

INTRODUCTION

Carbohydrate, with protein and fat, comprise the triad of major metabolic fuels. As a metabolic substrate, carbohydrate is present in organisms in its simple monomeric form, α -D-Glucopyranose, and as a branched polymer of α -Glucose namely glycogen. Disaccharides of glucose include lactose, maltose and sucrose but these are quantitatively less important. In normal healthy subjects, glucose circulates in plasma water at a basal concentration that ranges from 65 – 105 mg/dl. Following a meal, the plasma glucose concentration does not exceed 160 – 180 mg/dl in normal, healthy individuals. A sudden decrease in blood glucose will cause convulsions, as in insulin overdose, owing to the immediate dependence of the brain on the supply of glucose. Similarly, a sudden rise in blood glucose will lead to blindness, renal failure and nephropathy and a substantial rise will lead to diabetes which can be due to either lack of insulin or non-insulin dependent diabetes. Therefore the most important metabolic homeostasis is that concerned with glucose. 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 (Pictet 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 secretory 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 secretory 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 increase 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; Sjöholm, 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 with 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 secretory 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-secretory cells has been proposed to be one of the important features determining alloxan diabetogenicity. 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 secretory product of the pineal gland, is able to scavenge OH^\cdot formed by the interaction between alloxan, GSH and chelated ferrous ions and thus to diminish lipid peroxidation.

Melatonin 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-methyltransferase. 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). 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 pertussis-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 receptor subtypes (Mel_{1a} , Mel_{1b} , Mel_{1c}) exist (Reppert et. al., 1994, 1995a, b, 1996a, 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, 1988; Becker-Andre et. al., 1994). 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). Melatonin influences circadian and seasonal behavior and physiology (Reiter1991; 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 (Arendt 1995; Brown 1992), 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 (Langneux et. al., 2000), thermoregulation

(Saarela et. al., 1994) and moulting (Allain et. al., 1980). 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). Furthermore, the evidence for a melatonin receptor within the pancreatic islets of neonate rats has also been confirmed (Peschke et. al., 2000). The effect of 'pinealin', a 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 & 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 Casaba and Barth (1971) had demonstrated a suppressive influence of pineal on the B cells of pancreas in rats. Also, melatonin has been shown to suppress glucose induced insulin secretion in rats and mouse (Bailey *et al.*, 1974) and reduce glucose tolerance in rabbits (Muralidhar *et al.*, 1983). Further,

Gorray and Quay (1979) have isolated, two insulinotrophic and, one-insulinostatic, protein fractions from the mammalian pineal. 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). Also 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 & Peschke, 1998). 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 affect body weight, adiposity and food intake in seasonal animals (Himms-Hagen J., 1984; Wade and Bartness, 1984; Mc Elory 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 Elory 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 levels. 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 stimulate or lower 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 cortisol, 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 clinical implications of pineal dysfunction leading to hypoglycemia and insulin resistance have also been observed. Recent reports suggest that melatonin can not only affect the secretory action of B cells (Lima *et al.*, 2001), but can have a general protective action against the effect of streptozotocin – induced hyperglycemia (Anderson & Sandler, 2001) and alloxan induced destruction of B cells (Bromme *et al.*, 1999). Melatonin has also been thought to have a putative role in glucose metabolism via its actions on the suprachiasmatic nucleus and sleep regulation (Vancauter, 1998). Mazepa *et al.*, (2000) had reported that high melatonin administration increased liver glycogen contents of exercised and non-exercised rats. Exogenous melatonin enhanced utilization of liver carbohydrates but suppressed hepatic lipolysis (Mustonen *et al.*, 2002). Melatonin administration decreased significantly the serum triacylglycerol concentrations and liver glycogen content in male rats, and it increased the liver phospholipid content in females (Markova *et al.*, 2003). It has been recently reported that melatonin plays a physiological role in glycemic homeostasis, which

involves the GLUT-4 protein (Melissa *et al.*, 2003). Peschke (1998) has reported that insulin secretion from isolated pancreatic rat islets exhibits a circadian rhythm which is apparently generated within the islet and that the pineal secretory product melatonin influenced the rhythm generating oscillator and induced a phase shift in insulin secretion. Melatonin treatment augmented significantly the concentrations of total, free and esterified cholesterol, as well as high-density lipoprotein cholesterol in blood. Insulin secretion was enhanced and the level of free fatty acids in the blood decreased and surprisingly the blood glucose concentration was significantly elevated (Fabis *et al.*, 2002). Previous studies initiated in our laboratory have indicated certain definite alterations in carbohydrate metabolism as well as changes in the whole weight of pancreas of Feral Blue Rock Pigeon *Columba livia gemlin* (Patel *et al.*, 1982). Similar studies have indicated a season specific anti-insulin action of pineal in wild pigeons in terms of glycemic status (Patel *et al.*, 1983). The subsequent studies on domestic pigeons showed a species specific differential effect on the pineal-pancreas axis in the form of increased whole weight of pancreas (Patel & Ramachandran 1983) as compared to a decreased weight previously observed in wild pigeons (Patel *et al.*, 1982). On a seasonal basis, pinealectomy had a glycogen lowering effect and hypoglycemic tendency during breeding and regression phases and, an opposite effect was observed in the recrudescence phase (Patel *et al.*, 1988). Also, an anti-insulinic role of pineal in the breeding season of wild pigeons was reported by Ramachandran &

Patel (1989). These studies show a possible significance of pineal gland in modulating seasonal changes in carbohydrate metabolism in birds. Moreover, the muscle slices of pinealectomized pigeons showed an altered sensitivity to insulin and adrenalin in relation to uptake and release of glucose and, deposition and depletion of glycogen which corroborate the previous observations (Patel & Ramachandran, 1992). The literature available on the role of pineal and glucoregulation in mammals is scant. Our laboratory has attempted to evaluate the influence of melatonin on glycemic status and its response to glucoregulatory hormones. The observations on GTT & IRT taken together suggest an already existing glucagons responsiveness in melatonin treated rats which could be due to increased insulin secretion/sensitivity as indicated by the hypoglycemic state. This is probably the reason for the higher glucose elevation subsequent to glucose loading and slightly lesser percentage of hypoglycaemia and quicker recovery to normal glycemic state on insulin challenge in the experimental rats (Ramachandran, 2002). Overall, it can be said that pineal and its principle, melatonin has a definite glucoregulatory role and influence on carbohydrate metabolism across vertebrate series. 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). 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. Receptor antagonists are an important, widely used pharmacological tool in identifying receptor subtypes and studying neurotransmitter or neuromodulator mechanisms. In general, antagonists are not biological activators themselves, but rather they compete with ligands for a common binding site and interrupt the biological effect of the displaced ligand.

The competitive melatonin receptor antagonist luzindole blocked both the melatonin mediated phase advances of circadian rhythm of neuronal firing and increase in PKC activity in the rat supra chiasmatic nuclei (Amanda *et. al.*, 2001). These results are compatible with activation of a G protein coupled melatonin receptor (McArthur *et. al.*, 1997; Starkay *et. al.*, 1995), Luzindole shows 15-20 time higher affinity for MT₂ than the MT₁ receptor (Dubocovich *et. al.*, 1997, 1998; Nonno *et. al.*, 1999). In the mammalian retina, it antagonizes melatonin mediated inhibition of dopamine release through activation of MT₂ melatonin receptor (Dubocovich *et. al.*, 1997, 1998). Since melatonin administration in the early neonatal period has been shown to influence body and organ growth, reproductive axes as well as metabolic functions, it is relevant to study long term effects of altered neonatal melatonin status on adult carbohydrate and lipid metabolism, pancreatic functions and alloxan induced diabetes in the pubertal

period. In the present study, the consequences of neonatal hypomelatonemia (by use of melatonin receptor antagonist, luzindole) have been assayed.

The objectives defined are:

- To assess the influence of pre-weaning blockage of melatonin action on adult carbohydrate and lipid metabolism
- To evaluate the influence of pre-weaning blockage of melatonin action on serum titers of insulin
- To study the influence of pre-weaning blockage of melatonin action on *in vitro* tissue uptake of glucose and C¹⁴ glucose oxidation in response to different secretagogues.
- To evaluate the influence of pre-weaning blockage of melatonin action on aspects of alloxan induced diabetes in pubertal and adult animals.