CHAPTER – 2

NEONATAL MELATONIN RECEPTOR ANTAGONISM BY LUZINDOLE DECREASES PERIPHERAL GLUCOSE UPTAKE IN THE RAT: AN *IN VITRO* STUDY ON LIVER AND MUSCLE SLICES.

INTRODUCTION:

Previous evaluation on blockage of melatonin action in the neonatal period by the use of MT_2 receptor antagonist, luzindole had shown hypoglycemia with hyperinsulinemia and increased hepatic and muscle glycogen contents (Jani, 2004). The hyperinsulinemic state coupled with hypoglycemia and increased glycogen synthetase: phosphorylase activity ratios were taken to indicate increased peripheral conversion of glucose to glycogen. Though there are no studies involving assessment of carbohydrate metabolism under conditions of suppressed melatonin action, there are studies in relation to pinealectomy which have provided evidences for a role for melatonin in carbohydrate metabolism. The role of pineal gland and its hormone in the regulation of carbohydrate metabolism has been suspected quite early (Alcozer *et al.*, 1956; Milcu *et al.*, 1971). Pinealectomy in rats has been shown to decrease hepatic and muscle glycogenesis and increase blood pyruvate concentration (Milcu *et al.*, 1971).

Hyperglycemia and further a far higher increase in blood glucose after alloxan administration have been demonstrated in pinealectomized rats (Csaba and Barath, 1971). Modification of many other parameters involved in carbohydrate metabolism has also been recorded post-pinealectomy (Diaz and Blazquez, 1986). Converse results of hypoglycemia and increased glucose tolerance and hepatic and muscle glycogenesis after a glucose loading due to administration of pineal extracts have strengthened the pinealectomy induced effects (Milcu *et al.*, 1971). Some of the recent studies have shown that pinealectomy causes glucose intolerance, insulin resistance and decreased adipose cell responsiveness to insulin (Seraphim *et al.*, 1997; Lima *et al.*, 1998). Apart from a decrease in insulin response, pinealectomy also caused a fall in GLUT-4 content in adipose and muscle tissues (Lima *et al.*, 1998). Since luzindole induced melatonin receptor antagonism in the rat neonates showed hyperinsulinemia and increased tissue glycogen contents with hypoglycemia (Chapter-I), it was desirable to test the possibility of peripheral tissues depicting increased glucose uptake sensitivity. Hence the present *in vitro* study using liver and muscle slices from luzindole treated neonates for their ability to take up glucose in response to stimulants alone or in combination has been carried out.

MATERIAL AND METHODS: See page numbers 18-38.

RESULTS:

Hepatic tissue uptake in presence of insulin, acetylcholine and melatonin: Compared to control slices, luzindole treated slices showed significant (around 50%) reduction in glucose uptake. Though, the control slices showed highest uptake with insulin, followed by melatonin and acetylcholine, the experimental slices showed almost similar low uptake (Figure and Table; 2.1).

Hepatic tissue uptake in presence of combinations of insulin, melatonin and acetylcholine: Both acetylcholine and melatonin when present in combination with insulin reduced significantly the insulin induced uptake by control slices. Neither melatonin and acetylcholine nor, melatonin, acetylcholine and insulin showed any additive influence over that of melatonin alone. The experimental liver slices also showed no influence of any of the combinations; only melatonin and insulin showed a slightly higher additive influence over that of insulin or melatonin alone (Figure and Table; 2.1, 2.3).

Hepatic tissue uptake in presence of luzindole and luzindole+insulin and luzindole+acetylcholine and luzindole+acetylcholine+insulin: The liver slices of control animals showed some degree of glucose uptake in presence of luzindole equivalent to that shown with acetylcholine. Though neither luzindole+insulin nor luzindole+acetylcholine showed any difference in glucose uptake, a combination of luzindole+acetylcholine+insulin showed maximal uptake. The experimental slices showed significantly

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	✶
	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	✶	✶	⊙	✶	✶	✶	✶	

*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 luzindole treated weaning rats on 22nd day with combinations of insulin, acetylcholine and melatonin:

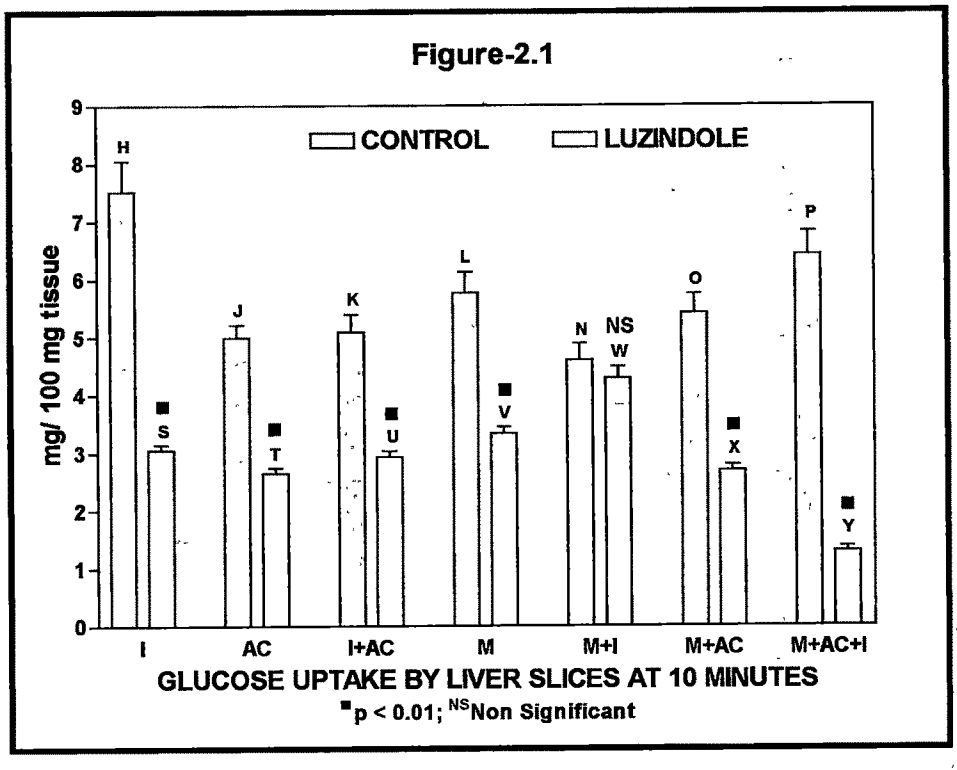

Table 2.1: Glucose uptake at 10 minutes by liver slices of control and luzindole treated weaning rats on 22nd day with combinations of insulin, acetylcholine and melatonin :

	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
LUZINDOLE	$^{*}3.05^{(S)}$ ±0.10	$^{*}2.65^{(T)}$ ±0.094	$^{*}2.94^{(U)}$ ±0.097	$^{*}3.34^{(V)}$ ±0.11	$^{NS}4.28^{(W)}$ ±0.20	$^{*}2.69^{(X)}$ ±0.094	$^{*}1.30^{(Y)}$ ±0.081

Values are expressed as mean ± SEM, $^{*}p < 0.01$; ^{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	*	*	*	*
	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	*	

*p<0.001; °P<0.05; NSNon Significant

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

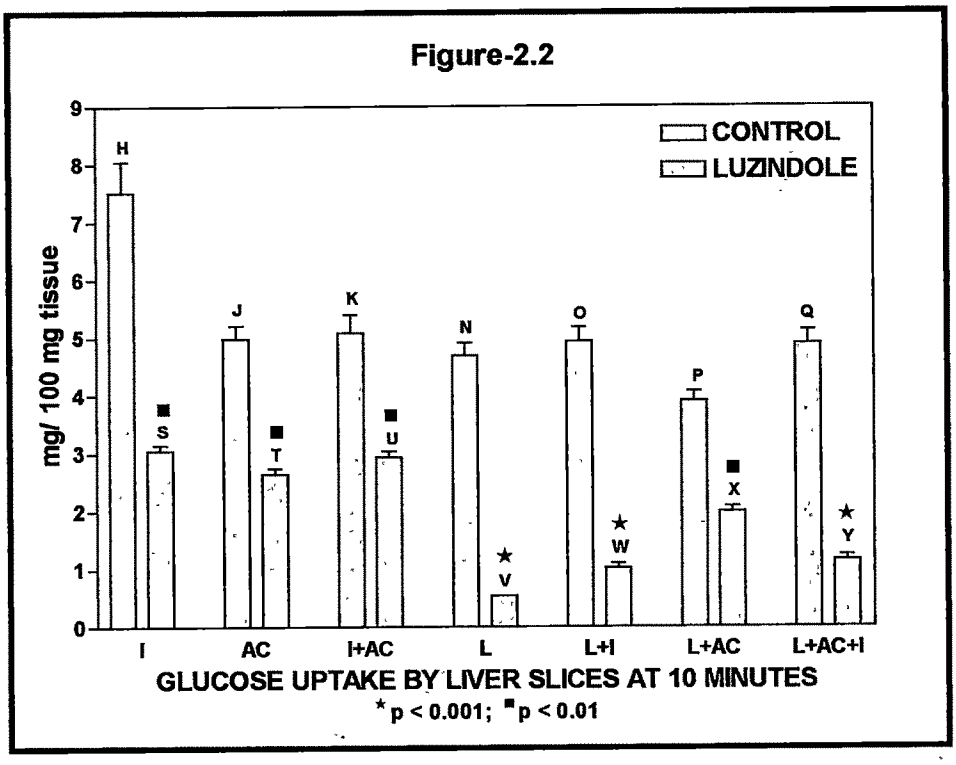

Figure 2.2: Glucose uptake at 10 minutes by liver slices of control and luzindole treated weaning rats on 22nd day with combinations of insulin, acetylcholine and luzindole:

	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
LUZINDOLE	$^{\blacksquare}3.05^{(S)}$ ±0.10	$^{\blacksquare}2.65^{(T)}$ ±0.094	$^{\blacksquare}2.94^{(U)}$ ±0.097	$^{*}0.54^{(V)}$ ±0.0032	$^{*}1.02^{(W)}$ ±0.080	$^{\blacksquare}1.99^{(X)}$ ±0.087	$^{*}1.16^{(Y)}$ ±0.080

Values are expressed as mean ± SEM, *p < 0.001; ■p < 0.01

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	✶	■	✶	✶	✶	✶	✶	✶	✶	✶	✶	✶	✶	✶
	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	✶

*p<0.001; ⊙P<0.05; NSNon Significant

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

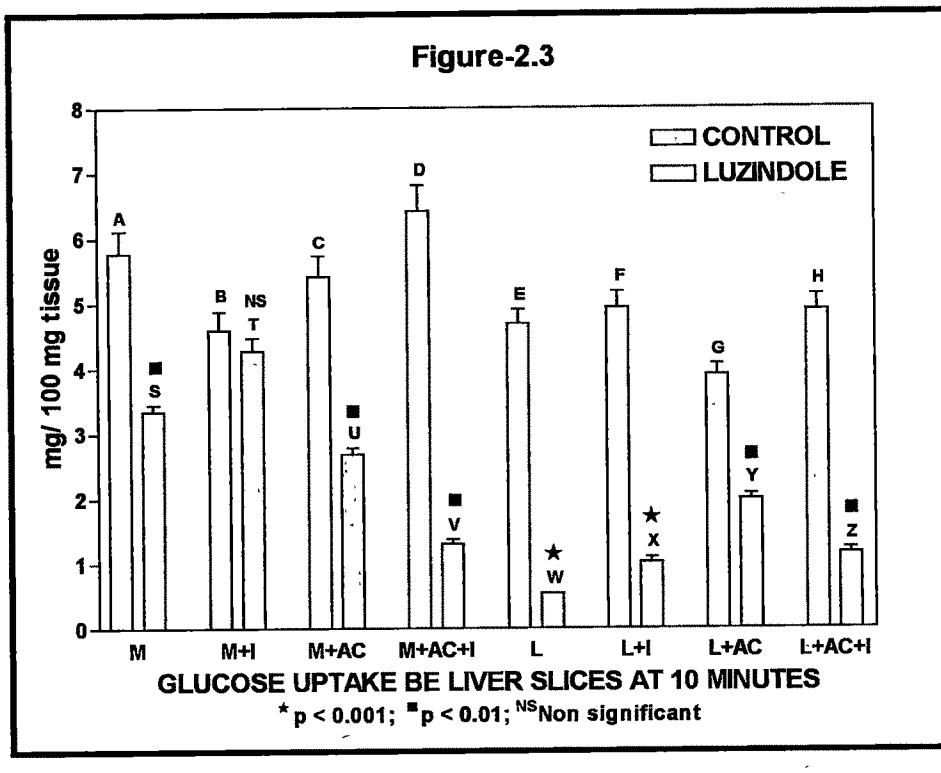

Table 2.3: Glucose uptake at 10 minutes by liver slices of control and luzindole treated weaning rats on 22nd day with combinations of insulin, acetylcholine, melatonin and luzindole:

	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
LUZINDOLE	■$3.34^{(S)}$ ±0.11	NS$4.28^{(T)}$ ±0.20	■$2.69^{(U)}$ ±0.094	■$1.30^{(V)}$ ±0.081	*$0.54^{(W)}$ ±0.032	*$1.02^{(X)}$ ±0.080	■$1.99^{(Y)}$ ±0.087	*$1.16^{(Z)}$ ±0.080

Values are expressed as mean ± SEM, *p < 0.001; ■p < 0.01; 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	✶	✶	✶	✶	✶
	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 2.4: Glucose uptake at 10 minutes by muscle slices of control and luzindole treated weaning rats on 22nd day with combinations of insulin, acetylcholine and melatonin:

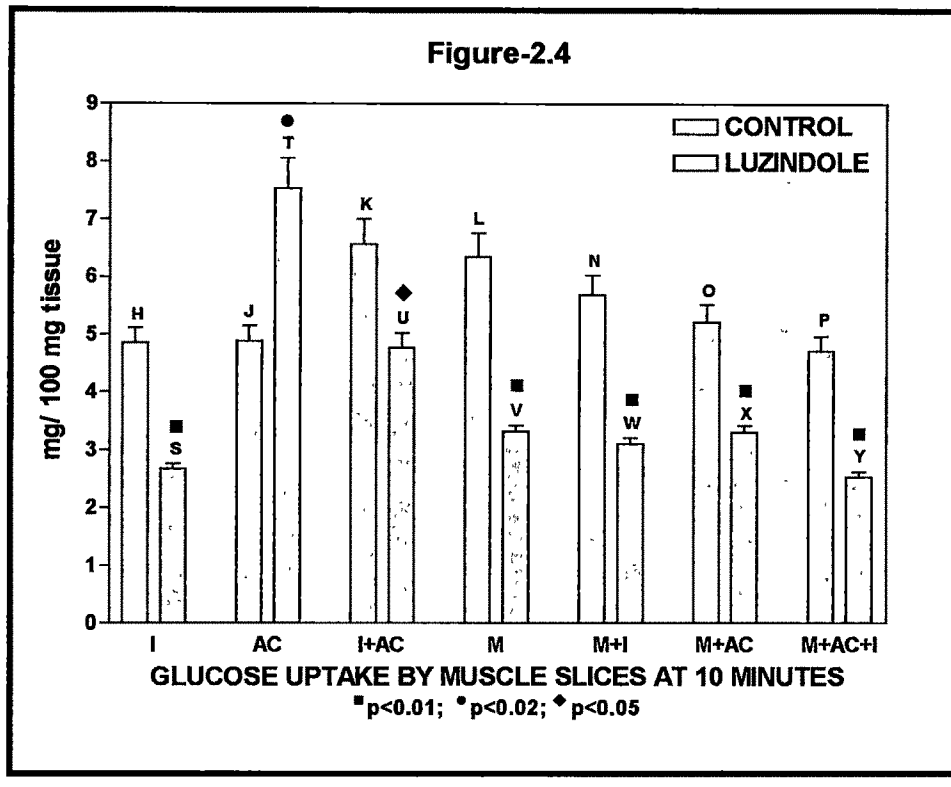

Figure 2.4: Glucose uptake at 10 minutes by muscle slices of control and luzindole treated weaning rats on 22nd day with combinations of insulin, acetylcholine and melatonin:

	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
LUZINDOLE	$\blacksquare 2.67^{(S)}$ ±0.094	$\bullet 7.53^{(T)}$ ±0.53	$\blacklozenge 4.77^{(U)}$ ±0.25	$\blacksquare 3.32^{(V)}$ ±0.11	$\blacksquare 3.11^{(W)}$ ±0.10	$\blacksquare 3.31^{(X)}$ ±0.11	$\blacksquare 2.54^{(Y)}$ ±0.093

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

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	✶	✶	✶	✶	✶
	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 luzindole treated weaning rats on 22nd day with combinations of insulin, acetylcholine and luzindole:

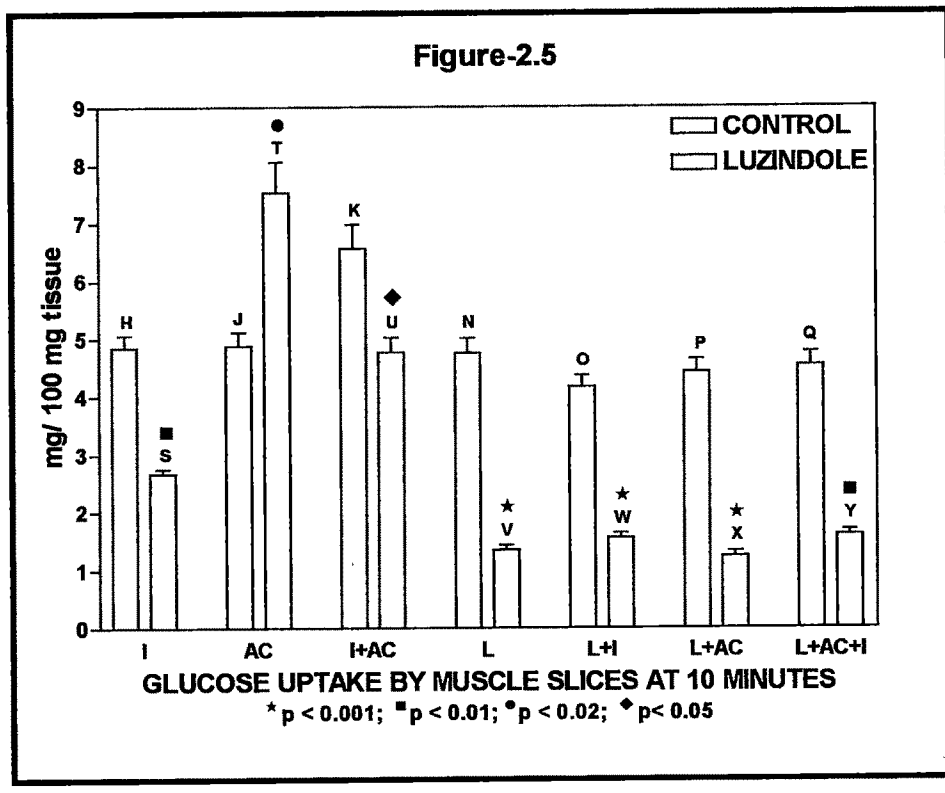

Table 2.5: Glucose uptake at 10 minutes by muscle slices of control and luzindole treated weaning rats on 22nd day with combinations of insulin, acetylcholine and luzindole:

	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
LUZINDOLE	$■2.67^{(S)}$ ±0.094	$•7.53^{(T)}$ ±0.53	$◆4.77^{(U)}$ ±0.25	$*1.35^{(V)}$ ±0.081	$*1.56^{(W)}$ ±0.083	$*1.25^{(X)}$ ±0.080	$■1.61^{(Y)}$ ±0.084

Values are expressed as mean ± SEM, *p < 0.001; ■p < 0.01; •p < 0.02; ◆p< 0.05

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	■	✶	✶	✶	✶	✶
	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; NSNon Significant

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

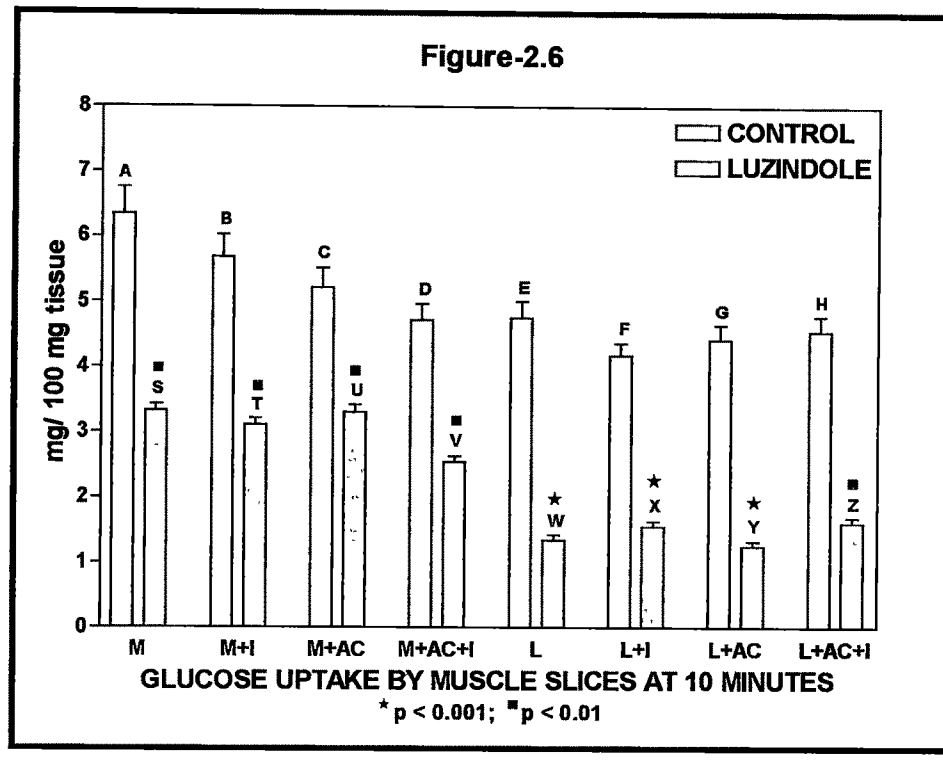

Table 2.6: Glucose uptake at 10 minutes by muscle slices of control and luzindole treated weaning rats on 22nd day with combinations of insulin, acetylcholine, melatonin and luzindole:

	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
LUZINDOLE	■3.32$^{(S)}$ ±0.11	■3.11$^{(T)}$ ±0.10	■3.31$^{(U)}$ ±0.11	■2.54$^{(V)}$ ±0.093	*1.35$^{(W)}$ ±0.081	1.56$^{(X)}$ ±0.083	1.25$^{(Y)}$ ±0.08	■1.61$^{(Z)}$ ±0.084

Values are expressed as mean ± SEM, *p < 0.001; ■p < 0.01

low uptake with luzindole alone as well as the combinations with insulin and acetylcholine (Figure and Table; 2.2, 2.3).

Muscle tissue uptake in presence of insulin, acetylcholine and melatonin: The control muscle slices showed maximal glucose uptake in presence of melatonin followed by, insulin and acetylcholine in the same range. Most markedly, luzindole treated muscle slices showed significantly maximal uptake with acetylcholine and significantly lower uptakes with melatonin and insulin (Figure and Table; 2.4).

Muscle tissue uptake in presence of combinations of insulin, acetylcholine and melatonin: Whereas the control slices showed a significantly higher uptake in presence of insulin+acetylcholine, equaling uptake promoted by melatonin alone, no other combination had any significant effect in increasing the uptake. The experimental muscle slices did not show any significant difference and showed consistently low uptake (Figure and Table; 2.4, 2.6).

Muscle tissue uptake in presence of luzindole and luzindole+acetlcholine, luzindole+insulin and luzindole+acetylcholine+insulin: Except for luzindole+insulin which showed a significantly increased uptake, no other combination had any effect in the control muscle slices. In contrast the experimental muscle slices showed almost negligible uptake with luzindole alone as well as the combinations with it (Figure and Table; 2.5, 2.6).

DISCUSSION:

The present results clearly show that neonatal blockage of melatonin action for 21 days can surely hamper the ability of liver for glucose uptake, as all agonists as well as their combinations showed a pronouncedly low ability to stimulate uptake. Both insulin and melatonin are potent promoters of hepatic glucose uptake but chronic luzindole treatment completely attenuates their ability. Melatonin seems to be more potent than insulin in promoting muscle glucose uptake and only acetylcholine+insulin could equal it. Interestingly, blockage of melatonin action increased significantly the muscle sensitivity to acetylcholine and maximal uptake was evident with acetylcholine in luzindole treated muscle slices. A remarkable observation is the ability of luzindole itself to promote glucose uptake in both liver and muscle and its potentiating influence with insulin in control animals similar to that with insulin and acetylcholine (Fig. and Tab; 2.2, 2.5)..

The presently noted significantly reduced tissue glucose uptake in luzindole treated neonates does not agree with the hyperinsulinemic glycogenic effect recorded previously (Chapter-I). Though the present results clearly show reduced tissue sensitivity towards promoters of glucose uptake, the glycogenic effect seen could be more a consequence of the quantitative effect of hyperinsulinemia rather than a qualitative effect. Since the muscle tissue of luzindole neonates has shown significantly increased glucose uptake in presence of acetylcholine, this could account for the glycogen loading of the muscle

tissue. The significantly increased response to acetylcholine in luzindole treated neonates as against response to melatonin or insulin+acetylcholine in control animals suggests increased sensitivity to parasympathetic modulation in the absence of melatonin action. In this context, modified tissue responsiveness to autonomic neurotransmitters as well as altered level of activity of the autonomic nervous system have been demonstrated in functionally pinealectomized animals (Carneiro *et al.*, 1991, 1994). Previously it has been shown that acetylcholine is a potent stimulator of glucose uptake in the pigeon (Patel and Ramachandran, 1992), while in rats though it is less potent than insulin has nevertheless has an enhancing influence on insulin induced 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 Ca^{+2} and increase the concentration of cAMP by decreasing phosphodiesterase activity (Rasmussen, 1975). Based on the present observations the possibility of functional pinealectomy/melatonin receptor antagonism in inducing modifications in the autonomic nervous activity and consequent effect on muscle glucose uptake cannot be ruled out and needs further studies.

The significantly high hepatic glycogen load in luzindole treated neonates (Chapter-I) as contrasted against the presently observed significantly reduced hepatic sensitivity and uptake of glucose with any of the agonistic agents need an explanation. In the light of the reported presence of melatonin receptors in the pancreas of neonates (Peschke

et al., 2000) the role of melatonin in reducing insulin secretion in the pre-pubertal period is supported by the previously recorded hyperinsulinemia in luzindole treated neonates (Chapter-I) and hypoinsulinemia in melatonin treated neonates (Jani, 2004). Despite inducing hyperinsulinemia by luzindole treatment, hepatic sensitivity to insulin is decreased and the reported reduced glucose tolerance, increased insulin resistance and decreased glucose uptake by pinealectomy support the same (Milcu *et al.*, 1971; Seraphim *et al.*, 1997; Lima *et al.*, 1998). The decreased hepatic sensitivity towards insulin, acetylcholine and melatonin suggests the possibility of some alternate mechanism of glucose uptake. It is clear that chronic luzindole treatment affects both melatonin and insulin receptors or downstream mechanism as well as acetylcholine action. In this context, the possible role of nitric oxide in increasing glucose uptake by the liver and muscle of luzindole treated neonates may have to be speculated as such a role for nitric oxide in increasing glucose transport independent of insulin action has been reported (Balon and Nadler, 1997). Their studies on nitric oxide synthetase inhibition as well as exposure to the nitric oxide donor have clearly shown decreased uptake with the former and increased uptake with the latter. This nitric oxide mediated pathway of glucose uptake was considered as a novel mechanism to increase glucose transport and was shown to be an independent pathway from that of insulin pathway (Balon and Nadler, 1997). In this context it is tempting to speculate that some such mechanism might be up-regulated by chronic luzindole treatment

as it obviously down-regulates both insulin and melatonin mediated glucose uptake.

Overall, the present study on glucose uptake by liver and muscle slices of luzindole treated rat neonates has revealed some novel aspects like increased acetylcholine response of muscle and an alternative pathway of glucose uptake independent of insulin, melatonin and acetylcholine. It is also interesting that luzindole itself has the potency to stimulate glucose uptake which may suggest of a function quite distinct from its role of melatonin receptor antagonism.

SUMMARY:

Since luzindole induced melatonin receptor antagonism in the rat neonates showed hyperinsulinemia and increased tissue glycogen contents with hypoglycemia, it was desirable to test the possibility of peripheral tissues depicting increased glucose uptake sensitivity. Hence the present *in vitro* study using liver and muscle slices form luzindole treated neonates for their ability to take up glucose in response to stimulants alone or in combination has been carried out. To this end, rat neonates have been treated with Luzindole (An MT_2 receptor blocker) (400 µg/Kg body weight) intra peritoneally from day 1 to day 21 and assessed on the 22^{nd} day. The luzindole treated rat liver slices showed significant reduction in glucose uptake induced by Insulin, Acetylcholine, Melatonin and their combinations. Also luzindole, as well as its combinations showed significantly decreased glucose uptake by the liver slices of experimental rats. Even the muscle slices of luzindole treated rats showed significantly decreased

glucose uptake induced by I, Ac, M as well as their combinations. The muscle slices of luzindole treated rats showed almost negligible glucose uptake in presence of luzindole and its combinations. The present study on glucose uptake by liver and muscle slices of luzindole treated rat neonates revealed some novel aspects like increased acetylcholine response of muscle and an alternative pathway of glucose uptake independent of insulin, melatonin and acetylcholine. It is also interesting to note that luzindole itself has potency to stimulate glucose uptake, which may suggest, of a function quite distinct from its role of melatonin receptor antagonism.