INTRODUCTION

Carbohydrate metabolism is centered on the provision and fate of glucose. Glucose is stored in the body in the form of glycogen in liver and muscle, however muscle contains about three to four times as much glycogen as does liver. Muscle glycogen is a readily available source of glucose for glycolysis within the muscle itself. The liver maintains blood glucose homeostasis by uptake of glucose in the absorptive state, which is converted to glycogen and triacylglycerol and by production of glucose from glycogenolysis and gluconeogenesis in the post absorptive state, wherein the concentration of blood glucose in most mammals is maintained between 4.5 – 5.5 mmol/L. After the ingestion of a carbohydrate meal, it may rise to 6.4 – 7.2 mmol/L. A sudden decrease in blood glucose will cause convulsions, as in insulin over dose, owing to the immediate dependence of the brain on a supply of glucose Similarly a sudden rise in blood glucose will lead to blindness, renal failure and nephropathy and a sustained rise will lead to diabetes, which can be due to either lack of insulin or non-insulin dependent. Therefore blood glucose concentration need to be maintained within narrow limits. This is accomplished by the finely tuned hormonal regulation of peripheral glucose uptake and hepatic

glucose production. Because the lipid membranes are impermeable to carbohydrates, carbohydrate transport systems are required. In recent years two distinct molecular families of cellular transporters of glucose have been cloned. The sodium linked glucose transporters are largely restricted to the intestine and kidneys where they actively transport glucose against a glucose-concentration gradient by using sodium co transport as an energy source (Wright *et al.*, 1991). The other group of transporters conveys glucose by facilitated diffusion down glucose-concentration gradients. This group consists of five homologous transmembrane proteins, GLUT – 1, 2, 3, 4 and 5 that are encoded by distinct genes. The GLUT – 1 is involved in constitutive glucose transport. Mutations in GLUT – 1 are associated with intractable seizures resulting from a reduction in glucose transport across the blood brain barrier (Seidner *et al.*, 1998). GLUT – 2 is a low affinity glucose transporter and has a role in sensing glucose concentration in the islets. Mutations in GLUT – 2 cause the Fanconi-Bickel syndrome, which is a rare autosomal disorder characterized by hepatic and renal glycogen accumulation, nephropathy and impaired utilization of glucose and galactose (Santer *et al.*, 1998). GLUT – 3 is a high affinity glucose transporter chiefly expressed in neurons and placenta. GLUT – 4 is the main insulin-responsive glucose transporter and is located primarily in muscle cells and adipocytes. Mutations in GLUT – 4 could cause insulin resistance (ORahilly *et al.*, 1992).

It is generally believed that pancreatic exocrine and endocrine cells develop from precursor cells present in the pancreatic duct (Pictel and

Rutter, 1972). Embryonic endocrine cells aggregate and form the islets of langerhans, which in mice, achieve a typical adult configuration after birth Insulin containing B cells form the core of the mature islets. Whereas the periphery contains lower numbers of the other endocrine cell types: the A, D and PP cells, which synthesize glucagon, somatostatin and pancreatic polypeptide respectively. The capacity of the pancreatic islets to respond to an elevated blood glucose level with increased insulin secretion obviously depends on a finely tuned short term regulation of the insulin secretary machinery by individual B cells. Over the past few decades, we have come to appreciate that the B-cell mass is dynamic, with a significant capacity for adaptation to changes in insulin demand (Bonner-weir S , 2000). Increase in B-cell mass may occur through increased B-cell replication, increased B-cell size, decreased B-cell death and differentiation of B-cell progenitors (neogenesis) (Finegood *et al.*, 1995). Control of insulin production at the cellular level is achieved in the B-cell through regulatory mechanisms operating at transcriptional, translational and post-translational levels. Islet content of insulin mRNA is tightly regulated both *in vitro* and *in vivo*, and has manifold variations during culture at different glucose concentrations (Howell and Bird, 1989; Welsh, 1989; Halban, 1990; Docherty and Clark, 1994). The mechanisms controlling the exocytotic release of insulin are finely tuned by a complex set of incoming signals; for example, nutrients and hormones carried via the blood, neuronal input from surrounding nerve terminals and paracrine influences from neighboring islet cells. Evidence is now accumulating

that insulin secretion is closely regulated by different intracellular signaling systems, encompassing phosphoinositide hydrolysis, increases in cytosolic Ca^{2+} and cAMP generation. Recent findings have revealed the existence in B-cell plasma membrane of ATP-regulated K^+-channels that control transmembrane K^+ fluxes (Rorsman *et al.*, 1990). Thus ATP may provide a link between glucose metabolism and changes in B-cell electrical activity elicited by glucose. More specifically, ATP generated by glucose metabolism may close K^+-channels resulting in depolarization and subsequent influx of Ca^{2+} through voltage activated Ca^{2+} channels. This influx of Ca^{2+} increases cytosolic Ca^{2+} concentration, an event that sets in motion secretary granule translocation and exocytotic release of insulin (Wollheim and Sharp, 1981). Parasympathetic nerve fibers are abundant in the pancreatic islets and parasympathetic stimulation enhances insulin secretion (Smith and Porte, 1976; Miller, 1981). Likewise, insulin release can be directly stimulated by addition of the parasympathetic transmitter acetylcholine or its non-hydrolysable analogue carbachol (Sjoholm *et al.*, 1993). Neogenesis is an important component of B-cell mass expansion during development and also has been shown to contribute to increases in B-cell mass in juvenile and adult rodent models (Finegood *et al.*, 1995; Rosenberg, 1995; Bouwens and Kloppel, 1996). Neogenesis from non ductal progenitors has been demonstrated in models of pancreas regeneration (Rosenberg, 1995; Fernandes *et al.*, 1997; Bouwens, 1998). Among the large number of protein hormones existing, growth hormone and the biologically related

lactogenic peptides prolactin and placental lactogen have been extensively investigated with regard to effects on B-cell proliferation (Hellerstrom and Swenne, 1985; Hellerstrom *et al.*, 1988; Sjoholm, 1993). Amino acids are also able to stimulate B-cell replication, and it appears as if these are more important than is glucose in this respect in early foetal life (Hellerstrom and Swenne, 1985). Interestingly, Lipsett and Finegood. (2002) showed that the increase in B-cell mass induced by continuous glucose infusion in rats was mainly due to acinar cell transdifferentiation into B-cells. It must be remembered that a factor does not have to act directly on the B-cell to effect the B-cell mass, an indirect effect that resulted in transient mild hyperglycemia could have an effect on the B-cell mass.

Diabetes mellitus, a metabolic disorder, is characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins with an increased risk of complication of vascular disease (Keen *et al.*, 1982; Pickup and Williams, 2003). The minimum defining characteristic feature to identify diabetes mellitus is chronic and substantiated elevation of circulating glucose concentration (Keen *et al.*, 1982; Ziv *et al.*, 1999). Diabetes mellitus may present as a relatively sudden, potentially lethal catastrophe or it can be associated few if any, symptoms or signs and may escape detection for many years. These extremes of clinical manifestations constitute the basis for subdividing diabetes mellitus into the insulin dependent (IDDM) and the non insulin dependent (NIDDM) types. In the recent past the term IDDM has been replaced by Type 1 diabetes mellitus. Type 1 diabetic

subjects have B-cell destruction, which is usually immune-mediated; the majority of the patients develop absolute insulin deficiency and are ketosis prone. The term NIDDM has been replaced by Type 2 diabetes mellitus, which encompasses the most prevalent form of the disease. Most subjects in Type 2 diabetes mellitus exhibit insulin resistance and ultimately develop concomitant insulin secretary defect (Keen *et al.*, 1982). Type 1 diabetes mellitus results from a severe absolute lack of insulin, caused by reduction in the B-cell mass. The three interlocking mechanisms responsible for the islet cell destruction are genetic susceptibility, acute auto immunity and environmental insult (Keen *et al.*, 1982, Pickup and Williams, 2003). The induction of experimental diabetes in the rat using chemicals which selectively destroy pancreatic B-cells is very convenient and simple to use. The most usual substances to induce diabetes in the rat are alloxan and streptozotocin. The understanding of changes in B-cells of the pancreas as well as in the whole organism after alloxan or streptozotocin treatment is essential for using these compounds as diabetogenic agents. Alloxan (2, 4, 5, 6-tetraoxypyrimidine; 5, 6-dioxyuracil) was first described by Brugnatelli in 1818. Wholer and Liebig used the name "alloxan" and described its synthesis by uric acid oxidation. The diabetogenic properties of this drug were reported many years later by Dunn, *et al.* (1943), who studied the effect of its administration in rabbits and reported a specific necrosis of pancreatic islets. Alloxan exerts its diabetogenic action when it is administered parenterally: intravenously, intraperitoneally or subcutaneously. The dose of alloxan required for

inducing diabetes depends on the animal species, route of administration and nutritional status. The most frequently used intravenous dose of this drug to induce diabetes in rats is 65 mg/kg body weight (Gruppuso *et al.*, 1990; Boylan *et al.*, 1992). When alloxan is given intraperitoneally or subcutaneously its effective dose must be 2 -3 times higher. The intraperitoneal dose below 150 mg/kg body weight may be insufficient for inducing diabetes in the rat (Katsumata *et al.*, 1992, 1993). Alloxan is a hydrophilic and unstable substance. Its half-life at neutral pH and 37°C is about 1.5 minutes and is longer at lower temperatures (Lenzen and Munday, 1991). On the other hand, when a diabetogenic dose is used, the time of alloxan decomposition is sufficient to allow it to reach the pancreas in amounts that are deleterious (Frerichs and Creutzfeldt, 1971; Lenzen and Panten, 1988; Malaisse, 1982; Malaisse *et al.*, 1982). The action of alloxan in the pancreas is preceded by its rapid uptake by the B-cells (Weaver *et al.*, 1978; Boquist *et al.*, 1983). Rapid uptake by insulin-secretary cells has been proposed to be one of the important features determining alloxan diabetogenecity. Another aspect concerns the formation of reactive oxygen species (Heikkila *et al.*, 1976; Grankvist *et al.*, 1979; Munday, 1988, Sakurai and Miura, 1989; Winterbourn *et al.*, 1989; Lenzen and Munday, 1991; Zhang *et al.*, 1992; Bromme *et al.*, 1999). One of the targets of the reactive oxygen species is DNA of pancreatic islets. Its fragmentation takes place in B-cells exposed to alloxan (Takasu *et al* , 1991; Sakurai and Ogiso, 1995). DNA damage stimulates poly ADP-ribosylation, a process participating in DNA repair.

Some inhibitors of poly ADP-ribosylation can partially restrict alloxan toxicity. This effect is, however, suggested to be due to their ability to scavenge free radicals rather than to a restriction of poly ADP-ribosylation initiated by alloxan (Sandler and Swenne, 1983; Le Doux *et al.*, 1988). Super oxide dismutase, catalase (Grankvist *et al.*, 1979; Grankvist, 1981; Jorns *et al.*, 1999) and non-enzymatic scavengers of hydroxyl radicals like melatonin (Ebelt *et al.*, 2000) were also found to protect against alloxan toxicity Melatonin, a secretary product of the pineal gland, is able to scavenge OH formed by the interaction between alloxan, GSH and chelated ferrous ions and thus to diminish lipid peroxidation.

Melatonin (N-acetyl-5-methoxy tryptamine) is synthesized in the pineal gland by the conversion of tryptophan to serotonin, which is acetylated by n-acetyltransferase (NAT) to N-acetylserotonin. N-acetylserotonin is subsequently converted to melatonin by the enzyme hydroxyindole-o-methyl transferase (HIOMT). The pineal hormone production is dependent on the light-dark cycle because of circadian changes in the activity of NAT, the pineal rate limiting enzyme (Puy *et al.*, 1996). A significant factor of endogenous melatonin availability is the age. It has been reported that aging is associated with progressive reduction of circadian melatonin synthesis in pineal gland. Equally the onset of many degenerative and proliferative diseases is associated with aging; what remains unclear is whether the increase of these diseases is related to reduced anti-oxidative protection potentially provided by melatonin (Reiter, 1992). The melatonin effects are mediated by the

specific high affinity receptors localized in plasma membranes and coupled to guanosinetriphosphate-glutamyl transpeptidase-binding proteins (Vanecek *et al.*, 1998). Furthermore, investigations of the ovine pars tuberalis demonstrated that melatonin receptors couple both to pertusis-toxin-sensitive and cholera-toxin-sensitive components, which are involved in the inhibition of cAMP mediated by the melatonin receptors (Morgan *et al.*, 1995). Recently cloning of several G-protein-coupled melatonin receptors has revealed that three melatonin subtypes (Mel_{1a}, Mel_{1b}, Mel_{1c}) exists (Reppert *et al.*, 1994, 1995 a, b; 1996 a, b; Reppert, 1997). It has also been demonstrated that melatonin effects are mediated through specific nuclear receptors (orphan ROR-RZR receptors) and in some cases melatonin can act without receptors, too (Dubocovich, 1998; Becker-Andre *et al.*, 1994). Melatonin is known to influence circadian and seasonal behavior and physiology (Reiter, 1991; Pang *et al.*, 1992; Reiter, 1993). The nocturnal release of melatonin alters the timing of mammalian circadian rhythms (Arendt, 1995) and regulates reproductive changes in response to deviations in day length in seasonally breeding mammals (Bartness *et al.*, 1993). Purported therapeutic capacities of melatonin extend to easing insomnia (Brown, 1995), alleviating jet lag (Brown, 1992; Arendt, 1995), protecting cells from free radical damage (Reiter *et al.*, 1994; Poeggeler *et al.*, 1996; Reiter *et al.*, 1996; Reiter, 1997), reducing tumor growth (Blask *et al.*, 1997), preventing cataracts (Pang *et al.*, 1996), displaying analgesic effects (Ebadi *et al.*, 1998), exerting a cardio-protective action (Lagneux *et al.*, 2000), thermoregulation

(Saarela *et al.*, 1994) and moulting (Allain *et al.*, 1980). The effect of pineal polypeptide as a potent and specific hypoglycemic factor in mammals has been reported as early as 1957 (Milcu *et al.*, 1957; Milcu *et al.*, 1963). It was believed that the pineal polypeptide was synergistically acting with insulin and was thought to have protective action on the pancreatic B-cells of animals treated with alloxan. Also hypertrophy of pancreatic islet was reported after chronic injections of pineal extract (Notario, 1956; Petronio and Tavazza, 1958). The seasonal effect of pinealectomy on liver glycogen stores and blood glucose was observed by Delahunty *et al*, (1978) on gold fish, whereas the reports of McKeown *et al.* (1975) on pigeon showed significant increase in plasma glucose after melatonin injections at different time periods. Mihail and Giurgea (1979) demonstrated hypoglycemic influence of pineal extracts in domestic pigeons and thus suggested pineal to be capable of compensating for the lack of endocrine pancreas. However, Csaba and Barath, (1971) had demonstrated a suppressive influence of pineal on the B-cells of pancreas in rats. It has been demonstrated that melatonin reduces pancreatic insulin secretion *in vitro* (Peschke *et al.*, 1997) and phase-response studies support the conviction that pancreatic B-cells may be targets for melatonin (Peschke and Peschke, 1998). Furthermore the evidence for a melatonin receptor within the pancreatic islets of neonate rats has also been confirmed (Peschke *et al.*, 2000). Recent reports suggest that melatonin not only affects the secretary action of B-cells (Lima *et al.*, 2001) but has a general protective action against the effect of

streptozotocin-induced hyperglycemia (Anderson and Sandler, 2001) and alloxan induced destruction of B-cells (Bromme *et al.*, 1999). Melatonin binding sites have been localized in several peripheral tissues e.g. in the gastrointestinal tract (Martin *et al.*, 1998), liver (Acuna-Castroviejo *et al.*, 1994), Kidney (Song *et al.*, 1995) and pancreas (Williams *et al.*, 1997). Melatonin has also been thought to have a putative role in glucose metabolism via its action on the suprachiasmatic nucleus and sleep regulation (Vancauter, 1998). Melatonin administration has been reported to increase (Delahunty *et al.*, 1978; Dhar *et al.*, 1983, Mahata *et al.*, 1988; Zemen *et al.*, 1993) or decrease (Mahata *et al.*, 1988) or have no effect on blood glucose level (John *et al.*, 1990; Ramachandran *et al.*, 2002). The mechanism by which melatonin modulates glycemic status is not clear. It is suggested that the hormone may act by interactions with other metabolic hormones like insulin, glucagon, growth hormone, corticosterone or catecholamines (Ramachandran, 2002). Melatonin has been shown to influence the plasma insulin level (Diaz and Blazquez, 1986), insulin secretion (Bailey *et al* , 1974; Peschke *et al.*, 1997) and even possibly insulin action (Frankel and Trandberg, 1991). It is also known to modulate the liver insulin and glucagon receptor concentrations (Rodriguez *et al.*, 1989) and increase the catecholamine content (Mahata *et al.*, 1988; Maitra *et al.*, 2000). Though melatonin is known to effect body weight, adiposity and food intake in seasonal animals (Himms-Hagen J., 1984; Wade and Bartness, 1984; Mc Elroy and Wade, 1986; Valtonen, M. *et al.*, 1995; Le Gouic *et al.*, 1996) these

effects may vary according to the species. Thus opposite results are observed in Siberian and Syrian hamsters in which melatonin decreases or increases body fat mass respectively(Wade and Bartness, 1984; Mc Elroy and Wade, 1984; Bartness and Wade, 1985; Bartness, 1995), the mechanism of melatonin action on energy metabolism in mammals is not well known. Compared to its effect on carbohydrate metabolism, effects on lipid metabolism have been less studied (de Vlaming *et al.*, 1974). Some studies have suggested an action of pineal gland on lipid metabolism and, administration of pineal extracts has been shown to lower the serum, hepatic, adrenal and testicular cholesterol level. In rabbits pineal extracts could decrease cholesterolemia, biliary cholesterol and serum phospholipids (Esquifino *et al.*, 1997). Cholesterol lowering effect of melatonin has been considered a potent effect as long term melatonin administration could significantly decrease the plasma cholesterol level and prevent fatty liver in genetic hypercholesterolemic rats (Aoyama *et al.*, 1988). Furthermore, a melatonin agonist and antagonist stimulates or lowers seasonal obesity in the garden dormouse (Le Gouic *et al* , 1996). The role of melatonin on lipid metabolism is also suggested by the observation of delayed post prandial clearance of triacylglycerol indicating possible lipid intolerance in human subjects under simulated nine hour phase-shifts (Hampton *et al* , 1996). Melatonin could also prevent hyperlipidemia caused by glucocorticoid administration in rats (Aoyama *et al.*, 1988) or by cholesterol rich feed (Mori *et al.*, 1989). It is also recorded that melatonin cannot prevent hypercholesterolemia in

old rats (Vaughan *et al.*, 1982). Also a circadian rhythm of low density lipoprotein (LDL) receptor activity has been demonstrated which is influenced by corticsol, but not mediated by it (Balasubramaniam *et al.*, 1994) Melatonin itself has been shown to inhibit LDL receptor activity and cholesterol synthesis in human mononuclear leucocytes (Muller-Wieland *et al* , 1994) Chapman, (1997) indicated that melatonin also influences lipoprotein lipase activity, a key regulatory enzyme in circulating triacylglycerol in adipose tissue. A recent study involving long term discontinuous melatonin treatment through drinking water, reduced serum triglyceride and cholesterol levels (Markova *et al.*, 2003). Also increased hepatic phospholipid and diacylglycerol concentrations due to melatonin administration have been reported (Mustonen *et al.*, 2002). Furthermore, melatonin can also reduce the serum levels of triglycerides and cholesterol in mammalian species (Rasmussen *et al* , 1999; Hoyos *et al* , 2000; Nishida *et al.*, 2002), and has an inhibiting effect on the uptake of plasma fatty acids for lipogenesis as well as fasting induced lipolysis in the inguinal fat pad perfused *in situ* in normal rats by a melatonin mediated mechanism (Sauer *et al* , 2001)

The influence of melatonin on reproductive development begins during the prenatal development and extends into postnatal life. The primary source of melatonin for the developing mammal is the maternal pineal gland. Maternal melatonin reaches the offspring via milk (Reppert & Klein, 1978). Rhythmic melatonin production from the developing pineal is first significant during the second & third week of postnatal life

in rodents (Tamarkin *et. al.*, 1980). The fetal environment is the key determinant of the adult phenotype, being linked to development of diseases and also the timing of puberty. Dependence of germ cells on thyroid hormone during the pre pubertal period has been shown (Chowdhary & Arora, 1984). Melatonin administration to immature rats has been reported to diminish ovarian and uterine weights (Wurtmann *et. al.*, 1963; Motta *et. al.*, 1967) and to retard testes and accessory sex organ development (Debeljuk, 1969; Kinson & Robinson, 1970; Kinson & Peat, 1971). Since the influence of melatonin on reproductive development has been known to commence during the pre-natal period and extend into the postnatal life (Weaver, 2000), melatonin administration either in the morning or in the evening in the infantile to pre pubertal period (10-25 days) has been tested in our lab. This study showed decreased body weight and testes weights in the period immediately after melatonin treatment, more pronouncedly in the evening treatment (Patel & Ramachandran, 1992). Apparently, melatonin administration in the early neonatal periods has definite influence on the body and organ growth, reproductive axis, as well as on metabolic functions. The effects of alterations in melatonin levels in the postnatal period remain still an unexplored avenue. Hence it was thought pertinent to study the long-term effects of experimental alterations in melatonin status on carbohydrate and lipid metabolism, pancreatic function and alloxan induced diabetes at pre pubertal, pubertal and adult stages along with hormonal profiles.

The objectives defined were

- To assess the influence of pre-weaning melatonin status on adult carbohydrate and lipid metabolism
- To evaluate the influence of pre-weaning melatonin status on serum titers of pancreatic hormones
- To study the influence of pre-weaning melatonin status on *in vitro* tissue uptake/release of glucose in response to different secretogogues.
- To evaluate the influence of pre-weaning melatonin status on aspects of alloxan induced diabetes in pubertal and adult animals.