CONCISE SUMMARY

of the thesis entitled

[redacted] OF INDUCED NEONATAL HYPERMELATONEMIA ON ADULT CARBOHYDRATE AND LIP[redacted] METABOLISM, PANCREATIC FUNCTION AND ALLOXAN INDUCED DIABETES

For the Degree of

Doctor of Philosophy
in
Zoology

Submitted by
Ms. Jennipher Jani

Division of Metabollic Endocrinology
Department of Zoology
Faculty of Science
The M S University of Baroda,
Vadodara-390 002India.

CONCISE SUMMARY

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 α, δ 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. 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 due mainly 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. 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 β-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 β-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 β-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 β-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 β-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 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 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 cortical, 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). 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 insulin.
- To study the influence of pre-weaning melatonin status on *in vitro* tissue uptake of glucose and C^{14} glucose oxidation 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.

The experimental paradigms included rendering animals hypermelatonemic , control alloxanised hypermelatonemic alloxanised, for these neonates were injected intra-peritoneally with melatonin in the evening from day 1 to day 21 and alloxan on 22^{nd} day and assessed on 45^{th} and 60^{th} day. The control alloxanised neonates were injected intra-

peritoneally with saline in the evening from day 1 to day 21 and alloxan on 22nd day and assessed on 45th and 60th day. The hypermelatonemic neonates were injected intra-peritoneally with melatonin in the evening from day 1 to day 21 and assessed on 22nd, 45th and 60th day.

Neonatal hypermelatonemia has induced favorable changes in the immediate weaning period clearly chronic melatonin treatment increased insulin sensitivity by a probable direct action of melatonin on insulin receptor kinetics or by way of GLUT gene expression. The increased tissue glycogen content is well supported by the increased glycogen synthetase activity and reduced glycogen phosphorylase activity with increased GS:GP ratio. The increased hepatic glucose-6-phosphatase activity seems to be a stimulatory effect on glucose-6-phosphatase synthesis by genetic activation either directly or indirectly by corticosterone. The purported increased insulin sensitivity as well as the increased tissue glycogen contents are supported by the observed higher glucose uptake by liver and muscle slices. Melatonin is a potent stimulator of glucose uptake along with insulin and acetylcholine Also melatonin treatment potentiates the glucose uptake by all the three stimulants as well as their combinations The presently observed increased serum free fatty acid level and decreased tissue total lipid content could suggest an adaptive increased lipid utilization to counteract the carbohydrate sparing effect of neonatal hypermelatonemia. The low tissue lipid stores could be easily correlated with the prevailing hypoinsulinemia. Neonatal

hypermelatonemia decreases lipid synthesis and increases lipid utilization as against increased lipid synthesis and decreased utilization in control weanings.

Neonatal hypermelatonemia has induced favorable changes in the pubertal period chronic pre-weaning hypermelatonemia has persistent glycogenic effect and increased glycogen synthetase activity. Apparently, neonatal hypermelatonemia has a protein anabolic influence as a long term effect in the pubertal period, it may suggest a potentiated influence of testosterone. Melatonin treatment in the neonatal period seems to hasten the postpartum development of insulin sensitivity. The relatively greater uptake capacity coupled with reduced glucose oxidation suggests an overall anabolic *milieu* in the neonatal hypermelatonemic rats. The reduction in serum levels of various lipid fractions other than free fatty acids suggests a hypolipidemic effect of chronic melatonin treatment Cholesterol lowering effect of melatonin seems to be a characteristic feature as at both weaning and in the pubertal stage the cholesterol content of the body is significantly less in neonatal hypermelatonemic rats. The effect of neonatal hypermelatonemia on the adult carbohydrate and lipid metabolism revealed significantly higher muscle glycogen and protein content in the hypermelatonemic rats as a consequence of the higher levels attained at the pubertal age itself. There is a similar degree of glycogenolysis in both control and experimental animals indicating utilization for energy purposes and the hypermelatonemic animals show a

The effect of neonatal hypermelatonemia with weaning alloxan treatment revealed certain favorable results in the pubertal period. The significant hypoglycemia seen in all alloxan treated rats, relatively more potent in nMT rats, suggests increased insulin action probably due to regeneration of the B cells. The purported regeneration of B cells is adequately supported by the histologically observable B cell regeneration and B cell density in the islets of alloxan treated control rats and increased islet density with regeneration of B cells in alloxan treated nMT rats. In the present study, nMT rats seem to resist the diabetogenic effect of alloxan, as noted by the relative healthy condition of the islet tissue compared to the non-nMT rats. This suggests a protective action of prior melatonin treatment in resisting the effects of B cell damaging agents. The body lipid profile shows a generalized ability of the weaning animals to resist diabetes related increase in lipid content The reduced tissue lipid and serum lipid fractions in both nMT and non-nMT rats relative to non alloxanised controls is probably indicative of reduced insulin level/action in the post alloxan treatment period. This is substantiated by the observed insulin status in these rats. The effect of neonatal hypermelatonemia with weaning alloxan treatment revealed certain favorable results in the adult stage. The increase in body lipid content is marked by a reciprocal decrement in serum lipid fractions. Since the nMT rats show relatively higher serum lipid profiles as well as tissue lipid loads, it is likely that a progressive insulin resistance is manifesting in these rats The present

study on weaning alloxan treatment seems to suggest development of insulin resistance and hyperinsulinemia as, long term effects, probably, more potentiated by a higher melatonin level in the neonatal period. A protein anabolic influence related to insulin resistance is also evident as the tissue protein contents are significantly increased in the rats. Protective action of neonatal melatonin on alloxan induced B cell damage is clearly seen from the histologically observable structural features of pancreatic islets. However, as a long term effect of weaning alloxan induced diabetes, neonatal melatonin treatment tends to potentiate hyperinsulinemia and insulin resistance and thereby predisposes these animals to NIDDM. Overall it can be conclude that melatonin administration in the neonatal period is of crucial significance in modulating the postnatal growth, weaning, pubertal and adult metabolic homeostasis and protection against alloxan induced free radical damage