CHAPTER - 1

Introduction
Carbohydrates are the most abundant biomolecules on the earth. Each year, photosynthesis converts more than 100 billion metric tons of CO\textsubscript{2} and H\textsubscript{2}O into cellulose and other plant products. These can then be consumed by other organisms and used as fuel for cellular respiration. Certain carbohydrates (sugar and starch) are dietary staples in most parts of the world and the oxidation of carbohydrates is the central energy-yielding pathway in most nonphotosynthetic cells. They fill numerous roles in living things, such as the storage and transport of energy (starch, glycogen) and structural components (cellulose in plants, chitin in animals). Additionally, carbohydrates and their derivatives play major roles in the working process of the immune system, fertilisation, blood clotting and development.

The most important carbohydrate is glucose, a simple sugar (monosaccharide). It occupies a central position in the metabolism of plants, animals and many micro-organisms. It is relatively rich in potential energy and thus a good fuel. By storing glucose as a high molecular weight polymer such as starch or glycogen, a cell can stockpile large quantities of hexose units while maintaining a relatively low cytosolic osmolarity. When energy demands increase, glucose can be released from these intracellular storage polymers and used to produce ATP either aerobically or anaerobically (Touster, 1962).

Glucose is not only an excellent fuel; it is also a remarkably versatile precursor, capable of supplying a huge array of metabolic intermediates for biosynthetic reactions. A bacterium such as \textit{Escherichia coli} can obtain from glucose the carbon skeletons for every amino acid, nucleotide, coenzyme, fatty acid or other metabolic intermediate it needs for growth. A comprehensive study of the metabolic fates of glucose would encompass hundreds or thousands of transformations. In animals and vascular plants, glucose has three major fates: it may be stored (as a polysaccharide or as sucrose); or oxidized to a three-carbon compound (pyruvate) via glycolysis to provide ATP and metabolic intermediates; or oxidized via the pentose phosphate (phosphogluconate) pathway to yield ribose 5-phosphate for nucleic acid synthesis and NADPH for reductive biosynthetic processes (fig.1).
Carbohydrates are major constituents of animal food and animal tissues. Glucose is the most important carbohydrate in mammalian biochemistry because nearly all carbohydrate in food is converted to glucose for further metabolism. The dependence of various tissues on glucose varies widely. Glucose is a major fuel of the tissues of mammals (except ruminants) and a universal fuel of the foetus.

Figure 1: Metabolic fates of glucose

Most tissues have at least a minimal requirement for glucose. In some cases, e.g., brain, the requirement is substantial, while in other, e.g., erythrocytes, it is nearly total. Indeed, glucose is so important to these specialised cells and the brain that several of the major tissues of the body work together to ensure continuous supply of this essential substrate. Tissues such as the brain need glucose constantly and low blood concentration of glucose can cause seizures, loss of consciousness and death. However, prolonged elevation of blood glucose concentrations, as in poorly controlled diabetes, can result in blindness, renal failure, cardiac and peripheral vascular disease and nephropathy. Glucose is continuously delivered to all tissues by the blood (Zierler, 1999).

The concentration of blood glucose is regulated within narrow limits. In the postabsorptive state, the concentration of blood glucose in humans and many mammals is set within the range 4.5-5.5 mmol/L. After the ingestion of a
carbohydrate meal, it may rise to 6.5-7.2 mmol/L. During fasting, the levels fall to around 3.3-3.9 mmol/L. Despite large changes in the input and utilization of glucose, the blood glucose levels are maintained at constant level in response to hormonal signals. So the concentration of glucose in the blood is subjected to tight regulation. Homeostasis of glucose in blood is mainly maintained by cooperative activity of liver, muscle, kidney and adipose tissue and by the endocrine glands at the cellular and enzymatic levels.

Blood glucose level is mainly determined by intake of glucose, absorption of glucose from gut, uptake of glucose by peripheral tissues like muscle, hepatic glucose output and insulin secretion from the pancreas (Zierler, 1999).

DISEASES ASSOCIATED WITH GLUCOSE METABOLISM:

The most frequently encountered disorder of carbohydrate metabolism is hyperglycemia (high blood glucose) due to diabetes mellitus and/or obesity. The incidence of hypoglycemia (low blood glucose) is unknown but is substantially lower. Glucose metabolism is defective in two very common metabolic disease, diabetes and obesity, which contribute in development of a number of major medical problems, including atherosclerosis, hypertension, small vessel disease, kidney disease and blindness.

Obesity:

The serious and frequent nutritional problem in the world is excessive energy consumption. In fact, obesity is the most frequent nutritional disorder. One striking clinical future of overweight individuals is a marked elevation of serum free fatty acids, cholesterol and triacylglycerols irrespective of the dietary intake of fat. Obesity is obviously associated with an increased number and/or size of adipose tissue cells. These cells overproduce factors such as leptin and tumor necrosis factor α, which cause cellular resistance to insulin by interfering with autophosphorylation of the insulin receptor and the subsequent phosphorylation of the insulin receptor substrate (Heidi et al., 2002).
Initially, the pancreas maintains glycemic control by overproducing insulin. Thus many obese individuals with apparently normal blood glucose control have a syndrome characterized by insulin resistance of the peripheral tissue and high concentrations of insulin in the circulation. This hyperinsulinemia appears to stimulate the sympathetic nervous system, leading to sodium and water retention and vasoconstriction, which increase blood pressure.

The insulin resistance in adipose tissue results in increased activity of the hormone-sensitive lipase, which along with the increased mass of adipose tissue is probably sufficient to explain the increase in circulating free fatty acids. These excess fatty acids are carried to the liver and converted to triacylglycerol and cholesterol. Excess triacylglycerol and cholesterol are released as very low density lipoprotein particles, leading to higher circulating levels of both triacylglycerol and cholesterol. For reasons that are not entirely clear this also results in a decrease in high density lipoprotein particles.

Eventually, the capacity of the pancreas to overproduce insulin declines, which leads to higher fasting blood sugar levels and decreased glucose tolerance. Fully 80% of adult-onset (type II) diabetics are overweight. Because of these metabolic changes, obesity is a primary risk factor in coronary heart disease, hypertension, and diabetes mellitus. This is nutritionally significant because all of these metabolic changes are reversible. Quite often reduction to ideal body weight is the single most important aim of nutritional therapy (Riccardi et al, 2004).

Approximately 17.5 percent of children (age 6 to 11) and 17 percent of adolescents (age 12 to 19) were overweight in 2001 to 2004. The prevalence of obesity during this same time period more than doubled among adults age 20 to 74 from 13.3 to 32.1 percent, with most of this rise occurring since 1980 (National Centre for Health Statistics, 2006). These facts presents statistics on the prevalence of overweight and obesity, as well as the health risks, mortality rates, and economic costs associated with these conditions.
Diabetes mellitus:

Diabetes mellitus is a major endocrine disorder characterized by elevated blood glucose levels resulting from absent or inadequate pancreatic insulin secretion with or without concurrent impairment of insulin action. In fact, diabetes now a days is a global problem affecting nearly 10% of population all over the world comes to 150 million people (Burke et al., 2003). According to World Health Organization (WHO) report the number of cases of diabetes mellitus will be from 171 million in 2000 to 360 million by 2030. By the year 2025, 75% of diabetics will reside in developing countries (Raman, 2002). As the number of people with diabetes multiplies worldwide, the disease takes an ever-increasing proportion of national and international health care budget.

The first widely accepted classification of diabetes mellitus was published by WHO in 1980 (WHO, 1980) and in modified form, in 1985 (WHO, 1985). The main classes of diabetes are Type 1 or insulin-dependent diabetes mellitus (IDDM), Type 2 or non-insulin-dependent diabetes mellitus (NIDDM), malnutrition related diabetes mellitus (MRDM), and gestational diabetes mellitus (GDM).

Diabetes has always been considered to be a disturbance in the metabolism of carbohydrates accompanied by alteration in the metabolism of fats and proteins. The changes are mainly the result of a low insulin/glucagon ratio. Basal levels of insulin and glucagon control hepatic glucose output. In NIDDM, fasting blood glucose is raised in direct proportion to hepatic glucose output (DeFronzo et al., 1987), and appears unlikely to be a result of decreased insulin action at the periphery as it has not been shown to correlate closely with insulin-stimulated glucose disposal (Bogardus et al., 1984; DeFronzo et al., 1982).

FOOD INTAKE AND SWEET TASTE SYSTEM:

Food intake is regulated by the complex interaction of psychological and physiological events associated with ingestion. While the energy content of foods has an important role in determining the amount eaten, a number of other properties of foods also may be important. These include palatability, macronutrient composition, form of the food (solid vs. liquid), how it is prepared, and its energy density.
Some sugars are of particular interest because of the sweet taste they provide. While sweetness increases the palatability of foods, particularly when combined with fat, and therefore may increase the probability that sweet foods will be selected for consumption (Drewnowski et al., 1992), there is no indication that sugar is associated with excessive food intake. Intake of sweet foods or drinks is limited by changes in the hedonic response to sweetness during consumption (Rolls, 1986). Thus, to a hungry individual a sweet food will be rated as extremely pleasant in taste, but as consumption proceeds this rating of pleasantness declines. Ratings of foods with different tastes, for example, salty foods, will be unaffected by consumption of sweet foods. This "sensory-specific satiety" limits consumption of one type of food and helps to ensure that a variety of foods is consumed (Rolls and Hetherington, 1989).

The taste system plays a critical role in determining whether a food is nutritious and should be ingested or is potentially toxic and should be rejected. Considerable attention has focused on the sweet taste of sugar because it is such a potent stimulator of eating in humans and many other animal species. A major advance in our understanding of sweetness perception was the discovery of two G-coupled receptor proteins, T1R2 and T1R3, which dimerize to form a broadly tuned sweet taste receptor (Breslin and Huang, 2006). Stimulation of the T1R2-T1R3 taste receptor by sugars or artificial sweeteners activates intracellular signalling elements, including α-gustducin, which stimulate peripheral gustatory nerves and in turn, brain gustatory pathways (Berthoud, 2002). The central processing of the sweet taste signal typically activates feeding circuits as well as brain reward systems that promote sweet appetite. Brain autonomic centres may also relay information via the vagus nerve to prepare the digestive system for the incoming carbohydrate-rich food. Digestive and absorptive processing of the ingested food is further coordinated by sugar sensing in the intestinal tract, which modulates nutrient absorption, hormone release, and gastrointestinal motility and generates satiation signals to the brain that terminate the meal (Steven et al., 2005).
The same T1R2-T1R3 sweet taste receptor that initiates sugar ingestion in the mouth also detects sugar in the intestinal lumen and triggers physiological responses that promote sugar absorption and metabolism (Margolskee et al., 2007).

DIGESTION AND ABSORPTION OF CARBOHYDRATES:

Simple sugars are the predominant carbohydrate absorbed in the digestive tract and in many animals the most important source of energy. Monosaccharides, however, are only rarely found in normal diets. Rather, they are derived by enzymatic digestion of more complex carbohydrates within the digestive tract.

Particularly important dietary carbohydrates include starch and disaccharides such as lactose and sucrose. None of these molecules can be absorbed for the simple reason that they cannot cross cell membranes unaided and unlike the situation for monosaccharides, there are no transporters to carry them across.

The digestion of starch begins with salivary amylase, but this activity is much less important than that of pancreatic amylase in the small intestine. Ingested starch and glycogen are partially digested by the action of salivary amylase in the mouth to form intermediate dextrins and maltose. The acid pH of the stomach inhibits amylase activity, but the alkaline pancreatic secretions increase the pH in the small intestine, allowing pancreatic amylase to complete digestion to oligosaccharides (Evenepoel, 2001).

Amylase hydrolyzes starch, with the primary end products being maltose, maltotriose, and α-dextrins, although some glucose is also produced. The products of α-amylase digestion are hydrolyzed into their component monosaccharides by enzymes expressed on the brush border of the small intestinal cells, the most important of which are maltase, sucrase, isomaltase and lactase (Southgate, 1995).

Then the monosaccharides are absorbed across the wall of the duodenum and ileum by different transfer processes. The rate of absorption for glucose and
Galactose is several times greater than for similar molecules absorbed by passive diffusion (e.g., xylose). Fructose is absorbed more slowly than glucose and galactose by a carrier-mediated process different from the glucose and galactose transport mechanisms. The monosaccharides are transported into the enterocyte facilitative transport system, such as Na⁺-dependent glucose transporter (SGLT1) and GLUT5 (one of the GLUT family transporter). The SGLT1 is responsible for the active transport of glucose and galactose with an equimolar amount of sodium against a concentration gradient into the cytoplasm of the enterocyte. Fructose is taken up by facilitated transport by the glucose transporter 5 (GLUT5). Glucose, galactose and fructose are pumped out of the enterocyte into the intracellular space by the glucose transporter 2 (GLUT2) (Lentze, 1995).

**GLUCOSE HOMEOSTASIS:**

Despite large changes in the input and utilization of glucose, the blood glucose levels are maintained at constant level. Maintenance of stable levels of glucose in the blood is one of the most finely regulated of all homeostatic mechanisms and one in which the liver, the extra hepatic tissues, and several hormones play a part.

Most digestible carbohydrates in the diet ultimately form glucose. The dietary carbohydrates that are actively digested contain glucose, galactose, and fructose residues that are released in the intestine. These are transported to the liver via the hepatic portal vein. Galactose and fructose are readily converted to glucose in the liver. The liver has the primary metabolic function of regulating the blood concentration of most metabolites, particularly glucose. In the case of glucose, this is achieved by taking up excess glucose and converting it to glycogen (glycogenesis) or to fat (lipogenesis). Between meals, the liver can draw upon its glycogen stores to replenish glucose in the blood (glycogenolysis) or, in company with the kidney, to convert noncarbohydrate metabolites such as lactate, glycerol and amino acids to glucose (gluconeogenesis).

Liver cells appear to be freely permeable to glucose (via the GLUT2 transport) (Fukumoto et al., 1989), whereas cells of extra hepatic tissues (apart
Glucose homeostasis is maintained in normal animals by the reciprocal regulation of insulin secretion by β-cells and glucagon secretion by α-cells of the Islets of Langerhans. Effects of insulin and glucagon on glucose homeostasis (Cherrington et al., 1976; Hjortoe et al., 2004) are shown in Table 1.

High blood glucose elicits the release of insulin, which speeds the uptake of glucose by tissues and favors the storage of fuels as glycogen and triacylglycerols, while inhibiting fatty acid mobilization in adipose tissue. Low blood glucose triggers release of glucagon, which stimulates glucose release from liver glycogen and shifts fuel metabolism in liver and muscle to fatty acid oxidation, sparing glucose for use by the brain. In prolonged fasting, triacylglycerols become the principal fuel; the liver converts the fatty acids to ketone bodies for export to other tissues, including the brain. In diabetes, insulin is either not produced or not recognized by the tissues, and the uptake of blood glucose is compromised. When blood glucose levels are high, glucose is excreted. Tissues then depend on fatty acids for fuel (producing ketone bodies) and degrade cellular proteins to provide glucogenic amino acids for glucose synthesis. Uncontrolled diabetes is characterized by high glucose levels in the blood and urine and the production and excretion of ketone bodies.
Table 1: Effects of insulin and glucagon on glucose homeostasis

<table>
<thead>
<tr>
<th>Metabolic effect</th>
<th>Target enzyme</th>
<th>Insulin</th>
<th>Glucagon</th>
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</thead>
<tbody>
<tr>
<td>Glucose uptake (muscle, adipose)</td>
<td>Glucose transporter (GLUT4)</td>
<td>↑</td>
<td>↓</td>
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<tr>
<td>Glucose uptake (liver)</td>
<td>Glucokinase (increased expression)</td>
<td>↑</td>
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<tr>
<td>Glycogen synthesis (liver, muscle)</td>
<td>Glycogen synthase</td>
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<tr>
<td>Glycogen breakdown (liver, muscle)</td>
<td>Glycogen phosphorylase</td>
<td>↓</td>
<td>↑</td>
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<tr>
<td>Glycolysis, acetyl-CoA production (liver, muscle)</td>
<td>PFK-1 (by PFK-2)</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Fatty acid synthesis (liver)</td>
<td>Acetyl-CoA carboxylase</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Triacylglycerol synthesis (adipose tissue)</td>
<td>Lipoprotein lipase</td>
<td>↑</td>
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<td></td>
<td>FBPase-2</td>
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<tr>
<td>Gluconeogenesis (liver)</td>
<td>Pyruvate kinase</td>
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<td></td>
<td>PEP carboxykinase</td>
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<tr>
<td>Fatty acid mobilization (adipose tissue)</td>
<td>Triacylglycerol lipase</td>
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<tr>
<td>Ketogenesis</td>
<td>Acetyl-CoA carboxylase</td>
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EFFECTS OF HYPERGLYCEMIA:

Hyperglycemia alone does not cause diabetic complications. It is rather the detrimental effect of glucose toxicity due to chronic hyperglycemia, which is mediated and complicated through oxidative stress. Diabetic hyperglycemia causes a variety of pathological changes in small vessels, arteries and peripheral nerves. Vascular endothelial cells become primary vulnerable targets of hyperglycemic damage as glucose continuously flows through them.

There is increasing evidence that complications related to diabetes are associated with oxidative stress induced by the generation of free radicals. Prolonged hyperglycemia, seen in uncontrolled diabetes leads to glucose oxidation, which is believed to be main source of free radicals. Free radical induced lipid peroxidation (Wilson et al., 1981) causes considerable changes in the structural organization and functioning of cell membrane and makes the membrane leaky. These lipid peroxides and prolonged hyperglycemia in turn stimulate glycation of protein, inactivation of enzymes and alterations in the structure and function of collagen, basement and other membranes thereby changing the receptor alignment and ion channels. These changes finally effect normal functioning of the cell by affecting the process of signal transduction. Chronic oxidative stress due to hyperglycemia may therefore play an important role in pancreatic β cell dysfunction (Mashino and Fridovich, 1987; Boynes, 1991).

Hyperglycemia induces oxidative stress, activation of protein kinase-C isoforms (Koya and King, 1998), increases formation of glucose-derived advanced glycation end products (Brownlee, 1995) and increases glucose flux through aldose reductase pathways are some of the known biochemical mechanisms of hyperglycemia-induced tissue/organ damage.

Nishikawa et al. (2000) has proposed a single unifying hypothesis linking these mechanisms by which elevated concentrations of glucose perturb cellular properties in a fundamental way. Hyperglycemia aggravates endothelial reactive oxygen species (ROS) generation by a variety of mechanisms. ROS increases the generation of tumor necrosis factor-α expression and aggravates oxidative stress (Pahl and Baeuerle, 1994) as shown in fig.2 (Ashok and Madhusudana Rao, 2002).
Figure 2: Sketch of pathways of carbohydrate metabolism and targets where imbalance/insufficiencies in function lead to hyperglycemia and resultant diabetic syndrome. S-Glut-1, Sodium glucose co-transporter-1; GIP, gastrointestinal peptide; VIP, Vasoactive intestinal peptide; EIA, Enteroinsular axis; glu-R, Glucose receptor; IR, Insulin receptor; IR-s, Insulin receptor substrate; Tk, Tyrosine kinase enzyme; PTP, Protein phosphotyrosine phosphatase; TNF, Tumour necrosis factor; Ald-Red, Aldose reductase; Hk, Hexokinase; LPL, Lipoprotein lipase; | |, basolateral membrane; (1) α-Glucosidase inhibitors; (2), Sulphonyl ureas; (3), Biguanides, and (4), Aldose reductase inhibitors are the available therapies at the particular points and synthetic medicines available. [A] Denotes points where phytochemicals of various or similar nature has been shown to demonstrate multiple activities (Ashok and Madhusudana Rao, 2002).
HYPERGLYCEMIA - THERAPY:

The epidemic of type 2 diabetes in the latter part of the 20th and in the early 21st century and the recognition that achieving specific glycemic goals can substantially reduce morbidity have made the effective treatment of hyperglycemia a top priority (Hirsch, 2005; Alberti and Aguilar, 1999). While the management of hyperglycemia has historically had center stage in the treatment of diabetes, therapies directed at other coincident features, such as dyslipidemia, hypertension, hypercoagulability, obesity and insulin resistance, have also been a major focus of therapy. Maintaining glycemic levels as close to the nondiabetic range as possible has been demonstrated to have a powerful beneficial impact on diabetes specific complications, including retinopathy, nephropathy, and neuropathy (Bastien, 2004). The development of new classes of blood glucose-lowering medications to supplement the older therapies, such as lifestyle directed interventions, insulin, sulfonylurea and metformin, has increased the treatment options for diabetes.

Controlled clinical trials, such as the Diabetes Control and Complications Trial (DCCT) (Nathan, 1993) and the Stockholm Diabetes Intervention Study (Reichard et al., 1993) in type 1 diabetes; and the UKPDS (Turner et al., 1998) and Kumamoto Study (Ohkubo et al., 1995) in type 2 diabetes have helped to establish the glycemic goals of therapy that result in improved long-term outcomes. Although the various clinical trials have had different designs, interventions, and measured outcomes, the trials, in concert with epidemiologic data (Klein et al., 1988; Chase et al., 1989), support decreasing glycemia as an effective means of reducing long-term microvascular and neuropathic complications.

CURRENT APPROACHES TO HYPERGLYCEMIA MANAGEMENT:
Diet control and exercise:

Lifestyle management including diet control and adequate exercise is essential to the successful treatment of Type 2 diabetes. Experts on diet and health and the American Diabetes Association (ADA) state that there is no single dietary regimen for diabetes. Dietary recommendations may be developed based on the individual’s requirements and treatment goals (Unwin and Marlin, 2004).
Persons with diabetes are treated with the diet include a reduction in simple carbohydrate intake with an increase in complex carbohydrates, dietary fiber, physical exercises like morning walk, yoga have been used with limited success. Some patients with NIDDM are satisfactorily treated with diet alone, others require a combination of diet and oral hypoglycemic drugs, a number will eventually come to require insulin.

**Insulin:**

Regulation of glucose metabolism is a key aspect of metabolic homeostasis and insulin is the dominant hormone influencing this regulatory system. One of the major effects of insulin is to enhance overall glucose disposal, and this is achieved by stimulation of glucose uptake into the target tissues. This task is facilitated by insulin-sensitive glucose transporter (GLUT4), which is uniquely expressed in skeletal muscles, cardiac muscles and adipose tissues (Olefsky, 1999). This action of insulin in the regulation of glucose homeostasis in post-absorptive state is a very important function in maintaining euglycemia and preventing hyperglycemia (Pessin et al., 1999). Insulin therapy affords effective glycemic control, yet its shortcomings such as ineffectiveness on oral administration, short half-life, and requirement of constant refrigeration and in the event of excess dosage-fatal hypoglycemia-limits its usage.

**Oral hypoglycemic agents:**

Unfortunately, insulin has to be taken as an injection simply because it is a protein which is broken up and digested by the enzymes of the gut if taken orally. Therefore, and oral insulin would both obviate the need for injections and more importantly, would closely mimic the physiological action of insulin by initial entry into the portal circulation. Oral therapies for diabetes mellitus have emerged out of this interest and are widely used still today. But rather than acting by directly mimicking insulin signaling, these drugs act by modifying the factors aiding in the control of hyperglycemia like stimulating insulin secretion (sulphonylureas), lowering hepatic glucose production (biguanides) or by interacting with other intracellular events (Balasubramanyam and Mohan, 2001; Inzucchi, 2002) as shown in fig.3 (ICMR, 2005).
Acarbose is an ADA accepted α-glucosidase inhibitor that slows down the breakdown of disaccharides and polysaccharides and other complex carbohydrates into monosaccharides. These agents slow the digestion so that glucose from starch enters the blood stream more slowly, and can be matched more effectively by an impaired insulin response or sensitivity. Acarbose when used in addition to insulin or sulfonylurea regimens hypoglycemia can occur in 2% (Martin and Montgomery, 1996). The most common side effects are gastrointestinal and include flatulence, borborygmi, abdominal pain and diarrhoea. Rarely elevated liver transaminases and decreases in serum iron concentrations have occurred (Martin and Montgomery, 1996).

Sulfonylureas are insulin secretagogues, triggering insulin release by direct action on the potassium-ATP channel of the pancreatic β-cells. Sulfonylureas are only useful in Type 2 diabetes mellitus they work best with patients over 40 years old, who have had diabetes mellitus for less than ten years. They have a characteristic profile of side effects (Prout, 1974). The primary side effect is hypoglycemia (Davis, 2004). Glyburide has the highest rate of serious hypoglycemia (16.6/1000 patient) and tolbutamide the lowest (3.5/1000 patients) (Shorr, 1996). Thirty one percent of patients treated with glibenclamide experience hypoglycemia (Van Staa, 1997). Meglitinides help the pancreas produce insulin and often called 'short-acting secretagogues'. Their mode of action is original, affecting potassium channels. By closing the potassium channels of the pancreatic β-cells, they open the calcium channels, hence enhancing insulin exocytosis. Repulginide from the meglitinide drug class acts like short acting sulfonylureas and are potentially useful as a sulfonylurea replacement. Repaglinide has been approved for use with metformin and the combination appears to be a very effective. Adverse reactions include weight gain and hypoglycemia.
Figure 3: Targets sites of action of oral hypoglycemic agents (ICMR, 2005)

- **Absorption** of glucose
- **Insulin secretion**
- **Hepatic output** of glucose
- **Insulin sensitivity**

- **Alpha-glucosidase inhibitors**
- **Sulphonylureas Meglitinides**
- **Biguanides**
- **Glitazones**
**Biguanides** reduces hepatic glucose output and increase uptake of glucose by the periphery including skeletal muscle. Among common diabetic drugs, metformin, a biguanide, is the only widely used oral drug that does not cause weight gain. Biguanides developed from a prototypic plant molecule is an excellent example of anti diabetic drug development from plants. Biguanides do not enhance the release of insulin but appear to inhibit gluconeogenesis (Alschuld and kruger, 1968; Lloyds et al., 1975). Lactic acidosis is the most serious side effect associated with metformin owing to decreased gluconeogenesis from precursors including alanine, pyruvate and Lactate (Lalau, 1995).

Contradictions to metformin use include congestive heart failure, metabolic acidosis, drug hypersensitivity and renal impairment. The most common feature associated with serious lactic acidosis is renal dysfunction, defined by an abnormal creatinine clearance or serum creatinine of 1.5 mg/dl or more (Jurovich, 1996).

From the brief overview of diabetic therapy, it can be seen that current methods of treatment for all types of diabetes mellitus fails to achieve the ideals of normoglycemia and the prevention of diabetic complications. Promising fields of research such as pancreatic transplants offer little hope to the majority of the world’s diabetics, for whom such procedures will be too expensive and difficult to obtain.

**Traditional plant therapies - diabetes:**

Before the introduction of insulin in 1922, the treatment of diabetes mellitus relied heavily on dietary measures, which included the use of traditional plant therapies. But after the advent of insulin and other hypoglycemic drugs (synthetic) this field of work largely remained unexplored. The yawning gap for additional agents to combat hyperglycemia and its accompanying complications presents an opening to revisit traditional antidiabetic plants (Gray and Flatt, 1997; Jachak 2002).

Medical plants play an important role in the management of diabetes mellitus especially in developing countries where resources are meager. Many
studies have confirmed the benefits of medicinal plants with hypoglycemic effects in the management of diabetes mellitus. The effects of these plants may delay the development of diabetic complications and correct the metabolic abnormalities. Moreover, during the past few years some of the bioactive drugs isolated from hypoglycaemic plants showed antidiabetic activity with more efficacy than oral hypoglycemic agents used in clinical therapy (Bnouham et al., 2006).

Indian plants that are most effective and most commonly studied in relation to diabetes and their complications are: *Allium cepa, Allium sativum, Aloevera, Cajanus cajan, Coccinia indica, Caesalpinia bonducella, Ficus bengalensis, Gymnema sylvestre, Monordica charantia, Ocimum sanctum, Pterocarpus marsupium, Swertia chirayita, Syzigium cumini* and *Tinospora cordifolia* (Shanmugasundaram, et al. 1990; Chattopadhyay, 1993; Chattopadhyay, 1999)

Many traditional plant treatments for diabetes mellitus are used throughout the world. Few of the medicinal plant treatments for diabetes have received scientific scrutiny, for which World Health Organization has also recommended attention (Bloom, 1980).

**MOLECULAR MODELLING – RATIONAL DRUG DESIGN:**

The availability of several crystal structures of receptors, enzymes and transporters involved in hyperglycemia provides the possibility to apply structure-based designing techniques for the development of specific and potent inhibitors of the target molecules. Thus *in silico* approaches have been providing additional tools to design important drugs that can be synthesized and tested later on in animal models.

In the recent past, the design of an otherwise nontoxic drug that inhibits a particular enzyme was largely carried out by trial-and error procedures: A very large number of diverse substances are screened for possible inhibitory effects and upon finding a suitable so called lead compound, a large number of its
variants are synthesized and likewise tested for drug efficacy. Although nearly all of the therapeutic drugs in use today were discovered in this way, it is an expensive and time-consuming method.

The use of recently developed molecular modelling technique promises to yield more efficacious drugs and to increase the rate at which they are developed. Molecular modelling has become a valuable and essential tool to the medicinal chemist working in the drug design enterprise. The advent of powerful computers and the evolution of high-quality software have resulted in a flexible array of methods that facilitate the rational drug design process. These modelling methods can speed the refinement of a lead compound from the laboratory bench to the pharmacy shelves.

Molecular modelling is based on several computational methods, ranging from quantum chemistry to simple parameterisation of experimental results. The rational drug design process has changed the way in which potential new drugs are discovered. In the past, many drugs were developed through a search of natural sources, most notably plants and microorganisms. Drugs such as morphine, penicillin G and digitalis are examples of efforts of this type. Subsequent synthetic modifications of these natural products extended the formulary by improving on the therapeutic profiles of the natural products.

The increasing cost of screening compounds and the decreasing yield of new and unique lead compounds from natural sources have made this approach less favoured in recent years. One estimate holds that only one compound in every 20,000 screened randomly will make it into the clinic, and this already low yield is expected to decrease even further in the future. The cost of taking a compound with potential therapeutic value from the laboratory bench to the pharmacy shelf has become almost prohibitive in recent years.

Although some progress has been made in recent years towards reducing the time and cost of drug product development by modifying the approval and clinical trial process, a successful drug product will never be either cheap or easy.
to produce. Certainly one way to make the drug product development process more cost effective is to improve the yield of promising drug candidates. This improvement can be accomplished by “working smarter” during the drug design process, applying both our knowledge of the mechanistic of target disease and our knowledge of the molecular characteristics of the compounds to have an effect on the disease state, thereby reducing the number of unsuccessful drug candidates. This approach to the therapeutic development is called the rational drug design approach. Molecular modelling in all of its forms is intimately linked to our ability to carry out rational drug design.

The rational drug design process starts with an understanding of the fundamental physiologic and biochemical aspects of the disease or targets, rather than a random screening process (Greer et al., 1994). As a first step, instead of just testing existing or newly synthesized compounds against a particular disease, rational drug design prescribes an investigation of the fundamental characteristics of the disease of interest. If the disease could be treated by the inhibition of a particular enzyme, then a study of the structure and detailed mechanism of action of that enzyme would be undertaken. If the crystal structure of the enzyme could be obtained, then we would have an exact model of the drug design target that we could approach directly. More often than not, we cannot obtain a model for a direct design process, so we must use the so-called indirect methods, which rely on structure-activity relationships and modelling of hypothetical active sites and target receptors.

The next step in the rational design process is the identification of a lead compound, that is, a compound to serve as the starting point for the drug design process. This lead compound could be the natural substrate for an enzyme or some other compound that has a side effect indicative of potential therapeutic activity. After development of one or more lead compounds, a congener set (a series of carefully designed analogs of the lead compound) is synthesized and tested for activity in an appropriate biologic test system. The resulting biologic activity is then analysed with statistical techniques and graphic and computational techniques are used to develop a quantitative model of its structure.
activity relationships. Based on this model, a new congener set is devised, synthesized, and tested.

Using structure-based drug design techniques a purine nucleoside phosphorylase inhibitor that shows great promise in treating both psoriasis and a form of T cell lymphoma has been designed in about 3 years, rather than about 10 years required by traditional trial-and-error techniques, and with much less expense. Moreover, since such rationally designed inhibitors are likely to bind to their enzyme more specifically and thus to be efficacious in lower doses than substances designed by trial-and-error, they are less likely to cause adverse side effects by interacting with other substances in the body.

The first unequivocal example of the application of structure-based drug design leading to an approved drug is the carbonic anhydrase inhibitor dorzolamide that was approved in 1995. Another important case study in rational drug design is imatinib, a tyrosine kinase inhibitor designed specifically for the bcr-abl fusion protein that is characteristic for Philadelphia chromosome-positive leukemias (chronic myelogenous leukaemia and occasionally acute lymphocytic leukemia). Imatinib is substantially different from previous drugs for cancer, as most agents of chemotherapy simply target rapidly dividing cells, not differentiating between cancer cells and other tissues (Greer et al., 1994).

The activity of a drug at its binding site is one part of the design. Another to take into account is the molecule's druglikeness, which summarises the necessary physical properties for effective absorption. One way of estimating druglikeness is Lipinski's Rule of Five (Lipinski et al., 2001).

NEED FOR NEW APPROACHES:

The present day diabetic treatment methods pose the following problems: (i) insulin therapy does not accurately emulate normal β-cell function, (ii) oral agents only partially correct the multiple cellular disturbances that characterize Type 2 diabetes, (iii) the present range of drugs do not completely normalize or reinstate glucose homeostasis, (iv) insulin resistance is still relatively impervious
to treatment and (v) diabetic patients are still at risk of chronic morbidity and premature mortality, despite the assiduous use of the available drugs. Therefore, it has become quite clear that elucidation of the molecular mechanisms of insulin signalling in diabetes would be necessary both to identify cellular targets and to aid in development of a ‘lead’ drug (Balasubramanyam and Mohan, 2001).

The United Kingdom Prospective Diabetes Study (UKPDS) compared three classes of glucose-lowering medications (sulfonylurea, metformin, or insulin) but was unable to demonstrate clear superiority of any one drug over the others with regard to complications (Turner et al., 1998). However, the different classes do have variable effectiveness in decreasing glycemic levels and the overarching principle in selecting a particular intervention will be its ability to achieve and maintain glycemic goals (Stratton et al., 2000; Nathan et al., 2006). Therefore, it is reasonable to investigate blood glucose lowering agents at different target levels responsible for hyperglycemia. Even though several drugs exist today in the market designed and synthesized traditionally as inhibitors at various target levels, all of them have several side effects in long-term usage.

Choosing specific antihyperglycemic agents is predicated on their effectiveness in lowering glucose, extraglycemic effects that may reduce long-term complications, safety profiles, tolerability, and expense. Apart from their differential effects on glycemia, there are insufficient data at this time to support a recommendation of one class of glucose-lowering agents, or one combination of medications, over others with regard to effects on complications. In other words, the salutary effects of therapy on long-term complications appear to be predicated predominantly on the level of glycemic control achieved rather than on any other specific attributes of the intervention(s) used to achieve glycemic goals. Blood glucose levels are mainly determined by intake of glucose, absorption of glucose from gut, uptake of glucose by peripheral tissues like muscle and adipose tissue, hepatic glucose output (glycogenolysis and gluconeogenesis), the insulin secretion from the pancreas. Compounds having selective and specific action are not very important for clinicians to prescribe certain medicines for chronic diseases because these medicines often cause
severe side effects. Multiple pharmacological actions become practical prerequisites for identifying highly efficacious drugs with multiple pharmacological actions for the simultaneous treatment of multifactorial symptoms of chronic diseases like diabetes and obesity.

Based on the above considerations the following proteins involved at different regulatory points are selected for the development of antihyperglycemic drug:

- Taste receptors – T1R2 and T1R3. Intake of glucose can be regulated by suppressing the sweet taste receptor activity without effecting the other olfactory GPCRs.
- Intestinal glucose transporters - GLUT2 and GLUT5. Absorption of glucose from gut can be inhibited.
- Peripheral tissue glucose transporters - GLUT4, GLUT6 and GLUT7. Uptake of glucose by peripheral tissues like muscle should be either stimulated or not effected by the drug.
- Glycogen phosphorylases - glycogen degradation regulating enzyme.

**G-PROTEIN COUPLED RECEPTORS:**

Chemosensory receptors have been identified as G-Protein Coupled Receptors (GPCRs). GPCRs are involved in a wide variety of physiological processes e.g., the visual sense, the sense of smell, behavioural and mood regulation, regulation of immune system activity, inflammation and cell density sensing (Salahpour et al., 2000). GPCRs are involved in many diseases and are also the target of around half of all modern medicinal drugs.

Tastant molecules act as agonists, binding to and stabilizing active conformations of receptors result in the initiation of signal transduction cascades. Taste signaling therefore, should be amenable to the methods of pharmacology (Palmer, 2007). GPCRs are only found in eukaryotes, including yeast, plants and animals. GPCRs constitute a large and diverse family of proteins whose primary function is to transduce extracellular stimuli into intracellular signals.
**GPCRs general structure:**

GPCRs are integral membrane proteins that possess seven membrane-spanning domains or transmembrane (TM) helices. The extracellular parts of the receptor can be glycosylated. These extracellular loops also contain two highly conserved cysteine residues which build disulfide bonds to stabilize the receptor structure (Milligan, 2004). All GPCRs have an extracellular N-terminal segment, seven TMs, which form the TM core, three exoloops, three cytoloops, and a C-terminal segment. A fourth cytoplasmic loop is formed when the C-terminal segment is palmitoylated at cysteine. Each of the seven TMs is generally composed of 20–27 amino acids. On the other hand, N-terminal segments (7–595 amino acids), loops (5–230 amino acids), and C-terminal segments (12–359 amino acids) vary in size, an indication of their diverse structures and functions. Interestingly, there is a weak positive correlation between an N-terminal segment’s length and ligand size (Ji et al., 1995), suggesting a role in ligand binding, in particular for large polypeptides and glycoprotein hormones. A notable exception is the 600-amino acid N-terminal segment of neurotransmitter receptors such as the calcium receptor. The crystal structure of only one member of this group, bovine rhodopsin, has been solved (Palczewski et al., 2000).

**The potential role of GPCR in drug discovery:**

GPCR accessory proteins represent potential future therapeutic targets for a range of conditions, however, their relative novelty and issues with the progressability of these proteins means that they may not enter the drug discovery pipeline in the short-term. The benefits of targeted family research, such as the concentration of knowledge and expertise in biology and chemistry fits with the current trend in the pharmaceutical industry to target family-based research as a means to meet the ever-growing need to fill the drug discovery pipeline (Muller, 2003).

Historically, the activation and inhibition of GPCR function has been a very successful avenue for drug discovery and development. However, it is clear that receptors do not function in isolation but are impacted by other proteins. These proteins may alter either binding or functional responses. Identification and
study of these interactions have grown rapidly in recent years and continue to do so, resulting in a plethora of potential receptor–protein connections. These associations can be regarded as alternative intervention points to modulate GPCR function and may not only provide alternative ways to modify receptor activity but also to exploit new chemical space for drug-like molecules. Such interactions may account for side effects or undesirable properties associated with otherwise well-validated GPCR targets (Hopkins and Groom, 2002).

Historically, the activation and inhibition of GPCR function has been a very successful avenue for drug discovery and development. Marketed drugs for both GPCR agonists (e.g., Sumatriptan for 5-HT1 receptor) and antagonists (e.g., Loratidine for H2 receptor) exist and there are a number of compounds in clinical trials exploiting allosteric binding sites present on GPCRs (Presland, 2004).

GPCRs constitute 15% of the druggable genome and 25% of molecular targets of experimental and marketed drugs, GPCR accessory proteins are an yet unexplored area that may offer the opportunity to expand the druggable genome (Hopkins and Groom, 2002).

Many accessory proteins, in particular cytosolic proteins, may be more amenable to crystal structure determination than GPCRs and so could benefit from this information for rational drug design. With the efforts currently being undertaken (e.g., www.mepnet.org), it is clear that this information is seen as key to improving rational drug design approaches for GPCRs (Ballesteros and Palczewski, 2001). Although the physiological importance of this interaction is being examined, the challenge for the pharmaceutical industry may be to integrate target family approaches rather than to maintain them is separate silos to progress them most optimally.

SWEET TASTE RECEPTOR – A GPCR FAMILY PROTEIN:

The sense of taste gives animals, the ability to evaluate what they should eat and drink. At the most basic level, this evaluation is to promote ingestion of nutritious substances and prevent consumption of potential poisons or toxins.
There is no doubt that animals, including humans, develop taste preferences. That is, they choose certain types of food in preference to others. Interestingly, taste preference often changes in conjunction with the needs of body. Similarly, animals often develop food aversions, particularly if they become ill soon after eating a particular dish, without any correlation between the food and the disease. Food preferences and aversions involve the sense of taste, but these phenomena are almost certainly mediated through the central nervous system (Scott and Timothy, 1999).

Taste receptor (TR) cells may selectively reside in various parts of the tongue and respond to different tastants and perceive taste modalities. TR cells are organized into taste buds that extend into different papillae in the tongue epithelium. Higher vertebrates are believed to possess at least five basic tastes: sweet, bitter, sour, salty and umami (Lloyd, 1996). Contrary to popular understanding that different tastes map to different areas of the tongue, taste qualities are found in all areas of the tongue (Huang et al., 2006; Margolskee, 2002). The original tongue map based on a mistranslation by Harvard psychologist Edward Boring (Hanig, 1901).

Sensitivity to all tastes occurs across the whole tongue and indeed to other regions of the mouth where there are taste buds (Collings, 1974). Circumvallated papillae found at the very back of the tongue contain hundreds to thousands of taste buds and are particularly sensitive to bitter substances. Foliate papillae found at the posterior lateral edge of the tongue, contain dozens to hundreds of taste buds and are sensitive to sour and bitter. Fungi form papillae found at the front of the tongue contain a few taste buds and specialize in sweet taste. These receptor molecules provide the unique specificity and selectivity of each sensory system. The colour receptors in our retinas allow us to see in colour and the olfactory receptors in our nose endow us with great olfactory discrimination. In the case of taste, they aid in responding of sweet cells to sweet substances, bitter cells to bitter compounds and so on. Mammals depend on their sense of taste for their very survival.
The taste receptors were also found on the palate, epiglottis, larynx, pharynx and oesophagus. The taste receptors that are localised in intestinal endocrine cells secrete a variety of gut hormones that control gut motility, nutrient absorption and metabolism.

For many years, it was believed that sweet taste sensation was likely to be mediated by specialised GPCRs found in the taste buds (Hoon et al., 1999). Sweet and umami taste involve T1R receptors, which belong to the subclass 3 of the GPCR super family and are distantly related to the metabotropic glutamate and calcium sensing receptors (Hoon et al., 1999; Jiang et al., 2004; Li et al., 2002). They have a large N-terminal extracellular domain that is linked by a cysteine-rich domain to the seven transmembrane domains (Jiang et al., 2004).

The receptors underlying sweet taste have been identified based on earlier genetic mapping of sweet taste response loci (Ninomiya et al., 1991; Bachmanov, 1997; Blizzard et al., 1999; Li, 2001) and recent data mining of human and mouse DNA sequence data bases (Max et al., 2001; Montmayeur et al., 2001). It has been known for some three decades that inbred strains of mice such as C57BL/6 and DBA/2 differ markedly in their ingestive responses to solutions containing the artificial sweetener saccharin (Capretta, 1970; Pelz et al., 1973).

The murine loci for sac (determines preference and electrophysiological responsiveness to saccharin, sucrose and other sweeteners) and dpa (determines preference and responsiveness to D-phenylalanine) were known to be the major genetic factors that determine differences between sweet-preferring and sweet-indifferent strains of mice (Lush et al., 1995; Fuller, 1974; Lush, 1989; Capeless and Whitney, 1995).

Two candidate taste receptors, called T1R1 and T1R2, were cloned and found to be selectively expressed in distinct taste bud regions, but neither of these receptors was found to be responsive to sweet stimuli when expressed in heterologous cells. In parallel studies, a number of labs studied the genetics of Sac mice; a strain that exhibits reduced sensitivity to sweet stimuli such as...
saccharin and sucrose. A gene in the *Sac* locus encodes a GPCR with extensive homology to T1R1 and T1R2. As with T1R1 and T1R2, new receptor termed T1R3 was also found to be not activated by sweet stimuli when expressed by it in heterologous cells. However, when T1R3 was co-expressed with T1R2, this resulted in the formation of receptors that were robustly activated by saccharin, sucrose and other sweet tastants. These findings suggest that heterodimerization of T1R2 and T1R3 is required for the formation of functional sweet taste receptors. If T1R3 is co-expressed with T1R1 instead of T1R2, this results in the formation of receptors preferentially responsive to amino acids instead of sweet stimuli (Steven et al., 2005).

**Sweet transduction:**

The sensations of bitter and sweet tastes are initiated by the interaction of tastants with GPCRs in the apical membranes of taste receptor cells (Margolskee, 2002). Transduction of sweet taste signals across plasma membranes in numerous cell types may also be induced by pathways involving a variety of lipid-derived second messengers produced from membrane phospholipids.

1. The phosphatidylinositol system activates a G-protein or a tyrosine kinase, which subsequently activates specific isoforms of phospholipase C resulting in the hydrolysis of the phospholipid phosphatidyl-inositol-4, 5-biphosphate (PIP2). The hydrolysis of PIP2 generates two products, the sugar phosphate inositol-triphosphate (IP3) as well as diacylglycerol (DAG). IP3 and DAG influence intracellular functioning and can lead to the subsequent depolarization of a cell. IP3 binds to a receptor on the endoplasmic reticulum which triggers the mobilization of calcium from intracellular stores. Calcium in concert with DAG activates protein kinase C, and protein kinase C phosphorylates key proteins that regulate the response of the target cell to the ligand. Calcium may also activate other cellular enzymes as well as ion channels. DAG can also be derived from membrane phospholipids in addition to PIP2. For example DAG can be produced directly from phosphatidylcholine (PC) by phospholipase C or produced indirectly from PC via another phospholipase called phospholipase D. Calcium mobilization
occurs in the phosphatidylinositol system but has not been shown in PC pathways.

Three modulators of lipid derived second messenger systems alter sweet taste responses: two membrane permeable analogs of DAG, 1-oleoyl-2-acetyl glycerol (OAG) and dioctanoyl glycerol (DiC8) as well as thapsigargin, which release calcium from intracellular stores (Schiffman et al., 1983).

2. The amiloride-sensitive sodium channels (Desimone et al., 1984; Simon et al., 1989; Naim et al., 1994) also appear to play a role in sweet taste transduction. Naim et al., (1994) found that several amphiphilic sweeteners stimulate cellular events through direct activation of G-proteins rather than by binding to receptors on the cell surface is intriguing. Several amphiphilic sweeteners were found to activate transducin and G-proteins. Na saccharin, neohesperidin dihydrochalcone, aspartame, Na cyclamate, and monellin significantly stimulated the GTPase activity of G-proteins. The concentrations of sweeteners required to activate G-proteins in vitro were highly correlated with concentrations that elicit taste in humans.

3. The adenylate cyclase system: The search for taste receptors that bind sweeteners has not been as successful as the pursuit of neurotransmitter and hormone receptors. In fact, no receptor for sweet taste has yet been isolated and characterised. The low affinity of tastants for receptors as well as the physiology of the gustatory system limits isolation of sweet taste receptors. Although the isolation of sweetener receptors has proven difficult, current consensus is that the sweet taste response is mediated by taste cell surface receptors that utilize the adenylate cyclase system as a second messenger system. The adenylate cyclase system, which is also the cellular signaling system for many hormones, involves the cascade of events. The sweetener molecule (agonist) binds to a receptor, which transmits a signal via the guanine nucleotide-binding protein resulting in activation of adenylate cyclase. It then induces hydrolysis of ATP to cAMP which leads to activation of the phosphorylating enzyme known as protein kinase A. The activated kinase then phosphorylates an ion channel in the taste cell membrane leading to depolarization of the taste cell. The validity of the model for sweet taste
transduction is supported by biochemical investigations that have established the existence and activation of components of the adenylate system in taste buds including adenylate cyclase (Kurihara and Koyama, 1972; Nomura, 1978; Striem et al., 1989; Naim et al., 1991; Striem et al., 1991). Certain sweeteners including sucrose and saccharin cause stimulation in adenylate cyclase activity leading to elevated levels of cAMP (Striem et al., 1991).

GLUCOSE TRANSPORTERS (GLUTs):

The transport of glucose across the plasma membrane of mammalian cells represents one of the most important cellular nutrient transport events, since glucose plays a central role in cellular homeostasis and metabolism. It has long been established that the plasma membranes of virtually all mammalian cells possess a transport system for glucose of the facilitative diffusion type; these transporters allow the movement of glucose across the plasma membrane down its chemical gradient either into or out of cells. These transporters are specific for the D-enantiomer of glucose and are not coupled to any energy-requiring components, such as ATP hydrolysis (Baldwin and Lienhard, 1981). The facilitative glucose transporters are called GLUT proteins, distinct from the Na+-dependent transporters, which actively accumulate glucose (Hediger et al., 1987; Bell et al., 1990; Gwyn and Geoffrey 1993).

Members of the GLUT protein family that belong to a much larger superfamily of 12-transmembrane segment transporters mediate facilitative glucose transport. The human GLUT family consists of 14 members, of which 11 have been shown to catalyze sugar transport. The individual isotypes exhibit different substrate specificity, kinetic characteristics and expression profiles thereby allowing a tissue-specific adaptation of glucose uptake through regulation of their gene expression as shown in table 2 (Joost et al., 2004).

GLUT isoforms differ in their tissue expression, substrate specificity and kinetic characteristics. GLUT1 mediates glucose transport into red cells, and throughout the blood brain barrier, and supply glucose to most cells (Mueckler et al., 1985). GLUT2 is a high \(K_m\) isoform expressed in hepatocytes, pancreatic beta
Table 2: GLUT proteins of homosapiens and their location
(Joost et al., 2004)

<table>
<thead>
<tr>
<th>Protein</th>
<th>Other Names</th>
<th>Gene Name</th>
<th>Chromosome Localization</th>
<th>Major Tissue Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLUT1</td>
<td>GTR1</td>
<td>SLC2A1</td>
<td>1p35-31.3</td>
<td>Erythrocytes, brain</td>
</tr>
<tr>
<td>GLUT2</td>
<td>GTR2</td>
<td>SLC2A2</td>
<td>3q26.2-27</td>
<td>Liver, islets</td>
</tr>
<tr>
<td>GLUT3</td>
<td>GTR3</td>
<td>SLC2A3</td>
<td>12p13.3</td>
<td>Brain (neuronal)</td>
</tr>
<tr>
<td>GLUT4</td>
<td>GTR4</td>
<td>SLC2A4</td>
<td>17p13</td>
<td>Muscle, fat, heart</td>
</tr>
<tr>
<td>GLUT5</td>
<td>GTR5</td>
<td>SLC2A5</td>
<td>1p36.2</td>
<td>Intestine, testis, kidney</td>
</tr>
<tr>
<td>GLUT6</td>
<td>GTR6</td>
<td>SLC2A6</td>
<td>9q34</td>
<td>Spleen, leukocytes, brain</td>
</tr>
<tr>
<td>GLUT7</td>
<td>GTR7</td>
<td>SLC2A7</td>
<td>1p36.2</td>
<td>Liver</td>
</tr>
<tr>
<td>GLUT8</td>
<td>GTR8, GLUTX1</td>
<td>SLC2A8</td>
<td>9</td>
<td>Testis, blastocyst, brain</td>
</tr>
<tr>
<td>GLUT9</td>
<td>GTR9, GLUTX</td>
<td>SLC2A9</td>
<td>4p15.3-16</td>
<td>Liver, kidney</td>
</tr>
<tr>
<td>GLUT10</td>
<td>GTR10</td>
<td>SLC2A10</td>
<td>20q12-13.1</td>
<td>Liver, pancreas</td>
</tr>
<tr>
<td>GLUT11</td>
<td>GTR11, GLUT10</td>
<td>SLC2A11</td>
<td>22q11.2</td>
<td>Heart, muscle</td>
</tr>
<tr>
<td>GLUT12</td>
<td>GTR12</td>
<td>SLC2A12</td>
<td>6q23.2</td>
<td>Heart, prostate</td>
</tr>
<tr>
<td>GLUT13</td>
<td>HMIT</td>
<td>SLC2A13</td>
<td>–</td>
<td>Brain</td>
</tr>
</tbody>
</table>

Cells and the basolateral membranes of intestinal and renal epithelial cells. It acts as a high-capacity transport system to allow the uninhibited (non-rate limiting) flux of glucose into or out of these cell types (Fukamoto et al., 1989). GLUT3 is a low Km isoform responsible for glucose uptake into neurons (Kayano et al., 1988). GLUT4 is expressed exclusively in the insulin-sensitive tissues, fat and muscle. It is responsible for increased glucose disposal into these tissues in the postprandial state and is important in whole-body glucose homeostasis (Fukamoto et al., 1989).

GLUT5 is a fructose transporter that is abundant in spermatozoa and the apical membrane of intestinal cells. Since the transport of glucose from the lumen into the epithelial cells is, under normal circumstances, mediated predominantly
by the unrelated sodium dependent glucose transporter (Hopfer and Johnson, 1987). The presence of a putative facilitative glucose transporter in the brush border is not easily explained. The explanation for the presence of GLUT5 in the brush border has been provided by the recent demonstration that GLUT5 is a high-affinity fructose transporter, with an apparently poor ability to transport glucose (Burant et al., 1992). Thus, on the luminal surface of the small intestine, the primary role of GLUT5 will be the uptake of dietary fructose (Kayano et al., 1990). Glut-6 is highly expressed in brain, spleen, and peripheral leukocytes (Doege et al., 2000).

GLUT7, expressed in liver and other gluconeogenic tissues, mediates glucose flux across endoplasmic reticulum membrane (Waddell et al., 1992). It is the transporter, which allows the flux of free glucose out of the lumen of this organelle after the action of glucose-6-phosphatase on glucose-6-phosphate (Mueckler et al., 1985). GLUT8 is found in adult testis and placenta (Carayannopoulos et al., 2000). Human GLUT9 is expressed in spleen, peripheral leukocytes and brain (Phay et al., 2000). Human GLUT10 has been identified as a candidate gene for NIDDM susceptibility. It is widely expressed with highest levels in liver and pancreas (McVie-Wylie et al., 2001). GLUT11 is expressed in heart and skeletal muscle (Doege et al., 2001). GLUT12 is expressed in skeletal muscle, adipose tissue, and small intestine (Roche et al., 2002). GLUT13 or proton myo-inositol transporter is highly expressed in glial cells and some neurons (Uldry et al., 2001).

Some isotypes (e.g., GLUT2) seem to be involved in the mechanisms of glucose sensing of pancreatic β-cells, neuronal or other cells, thereby playing a major role in the hormonal and neural control. Targeted disruption in mice has helped to elucidate the physiologic function of some isotypes (GLUT1, GLUT2, and GLUT4). Furthermore, several congenital defects of sugar metabolism are caused by aberrant transporter genes. In addition, a malfunction of glucose transporter expression or regulation appears to contribute to the insulin resistance syndrome (Scheepers et al., 2004).
Glucose transporters general structure:

Analysis of the predicted amino acid sequences of the mammalian glucose transporters showed that these are highly homologous with one another. The mammalian transporters possessed high levels of sequence identity with transporters found in many species including *Cyanobacteria* (Zhang et al., 1989), *Escherichia coli* (Maiden et al., 1988), *Zymomonas mobilis* (Barnell et al., 1990), yeast (Szkutnicka et al., 1989), algae (Sauer and Tanner, 1989), protozoa (Cairns et al., 1989) and plants (Sauer et al., 1990). This high level of sequence similarity is probably related both to a common mechanism of transport catalysis and to the transport of a common type of substrate (Maiden et al., 1988). Similarly, the range of preferred substrates includes hexoses, pentoses (Maiden et al., 1988) and disaccharides (Szkutnicka et al., 1989). The common features revealed by sequence alignment and analysis of all the above-mentioned transporters include 12 predicted amphipathic helices arranged so that both the N- and C- termini are at the cytoplasmic surface. There are large loops between helices 1 and 2 and between helices 6 and 7. The large loop between helices 6 and 7 divides the structure into two halves, the N-terminal domain and the C-terminal domain.

Infrared spectroscopy has suggested a high (over 80%) helical content for the GLUT1 protein (Chin et al., 1986; Alvarez et al., 1987). Conserved motifs in the glucose transporters include GRR (K) between helices 1 and 2 in the N-terminal half, and correspondingly between helices 7 and 8 in the C-terminal half. Similarly, EXXXXXXR occurs between helices 4 and 5 in the N-terminal half and correspondingly between helices 10 and 11 in the C-terminal half. These motifs may be conserved to maintain conformational stability of the protein and may be involved in salt bridging between helices. The repetition of these motifs between the two halves of the protein suggests that duplication of a gene encoding an ancestral six membrane spanning helical protein may have produced the two-domain 12-membrane spanning helical structure that is so highly conserved in the sugar transporter family. The constraints imposed by the short cytoplasmic loops suggest that a single group of 12 helices is unlikely, but instead the six helices in each of the N- and C-terminal domains may be
separately closely packed to produce a bilobular structure (Li, 1987). This packing arrangement has been incorporated into a molecular model of the hexose transporter GLUT1 (Hodgson et al., 1992).

Earlier studies suggested that most of the highly conserved residues in helical regions occur on the faces of helices that are directed to the centre of the protein and away from the membrane lipid. Conserved regions of particular interest occur in the C-terminal half of the protein and may be involved in ligand recognition. The motif QQXSGXNXXY in helix 7 is present in all the mammalian transporters and is highly conserved in all members of the wider glucose transporter superfamily (Hashirmoto et al., 1992). Residues QLS are highly conserved in the transporters (GLUT1, GLUT3 and GLUT4) which accept D-glucose with high affinity, but not in the transporters (GLUT2 and GLUT5) or the Zymomonas mobilis (Barnell et al., 1990) or Trypanosoma (Bringaud and Baltz, 1992; Fry et al., 1993) transporters which accept D-fructose. Molecular modelling and molecular dynamics studies suggest that prolines 383 and 385 are particularly important in facilitating an alternate opening and closing of the external site of these transporters (Hodgson, 1992). Ligand binding and labelling studies suggest some structural separation of external and internal binding sites (Davies et al., 1991; Schurmann et al., 1992).

Site-directed mutagenesis has already revealed some important features required for transport by GLUT1. Truncation of the C-terminal region results in a mutated transporter that is locked in an inward-facing conformation that has low affinity for exofacial ligands such as the photolabel ATB-BMPA (2-N-[4-(1-azido-2,2,2-trifluoroethyl)benzoyl]-1,3-bis-(D-mannos-4-yloxy)propyl-2-amine) results in a large reduction in sugar transport activity (Oka et al., 1990). Mutation of Gln 282 in transmembrane helix 7 also results in the complete loss of exofacial binding of ATB-BMPA, but in this case the mutation only results in a 50% reduction in sugar transport activity and the binding of the inside-specific binding ligand cytochalasin B (Hashirmoto et al., 1992). Mutation of the conserved Trp 412 in helix 11 of GLUT1 results in reduced transport activity but no loss of binding of cytochalasin B (Katagiri et al., 1991) or IAPS-forskolin (3-iodo-4-
azidophenethylamido-7-O-succinyl-deacetyl-forskolin) (Schumann et al., 1992). Mutation of Trp 388 of GLUT1 expressed in Chinese hamster ovary cells results in reduced transport and labelling with forskolin (Schumann et al., 1992). However, when this mutant was expressed in oocytes, it failed to insert correctly in the oocyte plasma membrane (Garica et al., 1992). Consistent with the proposed important role of Pro 385 in the mode of operation of the transporter, it has been observed that mutating this Pro 385 to isoleucine in GLUT1 markedly reduces glucose transport activity and ATB-BMPA labelling, but not cytochalasin B labelling (Tamori et al., 1993). Replacement of the C-terminal domain of GLUT1 with that of GLUT2 renders the mutated transporter with transport kinetics more like those of GLUT2 than GLUT1, but cytochalasin B binding, which is normally lower for GLUT2 than for GLUT1, remains unaffected (Katagiri et al., 1992).

GLYCOGEN PHOSPHORYLASE:

Excessive hepatic glucose production is a significant contributor to diabetic hyperglycemia. The liver produces glucose by two pathways, gluconeogenesis and glycogenolysis (breakdown of glycogen by phosphorylase, EC 2.4.1.1). The liver is the major regulator of plasma glucose levels in the postabsorptive state, and in type 2 diabetics. hepatic glucose production is significantly elevated relative to nondiabetics (Consoli, 1992; Gerich, 1992). In the postprandial state, where the liver has a proportionately smaller role in supplying glucose, the normal suppression of hepatic glucose production is not observed in type 2 diabetics (Gerich, 1992). The relative contribution of each to net hepatic glucose production in normal and diseased states has been difficult to quantitate (Rothman et al., 1991; Barrett and Liu, 1993; Pimenta et al., 1994), yet type 2 diabetics have been reported to display elevated gluconeogenic rates (Consoli, 1992; Landau et al., 1996). Attempts to modulate hepatic glucose production with gluconeogenesis inhibitors have yielded mixed results. Agents that suppress gluconeogenesis in vitro or in diabetic rodents by reducing gluconeogenic substrate availability or fatty acid metabolism have generally not been clinically efficacious or safe in humans (Bressler, 1992). With the exception
of metformin, an antidiabetic agent with multiple effects including gluconeogenesis inhibition, most inhibitors have failed to reduce hepatic glucose production and plasma glucose levels in humans caused by hepatic autoregulation, a compensatory increase in hepatic glycogenolysis that maintains a high rate of hepatic glucose production.

Glycogen phosphorylase (GP), the main regulatory enzyme in the liver responsible for the control of blood glucose levels by regulating glycogen degradation (Newgard et al., 1989; Schnier et al., 2003). One of several approaches to influence the action of GP is the use of glucose derivatives as active site inhibitors. This field of research commenced 10-15 years ago and, due to joint efforts in computer aided molecular design, organic synthesis, protein crystallography, and biological assays, resulted in glucopyranosylidene-spirohydantoins, N'-(β-D glucopyranosyl) ureas as the most efficient inhibitors of GP (Somsak et al., 2003).

The enzyme exists in two interconvertible forms: the dephosphorylated form, GPb (low activity, low substrate affinity), and the Ser 14-phosphorylated form, GPa (high activity, high substrate affinity) (Johnson et al., 1994). GP is controlled by phosphorylation and allosteric effectors (Oikonomakos et al., 1992). Allosteric activators, such as AMP or inhibitors such as ATP, glucose-6-P, glucose, and caffeine can alter the equilibrium between a less active T state and a more active R state or vice versa. The structures of T and R state GP have been characterized (Johnson and Kornfeld, 1992; Oikonomakos et al., 2000). GP contains at least 5 potential regulatory sites: the Ser 14-phosphate recognition site, the allosteric site that binds AMP, IMP, ATP, and glucose-6-phosphate, the catalytic site that binds the substrates glycogen and glucose-1-phosphate, and also glucose and glucose analogues, the inhibitor site, which binds caffeine and related compounds, the glycogen storage site (Oikonomakos et al., 2000). The stereochemical studies of Oikonomakos et al., (2000) showed that flavopiridol inhibited GP by binding to inhibitor site, the site where caffeine binds, located 10 Å from the catalytic site. The detailed interactions of flavopiridol with the protein provided a structural explanation for understanding the molecular basis of its high
affinity for GP and showed that the T state conformation is stabilized by flavopiridol as do caffeine and glucose (Oikonomakos et al., 2000).

Glycogenolysis inhibition could improve glycemic control. This proposal is based on patients with hepatic glycogen storage diseases, where episodic hypoglycemia is observed (Hers, 1959). Glucose production from the catalysis of glycogen to glucose-1-phosphate is rate-limited by phosphorylase a, well-studied enzyme that is regulated by multiple covalent, substrate, and allosteric effectors (Newgard et al., 1989). Inhibition of GP has been shown to lower blood glucose levels in an animal model of type 2 diabetes, supporting the hypothesis that inhibition of GP could be a potential pharmacological target for lowering hyperglycemia in patients with type 2 diabetes (Treadway et al., 2001; Oikonomakos, 2002).

**Structure of GP:**

Mammalian GPs are found in at least three isozymic forms. They are liver, muscle and brain, which can be distinguished by functional and structural properties as well as by the tissues in which they are preferentially expressed. The protein sequence deduced from the nucleotide sequence of the brain phosphorylase cDNA is 862 amino acids long compared with 846 and 841 amino acids for the liver and muscle isozymes, respectively. The greater length of brain phosphorylase is entirely due to an extension at the far C-terminal portion of the protein. The muscle and brain isozymes share greater identity with regard to nucleotide and deduced amino acid sequences, codon usage, and nucleotide composition with the liver's sequence, suggesting a closer evolutionary relationship between them (Newgard et al., 1989).

Although the activator AMP was required to bring the solution of GP to crystallization concentration, crystals were obtained only when the inhibitor glucose was also present in the experiment. Overall, the phosphorylated protein with glucose bound in the active site and AMP bound in the allosteric site as shown in the fig.4. It adopts a conformation that represents an intermediate in the transition between the T- and R-states. Glucose bound at the active site results in much of the protein adopting a T-state conformation, including the overall dimer
relationship. However, features such as the ordering of the N-terminus, the binding of AMP in the allosteric site, and the dimeric contacts at the interface near this site are consistent with the R-state conformation. In addition, the 313–325 loop is ordered around the bound AMP in a conformation only previously reported for AMP activated (R-state) GPb with pyridoxal pyrophosphate bound in place of the natural cofactor PLP (Sprang et al., 1991), resulting in a large and very specific set of contacts between AMP and the enzyme. The combination of AMP binding and phosphorylation of Ser 14 stabilizes the N-terminus and the cap-α-2 helix interface, creating a stable, local R-state conformation in this portion of the protein that prevents the completion of the transition to T-state initiated by glucose binding (Lukacs et al., 2006).

The corresponding structure of human liver GPa with both AMP and glucose bound has also been solved (1FA9) (Rath et al., 2000). They reported that the addition of AMP to liver GPa results in crystals that are of the R-state, even when glucose is included in the crystallization setup. Ser14 phosphorylation and the presence of AMP set conformation of the liver enzyme and it is not affected by glucose. In contrast, in the human muscle enzyme, the presence of AMP and phosphorylated Ser 14 are unable to prevent glucose from binding and initiating the conversion to T-state. AMP can bind to the muscle enzyme even if glucose is bound. The transition to R-state begins, but is stalled until glucose is released, that is, the ease of transition to R-state upon the drop in glucose levels is enhanced.

The implications that this has for drug development are very promising, since antidiabetic drugs should target liver GP. Because there are no sequence differences in the AMP binding site between the liver and muscle isoforms that can be exploited to gain selectivity, one can take advantage of kinetic differences instead. 5-chloroindole based compounds, which bind at the dimer interface, show two- to threefold lower activity against muscle enzyme than liver enzyme (Hoover et al., 1998; Martin et al., 1998). It has been shown via crystallography that these compounds bind to and stabilize the T-state of GP (Rath et al., 2000; Oikonomakos et al., 2000).
The muscle isoform of human GP has structural differences that are consistent with the long-known kinetic differences between the liver and muscle enzymes. The activator AMP can bind the muscle enzyme even when the binding of glucose locks it in the T-state conformation. Local changes around the AMP site prime the enzyme for the transition to the R-state, activating it in a way that does not happen in the liver isoform. The implications for the enzyme as a target in drug discovery continue to be promising (Lukacs et al., 2006).

SWEETENERS:

Which add the basic taste of sweetness to a food is a sweetener. Sweetness is one of the five basic tastes and is almost universally regarded as a pleasurable experience. Great divers chemical compounds exhibit sweet taste. Among common biological substances, all of the simple carbohydrates are sweet to at least some degree. Sucrose is the prototypical example of a sweet substance, although another sugar, fructose, is some what sweeter. Some of the amino acids are mildly sweet and some are sweetest; some other amino acids are perceived as both sweet and bitter. A number of plant species produce glycosides that are many times sweeter than sugar. The most well-known example is glycyrrhizin, the sweet component of licorice root, which is about 30 times sweeter than sucrose. Another commercially important example is stevioside, from the South American shrub Stevia rebaudiana. It is roughly 250 times sweeter than sucrose. Acesulfame potassium (Acesulfame K) is 180-200 times sweeter than sucrose, as sweet as aspartame, about half as sweet as saccharin and one-quarter the sweetness of sucralose. The artificial sweeteners that are in widespread use: saccharin, cyclamate, aspartame, acesulfame K, sucralose, neohesperidin dihydrochalcone, stevioside and rebaudioside A (Dyrskog et al., 2005). As with sucralose, aspartame, stevia, saccharin and other sweeteners that are sweeter than common sugars, there is concern over the safety of acesulfame K (Schiffman et al., 1987).
Figure 4: The overall fold of human muscle glycogen phosphorylase a. As there was one molecule in the asymmetric unit, the active dimer was generated using a symmetry related molecule. Glucose, AMP, Pyridoxal phosphate, P-Ser 14, and adenine are shown and labeled. The positions of amino acids that differ between the human and rabbit muscle enzyme are shown in the blue monomer as yellow spheres. Figures were made with MOLSCRIPT35 and Raster3D.36 (b) Stereoview of the interactions between human muscle GPa and AMP. These interactions include extensive water-mediated contacts from the phosphate groups, several direct hydrogen bonds between the adenine and the AMP binding loop, and one hydrogen bond between the ribose and the cap region of the dimeric molecule. Van der Waals contacts from the adenine include an edge-to-face interaction with Phe 316 and a stacking interaction with Tyr 75. 2Fo-Fc density for the AMP. (c) Superposition of human muscle glycogen phosphorylase a (yellow) with human liver GPa with AMP bound (1FA96) in orange and rabbit muscle GPb with AMP bound (7GPB27) in cyan, highlighting the difference in the allosteric site loop surrounding the AMP. Phe 316 is shown in for the viewer’s clarification (Lukacs et al., 2006).
INHIBITORS OF TASTE RECEPTORS:

+2-(4-methoxyphenoxy) propanoic acid (PMP) - Substituted phenol-xyalkanoic acid compounds have been reported to block sweet taste (Lindley, 1991; Johnson et al., 1994). The sodium salt of PMP (Na-PMP or lactisole) has been found to selectively block the sweetness intensity for 12 of 15 sweeteners at both 250 ppm and 500 ppm levels (Schiffman et al., 1995). These include 3 sugars (fructose, glucose, sucrose), 2 terpenoid glycosides (rebaudioside A, stevioside), 2 dipeptide derivatives (alitame, aspartame), 2 N-sulfonylamides (acesulfame K, sodium saccharin), 2 polyhydric alcohols (mannitol, sorbitol), and 1 sulfamate (sodium cyclamate). However, when the same concentrations of PMP were mixed with 3 of the 15 sweeteners (monoammonium glycyrrhizinate, neohesperidin dihydrochalcone, and thaumatin), there was little reduction in sweetness intensity. These data suggest that PMP is a selective competitive inhibitor of sweet taste and provide evidence for multiple sweet receptor types. Interestingly, PMP is almost tasteless (Johnson et al., 1994). PMP suppresses the sweet taste of various compounds in humans but not in rats (Li et al., 2002; Johnson et al., 1994; Kawai et al., 2002).

Tannic acid - The astringent compound tannic acid inhibits the intensity of a variety of sweet compounds to varying degrees providing further support for multiple sweet receptors. In mixtures with tannic acid, the greatest suppression was found for acesulfame K, Na saccharin, rebaudioside A, and stevioside; the least adaptation occurred with the sugars, polyhydric alcohols, and neohesperidin dihydrochalcone (Schiffman et al., 1994).

Sweetener derivatives - Several sweetener derivatives have been found to block the taste of sucrose when the inhibitor was combined with the sweetener. These include: chloramphenicol (Vlahopoulos and Jakinovich, 1986; Jakinovich, 1983; Schiffman et al., 1987) and N-(4-cyanophenyl)-N’- (sodiosulfo) methylurea (Muller et al., 1992). With chloramphenicol sodium saccharin was reduced by 21%, acesulfame K was reduced by 30%, sucrose was reduced by 32% and stevioside by 54%. Aqueous solutions of N- (4-cyanophenyl)-N’- (sodiosulfo) methylurea and sweeteners also show a range of suppression for
different sweeteners. Monoammonium glycyrrhizzate was inhibited by 24%; thaumatin by 30%; Na cyclamate by 40%; neohesperidin dihydrochalcone by 58%; aspartame by 61%; sucrose by 66%; acesulfame K by 82%; and sucralose by 83% (Schiffman et al., 1997).

**Thapsigargin** - Structurally, thapsigargin (a calcium ionophore) is classified as a sesquiterpene lactone, and is extracted from a plant, *Thapsia garganica*. It suppressed several sweet taste responses (Schiffman et al., 1995; Trubey et al., 2006).

**Amiloride** - The diuretic amiloride, a potent inhibitor of sodium transport, blocks the tastes of both sweet and salty (sodium salts) stimuli (Schiffman et al., 1983; DeSimone et al., 1984) depending on the species. Pretreatment of the human tongue with 500 mM amiloride reduced the intensity of all ten sweeteners tested but to varying degrees (Schiffman et al., 1983). The greatest suppression was for stevioside, which was blocked by 81%; fructose was the least affected and was reduced by 44%. These data showing differences in degree of suppression are consistent with multiple sweet receptors. Mixtures of amiloride and sucrose also reduced sweet responses (Simon et al., 1989).

**Gymnemic acid (GA)** - It is a mixture of triterpene glucuronides, which was found in the leaves of the Indian vine *Gymnema sylvestre*. GA was discovered in 1847 to temporarily reduce or abolish the sweet taste of sugar in humans (Hellekant and Gopal, 1976). Because GA has a specific (Bartoshuk et al., 1969; Oakley, 1985) and profound (Frank et al., 1992) effect on the sweetness of sugars and other sweet substances, the application of GA serves as a useful model of dissociated taste loss (Tomita and Horikawa, 1986). It shows reductions in sweet taste that recover with time but no reductions in bitterness, saltiness or sourness at any time following exposure to a wide range of GA concentrations (Suttisri et al., 1995).

Kurihara (1969) found that GA suppressed the taste of sucrose, Na Cyclamate, D- tryptophan, D-leucine, beryllium chloride, and lead acetate in
humans. However, it did not suppress the sweet taste of chloroform. Faurion et al. (1980) found that the degree of suppression of various sweeteners varied with chemical structure, and like Kurihara (1969), they found little suppression of the sweetness of chloroform. Hellekant and Gopal (1976) reported species differences in the effect of GA, further suggesting multiple sweet receptor types. They found that GA suppressed the taste of sucrose in hamsters with no effect on saccharin. The opposite was found in rats, i.e. there was suppression of saccharin with no effect on sucrose. In general, Hellekant and Roberts (1983) did not find any dramatic decrease of responses to either sweet or non-sweet substance in hamster after treatment with GA. In chimpanzee, Hellekant et al. (1996), like Faurion et al., found that the degree of suppression of the sweet response varied with the structure of the sweetener. GA completely abolished responses to acesulfame-K, aspartame, D-tryptophan, monellin, and thaumatin. However, there was 75% suppression of sucrose and only 50% suppression of xylitol.

GA was reported to suppress the taste of sucrose, Na Cyclamate, D-tryptophan, D-leucine, beryllium chloride, and lead acetate in humans (Kurihara, 1969; Hellekant and Gopal, 1976). The opposite was found in rats, i.e. there was suppression of saccharin with no effect on sucrose. In chimpanzee, Hellekant et al., (1996) found that the degree of suppression of the sweet response varied with the structure of the sweetener. GA completely abolished responses to acesulfame-K, aspartame, D-tryptophan, monellin, and thaumatin. However, there was 75% suppression of sucrose and only 50% suppression of xylitol. There is disagreement whether GA is an inhibitor for taste qualities other than sweetness (Diamant et al., 1965; Bartoshuk et al., 1969; Kurihara et al., 1969; Warren et al., 1969; Meiselman and Halpem, 1970).

It exists in 9 isoforms. Of these GA I, II, III and IV are anti-sweet substances from the leaves of Gymnema sylvestris. They contain a glucuronic acid moiety and the gymnemagenin aglycone esterified at position C-21 and C-28. Gymnemic acid is known to inhibit the intestinal absorption of glucose in human and rats (Liu et al., 1992).
INHIBITORS OF GLUCOSE ABSORPTION:

The flavonoid quercetin, a food component with an excellent pharmacology safety profile, might act as a potent luminal inhibitor of sugar absorption independent of its own transport, flavonols show promise as new pharmacologic agents in the obesity epidemic (Oran et al., 2007).

A recent multicenter, placebo-controlled randomized trial revealed that acarbose and voglibiose, an alpha-glucosidase inhibitor, improved postprandial hyperglycemia and subsequently reduced the risk of development of type 2 diabetes in-patients with impaired glucose tolerance (Yamagishi et al., 2005).

The gymnemic acid components are believed to block the absorption of glucose in the small intestine, the exact action being unknown. It could be involve one or more mechanisms (Nakamura, 1999). Concomitantly infused GA significantly depressed the increase in the portal immunoreactive gastric inhibitory peptide induced by glucose. These suggested a glucose receptor may interact with the GA, exists for the release of immunoreactive gastric inhibitory peptide and that the glucose receptor for gastric inhibitory peptide release is not likely to be identical with a glucose transporter or a vagal glucoreceptor in the lumen.

INHIBITORS OF GP:

Inhibition of GP has been proposed as a therapeutic strategy for the treatment of hyperglycemia (Aiston et al., 2001; Treadway et al. 2001). Several binding sites on the enzyme such as the catalytic, allosteric, inhibitor, and the new allosteric site have been identified as specific targets for inhibitor binding (Somsak et al. 2001; Treadway et al., 2001; Oikonomakos, 2002; Kurukulasuriya et al., 2003; Somsak et al., 2003). Inhibition of GP, the rate controlling enzyme of glycogenolysis, has been shown to lower blood glucose levels in an animal model of type2 diabetes, supporting the hypothesis that inhibition of GP could be a potential pharmacological target for lowering hyperglycemia in patients with type2 diabetes (Hellerstein et al., 1997).
In some cases detailed X-ray crystallographic studies have revealed key interactions responsible for inhibitor potency and have elucidated the structural mechanisms of enzyme inhibition (Oikonomakos et al., 2002). The allosteric site has lately attracted considerable interest (Zographos et al., 1997; Oikonomakos et al., 2005; Ogawa et al., 2003). Rational inhibitor design efforts have led to synthesis of phenyldiacid analogs (Lu et al., 2003) and phenoxy-phthalates (Kristiansen et al., 2004), which inhibited both the basal and the glucagon-induced glucose production when tested in cultured primary hepatocytes.

**Acyl ureas** - were reported as human liver GP inhibitors, which bind to the allosteric site of the enzyme. Acyl ureas induce conformational changes in the vicinity of the allosteric site. These inhibit GP by direct inhibition of AMP binding and by indirect inhibition of substrate binding (Oikonomakos, 2005).

**Caffeine** - is the most widely consumed central nervous system stimulant. Pretreatment of the tongue with caffeine enhances the taste of some sweeteners including thaumatin, stevioside, sodium saccharin, acesulfame K, neohesperidin dihydrochalcone and D-tryptophan with no effect on other sweeteners such as aspartame, sucrose, fructose and calcium cyclamate (Nehlig et al., 1992; Schiffman et al., 1994). Caffeine ingestion has been shown to decrease the rate of muscle glycogenolysis during moderate to intense aerobic exercise in endurance-trained subjects (Essig et al., 1980; Erickson et al., 1987; Spriet et al., 1992). It has been suggested that the glycogen-sparing effect of caffeine is due to enhanced intramuscular and/or extramuscular fat mobilization and oxidation (Costill et al., 1978; Essig et al., 1980; Spriet et al., 1992).

**Flavopiridol** ((2)-cis-5, 7-dihydroxy-2-(2-chlorophenyl)-8-[4-(3-hydroxy-1-methyl)-piperidinyl]-4H-benzopyran-4-one), a potential antitumor drug has been shown to be an inhibitor of muscle GP and to cause glycogen accumulation (Oikonomakos et al., 2000). The mode of binding of caffeine and flavopiridol is found to be same. GP–caffeine and GP–flavopiridol complex structures reveal the structural basis of the differences in the potencies of the these inhibitors and indicate binding residues in the inhibitor site that can be
exploited to obtain more potent inhibitors (Martin et al., 1991; Watson et al., 1994; Bichard et al., 1995; Krulle et al., 1995; Lundgren et al., 1997; Magda, 2004).

**1, 4-dideoxy-1, 4-d-arabinitol (DAB)** - This Glucose analogue has been shown to be an inhibitor of GPa both *in vitro* and *in vivo* (Andersen et al., 1999; Fosgerau et al., 2001). From a mechanistic point of view, DAB possesses features other than those of glucose with respect to GPa inhibition (Fosgerau et al., 2001).

**Ingliforib** - One of the inhibitors of GP is ingliforib (5-Chloro-N-\{(1S, 2R)-3-\{(3R, 4S)-3, 4-dihydroxy-1-pyrrolidinyl\}-2-hydroxy-3-oxo-(phenylmethyl) propyl\} -1H-indole-2-carboxamide) (Hoover et al., 1998; Martin, 1998; Rath, 2000; Hulin, 2001). It inhibits the GP isoforms expressed in the myocardium muscle GP and brain GP (Ross et al., 2004). This GP inhibitor is being investigated for the treatment of diabetes. Ingliforib reduced plasma glucose and lactate in our normal rabbits, and reduces plasma glucose in diabetic models (Hoover et al., 1998).

In recent years, evidence of cases of "insulin resistance" and the occurrence of side effects from prolonged administration of conventional drugs have triggered the search for safe and effective alternatives. From the above aspects of the diseases i.e., obesity, diabetes mellitus it is quite clear that, it is obvious to use same medicine for curbing of both the diseases. During the last two decades, traditional systems of medicine and medicinal plant derivatives research have become topics of global interest and importance. The multifactorial pathogenicity of diabetes and obesity demands multimodal therapeutic approach. Thus, future therapeutic strategies require a drug that acts at different levels with maximum therapeutic efficacy with minimum side effects.