"The Search for truth is one way hard and in another way easy. For it is evident that no one can master it fully nor miss it wholly. But each adds a little to our knowledge of nature, and from all the facts assembled, there arises a certain grandeur"

- Aristotle

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Review of Literature
In light of facts mentioned in the introduction, review of the literature has been drafted by giving a generalized description about gastrointestinal complications of diabetes, blood glucose homeostasis and physiology of gastrointestinal tract. In addition, an exhaustive survey of literature is presented for the interventions adopted or abandoned in the study.

3.1. Gastrointestinal complications of diabetes

Following gastrointestinal complications are known to be associated with untreated diabetes mellitus.

3.1.1. Gastroparesis: Gastroparesis is one of the commonly occurring forms of poor gastric emptying in diabetic patients. In 1958, Kassander\textsuperscript{13} introduced the term 'gastroparesis diabetorum' for the symptom involving delay in gastric emptying either in the absence of any mechanical obstruction of the pylorus or duodenum. The associated symptoms are postprandial nausea, epigastric burning or pain, bloating, vomiting of undigested food eaten several hours previously, anorexia and early satiety. These symptoms resemble those seen after truncal vagotomy and microscopic studies showed loss of unmyelinated axons\textsuperscript{14}. Pathophysiology involves the denervation of parasympathetic nervous system (PNS) of stomach, absence of gastric phase 3 of the migrating myoelectric complex (MMEC) and hypomotility of the antrum, as the most prominent features of gastroparesis\textsuperscript{15}.

3.1.2. Diarrhoea: Diarrhoea affect 10-20 % of diabetics, more common in men than women associated with passing frequent and loose consistency stools\textsuperscript{5}. It involves marked abnormality of motor pattern of small intestine. Phase 3 contractions are shorter and phase 2 activity is abnormal. Sympathetic denervation in the gut is common in diabetics which leads to abnormal absorption of fluids and electrolytes. Both truncal vagotomy and sympathectomy may cause diarrhoea. Small bowel is less sensitive to distention-induced pain, suggesting a defect in afferent nerve function\textsuperscript{5}. Transit is more rapid or there may be bacterial overgrowth in the small intestine. Impaired water and electrolytes absorption in small intestine was proposed as a key factor in the development of diarrhoea\textsuperscript{16}.
3.1.3. **Fecal incontinence**: A troublesome symptom involving accidental nocturnal passage of unformed stools is a characteristic symptom affecting 40% of diabetic patients with diarrhoea. The mean basal sphincter pressure was significantly reduced, which was generated by tonic contractions of internal anal sphincters. This indicates diabetics with fecal incontinence have a defect in sphincter function. A defect in anal sphincter function might cause loose stools and low-volume diarrhoea by allowing premature emptying of the colon\(^3\).

3.1.4. **Constipation**: This symptom was found to be the commonest GI symptom in diabetics. Severe constipation occurred in 20% of patients. Although the basal spike activity was normal with diabetes, the myoelectrical and colonic motor response to the meal were blunted and delayed and even absent in severe constipation. Colonic muscle was capable of responding to exogenous stimulation but ingested meal did not trigger appropriate neurohumoral signals to the colon\(^3\).

*The following significant scientific evidence were abridged and quoted in support of gastrointestinal complications in diabetic patients.*

**Gastric motility & small intestinal transit**

Rosa-e-Silva *et al* (1996)\(^6\) reported that patients with type 1 diabetes have abnormally rapid transit of liquid meal through the distal part of small intestine which may play a role in diarrhoea production. However, the gastric emptying and transit through the proximal small bowel were not different from control group.

**Gastric emptying & whole gut transit (WGT)**

Wegener *et al* (1990)\(^7\) observed in a clinical trial that gastric emptying was significantly prolonged in insulin treated DM and it was related to autonomic nerve dysfunction and peripheral neuropathy. However, no significant overall differences were observed between diabetes and control group, concerning mouth to cecum transit and whole gut transit time. Autonomic neuropathy and dyspeptic symptoms were the major parameters in predicting delayed gastric emptying.
**Intestinal transit**

Keshavarzian and Iber (1986) reported that there was no significant difference in intestinal transit between normal controls and type 1 diabetes. However, one third of diabetics had abnormal intestinal transit of liquid test meal. Their data suggest that abnormal intestinal motility is common in diabetics and altered intestinal transit is not a prerequisite for diabetic diarrhoea.

**Gastric emptying**

Keshavarzian *et al* (1987) observed that delayed gastric emptying of solids is common in patients with clinically detectable neuropathy and visceral autonomic neuropathy seems to be an important underlying factor in diabetic gastroparesis.

This part of the literature review express a common opinion that untreated diabetic patients suffer from rapid or abnormal intestinal transit associated with the visceral autonomic neuropathy. However, it is uncertain whether insulin treatment reverses the GI manifestations.
3.2. Streptozotocin animal model of diabetes mellitus

Streptozotocin (STZ) is an antibiotic obtained from *Streptomyces achromogenes*. Rakieten *et al.* (1963) were the first to report that when STZ was given intravenously to rats and dogs, caused diabetes mellitus. The model was further evaluated by Junod *et al.* (1967). After 7-10 h STZ-treatment, there was a massive β-cell degranulation and necrosis, associated with an increase in serum insulin levels and hypoglycemia. This was followed by a prolonged reduction in pancreatic insulin levels to <5% of normal values. Its action was dose related, ranging from a mild diabetes with a dose of 35 mg/kg to a severe ketotic state with 100 mg/kg, leading to death within 2-3 days. This animal model was assumed to display the features exhibited by uncontrolled DM in human subjects.

The following reports are the frequently cited and significant evidences for indicating deleterious effects of streptozotocin model on gut.

Lincoln *et al.* (1984) reported that in the myenteric plexus of the ileum from diabetic animals, adrenergic nerves displayed signs of degeneration. However, cholinergic nerves did not display any signs of reduction in the ileum. In contrast, in the proximal colon significant increases in noradrenaline and serotonin levels as well as choline acetyltransferase activity was observed.

Belai *et al.* (1996) observed a marked increase in nerve fibers containing sensory neuropeptides in mesenteric vessels of STZ-induced diabetic rats. They suggested an impaired sensorimotor function in the mesenteric vessels, which is likely to reflect a neuropathic change.
Fox et al (1999) evaluated STZ-induced diabetes in the rat as a model of painful diabetic neuropathy to assess the efficacy of potential agents. They noticed, STZ-induced DM animals were chronically ill, with reduced growth rate, polyuria, diarrhoea, enlarged bladder and showed markedly reduced motor activity and marked hyperalgesia. In view of these findings they questioned the predictive value of these animals as a model for the human condition of chronic diabetic pain seen in patients receiving long-term insulin treatment and ethical issues concerned with the use of the animals themselves.

Based on the evidence cited, we thought it is legitimate to abandon STZ-model and to adopt healthy animals to test the hypotheses of the study as the animals possess intact peripheral nervous system and precisely reflect any intervention-induced changes thereof.
3.3. Blood glucose homeostasis

In normal healthy subjects glucose circulates in plasma water at a basal concentration of 65-105 mg/dL, which is in rapid equilibration with red blood cells. Glucose undergoes multiple metabolic fates. It may be stored as glycogen, undergo glycolysis to pyruvate, then reduced to lactate and transaminated to form alanine or converted to acetyl coenzyme A, which in turn is oxidized to CO₂ and water via the tricarboxylic acid cycle.

Glucose is derived from three sources 1) intestinal absorption from dietary carbohydrates 2) glycogenolysis and 3) gluconeogenesis.

3.3.1. Glucoregulatory factors: Maintenance of plasma glucose concentration is critical for survival, despite the wide variations in glucose influx and efflux. This critical range is maintained by 1. Hormonal 2. Neural factors.

3.3.1.1. Hormonal factors: Insulin, glucagon, adrenaline, cortisol and growth hormone.

Insulin: It is a potent hormone, involved in the regulation of plasma glucose level. Its deficiency or excess can both cause metabolic imbalance of glucose. Insulin level rises in response to absorption of dietary carbohydrate, enhances glucose utilization and suppress endogenous glucose production, thereby lowering the glucose level.

Glucagon: It is a potent activator of glycogenolysis and gluconeogenesis and increase hepatic glucose production within minutes during hypoglycemic condition but its effect is transient.

Adrenaline: It is secreted during hypoglycemic condition and act within minutes to raise the blood glucose level. However, it has a complex action. It can stimulate both hepatic glucose production and limit glucose utilization by peripheral tissues.

Growth hormone: It stimulates gluconeogenesis, enhances lipolysis and antagonizes insulin stimulated glucose uptake. Its hyperglycemic effect does not appear for several hours.
**Cortisol:** It stimulates gluconeogenesis and increases the breakdown of protein and fat\(^9\). Similarly, cortisol causes an increase in the plasma glucose level only after 2-3 h\(^2\).

**Somatostatin:** Although somatostatin does not appear to have a direct effect on carbohydrate metabolism, it inhibits release of growth hormone from the pituitary, in addition, it inhibits secretion of glucagon and insulin by the pancreas.

**3.3.1.2. Neural factors:** Electrical stimulation of hepatic sympathetic nerves increases hepatic glucose release and cause hyperglycemia in animals. Parasympathetic stimulation decreases hepatic glucose release in animals\(^3\).

**3.3.2. Profile of blood glucose levels**

**3.3.2.1. Post prandial state**

The ingested starch is partially digested by salivary amylase in the mouth to form maltose. When the carbohydrates enter the duodenum the pancreatic amylase completes the digestion of carbohydrates to oligosaccharides. Maltose, lactose and sucrose are hydrolysed at brush border to form glucose, galactose and fructose. These monosaccharides are absorbed from the duodenum and ileum through carrier mediated transport, leading to elevation of blood glucose level and stimulation of insulin secretion\(^9\). Endogenous glucose production is markedly suppressed. Then the plasma glucose level decline rapidly as a result of accelerated glucose utilization coupled with diminishing glucose absorption, again rise in plasma glucose level occurs as a result of resumption of endogenous glucose production. Transition from exogenous glucose delivery to endogenous glucose production late after glucose ingestion is the result of coordinated diminution of insulin secretion and resumption of glucagon secretion\(^4\).

**3.3.2.2. Post absorptive state**

It occurs after a 10-14 h fasting or overnight fasting. Plasma glucose becomes relatively stable, thus glucose production and utilization rates are equal i.e., 1.8-2.6 mg/kg/min in normal adults after an overnight fast. About three fourths of hepatic glucose production results from glycogenolysis and remaining fourth from gluconeogenesis after an overnight fast\(^4\).
Maintenance of fasting plasma glucose concentration is primarily the responsibility of the liver. It is related to the presence of glucose-6-phosphatase which catalyse glucose-6-phosphate to glucose within the hepatocytes. Renal glucose release takes place only during prolonged starvation. During the fast, muscle can reduce glucose uptake to virtually zero, oxidize fatty acids for its energy. Reduced insulin secretion is fundamental for maintenance of the postabsorptive plasma glucose concentration and permits hepatic glucose production to proceed.

Free glucose in the extracellular fluid is about 15-20 gm in normal adults. Glycogen is mobilized to provide circulating glucose to about 24-130 gm. Thus in an adult, free glucose can provide as little as 3 h supply. Therefore, gluconeogenesis is important for maintenance of the postabsorptive plasma glucose concentration. If fasting is prolonged from 24-48 h plasma glucose level declines and then stabilizes and gluconeogenesis becomes the sole source of glucose production.

Boyle et al (1989) reported that overnight or prolonged fasting for 3 days in humans resulted in decrements in baseline glucose production, plasma glucose, insulin and increments in plasma glucagon, epinephrine, norepinephrine, growth hormone and cortisol. They concluded that glucagon plays a primary counter-regulatory role during fasting.

In animal species, basal rate of glucose turnover is higher than in humans eg. rats 7.5 mg/min/kg, the limited capacity of the liver to store glycogen confers an increasing role to gluconeogenesis for the maintenance of basal glycemia. The mechanism that prevents hypoglycemia during a more prolonged fast are similar to those operative after an overnight fast.

Fasting exerts stress on metabolic and physiologic processes in body. During fasting glucose-6-phosphate dehydrogenase is inhibited. In the initial stages of fasting, energy is obtained from fats, but as the time progresses both the fat and protein stores are depleted.
3.3.2.3. Hypoglycemia: When venous plasma glucose concentration obtained after an overnight fast, lies in between 45-60 mg/dL, is suggestive of hypoglycemia. Hypoglycemia suppresses the secretion of insulin and stimulates the release of glucagon, adrenaline, cortisol and growth hormone.

3.3.3. Hepatic glycemic control

Claude Bernard in the year 1854 observed that puncture of the floor of the fourth ventricle in dogs resulted in hyperglycemia. He concluded that it might have resulted from stimulation of nerve impulses passing via the splanchnic nerves and celiac plexus to the liver. Later, Cavazzani (1893) stimulated the celiac plexus in dogs and produced hyperglycemia with a reduction in hepatic glycogen content. Macleod (1907) stimulated left splanchnic nerve and produced similar results. Niijima and Fukuda (1973) using the toad showed clearly that decreased blood pressure did activate nerve fibres to the liver. This was the first demonstration of the reflex release of glucose via the hepatic nerves.

The liver is a major metabolic organ for the production of glucose and is richly innervated by the autonomic components from the splanchnic sympathetic nerves and vagal parasympathetic nerves. Liver receives neural input from sympathetic nerves (T7-T10) via the celiac plexus, which intermingle with parasympathetic nerves from the right (posterior) and left (anterior) vagus and from the right phrenic nerve.

3.3.3.1. Sympathetic innervation

The sympathetic nervous system (SNS) promotes hepatic glucose production by activating glycogenolysis in fed states and accelerating gluconeogenesis in fasted states. When hepatic nerves surrounding the common trunk the hepatic artery (anterior plexus) are stimulated, the glucose output is nearly doubled. Adrenaline is the strongest effector of hepatic glucose production.
Reflex release of glucose occurs during hemorrhage or other forms of acute stress and appears to be especially important in fasted animals where the response to adrenal discharge is greatly reduced or absent\textsuperscript{33}. In perfused rat liver, activation of the sympathetic liver nerves causes increase in glucose release and activity of glycogen phosphorylase and a decrease in activity of glycogen synthase.

The calculated total release of glucose from the liver with 8 Hz stimulation for a 4 min period of nerve stimulation varied widely between 10.5 and 55 mg/kg. Hepatic glucose output in anaesthetized cats rose from 10 mg/min/kg to a peak of about 33 mg/min/kg at 3 min after onset of the stimulation\textsuperscript{33}.

3.3.3.2. Parasympathetic innervation

The PNS is greatly stimulated by electrical stimulation of peripheral end of the vagus nerve, enhances glucose utilization and causes reopening of previously closed sinusoids in liver and stimulate glycogen synthetase phosphatase to produce active synthetase-a. This resulted in formation of glycogen and decreased output of glucose from the liver\textsuperscript{33}.

3.3.3.3. Central nervous system

Central nervous system (CNS) also has an important role in the maintenance of hepatic glucose production even when the counter regulatory hormones are inhibited. The effect of hypoglycemia is mediated via glucose sensors in the hypothalamic region of brain, which activates hepatic glycogenolysis via sympathetic innervation of the liver\textsuperscript{46}. 

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3.3.4.2. **Glucose as secretogogue:** The exact mechanism by which glucose stimulates insulin release is not fully understood, but its entry into β-cell and metabolism is required for insulin release. The β-cells do not need insulin for glucose transportation. Glucose enters the β-cell by facilitated transport, which is mediated by glucose transporter-2 (GLUT2). The sugar is then phosphorylated by glucokinase leading to production of ATP. ATP sensitizes closure of an ATP-sensitive potassium (K\(^\text{ATP}\)) channels. This results in decrease in K\(^+\) efflux which depolarizes the β-cell membrane. A compensatory activation of voltage dependent Ca\(^{2+}\) channels results in the influx of Ca\(^{2+}\) into β-cell. This increase in intracellular Ca\(^{2+}\) triggers the release of insulin by exocytosis.

3.3.4.3. **Insulin release:** Insulin is secreted at high frequency pulsatile bursts at a frequency of 5-15 pulse/min. The pulsatile release of insulin reflects the results of simultaneous metabolic processes in the β-cell population. These metabolic processes involve cyclic glycolysis with subsequent changes in intracellular ATP concentrations causing closure of K\(^{\text{ATP}}\) channels, depolarization of the β-cell membrane, a rise in intracellular calcium and exocytosis of insulin-containing granules.

It has been well documented that the release of insulin is biphasic in nature in response to prevailing plasma glucose. However, following phases were reported:

- **Cephalic phase:** This phase of insulin release represents the consequence of oropharyngeal stimulation producing a rapid (within 2 min) but small (5 μU/ml) increase in arterial plasma insulin which normally takes place on seeing the food. This rapid release represents the pool of insulin stored within β-cells which can be instantly mobilized upon stimulation. But this insulin has no effect on glucose clearance. This reflex is under vagal control.

- **First phase:** In this phase insulin release occurs slowly from 5-10 min after cephalic phase but in greater magnitude after ingestion of carbohydrates. Insulin released improves the plasma glucose profile by almost 50% and prevents the early accumulation of glucose in the vascular space.
Second phase: This phase of insulin release starts at the end of the first phase, improves the plasma glucose profile by 83% in 15-50 min. This phase has effect on glucose uptake by muscle and glucose production. Insulin release is directly related to the elevation in the glucose level[^8]. This phase continues until the normoglycemia is restored.

Third phase: Some *in vitro* studies using isolated islets have suggested a third phase of insulin secretion which commences 1.5-3 h after glucose exposure and is characterized by a spontaneous decline of secretion to 15-25% of peak values, a level that is subsequently maintained for more than 48 h[^7].

Insulin circulates in blood as the free monomer and its volume of distribution approximates the volume of extracellular fluid. Under fasting conditions in humans, pancreas secretes about 40 μg or 1 unit of insulin per hour into the portal vein, to achieve a concentration of insulin portal blood of 50 to 100 μU/ml and in the peripheral circulation of 12 μU/ml. After ingestion of a meal, there is a rapid rise in the concentration of insulin in the portal blood, followed by a parallel rise in the peripheral circulation as high as 100 μU/ml[^9].

Metabolism of insulin occurs primarily in liver, kidney and muscle. About 50% of the insulin that reaches the liver via the portal vein is destroyed and never reaches the general circulation. Several enzymes have been implicated in insulin degradation. The primary one is, thiol metalloproteinase located in hepatocytes. The released insulin has half life of 5 min.

Ultimately, insulin secretion depends on the intracellular concentration of Ca²⁺[^5]. Lowering extracellular concentrations of Ca²⁺ may inhibit secretion of insulin without affecting biosynthesis. In humans, the normal physiological plasma insulin ranges from 20-30 μU/ml[^9].
3.3.4.4. **Insulin receptors**: Insulin initiates its actions by binding to cell-surface receptors of cysteine rich region in the α-chain. In 1982, Kasuga et al.\(^5\) reported that insulin receptors possess tyrosine kinase activity. This was confirmed by c-DNA cloning which revealed that insulin receptor was homologues to the large family of tyrosine kinase receptors that control many aspects of cell growth and differentiation\(^6\). Such receptors are present in virtually all vertebrates, about 20,000 per cell. Concentration varies from as few as 40 receptors on circulating erythrocytes to more than 2 lakhs on adipocytes and hepatocytes. They are constantly being synthesized and degraded and has the half life of 7-12 h. There are no known competitive antagonists at the receptor level\(^5\).

Based on structural features the receptor tyrosine kinases is divided into three groups\(^4\). The insulin receptors represent the 3\(^{rd}\) family of receptor tyrosine kinases which is known to be composed of tetrameric receptors with a single cluster of cysteine residue in the extracellular α-subunits. The kinase domain is in the β-subunits and resembles prototype tyrosine kinase.

Insulin receptor is a large transmembrane glycoprotein composed of two 135 kDa α-subunits and two 95 kDa β-subunits which are linked by disulphide bonds to form a β-α·α-β heterotetramer. The α-subunits are placed extracellularly and contain the insulin-binding domain and the β subunits are embedded in cell membrane that possess tyrosine protein kinase activity. After insulin is bound, receptors aggregate and are rapidly internalized and undergoes autophosphorylation of several tyrosine residues in the β-subunits. The activated transmembrane receptor kinase initiates a cascade of events by first phosphorylating a protein called insulin receptor substrate-1 (IRS-1). Phosphorylated IRS-1 serves as a docking protein for variety of proteins that contain so called Src homology 2 (SH2) domain that are directly involved in mediating effects of insulin i.e., activation of membrane transport, cell metabolism or growth\(^5\).

3.3.4.5. **Insulin cellular actions**: Insulin action at cellular level was first suggested by Lundsgaard (1939-40)\(^3\). Insulin's anabolic actions include the stimulation of intracellular utilization and storage of glucose, amino acids and fatty acids, while it inhibits catabolic processes such as the breakdown of glycogen, fat and protein.
3.3.4.6. Principal actions of insulin

1. Rapid action (seconds): Increases the transport of glucose, amino acids and K⁺ into insulin sensitive cells.

2. Intermediate actions (minutes): A. Stimulation of protein synthesis B. Inhibition of protein degradation C. Activation of glycolytic enzymes and glycogen synthesis D. Inhibition of phosphorylase and gluconeogenic enzymes

3. Delayed action (hours): Increase in mRNA for lipogenesis and other enzymes.

Ozturk et al (1996) stated that insulin with other growth factors may play an important role in the development of GI tract and may be involved in pathophysiological conditions related to the intestine.

3.3.4.7. Insulin stimulators: Major stimulators are – Glucose, Amino acids, sulphonylureas, extracellular Ca²⁺, acetylcholine and vanadium.

**Glucose:** In addition to its prominent action as an insulin secretagogue, its action is mediated by K⁺_ATP channels, as mentioned previously. It can also increase insulin secretion independent of its action on K⁺_ATP channels. This finding was demonstrated using K⁺_ATP channel opener i.e., diazoxide in rodent islets.

**Sulphonylureas:** They bind to sulphonyl urea receptor-1 (SUR1), close K⁺_ATP channels, depolarize the β-cell membrane, raise [Ca²⁺]ᵢ, and increase insulin secretion. Once K⁺_ATP channels are blocked by maximally effective concentration of sulphonylureas these channels are unlikely to remain free for glucose.

**Extracellular Ca²⁺:** Ca²⁺ ions has an essential role in insulin secretion. Glucose does not increase insulin secretion in the absence of extracellular Ca²⁺. But, glucose can also stimulate insulin secretion even under Ca²⁺ depleted conditions. Ca²⁺ has a minute to minute triggering role.
Acetylcholine: Acts through $K'_{ATP}$ channel independent pathways. It produces the triggering signal (either by $Ca^{2+}$ mobilization or $Na^+$ dependent depolarization) and produces amplifying signals that act on protein kinase C to increase the efficacy of $Ca^{2+}$ on exocytosis. ACh and vasopressin both potentiate the acute effects of glucose on insulin secretion by generating $IP_3$ to release $[Ca^{2+}]_i$. Vasopressin potentiates sustained insulin secretion by depolarization.

3.3.4.8. Insulin inhibitors

Many inhibitors of insulin release decreases $[Ca^{2+}]_i$ by either hyperpolarizing the $\beta$-cell or by receptor-mediated, G-protein coupled effects to decrease voltage dependent calcium channels (VDCC) activity.

Cromakalim and pinacidil: They inhibit insulin release from $\beta$-cells by hyperpolarization, activation of $K^+$ channel and opening of them, and subsequent blocking of $Ca^{2+}$ influx.

Somatostatin: It is another hormone of pancreas, inhibit insulin secretion by opening $K'_{ATP}$ channels which is secondary to hyperpolarization of the cell membrane and also mediated by G-protein.

Catecholamines and clonidine: They suppress insulin secretion due to direct inhibition of $Ca^{2+}$ current through voltage-dependent $Ca^{2+}$ channels, resulting in a drop in $[Ca^{2+}]_i$. They have been shown to open $K'_{ATP}$ channels and also involves G-protein, because their effects were blocked by pertussis pretreatment. In addition to above effects they also act by inhibition of cAMP generation or acting at distal steps or exocytosis.

Tetraethylammonium ion (TEA): It inhibits high conductance $K'_{Ca^{2+}}$ activated channels in $\beta$-cells, leading to reduction of insulin secretion.

Cobalt, verapamil, and nifedipine: They inhibit insulin secretion by blocking $Ca^{2+}$ channels.
**Diazoxide**: It is an opener of $K^{+}_{\text{ATP}}$ channel, antagonize the effects of glucose by increasing $K^{+}$ permeability of the $\beta$–cell membrane.

3.3.4.9. **Insulin release regulator**

$[\text{Ca}^{2+}]_i$. Is the primary second messenger that regulates insulin release by $\beta$–cells. The rise in $[\text{Ca}^{2+}]_i$ triggers insulin release even in the absence of glucose.

In mouse islet cells, removal of extracellular $\text{Ca}^{2+}$ or addition of $\text{Ca}^{2+}$ channel blockers prevented glucose induced slow depolarization.
3.3.5. Conditions or agents altering glycemic state

Blood glucose (BG) level can be altered by physiological conditions or any agent affecting BG level. Fasting for different time periods (or consuming hypoglycemic drugs) can lower the blood glucose level which resembles hypoglycemic episodes experienced by diabetic patients. In experimental animals blood glucose level can be lowered by food deprivation or administering hypoglycemic drugs. Simultaneously, blood glucose can be elevated by dextrose administration or any drug possessing the action of blood glucose elevation eg. clonidine.

3.3.5.1. Glibenclamide

Glibenclamide is an oral hypoglycemic drug belonging to sulfonylurea (SU) group. It produces hypoglycemia by releasing insulin from β-cells of pancreas. It is an K\textsuperscript{ATP} channel antagonist, by binding to these channels selectively on β-cells, cause inhibition of efflux of potassium ions through the channel, which results in depolarization of membrane. Depolarization in turn opens a voltage gated Ca\textsuperscript{2+} channels, influx of Ca\textsuperscript{2+} ions, finally results in release of preformed insulin. Classical K\textsuperscript{ATP} channels are comprised of two subunits, the Kir6.2 subunits that form the K\textsuperscript{-} selective ion channel pore, and sulphonyl urea receptors subunits (SUR1 or SUR2). They also confer binding site for ATP. Binding of glibenclamide to SUR1 probably inhibits the cooperativity of the nucleotide binding folds (NBF)-1 and -2 of SUR1. It induces the closed state of the K\textsuperscript{ATP} channels.

The binding of glibenclamide to the SUR is of high affinity with K\textsubscript{s} of 0.24 nM. The SUR interaction is reflected by the biological activity of the drugs on either stimulating insulin secretion or inhibition of K\textsuperscript{+} efflux. SUR or a closely associated protein is the K\textsuperscript{ATP} channel. This hypothesis was first advanced by Sturgess et al (1985). The effect of SU on the K\textsuperscript{ATP} channel is a direct one and does not involve a second messenger. Sulphonylureas increase [Ca\textsuperscript{2+}] in a dose-dependent manner.
The following literature was frequently cited with regard to glibenclamide's hypoglycemic effect.

**Clinical trial evidence**

Johnson *et al* (1970) were the first to investigate glibenclamide (2.5 and 15 mg and maximum of 25 mg b.i.d) in randomized controlled clinical trial. Single dose administration lead to significant decrease in blood sugar / urinary sugar excretion and elevation of plasma insulin occurred between 2 and 6 h. They proposed the mechanism as, enhanced secretion and potentiation of insulin action. They also compared blood sugar level (p.c) between glibenclamide (25 mg) and chlorpropamide (250 mg) treated groups. They concluded that control of two hour postprandial blood glucose was better with glibenclamide (25 mg bid) than 250 mg of chlorpropamide. But these authors did not explore the effect on intestinal transit.

**Channels or receptors involvement**

Sturgess *et al* (1985) were the first to hypothesize that SUR is none other than the $K_{\text{ATP}}$ channel. They had demonstrated that tolbutamide at serum concentrations of (1 m mol/l) reduced $K^+$ movement through $K_{\text{ATP}}$ channels in the plasma membrane of an insulin producing cell line. Further, it was verified that glibenclamide had a similar effect but at concentrations one hundred times lower than tolbutamide (10 μ mol/l) and suggested that an $K_{\text{ATP}}$ or a protein closely associated with it may be the receptor through which sulphonylureas act to stimulate insulin secretion *in vitro*.

Gaines *et al* (1988) demonstrated that specific, high affinity sulphonylurea receptors were characterized on membranes of an insulin-secretion hamster beta cell line (HIT cells). Their study suggest that SUs initiates their biologic effect through a high affinity, specific interaction with SURs on the beta cell membrane.

Mikhailov *et al* (2001) suggested that SUR1 is the native $K_{\text{ATP}}$ channel close proximate of cytosolic loop (CL) 3 and 8 that leads to formation of glibenclamide binding site.
Selective $K_{ATP}$ channel or SU receptor antagonist

Schmid-Antomarchi et al. (1987) studied about properties of $K_{ATP}$ channels in RINm5F insulinoma cells. The most potent sulphonylureas (glibenclamide, glipizide and gliquidone) inhibit this channel in ATP depleted condition at nanomolar range of concentration has been compared and $[^{3}H]$Glibenclamide shown to be a potentially useful ligand for the identification of the SU receptors.

Insulin secretion

Groop et al. (1991) examined the relationship between plasma glibenclamide concentrations (50, 100, 200, 400 and 800 nM) and the insulin response and glucose metabolism during euglycemia (4.6±0.1 mM) and hyperglycemia (11.6±0.2 mM). They found that glibenclamide enhanced insulin secretion and glucose disposal only at the narrow range of 100-200 nM corresponding to an oral dose less than or equal to 10 mg.

At this stage of literature review we found that not many studies are available in exploring the effect of glibenclamide produced alterations in blood glucose, serum insulin and intestinal transit in integrated manner. Hence, one of the protocol was undertaken to evaluate the same.

3.3.5.2. Clonidine

Clonidine is an antihypertensive agent that reduces sympathetic tone by acting in the CNS. In addition to this effect, it is also known to induce hyperglycemia in experimental animals and in humans. A possible peripheral action on hepatic glucose release is suggested by the adrenergic properties of the drug, while the central action raise the possibility that its hyperglycemic action is an indirect effect resulting from stimulated secretion of a hormone involved in the regulation of glucose metabolism. The central hyperglycemic effect may depend on increased growth hormone secretion. In addition, its peripheral action may be exerted at the pancreas to inhibit insulin secretion and liver to activate hepatic glycogenolysis.

Review of Literature

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Literature update

Hoefke and Deckers 1968; Senft et al 1969; Rehbinder and Deckers 1968 (Cited from (Cf): Bock and Van Zwieten 1971) all these authors assumed that the hyperglycemic effect of clonidine was closely related to transient stimulation of peripheral adrenergic α-receptors.

Central effect

Bock and Van Zwieten (1971) were the first to report about CNS mediated hyperglycemic action of clonidine. Infusion of clonidine 10 μg/kg into the left vertebral artery of anaesthetized cat, provoked significant hyperglycemia. The most likely site of action is the hypothalamic region which in turn may activate the anterior pituitary gland to release somatotropin. The increased production of somatotropin might be reason for hyperglycemia (from 2 to 4 mU/ml).

May et al (1990) demonstrated in rabbits by autoradiographic technique that clonidine acts on α₂ adrenoceptors localized at two sites in brain i.e., in the nucleus of the tractus solitarius and dorsal motor nucleus of the vagus nerve.

Peripheral effect

Humphreys and Reid (1979) examined the metabolic effects of clonidine on plasma glucose concentration in anaesthetized dogs. Clonidine (30 μg/kg; i.v.) produced a prompt elevation of blood glucose level after 2 h. When the same dose was administered to hypophysectomized dogs, no alteration in blood glucose level was observed. They presumed that the hyperglycemic action of clonidine might have resulted from stimulation of growth hormone secretion.

Di Tullio et al (1984) reported that clonidine induced a dose-dependent hyperglycemic response in fed rats. At a dose of 0.1 mg/kg (p.o; or i.v. or i.p) clonidine produced an equivalent hyperglycemic response. The hyperglycemic response was partially inhibited by the selective α₂ antagonists (yohimbine or rauwolscine) and non-selective antagonist (phentolamine). Hyperglycemic response was partially inhibited by 3-mercaptopicolinic acid (inhibitor of gluconeogenesis). Their results suggested
hyperglycemic response induced by clonidine is mediated by $\alpha_2$ receptors located within
the CNS and hyperglycemia is produced in part by enhanced gluconeogenesis. They
proposed a major role for growth hormone which can stimulate gluconeogenesis and
decrease peripheral glucose utilization.

Angel et al (1988) demonstrated in mice that the selective $\alpha_2$ adrenoceptor agonist
(clonidine and guanabenz) produced a dose dependent hyperglycemic response which
was accompanied by a concomitant inhibition of insulin secretion. Their hyperglycemic
effect was antagonised by centrally acting $\alpha_2$ antagonist (yohimbine, idazoxan) and pe-
ripherally acting antagonist (benextramine).

In the same year Moratinos et al (1988) demonstrated in conscious rabbits, that an
intravenous infusion of clonidine (2 $\mu$g/kg/min) induced hyperglycemia accompanied by
an inhibition of insulin secretion. Yohimbine, when infused at a rate 20 $\mu$g/kg/min sup-
pressed clonidine induced hyperglycemia and blocked the inhibitory effect on insulin
secretion mediated by clonidine indicating an $\alpha_2$ action.

Angel and Langer (1988) also concluded from their study in rats, that $\alpha_2$ –receptor
stimulation induces hyperglycemic response through a peripheral effect that involves
inhibition of insulin release through stimulation of post synaptic $\alpha_2$ receptor in pancreatic
$\beta$–cells.

Gotoh et al (1988) evaluated whether clonidine exerts its action in CNS or outside the
CNS to induce hyperglycemia in rats. All the tested doses (5, 50 and 100 nmol), the
hyperglycemic response of intravenous clonidine, was not significantly different from
those injected into the 3rd cerebral ventricle. The inhibitory effects of $\alpha$– adrenergic
antagonists i.e., yohimbine (50 nmol/0.2 ml) on clonidine induced hyperglycemia were
manifested peripherally but not centrally, suggesting clonidine induced hyperglycemia
is mainly due to a peripheral $\alpha$– adrenergic mechanisms. This finding is in contrast to
the study reported by Angel et al (1988).
Hormonal release

DiTullio et al (1984) proposed a major role for growth hormone for hyperglycemic effect which can stimulate gluconeogenesis and decrease peripheral glucose utilization and is known to be elevated during clonidine treatment.

Gotoh et al (1988) found an evidence of insignificant rise of plasma-immuno reactive insulin levels despite hyperglycemia suggesting that clonidine acts directly on the pancreas to suppress insulin secretion. The suppression of insulin secretion would be expected to contribute to hyperglycemia by impairing peripheral glucose utilization. Clonidine administration also resulted in elevation of plasma immuno reactive glucagon levels it may be due to α-agonism of clonidine on pancreas.

At biochemical mediators

Panagiotidis et al (1993) had proposed an alternate mechanism for clonidine induced hyperglycemia by studying monoamines located in the secretory granules of pancreatic β-cells which have an inhibitory influence on insulin secretion. Monoamines are inactivated by the enzyme monoamine oxidase. Clonidine produced a moderate inhibition (12-18%) of islet monoamine oxidase activity accompanied by reduced plasma insulin and elevated plasma glucose level in mice.
3.4. Gastrointestinal tract

3.4.1. Gastrointestinal nervous system

Studies about extrinsically denervated intestine by Bayliss and Starling (1899)\textsuperscript{101}, Langley and Magnus (1905)\textsuperscript{102} and Trendelenburg (1917)\textsuperscript{103} revealed that intestine possess its own nervous system called enteric nervous system (ENS) to coordinate inherent contractions or peristaltic reflex. It is probably because of this self-contained neural apparatus that still functions even after surgical interruption of the extrinsic innervation of the gut such as vagotomy or sympathectomy and or spinal cord injury. The ENS may actually be the ‘talk back’ to ganglia relaying input from the CNS. The independence of the ENS was not only established but actually recognized long ago, Langley (1921)\textsuperscript{104} in his classical description classified the ENS as a third division of the ANS. In humans, there are about 2x10\textsuperscript{3} efferent fibers in the abdominal vagus nerves\textsuperscript{105}. While there are more than 10\textsuperscript{8} ganglion cells in the intestine, a number that is of the same order of magnitude as the number of neurons in the human spinal cord\textsuperscript{106}. It seems improbable that 2x10\textsuperscript{3} nerve fibers can innervate 10\textsuperscript{8} cells in a useful manner\textsuperscript{107}. The ENS is primarily derived from cells of the vagal segment of the neural crest that migrate to the cranial portion of the gut and subsequently move caudally to populate the entire gastrointestinal (GI) tract\textsuperscript{108}.

3.4.1.1. Clinical targets

The ENS is central to normal gut function and is involved in most, if not all, disorders of GI tract\textsuperscript{108}. Even when the primary pathology lies in another part of the gut, the ENS still serves as the effector neural controller, leading to a disturbance in gut function and generation of symptoms. Therefore, ENS serves as a useful therapeutic target for disorders of GI tract, for eg: in the treatment of distal ulcerative colitis by enemas containing the neural active membrane stabilizer lignocaine was shown to be effective, which implied possible involvement of neural processes in the inflammatory process. Opioid analogues act on enteric neurons to diminish large bowel motor and secretory function.
Vomiting and disorders of gastric emptying have been successfully targeted using $5\text{-HT}_3$ antagonists and $5\text{-HT}_4$ agonists. Constipation is predominantly a disorder of motor function indicated by slowing of gut transit due to impaired rectal and anal coordination. Characterization of neurotransmitters involved in peristalsis, has led to the development of specific drugs which may enhance peristaltic activity. Selective $5\text{-HT}_4$ agonist appear to be promising in treating constipation. Loperamide can be used in the treatment of diarrhoea. This $\mu$ opioid analogue effectively diminished both secretory and hypermotility. Octreotide (Somatostatin analogue) inhibits GI motor and sensory function and has effect on visceral blood flow. It controls diabetic diarrhoea and AIDS diarrhoea. The $\alpha_2$-adrenergic agonist clonidine has been used successfully in the therapy of diabetic diarrhoea presumably by reversing the peripheral adrenergic abnormalities in the diabetic diarrhoea. The ultimate goal in managing patients with GI disorders is to relieve symptoms and thereby improving the quality of life.

The enteric nerves of the gut have at least four primary targets. 1. Smooth muscle cells responsible for GI motility 2. Mucosal secretory cells 3. Endocrine cells 4. Microvasculature that maintains mucosal blood flow during intestinal secretion.

3.4.1.2. Central nervous system – Enteric nervous system circuits

The ENS is well connected to the CNS through both motor and sensory pathways of the sympathetic and the parasympathetic nervous system.

3.4.1.2.1. Sympathetic nervous system (SNS)

The sympathetic fibers entering the gut are adrenergic, postganglionic fibers with cell bodies in the prevertebral ganglia (nodose ganglia), comprising of coeliac, superior and inferior mesenteric ganglia, their input regulates intestinal motility, blood flow, water & electrolyte secretion. Falck-Hillarp technique revealed that sympathetic innervation was very scarce to the major part of smooth muscle layers, and most of the adrenergic fibers contact with one of the two major plexuses, innervation is seen in the mucosa in particular around the crypt epithelium. There is experimental evidence for adrenergic inhibition of local excitatory motor reflexes and /or
extrinsic excitatory parasympathetic nervous activity\textsuperscript{113}. Recent studies have identified two transmitters mediate afferent input to perivertebral ganglia (PVG) and modulate sympathetic efferent output. 1. Carbon monoxide (CO), a gaseous molecule suggested to be an important signaling molecule in the body. GI transit is markedly altered in mice lacking the haeme oxygenase 2 (HO-2), the biosynthetic enzyme for CO biosynthesis in nervous system, suggesting CO may play a role in regulating the gut\textsuperscript{114}. The second transmitter is leptin hormone produced by adipocyte, acts on neurons in the hypothalamus to control food intake. Confocal microscope identified leptin receptors in PVG neurons\textsuperscript{117}. These findings indicate sympathetic control of smooth muscle to a large extent is an indirect action and may act via luminal sensory receptors.

3.4.1.2.2. Parasympathetic nervous system (PNS)\textsuperscript{112}

The parasympathetic motor pathways consists of the vagus nerves that control upper gastrointestinal tract and the sacral nerves that regulate the distal colon and rectum. However, in small bowel, vagal preganglionic neurons innervate only small clusters of select myenteric neurons. These differences in the intensity of innervation by parasympathetic (PS) fibers reflect the fact that the CNS exerts direct control of the oesophagus and stomach and rectosigmoid parts of GIT and less direct control on the small intestine and proximal colon. The preganglionic neurons are all cholinergic and exert excitatory effects on enteric neurons through nicotinic and in some regions, muscarinic receptors.

These circuits indicate that the ENS and CNS are closely linked and have profound influences on each other.

3.4.1.3. Enteric nervous system\textsuperscript{112}

In gastro intestinal tract, an array of ganglia occurs in two layers. One, as the myenteric plexus (plexus of auerbach's) that occupies at intermuscular space between circular and longitudinal layers, provides motor innervation to the two muscle layers and secre-tomotor innervation to the mucosa. Ratio of preganglionic vagal fibers to ganglion cells in myenteric plexus (auerbach's plexus) estimated to be 1:8,000\textsuperscript{118}. The other one as submucous plexus (meissner's) lies within the submucosa between the circular muscle
and muscularis mucosa, which plays an important role in secretory control. Process from these ganglion cells form dense networks in these two planes and also extend to interconnect the two main plexuses, to innervate the three muscle layers. In human's intestine, even a third intermediate submucous nerve network has been reported\(^{119}\).

Extensive studies over last 20 years have led to identification of all major neuronal types in small intestine of guinea pig (G-pig) but similar studies in histology of mice intestine are lacking, however G-pig has become a model of the organization of EN circuits. Kunze and Furness (1999)\(^{120}\) depicted circuit of neurons being in series, whereas numerous connections between neurons mean that they act as assemblies with both in-parallel and in series connections.

There are 2,500 nerve cells/mm. length of G-pig gut and 1 mm containing the cell bodies of 400 inhibitory motor neurons and 300 are of excitatory motor neurons as well as the cell bodies of about 120 ascending and 120 ChAT (Choline Acetyl Transferase) /NOS (Nitric Oxide Synthase) descending interneurons. A large proportion of the remaining neurons are longitudinal muscle motor neurons account for 500 nerve cell bodies/mm. There are also small population of ChAT/Somatostatin (4%), ChAT/5-HT (serotonin) (2%) and ChAT/VP (vasoactive intestinal polypeptide) (3%) as descending interneurons\(^{120}\).

### 3.4.1.4. Receptors in enteric nervous system\(^{121}\)

Most drugs affect GI motility by acting as an agonist or antagonist at specific cellular receptors. The presence of receptors on enteric neurons is another expression of their heterogeneity. Stimulation or inhibition of contractile activity through receptors on enteric neuronal circuits offers potential of achieving higher specificity. 5-HT\(_3\) receptor antagonists inhibit colonic motor activity in humans via neural pathways. Myenteric neurons can express receptors for both peptides and non-peptides. Generally, expression of a receptor is limited to a subset of myenteric neurons, with probably the only exception being expression of nicotinic cholinergic receptors on all myenteric neurons in the G-pig stomach\(^{122}\). A number of clinically used drugs that alter gastrointestinal motility act via the ENS. Prokinetic drugs, metoclopramide, cisapride or domperidone stimulate
GI motility at least in a part through release of ACh from intrinsic cholinergic neurons. Motilin and erythromycin can induce prolonged depolarization in a subset of neurons in the G-pig stomach, possibly by activation of motilin receptors.

**Opioid neurons & receptors**\(^ {123} \): Opioid neurons constitute the largest population of peptide-containing neurons in the myenteric plexus of the gut. They contain either Met-enkephalin or Leu-enkephalin. They are, Dogiel Type I / S neurons which possess several dendritic processes extending oral (towards mouth) within the plexus and one long axonal process extending into the underlying circular layer\(^ {124} \). Opioid receptors in the gut was first identified by classical pharmacological methods initiated by pioneering work of Paton (1957)\(^ {125} \). Martin *et al* (1976)\(^ {126} \) have postulated the existence of three subspecies of opioid receptors designated \( \mu \), \( \delta \) and \( \kappa \) receptors based on pharmacological action in man and experimental animals. In humans \( \mu \), \( \delta \) and \( \kappa \) receptors contribute to opioid induced inhibition of motor activity\(^ {127} \). Porreca and Burks (1983)\(^ {128} \) observed that selective \( \mu \) (morphine) and \( \delta \) D-Ala\(^2\)-D-Leu\(^5\)-Enkephalin (DADLE) agonists were effective in inhibiting GI transit but \( \kappa \) agonist (ketocyclazocine) did not affect the transit. Their result suggest spinal cord is a discrete site of opioid effects of GI motility. Bitar and Makhlouf (1985)\(^ {129} \) reported that opioid receptors are capable of mediating contractions are present on circular muscle cells and selectively absent in longitudinal muscle cells of G-pig ileum and human jejunum. Opioid peptides act directly to cause contraction of circular but not longitudinal muscle cells.

### 3.4.2. Gastrointestinal motility

**3.4.2.1. Electrical and mechanical activity of smooth muscle**\(^ {130} \)

Visceral smooth muscle is characterized by the instability of its membrane potential and by the fact that it shows continuous irregular contractions that are independent of its nerve supply, this maintained state of partial contractions is called tonus or tone. Thus the excitation-contraction coupling in visceral smooth muscle is a very slow process compared with that in skeletal or cardiac muscle.
Calcium is involved in the initiation of contraction of smooth muscle (SM). However, visceral SM generally has a poorly developed sarcoplasmic reticulum and the increase in intracellular calcium concentration that initiates contraction is primarily due to Ca\textsuperscript{2+} influx from the extracellular fluid via voltage gated Ca\textsuperscript{2+} channel\textsuperscript{130}.

Visceral SM is unique, unlike other types of muscle, it contracts when stretched, in the absence of any extrinsic innervation. Stretch is followed by a decline in membrane potential, an increase in the frequency of spikes and a general increase in tone. If adrenaline or nor-adrenaline added to a intestinal SM preparation, membrane potential becomes larger, the spikes decrease in frequency and muscle relaxes. Adrenaline exerts α and β actions on the muscle. When ACh is added \textit{in vitro}, the membrane potential decreases and spikes become more frequent, muscle become more active with an increase in tonic tension and the number of rhythmic contractions. This effect is mediated by phospholipase C4, IP\textsubscript{3}, which increases the intracellular Ca\textsuperscript{2+} concentration\textsuperscript{131}.

3.4.2.2. Excitatory-inhibitory mechanisms of smooth muscle

The motor unit of the intestinal circular muscle is regarded as a circumferential strip that is electrically coupled with its innervating neurons. Most excitatory nerves those that increase contraction force are cholinergic i.e., acetylcholine is the responsible neurohormone but not the sole excitatory neurohormone substance\textsuperscript{120}. Most inhibitory nerves that depress contraction force, release a transmitter that is not yet known. It was once proposed and widely accepted that adrenaline is responsible, but this idea was thoroughly discredited about 40 years ago. From that time the inhibitory nerves to the intestinal muscle came to be called the non-adrenergic -non-cholinergic (NANC) nerves and that term is still current. Various substances for this unknown inhibitory transmitter are now under study. They include, vasoactive intestinal polypeptide (VIP), ATP, NO or some nitrogenous compounds\textsuperscript{130}. Acceleration of GI motility can be achieved by direct stimulation of gastrointestinal muscle, by activation of excitatory neural pathways or by inhibition of inhibitory pathways. Deceleration can be produced by a direct relaxant effect on GI smooth muscle, by inhibiting the excitatory neural pathways, or by activation of inhibitory pathways. Inhibitors of GI motility such as, botulinum toxin, or
sumatriptan, may inhibit release of ACh or stimulate the release of NO from the intrinsic neurons.

**Hormonal control:** The discovery of hormones of enteric origin such as secretin and gastrin gave rise to the idea that the gut can influence its own activity by means of substances it releases into the systemic circulation. The recent discovery of many other circulating peptides of enteric origin has strongly re-inforced the idea. The major enteric hormones currently under consideration in the normal regulation of contractile force are cholecystokinin and motilin. The major non-enteric hormone under consideration is progesterone, which in combination with oestrogen may be responsible for the slight inhibition of intestine seen in pregnancy[113].

**Other muscle excitation mechanisms:** Intestinal smooth muscle cells have stretch-activated channels and stretch of muscle causes their excitation[132]. The smooth muscle cells are electrically coupled through gap junctions. It is thus possible to control an entire layer of smooth muscle by releasing the transmitter only at the myenteric plexus-smooth muscle interface[107].

**Sequence of events occurring in contraction and relaxation of visceral smooth muscle**[118]

1. Binding of acetyl choline to muscarinic receptors
2. Increased influx of Ca\(^{2+}\) into the cell
3. Activation of calmodulin - dependent myosin light chain kinase
4. Phosphorylation of myosin of light chain at position 9
5. Increased myosin ATPase activity and sliding actin over myosin
6. Contraction
7. Dephosphorylation of myosin by myosin phosphatase
8. Relaxation or sustained contraction due to the latch bridge mechanism
9. Relaxation of smooth muscle following dissociation of Ca\(^{2+}\)-Calmodulin complex

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3.4.2.3. Patterns of gastrointestinal motility

Two patterns of GI motility were well recognized in the mammalian small intestine.\(^\text{118}\)

3.4.2.3.1. Motility during fasting (interdigestive state): It occurs about 4-6 h after the meal is taken. It is characterized by the migrating myoelectric complex (MMEC), which passes along the intestine every 80-110 min in humans, and takes about 6-10 min to pass any point in the intestine and characterized by intense rhythmic contractions of the circular muscle. The MMEC is consisted of four distinct phases. The MMEC is also referred to as phase III (5% of cycle lasts for 10 min, consist of ring contractions occurs at the maximum possible frequency and amplitude). Phase III is followed by a period of less intense activity called transition state or Phase IV, then by quiescence (Phase I) lasting for 40 - 60 min and by irregular contractions of Phase II (20% of cycle lasts for 35-45 min, appears as jumping from one to two rambling of rhythmic ring contractions) that is followed by reoccurrence of Phase III.\(^\text{115}\) The MMEC is still observed after total extrinsic denervation of small intestine. Each phase begins earlier at more proximal sites than at more distal sites. Because of its propagation along the intestine this pattern is called the migrating motor complex (MMC) or as fasted pattern. Sometimes MMC is used to refer especially to phase III, simply because it is well demarcated and conspicuous component of the fasted pattern. In continuously feeding animals like sheep, ox and G-pig, MMC passes down the intestine at regular intervals even when the animal is digesting. The MMC can be abolished from the segment of intestine that is infused through the vasculature with tetrodotoxin or hexamethonium.

3.4.2.3.2. Motility during fed state: Intermittent feeders e.g. humans and dogs the MMC disappears soon after a meal is taken and replaced by the fed pattern which is difficult to define their frequency (approx. 12-15 min). Fed pattern helps in mixing and propulsion of chyme. In a human study, Dusdieker and Summers (1979)\(^\text{133}\) reported that 45% of individual contractions did not propagate and about 35% propagated for less than 9 cm. The propagative contractions are peristaltic waves that die out after traveling a short distance. Bayliss and Starling (1899)\(^\text{181}\) defined peristaltic contractions as ‘true coordinated reflexes, started by mechanical stimulation of the intestine and
carried out by the local nervous mechanism travel in one direction, they are independent of the connection of the gut with the CNS and are abolished on paralyzing local nervous apparatus. Mall (1896)\textsuperscript{114} deduced that irritation of the mucosa by a bolus was the stimulus for peristalsis. Peristalsis neurohumorally mediated local reflexes involving the release of 5-HT by mucosal stimulation or mechanical distention of the gut lumen triggers activity in the intrinsic afferent neurons. Above the site of the stimulus, ascending cholinergic interneurons relay this signal to excitatory motor neurons containing ACh and substance P. As a result, the circular-muscle layer above the stimulus contracts. At the same time below the stimulus site, descending cholinergic interneurons activate inhibitory motor neurons that contain NO, VIP and ATP, causing relaxation. The resultant forces propel the bolus in an antegrade direction. As the bolus moves, it triggers similar local peristaltic reflexes at successive sites along the gut\textsuperscript{112}.

Read et al (1982)\textsuperscript{135} demonstrated that transit time of a meal through small intestine is not related to the rate at which meal leaves the stomach in normal volunteers. Using non-invasive technique varying size or composition of a meal is altered when it passed through the stomach and small intestine. Increasing size of the meal by doubling the absorbable components delayed gastric emptying but time taken for the head of the meal to reach the caecum is not significantly altered.

3.4.2.4. Types of intestinal contractions

For a century it has been commonly recognized\textsuperscript{136} that the intestinal muscularis externa displays two distinct motility patterns.

3.4.2.4.1. Ring contractions: Ring contractions are what we exclusively imagine when we think of SI motility. The ring contractions are generally called peristalsis or peristaltic contractions that involves circular muscle. A ring contraction can begin at any level of small intestine and involve the whole circumference of the intestine at once eg. in humans, duodenum, contractions occur at 12 per min with a five second period.
3.4.2.4.2. Sleeve contractions: They produce shortening of the small intestine by contracting longitudinal muscle layer with very little alteration in luminal diameter. The function of sleeve contractions is mainly mixing of intestinal contents i.e., shifts of fluid between the core and the periphery of the fluid filled lumen, thereby optimizing absorption137.
Table I. Physiological actions of gastrointestinal hormones and neurotransmitters on GI motility

<table>
<thead>
<tr>
<th>Hormone /Transmitter</th>
<th>Major action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholine</td>
<td>Major neuron-to-neuron (ganglionic) transmitter and a primary excitatory transmitter to smooth muscle in ENS. Involved in stimulation of spontaneous phasic and tonic contractions of intestine</td>
</tr>
<tr>
<td>Adenosine</td>
<td>Induce direct relaxation of longitudinal muscle of duodenum, ileum and colon and has antiperistaltic effect</td>
</tr>
<tr>
<td>Adrenaline</td>
<td>Inhibition of intestinal motility</td>
</tr>
<tr>
<td>ATP</td>
<td>It has both excitatory and inhibitory effects. Cause contraction of intestine</td>
</tr>
<tr>
<td>Anandamide</td>
<td>Depresses intestinal peristalsis via cannabinoid (CB, receptors</td>
</tr>
<tr>
<td>Calcitonin gene related peptide (CGRP)</td>
<td>Involved in sensory transmission of gut reflexes, enhances intestinal motility</td>
</tr>
<tr>
<td>Cholecystokinin (CCK)</td>
<td>Produces excitatory response in duodenum and inhibitory response in jejunum</td>
</tr>
<tr>
<td>Dopamine</td>
<td>A modulatory transmitter, inhibits intestinal motility</td>
</tr>
<tr>
<td>Enkephalins</td>
<td>Inhibit ACh release, thereby inhibit peristalsis</td>
</tr>
<tr>
<td>Endothelins</td>
<td>Stimulates intestinal peristalsis through endothelin (ET, receptors, inhibits through ET, receptors</td>
</tr>
<tr>
<td>Gamma-amino butyric acid (GABA)</td>
<td>Produce a transient concentration dependent contraction of intestine followed by relaxation</td>
</tr>
<tr>
<td>Histamine</td>
<td>At higher concentration activates motility</td>
</tr>
<tr>
<td>Melatonin</td>
<td>Enhances frequency of intestinal migrating motor complex</td>
</tr>
<tr>
<td>Motilin</td>
<td>Initiates interdigestive intestinal motility</td>
</tr>
<tr>
<td>Neuropeptide Y</td>
<td>Inhibits spontaneous contractions of ileum</td>
</tr>
<tr>
<td>Nitric Oxide (NO)</td>
<td>Cotransmitter at inhibitory neuromuscular junctions, inhibits motility of intestine</td>
</tr>
<tr>
<td>Prostaglandins</td>
<td>Contracts longitudinal sections of gastric fundus and ileum</td>
</tr>
<tr>
<td>Serotonin (5-HT)</td>
<td>Cotransmitter at excitatory neuron-to-neuron junction, stimulates intestinal motility</td>
</tr>
<tr>
<td>Somatostatin</td>
<td>Neuronal inhibitory effect</td>
</tr>
<tr>
<td>Tachykinins (Substance P &amp; Neurokinin A)</td>
<td>Excitatory cotransmitters with ACh at neuromuscular junction. Produce dose dependent contractions of intestine. Involved in peristaltic contractions</td>
</tr>
<tr>
<td>Thromboxane</td>
<td>Involved in spontaneous contractions of intestinal smooth muscle</td>
</tr>
<tr>
<td>Vasoactive intestinal polypeptide (VIP)</td>
<td>It has both excitatory secretomotor action and inhibitory cotransmitter role at neuromuscular junction. Produce smooth muscle relaxation</td>
</tr>
</tbody>
</table>
3.4.3. Smooth muscle cell

Smooth muscle cells are elongated cylindrical cells in which a single nucleus lies in a homogenous cytoplasm. This apparent homogeneity gives visceral muscle its common name as smooth muscle. Cytoplasmic filaments are made of three different proteins actin, myosin and desmin. Actin and myosin are contractile filaments whereas desmin is a cytoskeletal filament of visceral muscle. The two contractile filaments operate in the same as they do in skeletal muscle to cause contractions. The sliding together of actin and myosin filaments is translated to shortening of the cell through the attachments of these filaments to the dense bodies and bands. These dense bodies/bands are analogs of the 'Z' lines of skeletal muscle. The cytoskeletal filaments of desmin organizes the cell interior."".

3.4.3.1. Ion channels in smooth muscle cells

Elucidation of ionic channels mechanism may pave the way for the development of selective neuronal or muscle channel blocking drugs. In general excitatory transmitters increase Ca\(^{2+}\) influx through non-selective cation channels, while inhibitory transmitters enhance the open probability of a variety of K\(^{+}\) channels.\(^{130}\)

GI smooth muscle cells operate at potencies negative to 0 mv. The net current through non-selective cation channels is always inward and carried predominantly by Na\(^{+}\). However, the entry of Na\(^{+}\) has no known effect in the contractile process, but the depolarization produced as a result of entry of +ve charge, enhances the open probability of Ca\(^{2+}\) channels.\(^{134}\)

Recent studies have begun to utilize reverse transcriptase polymerase chain reaction (RT-PCR) to identify cell-specific ionic species on GI smooth muscle cells.

**Voltage-dependent K\(^{+}\) channels:** The responses of smooth muscle cells to slow wave depolarization depend to a significant extent on the types of K\(^{+}\) channels expressed. The most diverse group of conductance in GI muscles is the voltage dependent, Ca\(^{2+}\) -insensitive K\(^{+}\) (Kv) channels. These conductance have a wide variety of
pharmacological and electrical properties. A variety of K+ channel blocking drugs can dramatically affect the pattern of electrical activity in GI smooth muscle. In particular, $I_{art}$ is a fast activating current blocked by micromolar concentration of 4-aminopyridine. Muscarinic stimulation activates a nonselective cation current in GI muscles but suppression of delayed rectifier K+ currents is another possible means of excitable regulation

**Calcium-activated K+ channels:** These channels are activated by purinergic stimulation and are important in GI motility because they mediate a portion of the inhibitory response to nerve stimulation

**Inward rectifier and ATP-sensitive K+ channels:** Whole cell patch clamp studies on isolated canine colonic myocytes detected small, inwardly rectifying, Ba2+ -sensitive K+ currents, indicating the presence of Kir2-like currents. Molecular evidence demonstrated that Kir2.1 was expressed in cells from canine colon muscle as well as other canine GI smooth muscle. In GI tract this type of K+ current may act to assist repolarization between slow waves during muscarinic responses, thus preserving phasic electrical and mechanical activity. K+$_{ATP}$ channel agonists such as cromakalim and nicorandil, have been shown to hyperpolarize and relax stomach, ileum and colon. A recent study using RT-PCR on dispersed and isolated mouse colon smooth muscle cells identified Kir6.2.

**Ca2+ channels:** The predominant Ca2+ channels in smooth muscle are L-type channels, which provides Ca2+ influx that initiates contractions. In human jejunum, the predominant Ca2+ current is dihydropyridine sensitive and found to be regulated by G-proteins.
3.4.4. Agents altering gastrointestinal motility

Diabetic patients are known to be associated with alterations in GI transit which contribute to episodes of hypoglycemia. In experimental animals the association between GI transit and glycemic state can be evaluated by administering drugs having accelerating or attenuating effect on GI motility and measurement of blood glucose level.

3.4.4.1. Metoclopramide

Metoclopramide (Met) was first introduced in Europe during 1970s as a medication for the treatment of nausea and vomiting\textsuperscript{42} by virtue of its effect on medullary chemoreceptor trigger zone, makes it a useful antiemetic drug. It was approved by FDA, USA, in 1980 for the treatment of diabetic gastroparesis and later for emesis associated with cancer chemotherapy. Metoclopramide's gastrointestinal smooth muscle excitation effects are related to its ability to enhance acetylcholine release and to antagonize the inhibitory neurotransmitter, dopamine, thereby effectively coordinating gastric-pyloric-small intestinal motor function\textsuperscript{43}. In humans, atropine (10 μg/kg) blocks the effect of metoclopramide on GIT\textsuperscript{44}.

Literature update

Metoclopramide action on GIT was first described by Justin-BesaÇon et al in 1964\textsuperscript{45}. They found that metoclopramide accelerated the transit of radio-opaque material from the stomach. They ascribed this finding to the occurrence of forceful and orderly antral contractions and to simultaneous dilatation of pyloric sphincter and duodenal bulb.

Following evidences were described briefly, as some authors tried to distort the original findings for their convenience and to contradict the original works.

In vivo experiment

Jacoby and Brodie (1967)\textsuperscript{46} demonstrated effect of metoclopramide in various animal species. (1) Gastric emptying (GE) of amberlite pellets in rats: Metoclopramide (16 or 32 mg/kg; s.c) significantly accelerated the GE. (2) GE of barium sulphate meal in monkeys: Metoclopramide (2 mg/kg; i.m) accelerated GE by more than 50%.
Gastrointestinal motility (GIM) in unanaesthetized dogs: metoclopramide (1.2 or 4 mg/kg; i.v) showed a graded increase in action, 4 mg effect lasted almost 2 h. (4) GIM in anaesthetized dogs: metoclopramide was found to stimulate gastric antral motility (GAM) but not duodenal, ileal or colonic motility, lasted from 2 to 10 min. (5) Gastric acid secretion (GAS) in oesophageal and pyloric ligated rat model: Metoclopramide had not shown any effect on volume or acidity of gastric secretion in a dose which accelerated gastric emptying. GAS in gastric fistula of dog: metoclopramide (2 mg/kg; i.m) had increased volume of secretion but not affected titratable acidity. (6) Locus and mechanism of action of metoclopramide: (a) Cholinergic mediation: metoclopramide (2 or 8 mg/kg; i.v) produced a dose related increase in GAM index. Series of doses of ACh at 3 min. intervals before and after metoclopramide (2 mg/kg) did not potentiate the ACh induced response. However, neostigmine (0.01 mg/kg; i.v) produced a marked potentiation of ACh-induced response. Moreover, atropine (0.01 mg/kg) prevented metoclopramide induced GAM. (b) Opioid mediation: Morphine (0.4 mg/kg; i.v) pretreatment had also abolished. (c) At ganglion level: A long acting ganglionic blocker, chlorisondamine (1 mg/kg; i.v) pretreatment of metoclopramide in vagotomized dogs, a significant increase in GAM index with 0.5 or 2 mg/kg of metoclopramide was observed indicating that the stimulation was not blocked and might have potentiated by ganglion blocker. Their findings indicate metoclopramide stimulatory action was not dependent on the integrity of the vagus nerve nor it dependent on other ganglionated extrinsic nerve pathways and acting by a peripheral mechanism. They concluded that metoclopramide may involve the activation of intramural cholinergic neurons responsible for augmenting gastric motility but not acid secretion either by a direct stimulation or removal of inhibitory circuits, but direct evidence was not observed in their study.

In vitro experiment

Eisner (1968) was the first worker reporting about metoclopramide mode of action in vitro in various parts of human gastrointestinal smooth muscle. (a) Stomach: metoclopramide per se did not produce any effect with concentrations up to 100 µg/ml of bath volume. But in the presence of Met, the contraction to added ACh were increased. (b) Pylorus & Duodenum: Met(0.1-10 µg/ml) sensitized strips to ACh.
(c) Small intestine: Met (0.1-1 μg/ml) per se contracted longitudinal and circular strips
(d) Colon-circular muscle: Met (0.1-10 μg/ml) per se contracted (e) Colon-longitudinal muscle: Met (0.1-10 μg/ml) per se had no effect but sensitized strips to ACh. They concluded that human small intestine and colonic circular muscle are sensitive to Met and other parts of GIT were sensitized to ACh.

Birtley and Baines (1973)\textsuperscript{145} reported the effects of metoclopramide on isolated intestine from rabbit and G-pig and interaction between Met and various agonists of intestinal motility. ACh (excitatory neurotransmitter; 0.5 μg/ml), histamine (has direct spasmogenic effect; 0.2 μg/ml) and Serotonin (its role implicated in peristalsis; 1 μg/ml). They concluded that involvement of tryptaminergic mechanism for the control and maintenance of peristalsis. Met (3-10 μg/ml) certainly increased the frequency of peristalsis waves and at high conc. 30 μg/ml depressed activity due to the local anaesthetic action.

Hay (1977)\textsuperscript{146} reported that metoclopramide may act by increasing the amount of ACh released at the postganglionic cholinergic nerve endings in isolated longitudinal muscle strips of G-pig stomach.

Zar \textit{et al} (1982)\textsuperscript{150} have investigated effect of metoclopramide in G-pig ileum on (1) electrically stimulated cholinergic transmission (2) histamine induced contractions (3) non-cholinergic contractions (4) dopamine induced relaxation. Metoclopramide (0.3-300 μM) cholinergically mediated electrically evoked contractions in dose dependent manner. They concluded that metoclopramide (3-300 μM) at concentrations within the range of plasma with therapeutic doses in man does not seem to act by blocking dopamine receptors but selectively facilitates cholinergic mechanism.

Craig and Clarke (1990)\textsuperscript{181} demonstrated the prokinetic benzamides are partly agonistic at 5-HT\textsubscript{4} receptors in G-pig ileum. Linnik \textit{et al} (1991)\textsuperscript{181} proposed in similar lines that metoclopramide and cisapride possess activity in common at 5-HT\textsubscript{4} receptors.
Briejer et al (1995) in their review article and Pasricha (2001) in his chapter stated that 'metoclopramide facilitates ACh release from enteric neurons and an action that may be mediated indirectly by several different mechanisms including suppression of inhibitory interneurons by antagonism of 5-HT$_{3}$ receptors and stimulation of excitatory neurons through activation of 5-HT$_{4}$ receptors (predominant agonist at 5-HT$_{4}$ receptors) with antagonism of dopamine receptors playing a minor role'.

3.4.4.2. Atropine

Belladonna alkaloids were recommended in wide varieties of conditions such as irritable bowel syndrome, increased tone or motility of GIT (diarrhoea). These agents reduce the tone and motility when administered in maximal tolerated doses involving muscarinic (M$_{3}$) receptors in sub mucosal plexus of GIT.

Atropine is a non-specific competitive antagonist of acetylcholine for muscarinic receptors and abolish the effects of acetylcholine completely on the gastrointestinal tract. Both in normal subjects and in patients with gastrointestinal diseases, full therapeutic doses of atropine (0.5-1 mg) produce definite and prolonged inhibitory effect (along the length of GIT) on the motor activity of the stomach, duodenum, jejunum and ileum. Larger doses of atropine (5-10 mg) in humans, inhibits the parasympathetic control of the GIT and decrease the tone and motility of the gut. However, it inhibits only incompletely the effects of vagal impulses.

Literature update

Atropine is an established non-specific muscarinic receptor blocker. Many workers used atropine as a tool for identifying cholinergically mediated mechanisms. The first report was available using atropine by Gaddum in 1937 about its cholinergic antagonism. In 1965 Paton and Rang studied about ACh receptors by measuring the amount of tritium labeled atropine taken up the longitudinal muscle layer of G-pig ileum.
Mittal et al. 1995 reported in healthy subjects that atropine reduces the frequency of gastroesophageal reflex by its inhibitory effect on the frequency of lower oesophageal sphincter.

Qian et al. (1999) assessed effects of atropine quantitatively and found persistent tachygastria in 25% and bradygastria in 75% of dogs after atropine (0.25 mg/kg) administration. They found that gastric pacing was able to normalize gastric dysrhythmia.

Chaudhuri et al. (2000) reported that atropine (1 mg/kg; i.p) per se did not produce significant inhibition of SIT when compared to control in normal mice.

Lidums et al. (2000) investigated the effect of atropine (15 μg/kg bolus, 4 μg/kg/h i.v infusion) on fasting gastric compliance and postprandial gastric tone in healthy subjects. Atropine significantly reduced minimum distending pressure and increased proximal gastric compliance. Atropine also significantly reduced the rate of postprandial transient lower oesophageal sphincter relaxation. They concluded that atropine produced this effect by a central action on the integrating mechanisms in the brain stem.

Barajas-Lopez et al. (2001) reported that nicotinic receptors expressed by submucosal neurons in G-pig are also inhibited by atropine at concentration equal or greater than 1 μM.

This part of the literature review indicates the GI effects of metoclopramide and atropine were well studied.
3.4.5. Effect of Insulin on gastrointestinal motility

Insulin effect on digestive function dates from

1. Occurrence of hunger disease with insulin treatment in diabetic patients

2. Use of insulin hypoglycemic test to determine the technical success of vagotomy for peptic ulcers

Insulin in addition to control hyperglycemia in diabetes, Burnstock et al (1988) observed that in STZ-induced diabetic animals, insulin acute administration was found to restore abnormal distribution of phosphorylated neurofilaments and defective storage and release of VIP and CGRP. They suggested that presence of steady state level of insulin might prevent some of the changes which occurs in early stage of DM.

3.4.5.1. Gastric hypermotility effect

Bulatao and Carlson (1924) reported in dogs that upon insulin administration (20-40 U: s.c), an increase in gastric tonus and in the height and frequency of gastric contractions occurred from 10-60 min, gradually leading to gastric tetany which persisted until the hypoglycemic state was terminated by the injection of glucose.

Pavel and Milco (1932) reported that prolonged insulin therapy in undernourished humans was found to induce hyper motility in vivo and a direct stimulatory effect of insulin on intestinal muscle tone was described as a possible mechanism.

Schvarcz et al (1995) compared gastric emptying rate during normoglycemia with that during insulin-induced hypoglycemia in healthy subjects. They recorded augmentation of gastric emptying in insulin administered group and proposed that it is due to hypoglycemic effect induced by insulin.
3.4.5.1.1. Involvement of vagus nerve

Quigley and Templeton (1929-30) demonstrated that insulin injection do not increase the motility of stomach in bilaterally vagotomized dogs. This clear-cut observation prompted these investigators to suggest that insulin injection could be used as a challenge to test the functional integrity of the vagus fibers to the stomach. An inhibitory effect of insulin on stomach observed in vagotomized dogs was also thought to be mediated by the sympathetic pathways as an alternative pathway used by insulin. They proposed that the final action of insulin on the motor activity of stomach depends on the algebraic summation of the excitatory impulses over the vagus nerve and the inhibitory impulses over the sympathetic nerves, usually the excitatory effect predominating over inhibitory effect. Postlethwait et al (1948) demonstrated that gastric hypermotility produced by insulin in rabbits was immediately and completely abolished by cervical vagotomy.

3.4.5.1.2. Involvement of changes in blood glucose level

Lorber and Shay (1962) demonstrated in dogs that i.v administration of insulin (0.25 U/kg) produced motor stimulation of main stomach, response averaged to 68.1 min (range from 24 to 157 min). This effect was consistent with a lower dose without affecting BG level. The motor effect of insulin was reversed by i.v administration of 5% dextrose. They concluded that gastric function is influenced by changes in blood sugar concentration rather than by absolute blood sugar levels.

3.4.5.1.3. Insulin as a model of hyper motility of gut

Lish and Peters (1957) evaluated antispasmodic potencies of various drugs in rats, in terms of relative degree of antipropulsive action with charcoal meal test. They had used insulin (40 U/ml) at the dose of 2 ml/kg; s.c., volume injected in to rats. Thirty minutes later 5 ml/kg of charcoal mixture was administered orally. 40 min later gastrointestinal tract was dissected out. They found stomach is small and firm with little retained material. No charcoal is found in proximal intestine and the farthest point containing one cm or more of the charcoal is 70-80% of length of small intestine. This clearly indicates
This clearly indicates insulin administration produced an increase in distance traversed by the charcoal front. The most consistent result of insulin administration was the 'cleaning out' of stomach and proximal 34% of small intestine. They conceived this result as predominant effect of insulin via autonomic nerves from hypoglycemic stimulation of vagal centres in the CNS and that stomach and more proximal portions of the GI tract are richly supplied by vagal fibres. They had developed a hypermotility model in intact animals model using insulin for comparing activity of new antipropulsive agents.

Jacoby and Brodie (1967) used insulin (0.5 U/kg; i.v) as a model agent to produce duodenal motility comparable to 2 mg/kg; i.v., of metoclopramide in dogs. Benztropine (0.1 mg/kg; i.m) given 30 min prior to insulin completely antagonized the duodenal stimulation, indicating involvement of cholinergic mechanisms.

Bueno and Ruckebusch (1976) reported that insulin administration (3 U/kg; i.v) in sheep stimulated irregular spiking activity for 1 h, suggesting that insulin may have a permissive role in regulating the basal pattern of motility.

3.4.5.2. Involvement of adrenergic system

Anderson and Mark (1993) reported that insulin produced marked sympathetic activation. Edwards and Tipton (1989) reported that insulin elevated plasma noradrenaline in rats. Cheng et al (1997) had evaluated effect of insulin on release of noradrenaline from G-pig ileal myenteric synaptosomes. Insulin had stimulated the secretion of noradrenaline in a concentration dependent manner from 0.01 U/ml.

3.4.5.3. Route of administration

Experience has shown that a more reliable effect on blood sugar will be obtained if the insulin is given intravenous rather than by s.c or i.m., route. However, for the purpose of studying the effects of the rate of fall of blood sugar, s.c., may be desirable in certain experiment.
3.4.5.4. Effect on isolated smooth muscle

It has been demonstrated that insulin has a direct inhibitory action on intestinal muscle strips. Abderhalden and Gellhorn (1925)\textsuperscript{174} attributed this effect to the phenol content of commercial insulin. However, following reports are available. The direct action of insulin on isolated intestinal strips of rat and colon of G-pig consist of a transitory inhibition followed by marked increase in tonus\textsuperscript{173}.

Altan \textit{et al} 1989\textsuperscript{175}, investigated short term effect of insulin on different smooth muscles isolated from non-diabetic animals. Insulin produced dose dependent relaxation of the isolated rat duodenum. This effect was inhibited by sodium orthovanadate, trifluoperazine, verapamil, aspirin and dexamethasone non-competitively. They suggested prostanoid mechanism may be involved in relaxing effect of insulin, further due to activation of Ca\textsuperscript{2+} pump-ATPase.

Ozturk \textit{et al} (1996)\textsuperscript{144} stated in their review article that inhibitory effect of insulin on smooth muscles may accompany hyper polarization as well.

This part of literature review indicates that in addition to insulin’s effect on glucose clearance, insulin prevents neurodegeneration induced by streptozotocin. When we reviewed the literature about insulin effect on GI motility we found conflicting reports against the well reported effect of acceleration of GI motility, particularly studies done by Altan \textit{et al} \textsuperscript{175} and Ozturk \textit{et al} \textsuperscript{144}. In view of these varied reports we have planned the studies both \textit{in vivo} and \textit{in vitro} to establish the inherent effect of insulin at various doses on intestinal transit in mice.
3.4.6. Centrally mediated effect of insulin on GI motility

Generally, drugs are administered into the brain to find any specific action exerted through CNS when no action is produced through any peripheral route. Some drugs fail to gain access through blood brain barrier (BBB), such drugs can be administered directly into ventricles of brain to study any action.

Insulin receptors are widely distributed all over peripheral tissues (InsR_p) and their function is well characterized as mediators of glucose transport into the cells. Presence of insulin receptors within the brain (InsR_b) and their functions are not known much. It is well-known that brain tissue does not depend upon insulin for glucose transportation. Then the definite role of brain insulin and receptors are still enigmatic.

3.4.6.1. Literature update

3.4.6.1.1. Existence of insulin in CNS

The first report about the presence of insulin in brain available in 1967\textsuperscript{177}. Later it was found that insulin in whole brain extracts averaged about 25 fold greater than plasma insulin level\textsuperscript{178}. Two possibilities were suggested for such high levels in brain namely 1) movement of insulin from general circulation into brain against concentration gradient through active transport process 2) synthesis of insulin within the brain tissue.

Adamo \textit{et al} (1989)\textsuperscript{179} suggested that insulin and insulin-like growth factors (IGF)-I and II are homologous peptides synthesized by nervous tissue. The hypothesis of adult mammalian brain synthesizes its own insulin is yet to be strengthened\textsuperscript{180,176}. Till recently, based on insulin presence in foetal and cultured brain cells led to inferring \textit{de novo} synthesis of insulin in CNS.

3.4.6.1.2. Transportation of insulin

Transportation of insulin across BBB into specific areas of brain was reported by Banks and Kastin (1998)\textsuperscript{181}, highly significant levels in pons-medulla, hypothalamus and hippocampus. But insulin levels do not have similar InsR_b density in those regions of brain. Endothelial cells internalize the insulin and deliver to other side with minimum
degradation\textsuperscript{182}. This transportation was reported to be dependent on an endothelial InsR\textsubscript{b}-facilitated transport process\textsuperscript{183}, which are located on brain microvasculature\textsuperscript{184}.

Baura \textit{et al} 1993\textsuperscript{185} concluded that transportation of plasma insulin into brain is saturable and is likely facilitated by an insulin receptor mediated transport process and suggested that central insulin is responsible for regulation of food intake and body weight.

\textbf{3.4.6.1.3. Role of central insulin and InsR\textsubscript{b}:} Putative functions of insulin in the brain\textsuperscript{176}

1. Neurotrophic: Following trophic actions were reported, potentiation of neurite outgrowth, stimulation of protein synthesis and increased phosphorylation of ribosomal protein S6 in cultured foetal neurons.

2. Neuromodulatory: In neuronal cells, insulin produces inverse effects on norepinephrine and serotonin reuptake. Insulin increase in norepinephrine concentration within the synaptic cleft by its inhibitory action on norepinephrine reuptake. Subsequently, norepinephrine stimulates β-receptors, which results in elevation of c-AMP levels in glial cells, further leads to release of glucose from glycogen stores, providing sugar for glial cells. This hypothesis may indicates a crucial role for insulin in brain, deserves either active transportation into brain or \textit{de novo} synthesis in brain. This suggests insulin might possess a neuromodulatory action in CNS and in pathological conditions like Alzheimer disease, Huntington disease, Parkinsonism, Schizophrenia, Depression and Anxiety.

Body weight control: Lateral ventricular infusion of insulin in baboons causes a dose-dependent decrease in caloric intake and body weight lasting up to 3 weeks.

Takeshita and Yamaguchi (1997)\textsuperscript{8} demonstrated effect of intracerebroventricular (i.c.v.), administration of insulin, attenuated the second phase of the formalin-induced nociception in mice. Based on this, they speculated that CNS is the site of the antinociceptive action of insulin.
3.4.6.1.4. Role of CNS in insulin secretion

Chen et al (1975) reported that central administration of insulin in dogs (0.2 U/kg) showed stimulation of pancreatic insulin secretion in 5 min and they concluded that the pancreatic beta cells might be under the influence of insulin sensitive cells of the CNS.

This part of literature review indicates insulin is present in the brain at greater quantities over peripheral levels in normal conditions. Brain insulin is involved in food intake and body weight control. It has the following suggested actions namely neurotrophic, neuromodulatory, antinociceptive and secretagogue of pancreatic insulin. But no report is available in evaluating effect of centrally administered insulin on intestinal transit. Hence, one of the protocol was designed to evaluate the same.
3.5. Methodology

3.5.1. Blood glucose (BG) measurement

*Methods:* The normal fasting blood glucose level may vary considerably depending on whether the blood is obtained from capillaries or veins, macro or micro technique used and type of biochemical procedures used for analysis of glucose. During fasting, capillary blood glucose concentration is only about 2-5 mg/dL higher than that of venous blood in humans\(^30\).

Blood glucose level is measured by many analytical techniques. In the past, BG was analysed with relatively non-specific methods that resulted in faulty results. Older techniques *viz.*, oxidative-reduction, photometric are rarely used. Commonly used methods involve specific enzymatic reaction with glucose *viz.*, hexokinase, glucose oxidase, glucose dehydrogenase\(^30\).

*Glucose dehydrogenase method*\(^30\): This enzyme catalyses the oxidation of glucose to gluconolactone. This reaction is highly specific for glucose and provides results similar to hexokinase technique. This method was adopted for continuous flow analyzer\(^187\).

*Validity of control BG level:* Since the relative degree of hypoglycemia depends upon the control BG value, it is important to know whether the latter is valid. The excitement or discomfort by handling or surroundings, experienced by animal may provoke an elevation of fasting blood glucose. However, such variation can be minimized by exposing control group to similar experimental conditions\(^173\).

3.5.2. Insulin estimation

Measurement of circulating insulin concentration is useful in the clinical evaluation of several conditions. At least forty years ago Yalow and Berson (1960)\(^188\) developed a specific and sensitive radio immunoassay technique for plasma insulin estimation. Based on this protocol, numerous papers were published and helped in the understanding of the physiology of insulin secretion and in disease conditions like DM\(^189\). In fact, it was the first hormone for which radioimmunoassay was developed\(^80\). The basic principle
involves a competition between an unlabeled insulin in the standard or samples and radioiodinated (I-125) insulin for the fixed number of binding sites on a specific antibody. The amount of labeled insulin bound to the antibody is inversely proportional to the concentration of the unlabeled insulin present. Practically, insulin concentration of samples is quantitated by measuring the radioactivity associated with the bound fraction of sample and standard.


The half-life of insulin in plasma is about 5 to 6 min in normal subjects. Whereas proinsulin half-life is about 17 min and accounts for about 10% of the immunoreactive insulin in plasma but the proinsulin is only 2% as potent as insulin. Therefore the biologically effective concentration of insulin is somewhat lower than estimated by immunoassay.

In humans, under basal conditions, the pancreas secretes about 40 μg or 1 unit of insulin per hour into the portal vein, resulted in 50 to 100 μU/ml and in the peripheral circulation of 12 μU/ml. After ingestion of a meal, rapid rise in insulin level occurs in the portal blood, followed by a parallel but smaller rise in the peripheral circulation as high as 100 μU/ml.

In any 24 h period, about 50% of the total insulin secreted by the pancreas is released under basal conditions and reminder is secreted in response to meal.

### 3.5.3. C-peptide of insulin estimation

After insulin secretion into the portal vein, insulin undergoes variable hepatic extraction and peripheral clearance. As a result, the peripheral concentration of insulin do not reflect the changes in the pancreatic secretion. To avoid these problems, many investigators used plasma C-peptide level as a surrogate for the assessment of insulin secretion in insulin treated patients.
This hypothesis is based on the fact that C-peptide (31 amino acid connecting peptide) is co-secreted with insulin in an equimolar ratio is not taken by the liver and has a long peripheral clearance and analytically more sensitive than insulin assay. C-peptide has longer half life (30 min)\textsuperscript{42}.

C-peptide levels are better indicators of β-cell function than peripheral insulin secretion. Further C-peptide assay do not measure exogenous insulin and do not cross-react with insulin antibodies which interfere with the insulin immunoassay\textsuperscript{40}.

Human serum / plasma C-peptide in fasting state ranges from 1.1 to 3.5 ng/ml and normal levels are 0.9 to 4.2 ng /ml. C-peptide is estimated by radio immuno assay\textsuperscript{41}.

### 3.5.4. Upper gastrointestinal transit measurement

**The evaluation of GI motility may be helpful in**

1. Determining alteration in motility secondary to physiological or pharmacological stimuli.

2. Judging the therapeutic potential of drugs in motility disorders.

3. Determining antispasmodic potencies of many drugs as they relax intestinal muscle or contractility.

**The in vivo methods to study GI motility in experimental animals include**

Charcoal, dyes, radio-opaque pellets or radio active markers instilled into the stomach or intestinal lumen. The movement of non-radioactive marker is quantified by measuring the distance traveled by the leading edge of marker. This assumes that the pattern by which the marker is distributed within the intestine is identical in all animals.

*Roentgenological visualization*: Barium meal has been used extensively in dogs and cats but not of much value in smaller laboratory animals.
**Small intestinal transit (SIT) - Physical marker method**

Markers that are inert, non-digestible having distinct colour with suitable viscosity (e.g.: phenol red, charcoal meal and chromic acid) are administered orally or intragastrically (0.3-0.5 ml). They are ideally suited for the measurement of propulsion since they do not produce changes in motility themselves. After a prescribed time period, animals are sacrificed and marker front can be visualized easily in the intestine. Distance traveled by the marker front is noted and expressed as % transit with reference to the whole length of intestine. It gives a qualitative information about propulsion by visualized interpretation. This is generally performed in smaller animals like mice.

Use of marker can be traced back to 1931 by Macht and Barbara\(^2\), their meal consist of aq suspension of purified animal charcoal (5%) and gum tragacanth (5%). Mice were given 0.2 ml of the meal p.o., route and were killed by cervical dislocation 15 min later. The intestine was excised immediately, the distance traveled by the meal was measured. The method quoted by Janssen and Jageneau (1957)\(^3\) received the highest citations, involves 2-4 months old mice that have been fasted overnight, after one hour of drug administration (i.p), animals were given by stomach tube 0.3 ml of an aq suspension containing 10% charcoal and 5% gum acacia. Two hours later, the animals were killed and intestine excised immediately from cardia to anus and distance measured from 'pylorus to anus' and pylorus to appendices.

The standard procedure consist of: Fasting of mice atleast for 18 h prior to experimental protocol but having free access to water, placed in wire mesh laid flooring cages to prevent coprophagy and also to avoid interference by food material. The drug under the investigation may be given 10-30 min prior to test meal, when the route of administration is intraperitoneal or intravenous. If the drug is to be given orally, it must be given atleast 8 h prior to meal. Control group should be treated with vehicle. Any oral intake should not be allowed within that period of experiment as it may vitiate the results. Handling of the intestine should be minimal to avoid false displacement of the test meal.
The change in % intestinal transit noted under the influence of test drug as compared to control values will give indication of prokinetic or anti-kinetic effect of the drug. This can be further compared with agents having known action.

This assay procedure measures the combination of gastric emptying and intestinal transit. Summers et al (1970) proposed surgically modified method to study effect of drugs on gastric emptying and small intestinal transit. This model may eliminate the influence of the stomach emptying on small bowel transit. But this surgically altered model has a factor of surgical stress and may interfere the results.

Although SIT transit measurement with charcoal is not a quantitative method as compared to radioactive methods, it is still widely used as useful visible marker to estimate gastrointestinal motility in vivo.

3.6. Conclusion of literature review

There are no studies available in dealing the relationship between glycemic, insulinemic effects and SIT, further importantly the inherent effect of insulin on SIT deserves a scientific attempt.

Therefore, it will be interesting to construct a composite picture by exploring (a) the effect of hypoglycemia or hyperglycemia on small intestinal transit (b) effect of intestinal transit on glycemic state (c) relationship between insulin level and SIT (d) effect of exogenous insulin on SIT (e) effect of centrally administered insulin on SIT and (f) effect of insulin on isolated small intestine.