CHAPTER – 2

NEONATAL HYPERMELATONEMIA POTENTIATES PERIPHERAL GLUCOSE UPTAKE IN THE RAT: AN *IN VITRO* STUDY ON LIVER AND MUSCLE SLICES.

INTRODUCTION:

Previous study on induced neonatal hypermelatonemic status for the entire pre-weaning period had revealed increased hepatic and muscle glycogen contents with hypoglycemia and hypoinsulinemia compared to age matched controls (Chapter I). These changes were accompanied by increased glycogen synthetase activity with decreased phosphorylase activity Melatonin was purported to induce increased insulin sensitivity to generate a glycogenic and hypoglycemic state. A parallel study on functional hypomelatonemia induced by luzindole treatment has shown hyperinsulinemia and decreased insulin sensitivity of liver and muscle (Adi, 2004). Many past studies had indicated some undefined but definite role for pineal and its hormone in regulating glycemic status and carbohydrate metabolism. Quay (1970) opined "One of the areas of pineal endocrinology most difficult to evaluate is that relating to associated phenomena in liver, tissue metabolism and blood chemistry". Some of the earlier reports have

suggested "pinealin" a pineal polypeptide to be a potent and specific hypoglycemic factor in mammals (Milcu *et al.*, 1957, 1963). The workers of this school believed the pineal polypeptide to be acting synergistically with insulin and to have protective action on the pancreatic B cells of animals treated with alloxan (Damien, 1989). Some workers reported hypertrophy of pancreatic islets following chronic injection of pineal extracts (Notario, 1956, Petronio and Tavazza, 1958). Related reports in this regard are pinealectomy induced hyperinsulinemia (Milcu *et al.*, 1971; Gorray *et al.*, 1979) and the decreased glucose tolerance in rats (Milcu *et al.*, 1971). The above reports indicate a role for pineal hormone in glucose homeostasis. Melatonin administration has been shown to increase, decrease or even have no effect on blood glucose level (Delahunty *et al.*, 1978; Dhar *et al.*, 1983; Mahata *et al.*, 1988; John *et al.*, 1990; Zemen *et al.*, 1993). How exactly melatonin modulates plasma glucose is not yet clear. It is suggested that melatonin may act indirectly through other hormones involved in carbohydrate metabolism like insulin, glucagon, growth hormone or catecholamines. Melatonin has been shown to influence plasma insulin level (Diaz and Blazquez, 1986), insulin secretion (Bailey *et al.*, 1974; Peschke *et al.*, 1997) and possibly insulin action (Frankel and Trandberg, 1991). The hormone is also known to influence liver insulin and glucagon receptor concentrations (Rodriguez *et al.*, 1989) and even increase the catecholamine content (Mahata *et al.*, 1988; Maitra *et al.*, 2000). A direct effect of melatonin on sensitization of peripheral tissues to insulin action was also suggested

by the observations that, EC_{50} of insulin stimulated (3H)-2-deoxy-glucose uptake in isolated adipocytes previously incubated with melatonin shifted to the left (Lima *et al* , 1994). It is in this background and the observed effects of neonatal hypermelatonemia on carbohydrate homeostasis, that the present *in vitro* study on glucose uptake by liver and muscle slices of hypermelatonemic animals has been conducted in presence of various stimulants and a melatonin antagonist (Luzindole) to test the inferred increased tissue sensitivity to glycogenic stimuli.

MATERIAL AND METHODS: See page Nos. 16 to 37.

RESULTS:

Liver Slices:

- **Uptake in presence of Insulin, Melatonin and Acetylcholine:** The liver of control rats showed glucose uptake by all the three agents in the order insulin>melatonin>acetylcholine. The liver slices of melatonin treated rats also showed uptake with all the three. Though insulin induced glucose uptake was not significantly different from the control slices, both melatonin and acetylcholine promoted significantly greater uptake in that order in the experimental slices (Figure and Table; 2.1)
- **Uptake by Insulin + Acetylcholine, Insulin + Melatonin, Melatonin + Acetylcholine and Insulin + Melatonin + Acetylcholine:** All combinations of the three stimulants did not induce glucose uptake more than that promoted by insulin alone in the control liver slices. I+Ac, I+M and M+Ac all showed

uptake similar to that by melatonin or acetylcholine alone. Only the combination of all the three showed a higher uptake though still lesser than that induced by insulin alone. The liver slices of hypermelatonemic rats showed significantly greater glucose uptake involving all combinations of melatonin i.e. M+I, M+Ac and M+I+Ac even significantly greater than that shown by insulin or melatonin alone. I+Ac was not effective in promoting uptake more than that of Ac alone and was less than with insulin alone (Figure and Table; 2.1, 2.2).

- **Uptake by Insulin, Melatonin and Acetylcholine in presence of luzindole:** In the control slices even luzindole had induced glucose uptake similar to that of Ac. Though luzindole had reduced the uptake promoted by either insulin or acetylcholine alone, a combination of all the three i.e. L+I+Ac showed maximum uptake. In contrast, in the liver slices of experimental rats luzindole alone registered glucose uptake equivalent to that by insulin or melatonin. However, insulin, acetylcholine and insulin and acetylcholine did not show any increment in glucose uptake over those promoted by these agents alone. Even a combination of L+I+Ac showed lesser uptake (Figure and Table; 2.2, 2.3)

Table and Figure: 2.1

Bonferroni's Multiple Comparison Test Control Groups

	H vs J	H vs K	H vs L	H vs N	H vs O	H vs P	J vs K	J vs L	J vs N	J vs O	J vs P
p	■	■	⊙	✶	■	NS	NS	NS	NS	NS	NS
	K vs L	K vs N	K vs O	K vs P	L vs N	L vs O	L vs P	N vs O	N vs P	O vs P	
p	NS	NS	NS	NS	NS	NS	NS	NS	⊙	NS	

Bonferroni's Multiple Comparison Test Melatonin Groups

	S vs T	S vs U	S vs V	S vs W	S vs X	S vs Y	T vs U	T vs V	T vs W	T vs X	T vs Y
p	NS	NS	NS	NS	NS	NS	NS	NS	NS	⊙	NS
	U vs V	U vs W	U vs X	U vs Y	V vs W	V vs X	V vs Y	W vs X	W vs Y	X vs Y	
p	NS	NS	⊙	NS	NS	NS	NS	NS	NS	NS	

*p<0.001; ■P<0.01; ⊙P<0.05; NSNon Significant

Figure 2.1: Glucose uptake at 10 minutes by liver slices of control and melatonin treated weaning rats on 22nd day with combinations of insulin, acetylcholine and melatonin:

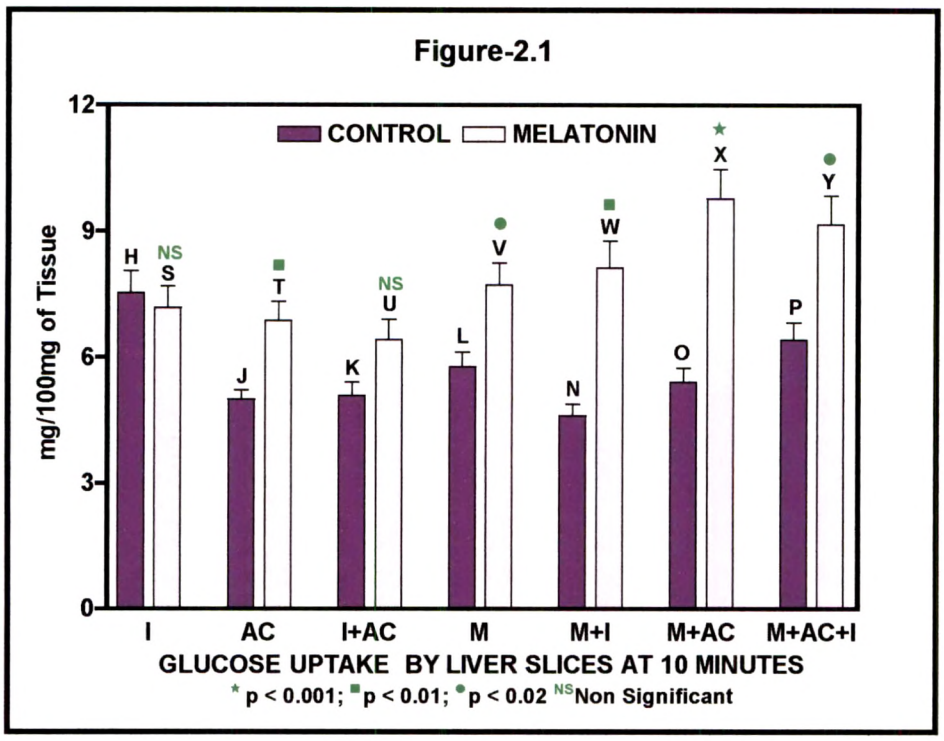

Table 2.1: Glucose uptake at 10 minutes by liver slices of control and melatonin treated weaning rats on 22nd day with combinations of insulin, acetylcholine and melatonin:

	I	AC	I+AC	M	M+I	M+AC	M+AC+I
CONTROL	7.52(H) ±0.53	4.99(J) ±0.22	5.09(K) ±0.31	5.77(L) ±0.35	4.60(N) ±0.28	5.41(O) ±0.33	6.42(P) ±0.40
MELATONIN	NS7.17(S) ±0.51	■6.87(T) ±0.45	NS6.41(U) ±0.48	●7.71(V) ±0.53	■8.12(W) ±0.64	*9.77(X) ±0.71	●9.16(Y) ±0.69

Values are expressed as mean ± SEM, *p < 0.001; ■p < 0.01; ●p< 0.02; NS Non Significant

Table 2.2

Bonferroni's Multiple Comparison Test Control Groups

	H vs J	H vs K	H vs N	H vs O	H vs P	H vs Q	J vs K	J vs N	J vs O	J vs P	J vs Q
p	*	*	*	*	*	*	NS	NS	NS	NS	NS
	K vs N	K vs O	K vs P	K vs Q	N vs O	N vs P	N vs Q	O vs P	O vs Q	P vs Q	
p	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	

Bonferroni's Multiple Comparison Test Melatonin Groups

	S vs T	S vs U	S vs V	S vs W	S vs X	S vs Y	T vs U	T vs V	T vs W	T vs X	T vs Y
p	NS	NS	NS	NS	⊙	NS	NS	NS	NS	NS	NS
	U vs V	U vs W	U vs X	U vs Y	V vs W	V vs X	V vs Y	W vs X	W vs Y	X vs Y	
p	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	

*p<0.001; ⊙P<0.05; NSNon Significant

Figure 2.2: Glucose uptake at 10 minutes by liver slices of control and melatonin treated weaning rats on 22nd day with combinations of insulin, acetylcholine and luzindole:

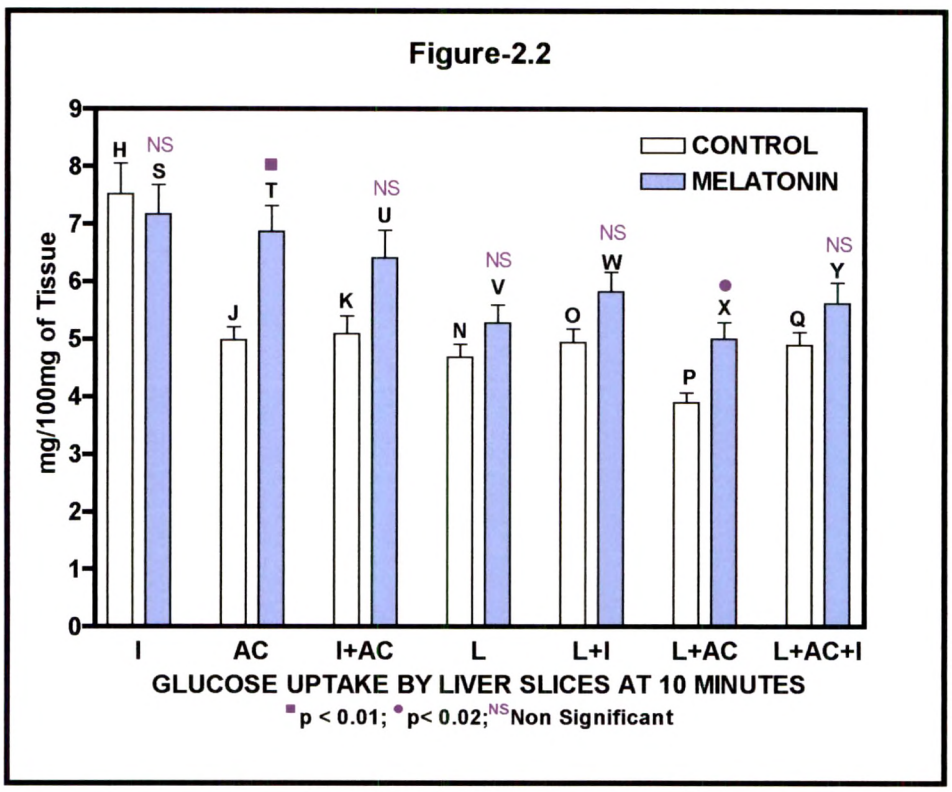

Figure 2.2: Glucose uptake at 10 minutes by liver slices of control and melatonin treated weaning rats on 22nd day with combinations of insulin, acetylcholine and luzindole:

	I	AC	I+AC	L	L+I	L+AC	L+AC+I
CONTROL	7.52(H) ±0.53	4.99(J) ±0.22	5.09(K) ±0.31	4.69(N) ±0.22	4.94(O) ±0.24	3.90(P) ±0.17	4.90(Q) ±0.23
MELATONIN	NS 7.17(S) ±0.51	■ 6.87(T) ±0.45	NS 6.41(U) ±0.48	NS 5.28(V) ±0.31	NS 5.82(W) ±0.35	• 5.00(X) ±0.29	NS 5.63(Y) ±0.36

Values are expressed as mean ± SEM, ■ p < 0.01; • p< 0.02; NS Non Significant

Table 2.3

Bonferroni's Multiple Comparison Test Control Groups

	A vs B	A vs C	A vs D	A vs E	A vs F	A vs G	A vs H	B vs C	B vs D	B vs E	B vs F	B vs G	B vs H	C vs D
p	NS	NS	NS	NS	NS	■	NS	NS	■	NS	NS	NS	NS	NS
	C vs E	C vs F	C vs G	C vs H	D vs E	D vs F	D vs G	D vs H	E vs F	E vs G	E vs H	F vs G	F vs H	G vs H
p	NS	NS	⊙	NS	■	⊙	✶	⊙	NS	NS	NS	NS	NS	NS

Bonferroni's Multiple Comparison Test Melatonin Groups

	S vs T	S vs U	S vs V	SvsW	S vs X	S vs Y	S vs Z	T vs U	T vs V	TvsW	T vs X	T vs Y	T vs Z	U vs V
p	NS	NS	NS	NS	NS	⊙	NS	NS	NS	⊙	NS	■	NS	NS
	UvsW	U vs X	U vs Y	U vs Z	VvsW	V vs X	V vs Y	V vs Z	WvsX	WvsY	WvsZ	X vs Y	X vs Z	Y vs Z
p	✶	✶	✶	✶	✶	■	✶	■	NS	NS	NS	NS	NS	NS

*p<0.001; ⊙P<0.05; NS Non Significant

Figure 2.3: Glucose uptake at 10 minutes by liver slices of control and melatonin treated weaning rats on 22nd day with combinations of insulin, acetylcholine, melatonin and luzindole:

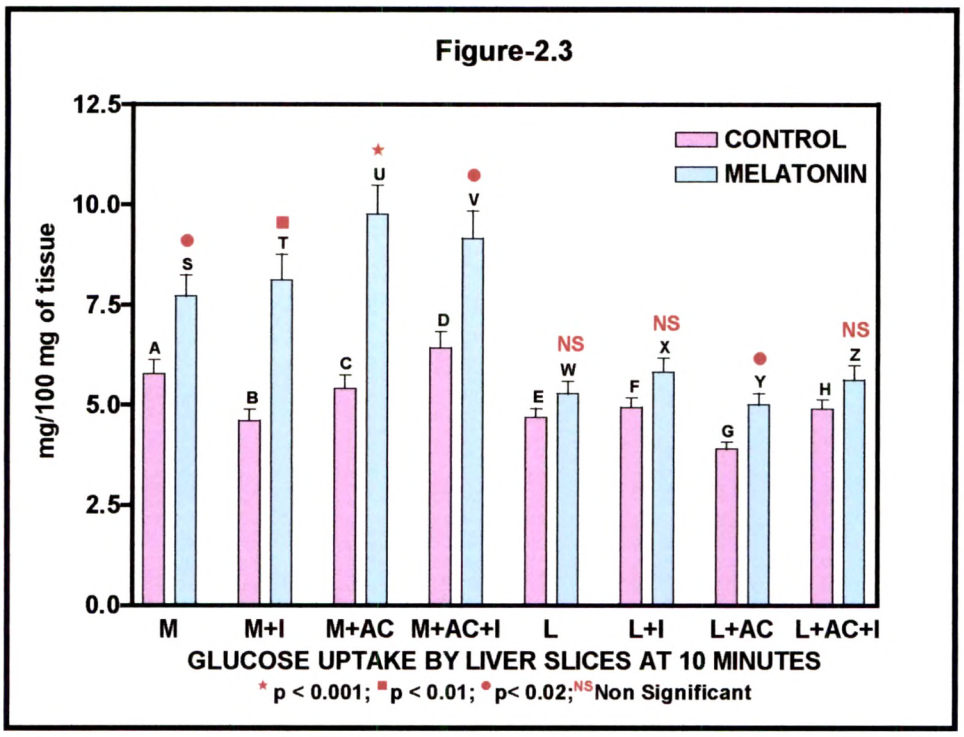

Table 2.3: Glucose uptake at 10 minutes by liver slices of control and melatonin treated weaning rats on 22nd day with combinations of insulin, acetylcholine, melatonin and luzindole:

	M	M+I	M+AC	M+AC+I	L	L+I	L+AC	L+AC+I
CONTROL	5.77 (A) ±0.35	4.60 (B) ±0.28	5.41 (C) ±0.33	6.42 (D) ±0.40	4.69 (E) ±0.22	4.94 (F) ±0.24	3.90 (G) ±0.17	4.90 (H) ±0.23
MELATONIN	● 7.71 (S) ±0.53	■ 8.12 (T) ±0.64	* 9.77 (U) ±0.71	● 9.16 (V) ±0.69	NS 5.28 (W) ±0.31	NS 5.82 (X) ±0.35	● 5.00 (Y) ±0.29	NS 5.63 (Z) ±0.36

Values are expressed as mean ± SEM, * p < 0.001; ■ p < 0.01; ● p< 0.02; NS Non Significant

Table and Figure: 2.4

Bonferroni's Multiple Comparison Test Control Groups

	H vs J	H vs K	H vs L	H vs N	H vs O	H vs P	J vs K	J vs L	J vs N	J vs O	J vs P
p	NS	⊙	NS	NS	NS	NS	⊙	NS	NS	NS	NS
	K vs L	K vs N	K vs O	K vs P	L vs N	L vs O	L vs P	N vs O	N vs P	O vs P	
p	NS	NS	NS	■	NS	NS	⊙	NS	NS	NS	

Bonferroni's Multiple Comparison Test Melatonin Groups

	S vs T	S vs U	S vs V	S vs W	S vs X	S vs Y	T vs U	T vs V	T vs W	T vs X	T vs Y
p	✶	NS	■	NS	⊙	■	✶	NS	⊙	NS	NS
	U vs V	U vs W	U vs X	U vs Y	V vs W	V vs X	V vs Y	W vs X	W vs Y	X vs Y	
p	⊙	NS	NS	⊙	NS	NS	NS	NS	NS	NS	

*p<0.001; ■P<0.01; ⊙P<0.05; NSNon Significant

Figure 2.4: Glucose uptake at 10 minutes by muscle slices of control and melatonin treated weaning rats on 22nd day with combinations of insulin, acetylcholine and melatonin:

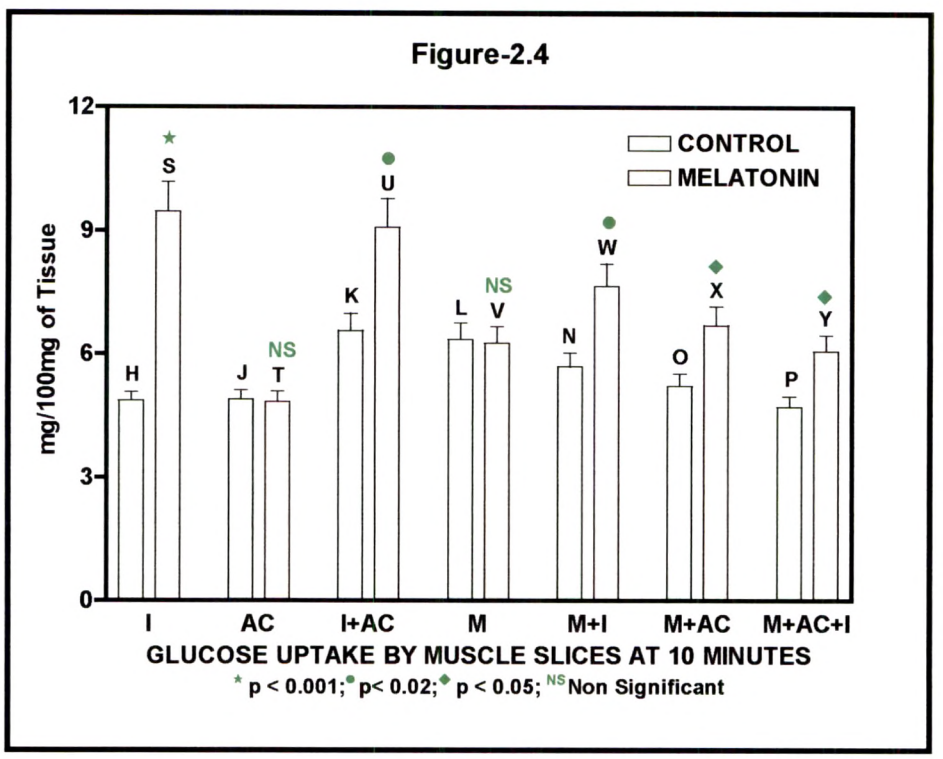

Figure 2.4: Glucose uptake at 10 minutes by muscle slices of control and melatonin treated weaning rats on 22nd day with combinations of insulin, acetylcholine and melatonin:

	I	AC	I+AC	M	M+I	M+AC	M+AC+I
CONTROL	4.86 (H) ±0.21	4.89 (J) ±0.23	6.57 (K) ±0.41	6.35 (L) ±0.40	5.69 (N) ±0.34	5.22 (O) ±0.30	4.72 (P) ±0.25
MELATONIN	*9.46 (S) ±0.72	NS 4.83 (T) ±0.26	•9.08 (U) ±0.70	NS 6.26 (V) ±0.40	•7.65 (W) ±0.54	◆6.70 (X) ±0.45	◆6.07 (Y) ±0.39

Values are expressed as mean ± SEM, *p < 0.001; •p< 0.02; ◆p < 0.05; NS Non Significant

Table 2.5

Bonferroni's Multiple Comparison Test Control Groups

	H vs J	H vs K	H vs N	H vs O	H vs P	H vs Q	J vs K	J vs N	J vs O	J vs P	J vs Q
p	NS	■	NS	NS	NS	NS	■	NS	NS	NS	NS
	K vs N	K vs O	K vs P	K vs Q	N vs O	N vs P	N vs Q	O vs P	O vs Q	P vs Q	
p	■	✶	✶	✶	NS	NS	NS	NS	NS	NS	

Bonferroni's Multiple Comparison Test Melatonin Groups

	S vs T	S vs U	S vs V	S vs W	S vs X	S vs Y	T vs U	T vs V	T vs W	T vs X	T vs Y
p	✶	NS	✶	✶	✶	■	✶	NS	NS	NS	NS
	U vs V	U vs W	U vs X	U vs Y	V vs W	V vs X	V vs Y	W vs X	W vs Y	X vs Y	
p	✶	✶	✶	⊙	NS	NS	NS	NS	NS	NS	

*p<0.001; ■P<0.01; ⊙P<0.05; NSNon Significant

Figure 2.5: Glucose uptake at 10 minutes by muscle slices of control and melatonin treated weaning rats on 22nd day with combinations of insulin, acetylcholine and luzindole:

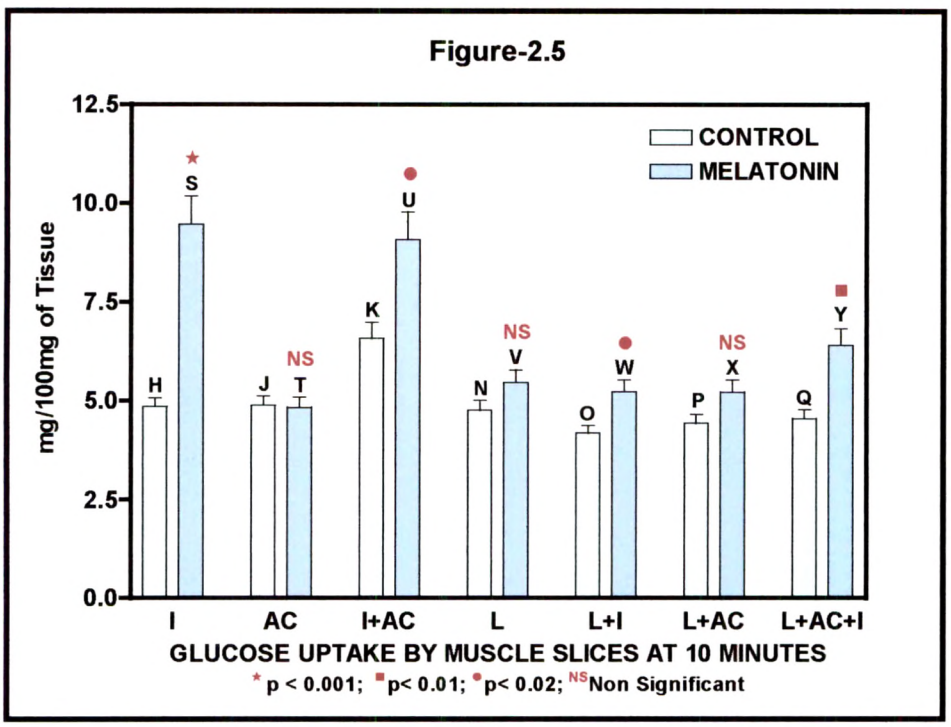

Table 2.5: Glucose uptake at 10 minutes by muscle slices of control and melatonin treated weaning rats on 22nd day with combinations of insulin, acetylcholine and luzindole:

	I	AC	I+AC	L	L+I	L+AC	L+AC+I
CONTROL	4.86 (H) ±0.21	4.89 (J) ±0.23	6.57 (K) ±0.41	4.76 (N) ±0.25	4.18 (O) ±0.19	4.43 (P) ±0.22	4.55 (Q) ±0.23
MELATONIN	*9.46 (S) ±0.72	NS 4.83 (T) ±0.26	●9.08 (U) ±0.70	NS 5.46 (V) ±0.32	●5.23 (W) ±0.30	NS 5.22 (X) ±0.30	■6.41 (Y) ±0.42

Values are expressed as mean ± SEM, *p < 0.001; ■p< 0.01; ●p < 0.02; NS Non Significant

Table 2.6

Bonferroni's Multiple Comparison Test Control Groups

	A vs B	A vs C	A vs D	A vs E	A vs F	A vs G	A vs H	B vs C	B vs D	B vs E	B vs F	B vs G	B vs H	C vs D
p	NS	NS	⊙	⊙	✶	■	■	NS	NS	NS	⊙	NS	NS	NS
	C vs E	C vs F	C vs G	C vs H	D vs E	D vs F	D vs G	D vs H	E vs F	E vs G	E vs H	F vs G	F vs H	G vs H
p	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Bonferroni's Multiple Comparison Test Melatonin Groups

	S vs T	S vs U	S vs V	SvsW	S vs X	S vs Y	S vs Z	T vs U	T vs V	TvsW	T vs X	T vs Y	T vs Z	U vs V
p	NS	NS	NS	NS	NS	NS	NS	NS	NS	⊙	■	■	NS	NS
	UvsW	U vs X	U vs Y	U vs Z	VvsW	V vs X	V vs Y	V vs Z	WvsX	WvsY	WvsZ	X vs Y	X vs Z	Y vs Z
p	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

*p<0.001; ⊙P<0.05; NSNon Significant

Figure 2.6: Glucose uptake at 10 minutes by muscle slices of control and melatonin treated weaning rats on 22nd day with combinations of insulin, acetylcholine, melatonin and luzindole:

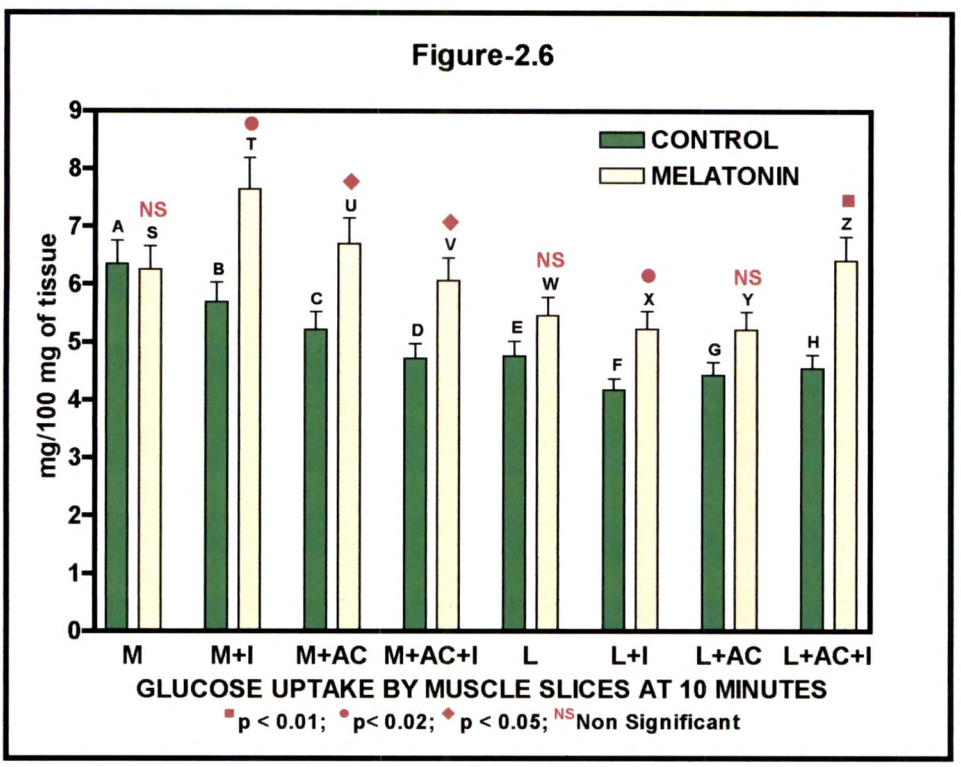

Table 2.6: Glucose uptake at 10 minutes by muscle slices of control and melatonin treated weaning rats on 22nd day with combinations of insulin, acetylcholine, melatonin and luzindole:

	M	M+I	M+AC	M+AC+I	L	L+I	L+AC	L+AC+I
CONTROL	6.35(A) ±0.40	5.69(B) ±0.34	5.22(C) ±0.30	4.72(D) ±0.25	4.76(E) ±0.25	4.18(F) ±0.19	4.43(G) ±0.22	4.55(H) ±0.23
MELATONIN	NS6.26(S) ±0.40	●7.65(T) ±0.54	◆6.70(U) ±0.45	◆6.07(V) ±0.39	NS5.46(W) ±0.32	●5.23(X) ±0.30	NS5.22(Y) ±0.30	■6.41(Z) ±0.42

Values are expressed as mean ± SEM, ■p < 0.01; ●p< 0.02; ◆p < 0.05; NSNon Significant

Muscle Slices:

- **Uptake in presence of Insulin, Acetylcholine and Melatonin:** Control muscle slices showed maximal uptake in presence of melatonin and lower but equal uptake with both insulin and acetylcholine. The muscle slices of experimental rats showed no difference in uptake either with melatonin or acetylcholine compared to controls while the uptake with insulin was significantly greater (Figure and Table; 2.4)
- **Uptake with combinations of Insulin, Melatonin and Acetylcholine:** Except for I+Ac which showed significantly higher uptake equal to that of melatonin alone, no other combination with melatonin i.e. M+I, M+Ac and M+I+Ac showed any further increase in glucose uptake promoted by any of them individually. In the experimental muscle slices, I+Ac showed increased glucose uptake similar to that of insulin while all combinations with melatonin showed similar uptake to that of melatonin alone and, M+I more than melatonin but less than insulin (Figure and Table, 2.4, 2.5).
- **Uptake by Insulin, Acetylcholine and Melatonin in presence of Luzindole:** Interestingly, luzindole promotes glucose uptake similar to that promoted by insulin or acetylcholine. Except for L+I which showed a lower uptake, no other combination (L+Ac or L+Ac+I) showed any increase over that shown by individual ones. Luzindole induced uptake was found to be higher in hypermelatonemic muscle slices compared to control slices.

Combinations of insulin, acetylcholine or insulin and acetylcholine with luzindole did not show any increase in uptake over that of luzindole alone (Figure and Table; 2.4, 2.5)

DISCUSSION:

The results of the present study clearly show that neonatal hypermelatonemia for twenty one days has significant influence on tissue sensitivity to agonistic agents which promote glucose uptake like insulin and acetylcholine. The direct effect of melatonin in inducing glucose uptake is also evident. Differential effects with reference to liver and muscle are also evident Another noteworthy feature is the ability of luzindole, a melatonin antagonist also to promote glucose uptake as well as its ability to reduce melatonin mediated indirect mode of glucose uptake. The liver of control rats show maximum glucose uptake in presence of insulin followed by melatonin and acetylcholine (Fig. and Tab ; 2.1). It is also clear that the effect of insulin is masked when in combination with acetylcholine or melatonin (Figure and Tab.; 2.1). A greater uptake though still less than insulin occurs only in a combination of all the three In contrast, melatonin induced glucose uptake is significantly increased in hypermelatonemic liver equal to that induced by insulin (Fig. and Tab. 2.1). Even acetylcholine induced uptake was significantly higher. Unlike the control liver, the hypermelatonemic liver showed increased sensitivity and significantly higher uptake with all combinations of melatonin i.e. melatonin and insulin, melatonin and acetylcholine and melatonin, insulin and acetylcholine.

Maximal uptake of glucose was seen in presence of melatonin in control muscle with both insulin and acetylcholine showing similar lower uptake. Only a combination of I+Ac could show some uptake as that shown by melatonin. On the other hand, whereas melatonin or acetylcholine did not show any increase in glucose uptake in hypermelatonemic muscle, insulin and insulin and acetylcholine showed maximal uptake suggesting increased potency. All combinations with melatonin showed greater uptake in experimental muscle compared to control muscle (Fig. and Tab. 2.4). Taken as a whole the striking points are:

1) Liver of control animals shows maximal uptake with insulin.
2) Liver of melatonin treated rats shows maximal uptake with melatonin and, almost similar uptake with insulin and acetylcholine; combinations show further increase.
3) Muscle of control rats shows maximal uptake with melatonin and, insulin and acetylcholine.
4) Muscle of hypermelatonemic rats shows maximal uptake with insulin followed by melatonin. Combinations of insulin or melatonin also showed higher uptake. Clearly hypermelatonemic liver and muscle show greater sensitivity to glucose uptake compared to control tissues.

The increased hepatic response to acetylcholine may suggest chronic melatonin induced sensitivity to parasympathetic modulation. Previously it has been shown that acetylcholine is a potent stimulator of hepatic glucose uptake in the pigeon (Patel and Ramachandran, 1992).

In contrast, rats are known to show relatively more sensitivity to insulin but, acetylcholine can exert an enhancing influence on insulin induced glucose uptake (Mondon and Burton, 1971). It is likely that acetylcholine induces glucose uptake by flow coupled transport as it is known to alter membrane permeability and bring about release of calcium and increase the concentration of cAMP by decreasing phosphodiesterase activity (Rasmussen, 1975). Conceivably, chronic melatonin treatment induced increased sensitivity of liver, to acetylcholine, melatonin and combinations of melatonin, as well as, the increased sensitivity of muscle to insulin and, insulin and acetylcholine, as well as to melatonin and, combinations of melatonin, indicate increased peripheral glucose utilization which is corroborated by the observed hypoglycemia and increased tissue glycogen content (Chapter-I)

The present results clearly show that melatonin by itself is a potent promoter of glucose uptake which could be related with the demonstrated presence of melatonin receptors of the Mel_{1b} or MT_2 subtype (Poon *et al.*, 2001). This is confirmed by the decreased melatonin induced glucose uptake by tissue slices of rat neonates treated with luzindole, a melatonin receptor antagonist (Adi, 2004). A direct action of melatonin on hepatocytes to modulate blood glucose level is shown by the elevation of plasma glucose level within the 1^{st} hour after a mid-light intraperitoneal injection of melatonin in mice (Poon *et al.*, 2001). This melatonin induced hyperglycemia was shown to be associated with a decrease in hepatic melatonin binding affinity.

A direct independent action of melatonin on hepatocytes is also indicated by the inability of insulin induced hypoglycemia to bring about any significant changes in melatonin binding. Based on these observations, Poon *et al*, (2001) have suggested that melatonin binding may be a result of both the high melatonin level and high glucose level. Incidentally, a high melatonin level has been shown to down regulate melatonin binding in a number of tissues (Poon and Pang, 1994). In contrast to these observations involving acute melatonin treatment, is the present one, which involves chronic melatonin treatment wherein, hypoglycemia (Chapter-I) and increased glucose uptake have been recorded. Obviously, responses to chronic treatment vs. single injection of acute treatment needs to be clearly demarcated and differentiated. A related study involving 30 day melatonin replacement in pinealectomized rats showed restoration of GLUT-4 protein content as well as *in vivo* insulin sensitivity. It was suggested that there is a physiological role of melatonin in glucose homeostasis which includes GLUT-4 gene expression. It is clearly inferable that, chronic melatonin treatment has a probable ability to not only to maintain melatonin receptors but also stimulate glucose uptake in presence of insulin, melatonin or acetylcholine. Further support towards for a direct melatonin mediated action comes from Picinato *et al.* (2002) who postulated that melatonin may act peripherally by regulating insulin secretion (Peschke *et al.*, 1997; Peschke *et al.*, 2002).

The uptake studies carried out in presence of luzindole (MT_2 blocker) have shown no significant effect Basal level of uptake was

seen in presence of luzindole alone as well as in presence of combinations of luzindole like, insulin+luzindole, acetylcholine+luzindole and even, acetylcholine+insulin+luzindole, though, a slight inhibitory effect on acetylcholine and insulin induced uptake could be seen (Fig. and Tab. 2.2, 2.6). Similar observations are made by Adi (2004) as well, in his studies on melatonin receptor antagonism and carbohydrate metabolism. Overall, the results of present study suggest that:

1) Melatonin is a potent stimulator of glucose uptake along with insulin and acetylcholine and that, chronic exposure to melatonin potentiates the glucose uptake by all the 3 stimulants as well as their combinations.
2) There is potentiated insulin sensitivity in the muscle tissue of hypermelatonemic animals.
3) There could be increased parasympathetic tone/sensitivity in chronically melatonin treated animals

SUMMARY:

Previous study on induced neonatal hypermelatonemic status for the entire pre-weaning period had revealed increased hepatic and muscle glycogen contents with hypoglycemia and hypoinsulinemia compared to age matched controls (Chapter I). It is in this background that the present *in vitro* study on glucose uptake by liver and muscle slices of hypermelatonemic animals has been conducted in presence of various stimulants and, a melatonin antagonist (luzindole), to test the inferred increased tissue sensitivity to glycogenic stimuli. To this end, rat

neonates have been treated with melatonin in graded doses of 200 µg/animal from day 1 to day 7; 400 µg/animal from day 8 to day 14 and 600 µg/animal from day 15 to day 21 and assessed on the 22nd day. The liver slices of experimental animals showed significantly increased glucose uptake with melatonin (M) and acetylcholine (Ac); however the combination of Insulin (I), Ac and M showed maximum glucose uptake in liver slices of experimental rats. In muscle slices of experimental rats, insulin induced glucose uptake was significantly greater as compared to M or Ac, and even the combination of I and Ac induced glucose uptake was similar to that of I alone as compared to any other combination. Luzindole (L) induced glucose uptake was higher in the muscle slices of experimental rats. The results of the present study suggest that melatonin is a potent stimulator of glucose uptake along with I and Ac and that chronic exposure to melatonin potentiates the glucose uptake by all the 3 stimulants as well as their combinations. There is a potentiated I sensitivity in the muscle tissue of hypermelatonemic rats and there could be in this context increased parasympathetic tone/sensitivity in chronically melatonin treated animals.