.I functions (gastric emptying, G.T. motility), its major function may be to regulate nutritional influx at the level of GI tract.