Introduction

Diabetes mellitus is undoubtedly the oldest known endocrine disorder, usually defined as a chronic disturbance of carbohydrate metabolism, represented by hyperglycemia and glycosuria. It was aptly characterized over 1800 years ago by Aretaeus of Cappadocia, who described it as "a moist and cold wasting of flesh and limbs into urine" and called it diabetes from the Greek word for 'siphon'. Over a period of years, the earlier definition of diabetes has been modified, being more widely described as new discoveries flooded the clinical scenario. The term 'diabetes mellitus' now designates a group of diseases characterized by impaired glucose tolerance resulting in a hyperglycemia and glucosuria as a consequence of absolute or relative insulin deficiency. The early manifestations of this disorder include polyuria, polydipsia and hyperphagia, weakness and fatigue and also some impairment of mental functions, including inability to concentrate. The end of this grave illness comes through severe metabolic derangement including ketosis, acidosis and diabetic coma. These disturbances occur because of the basic factor of impaired glucoregulation.

The maintenance of a constant blood glucose concentration results from a dynamic equilibrium of many factors including intestinal absorption, storage, utilization as well as release of glucose from endogenous stores. Endocrine glands and nervous system are two prime stimuli that can influence individual tissues to respond metabolically for the benefit of the whole organism rather than for the tissue per se. Although metabolic controls are not a monopoly of higher animals, the evolution of the mammalian brain with its major dependency on glucose for energy, presumably has necessitated a highly responsive autonomic nervous
system capable of analyzing and integrating information from the internal milieu and rapidly reacting to environmental perturbations by transmission of signals to various fuel compartments for the mobilization of appropriate substrate. Along with the nervous system, the endocrine system assumes the part of the other regulator of the intermediary metabolism by elaborating a wide range of hormonal secretions.

The autonomic nervous system (ANS) regulates the visceral activities through its two functional divisions-the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS). These components are anatomically represented by the splanchnic and vagal innervations to the viscera. In the central nervous system (CNS), it is the hypothalamus that governs the actions of the ANS, it integrates the visceral activities in response to the fluctuating external and internal conditions. The hypothalamic nuclei have autonomic functions, out of which, the ventromedial (VMH) and the lateral hypothalamic (LH) nuclei have reciprocal actions on metabolism, VMH being sympathetic and LH being parasympathetic in nature (Ban, 1966; Frohman and Bernardis, 1971; Oomura, 1973; Shimazu, 1983). The axons emerging from VMH neurons communicate with the splanchnic nerves at the level of the thoracic cord. Fibers from the LH neurons connect with the vagus. These VMH-splanchnic and LH-vagal circuits have metabolic influences on various peripheral tissues. Glucose being the most important metabolic substrate of the CNS, glucose sensing neurons are scattered within the brain (Shimazu, 1981), thus providing for glucose modulation at the hypothalamic level (Rohner-Jeanrenaud, 1983).

The SNS and the PNS have antagonistic effects on the visceral organs that they innervate. They control the metabolic functions and maintain the homeostasis by causing the release of hormones through their innervations to the endocrine glands such as pancreas, adrenal etc. Thus neural signals can contribute to the regulation of the blood glucose level both by having direct effects on tissues that produce or consume glucose and through the control of hormonal secretion.

The pancreatic hormones, insulin and glucagon, are the main agents involved in maintaining the metabolic homeostasis of carbohydrates in the body. Insulin has an anabolic action causing the uptake of glucose from the blood and its deposition in the form of glycogen in the storage organs of the body. Glucagon on the other hand, has an antagonistic
effect. It induces glucose production by activating enzymes of the glycogenolytic and gluconeogenic pathways. An alternate control of insulin and glucagon levels is via direct action of the autonomic nerves (Woods and Porte, 1974). Parasympathetic effects on the pancreas result in elevated insulin levels (Girardier et al., 1976). Splanchnic nerve stimulation in calves raised glucagon induced hyperglycemia and prevented the insulin increase expected with hyperglycemia (Bloom et al., 1973). The regulation of insulin and glucagon release from endocrine pancreas by circulating nutrients, plays an essential role in the hormonal control of fuel homeostasis. An increase in extracellular glucose lowers circulating glucagon levels in man and other animals (Farah, 1983).

The adrenal medulla is an ally of the sympathetic system. The catecholamines, epinephrine and norepinephrine are produced and released by the medulla, as well as by the sympathetic pre and post ganglionic nerve endings. These hormones/neurotransmitters induce hyperglycemia through glucose mobilization from the glycogen stores of the body. Plasma norepinephrine levels are an indicator of sympathetic neural activity, epinephrine on the other hand has been used as a measure of secretion from the adrenal medulla (Coupland, 1965; Axelrod and Weinshilbaum, 1972). In rats it has been demonstrated that the release of norepinephrine and epinephrine from the adrenal medulla is under both neuronal and non neuronal control (Khalil et al., 1986). The importance of catecholamines in the counterregulation of insulin induced hypoglycemia has long been demonstrated in several animal models of sympathectomy (Sachater, 1951; Sacca' et al., 1975; 1977). They are strongly able to antagonize insulin action of glucose disposal (Deibert and Depronzo, 1980; Rizza et al, 1980). Catecholamines cause a significant reduction in plasma insulin levels (Hooper et al., 1994); this reduction was observed while the glucose levels were high, indicating that catecholamines may have a direct suppressive effect on insulin secretion by B cells. Moreover, small increases in the plasma epinephrine level during insulin deficiency can significantly worsen the resulting hyperglycemia (Goldstein et al., 1995). Catecholamines can act directly by increasing cAMP concentration in the cell (Hooper et al., 1994), or indirectly by stimulating an increase in glucagon release, via actions on α-adrenergic receptors. Glucagon in turn may increase intracellular cAMP concentration and cause glucose release (Foster and McGarry, 1992)
Apart from the catecholamines, adrenal cortical steroids, especially glucocorticoids have many actions that promote glucose production in the body by increasing the rate of gluconeogenesis, by releasing glucogenic amino acids from peripheral tissues (Noall et al., 1957). Thus, their catabolic effects on protein are in keeping with general action of these hormones to provide optimal milieu for gluconeogenesis. Glucocorticoids impair the stimulation by insulin of whole body glucose uptake and oxidative and non-oxidative glucose disposal in vivo (Nosadini et al., 1983; Baron et al., 1987; McMahon et al., 1988). They also interact with several aspects of sympatho adrenal system. They increase the synthesis of the enzyme phenylethanolamine N-methyltransferase (PNMT) in several tissues, including skeletal muscle leading to an increased local synthesis of E (Kennedy and Zeigler, 1990, 1991; Kennedy et al., 1993). Furthermore, they enhance the actions of catecholamines by increasing the synthesis and affinity of β- adrenoceptor (Harrison et al., 1968; Davies et al., 1981; Malbon and Hadcock, 1988). Both glucocorticoids and adrenergic agents may increase lipolysis and decrease glucose oxidation by enhancing the activity of the glucose-fatty acid cycle (Randle et al., 1963). Both agents have also been shown to decrease glucose transport (Carter-Su and Okamoto, 1985). Elevated corticosteroid levels have been implicated in diabetic condition (Walker et al., 1989). The hyperglycemic effect is mediated by the action of corticosteroids in mobilizing glucose from its stores. Beside this, glucocorticoids are known to influence binding and action both in vivo and in vitro, by inhibiting insulin binding in a time and dose dependent manner (Montiel et al., 1987). Acute increases in cortisol in conjunction with simultaneous increases in glucagon or epinephrine can have additive or synergistic effects on carbohydrate metabolism.

An important regulator of adrenal cortical secretion is ACTH. It stimulates the growth of the adrenal gland in vivo. An elevated concentration of ACTH causes enlargement of the gland and increases the synthesis and content of DNA in adrenal cortex (Liddle et al., 1962; Masui and Garren, 1970). If other things are equal, the higher the level of steroids, the less ACTH is secreted. If cortisol levels are supraphysiologic, ACTH secretion is suppressed and the adrenal ceases its secretory activity until cortisol levels return to normal. On the contrary, if cortisol levels are subnormal, the anterior pituitary is released from this suppressive influence. ACTH levels rise, and the adrenal secretes cortisol until normal blood levels are restored. In addition to the regulation by ACTH, adrenal corticosteroid release is also augmented by adrenergic mechanisms (Bornstein and Erhart-Bornstein, 1992).
In addition to glucagon, catecholamines and corticosteroids, GH has also been implicated in diabetes mellitus as one of the counterregulatory hormones (Gerich, 1984, 1986; Holly et al., 1988). The principal physiological action of GH is believed to be the anti insulin activity, which results in hyperglycemia, hyperinsulinemia, increased lipolysis, and decreased glucose metabolism (Goodman and Schwartz, 1974; Davidson, 1987). Increased GH levels are involved with insulin resistance, which adversely affects diabetic control (Holly et al., 1988). It has been proposed that hypersecretion of GH may be the cause as much as consequence of poor diabetic control (Press et al., 1984a). Hypoglycemia stimulates GH secretion in humans (Roth et al., 1963a). Early studies with the GH lines of rat pituitary tumor cells showed that glucocorticoid hormones stimulate cellular production of GH (Bancroft, 1981). It has subsequently been shown that glucocorticoids increase cellular levels of GH mRNA in GH cells or rat pituitary in vivo (Nyborg et al., 1985). Conversely, Ivarie et al. (1981) found that high concentration of insulin inhibited glucocorticoid stimulation of rGH synthesis.

The major regulators of metabolic homeostasis are liver, muscle, kidney, and adipose tissue. Much attention has been paid to the liver, muscle and adipose tissue regarding their involvement and importance in maintaining a homeostatic profile of metabolism. The kidney is usually considered to be involved in excretion and reabsorption. But it also plays an important role in blood glucose regulation. Renal contribution to systemic glucose production is 10-20% under normal physiological conditions and 30-35% during starvation (Adrogue, 1992). Both glucose formation and the activity of gluconeogenic enzymes have been shown in renal cortex (Krebs, 1963; Scrutton and Uttar, 1968; Exton, 1972; Wirthensohn, 1979; Guder and Ross, 1984). Rat kidney was found to synthesize glucose and release it into the blood stream (Kido et al., 1978). Therefore, it could be speculated that renal glucose production could be one of the factors contributing to the diabetic hyperglycemia.

Besides, in poorly controlled diabetes, nephropathy is a major complication, which ultimately result in kidney failure. It has been reported that one third of diabetics with insulin dependent diabetes mellitus (IDDM) develop nephropathy after 15 years of illness (Marks and Krall, 1971). Erasmus Darwin (1801) was the first to show diabetic nephropathy. He observed coagulation of urine when heated and attributed this to albuminuria. Kimmelstiel
(1966) showed that the structural changes arise in diabetics because of altered metabolism in the mesangial cells. Electron microscopic renal biopsy showed glomerulosclerosis and thickening of glomerular basement membrane (GBM) and Bowman's capsule (Lannigar, 1964). These changes have also been observed in animals with pancreactomy (Doyle, 1964). Thus, well recognized renal function and ultra structural changes occur in diabetes in man and experimental animals (Orskov et al., 1965; Mongensen, 1971; Osterby, 1974). Vargas et al. (1970) have shown both tubular and interstitial changes in the non protected kidney of alloxan diabetic rats

Patients with insulin dependent diabetes are increasingly treated with intensive insulin regimens in an attempt to achieve blood glucose levels close to those found physiologically. Intensive injection therapy is associated with periods of hyperinsulinemia and very commonly with low blood glucose concentrations (Rizza et al., 1980). Although more appropriate insulin delivery has been reported as improving insulin sensitivity, it is not normalized (Lager et al., 1983). Some animal studies suggest that overinsulinization should increase insulin responsiveness above normal (Wardzala et al., 1985). But this does not happen in the intensively treated diabetic man. There are reports suggesting that exposure of peripheral tissues to high insulin concentrations in the long term might be expected to decrease their sensitivity to insulin (Rizza et al., 1982a,b) with the potential for a damaging cycle of insulin-resistance and hyperinsulinemia. Moreover, it has been shown that insulin requirements of patients with diabetic nephropathy are higher (Ortved-Andersen, 1976).

Autonomic neuropathy has been associated with disturbances in the metabolism in diabetes (Holly et al., 1988; Dunger et al., 1991). Certain aspects of autonomic dysfunction and the subsequent alterations in the metabolic profile, both, in avian and mammalian systems, have been studied in our laboratory. Liver has been studied in detail by Patel and Pilo (1977), Pilo et al. (1984), Mehan (1985), Parikh (1990) and Oommen (1992) and a coworker (Yadav, 1997). Cholinergic, and a part of adrenergic functions influenced by parasympathetic and sympathetic nerves respectively, have been demonstrated by these workers by curtailing the function of the divisions of the autonomic nervous system individually. Parasympathetic influence, anatomically mediated by the vagus, was removed by performing vagotony, at cervical level in pigeon and at subdiaphragmatic level in rat. The sympathetic system was partially abolished by administering 6-hydroxydopamine (6-OHDA) which destroys
adrenergic nerve terminals, consequently causing a decrease in circulating adrenergic neurotransmitters (Thoenen and Tranzer, 1973).

Autonomic influence on the renal metabolism has been examined by Mehta (1985), Oommen (1992) and Pillai (1993). The renal innervation by the ANS contributes significantly to the regulation of renal function (Dibona, 1982). Greene (1959) has described the renal autonomic innervation. The preganglionic sympathetic fibers arising from the lesser splanchnic nerve synapse at the celiac ganglion and form the celiac plexus. The post ganglionic sympathetic fibers arising from the celiac plexus and its sub plexus, the renal plexus innervate kidney. The parasympathetic innervation is through the preganglionic fibers coming through the vagus, which merge at the celiac plexus, and the post ganglionic fibers innervate the kidney. These autonomic influences are mediated by ACh release from parasympathetic fibers and epinephrine and norepinephrine from sympathetic fibers. Biochemical changes caused by vagotomy and chemical sympathectomy have been studied in avian kidney by Pilo et al. (1984). Metabolic effects of acetylcholine, insulin, glucagon and thyroid hormone administration in pigeon kidney have been characterized by Mehta and Pilo (1987; 1988; 1989). Work has been carried out in mammalian kidney by Pillai (1993) to examine the effects of vagotomy. Oommen (1992) has studied the effects of vagotomy and chemical sympathectomy by 6-OHDA on certain biochemical parameters of rat kidney. He has also observed the consequent changes in the glucose tolerance of these rats.

In the present study, vagotomy has been carried out to investigate the effects of parasympathetic denervation on the metabolism of kidney. This surgery would be able to simulate a condition functionally similar to diabetes, resulting in diminished insulin secretion due to cholinergic denervation. In previous studies, the adrenal gland has not been much attended to. Since it is a component of the sympathoadrenal system, adrenalectomy has been performed to observe the resulting effects on the metabolism. It is necessary to check the hypothesis that chronic hyperglycemia may in part be a result of adrenocortical or medullary influences or both on carbohydrate metabolism. Beyond the decreased insulin sensitivity of peripheral tissues, there could be some accentuated source of glucose such as enhanced gluconeogenesis, that could possibly exacerbate the chronic hyperglycemia of the diabetics. The surgeries have been carried out in single to characterize their individual effects. Adrenalectomy has also been carried out simultaneously with vagotomy to observe the
metabolic alterations in the kidney, when only the sympathetic system is persistent. The results of these experiments have been discussed in chapters 2 and 3.

To search for the specific role of the sympathetic system in metabolic regulation, chemical sympathectomy has been performed to curtail this influence. Guanethidine, an antihypertensive drug used by clinicians, depletes the catecholamine store of the neurons (Burnstock et al., 1971) by causing autoimmune destruction of the cell body (Zochodne et al., 1988). This causes loss of CA, which in turn leads to an inhibition of the adrenergic influence on the tissues. Guanethidine thus offers a definite advantage over 6-OHDA as a sympatholytic agent, which leaves the nerve cell body intact (Thoenen and Tranzer, 1973). Therefore, guanethidine has been used in the present study to induce a total chemical sympathectomy. chemical sympathectomy has also been performed in combination with adrenalectomy and vagotomy to obtain a clear picture of the metabolic consequences these systems could produce by their inhibition (Chapters 4 and 5).

An attempt has therefore been made to elucidate the part that the autonomic nervous system plays in the metabolic regulation. The metabolic alterations in an animal would first and foremost be reflected by the changes in body weight, and also by the changes in the weight and water content of the organs undergoing disturbances in metabolism. Therefore, body weight, renal weight and renal water content have been studied (Chapter 1).

Since the analysis of enzyme patterns would be able to provide an insight into the metabolic organization of the tissue during dynamic physiological conditions, assays of major enzymes have been carried out in the kidney. Different pathways of carbohydrate, protein and lipid metabolism, have been investigated for the purpose of defining the contribution of the kidney in the glycemic status of an animal.

The endocrine glands which play an eminent role in the precise regulation of the intermediary metabolism have also been examined. Insulin regulation after autonomic manipulation has been studied by a coworker (Yadav, 1997). New discoveries have demonstrated that the concept placing the entire responsibility for glucose homeostasis on the insulin was not complete. Some other hormones also have been implicated in diabetic condition as previously discussed, viz., corticosterone, ACTH and GH. These have been
assayed after the neural and endocrine manipulations (Chapters 6 and 7) to find out if these maneuvers can normalize the altered hormonal profile found to accompany and aggravate the diabetic condition.