CHAPTER – 8

DOWN REGULATION OF GLUCOSE UPTAKE BY LIVER AND MUSCLE OF YOUNG RATS SUBJECTED TO NEONATAL MELATONIN ANTAGONISM: AN *IN VITRO* STUDY

INTRODUCTION:

Recently it has been showed that melatonin reduces pancreatic insulin secretion *in vitro* (Peschke *et al.*, 1997) and phase-response studies support the conviction that pancreatic beta cells may be targets for melatonin (Peschke and Peschke, 1998). Melatonin has also 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 Strandberg, 1991). Melatonin administration has been reported to have both hyper and hypoglycemic effects in a variety of animals (Ramachandran, 2002). The effects of neonatal melatonin administration on weaning and pubertal rats have shown significant higher insulin sensitivity with hyperinsulinemia and stimulated glycogenesis in the weaning period and persistent higher glycogenic effect even in the pubertal period due to transient pre-weaning hypermelatonemia (Jani, 2004). It has been demonstrated that pinealectomized rats show decreased hepatic and muscle

glycogenesis and an increase in blood pyruvate concentration (Milcu *et al.*, 1971; Mellado *et al.*, 1986). Further, pinealectomy has been shown to increase blood sugar levels in normal as well as alloxan treated rats (Csaba and Barath. 1971). Pinealectomy has been shown to decrease insulin response and manifest a fall in GLUT-4 content in adipose and muscle tissues (Lima *et al.*, 1998). 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). Biochemically usage of selective agonist and antagonist is not only important in identifying melatonin receptor subtypes but can also be used as an important tool for identifying specific physiological functions of melatonin an added advantage over the generalized effects of pinealectomy.

MATERIAL AND METHODS: See page numbers 18-38

RESULTS:

Liver Slices:

- **Uptake in presence of Insulin, Acetylcholine and Melatonin:** The liver slices of control rats showed significantly higher glucose uptake in presence of all the three agents individually as compared to the experimental rat liver slices. There was no significant difference between the uptake induced by insulin, acetylcholine or melatonin in the liver slices of control rats. However, the uptake induced by insulin was significantly higher as compared to that of acetylcholine or melatonin in the experimental rat liver slices. There was no significant difference

in the uptake promoted by acetylcholine and melatonin in the liver slices of experimental rats (Figure and Table; 8.1, 8.2)

- **Uptake by combinations of Insulin, Acetylcholine and Melatonin:** The uptake induced by M+Ac was significantly higher (almost double) as compared to that by any other combination or any of the agents individually in the liver slices of control rats. Whereas the uptake promoted by I+Ac and M+I were similar to each other and less than that by M+Ac+I as well as any of the agents individually in the liver slices of the control rats. The liver slices of luzindole treated rats showed significantly decreased glucose uptake in presence of any of the combinations as compared to the control rat liver slices. While M+Ac induced maximum glucose uptake in the liver slices of experimental rats I+Ac promoted minimal uptake as compared to other combinations or any of the agents individually (Figure and Table; 8.1, 8.2).
- **Uptake by combinations of Insulin, Acetylcholine and Luzindole:** In the liver slices of control rats the uptake induced by luzindole was similar to that of insulin. Whereas, the uptake induced by L+Ac+I was maximum that by L+I and L+Ac was minimum as compared to any of the agents individually in the liver slices of control rats.

Table and Figure: 8.1

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	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	*	

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	*	⊙	*	NS	*	

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

Figure 8.1: Glucose uptake at 10 minutes by liver slices of adult rats on 60th day with combinations of insulin, acetylcholine and melatonin subjected to neonatal luzindole treatment:

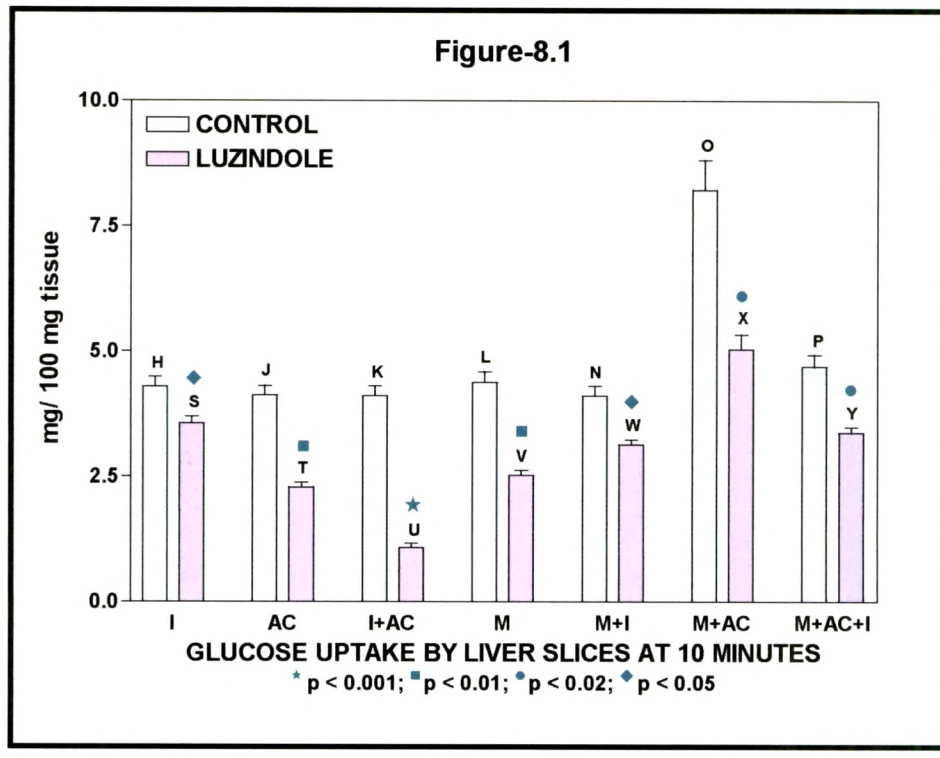

Table 8.1: Glucose uptake at 10 minutes by liver slices of adult rats on 60th day with combinations of insulin, acetylcholine and melatonin subjected to neonatal luzindole treatment:

	I	AC	I+AC	M	M+I	M+AC	M+AC+I
CONTROL	4.29(H) ±0.20	4.12(J) ±0.19	4.11(K) ±0.19	4.38(L) ±0.21	4.11(N) ±0.19	8.22(O) ±0.60	4.69(P) ±0.24
LUZINDOLE	◆3.56(S) ±0.13	■2.28(T) ±0.09	*1.08(U) ±0.08	■2.52(V) ±0.093	◆3.13(W) ±0.10	●5.04(X) ±0.30	●3.37(Y) ±0.11

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

Table and Figure: 8.2

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	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	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	✶	

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

Figure 8.2: Glucose uptake at 90 minutes by liver slices of adult rats on 60th day with combinations of insulin, acetylcholine and melatonin subjected to neonatal luzindole treatment:

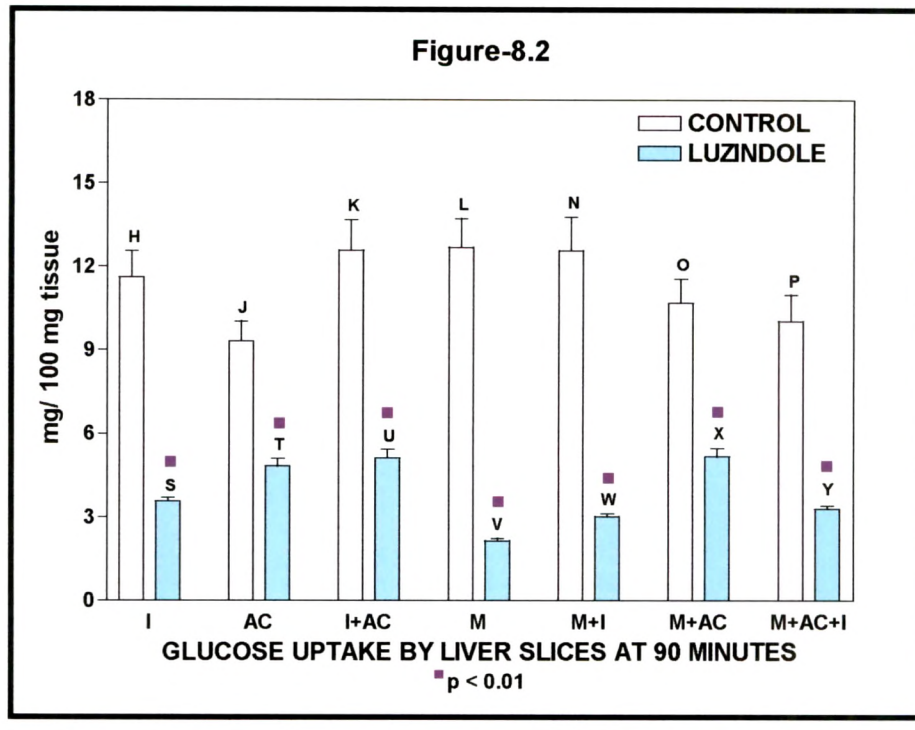

Table 8.2: Glucose uptake at 90 minutes by liver slices of adult rats on 60th day with combinations of insulin, acetylcholine and melatonin subjected to neonatal luzindole treatment:

	I	AC	I+AC	M	M+I	M+AC	M+AC+I
CONTROL	11.61$^{(H)}$ ±0.94	9.32$^{(J)}$ ±0.71	12.59$^{(K)}$ ±1.09	12.70$^{(L)}$ ±1.03	12.59$^{(N)}$ ±1.20	10.71$^{(O)}$ ±0.85	10.06$^{(P)}$ ±0.94
LUZINDOLE	▪3.57$^{(S)}$ ±0.13	▪4.84$^{(T)}$ ±0.26	▪5.14$^{(U)}$ ±0.30	▪2.15$^{(V)}$ ±0.09	▪3.03$^{(W)}$ ±0.10	▪5.19$^{(X)}$ ±0.30	▪3.31$^{(Y)}$ ±0.11

Values are expressed as mean ± SEM, ▪p < 0.01

Table 8.3

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	✶	✶	

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	✶	■	✶	✶	

*p<0.001; ⊙P<0.05; NSNon Significant

Figure 8.3: Glucose uptake at 10 minutes by liver slices of adult rats on 60th day with combinations of insulin, acetylcholine and luzindole subjected to neonatal luzindole treatment:

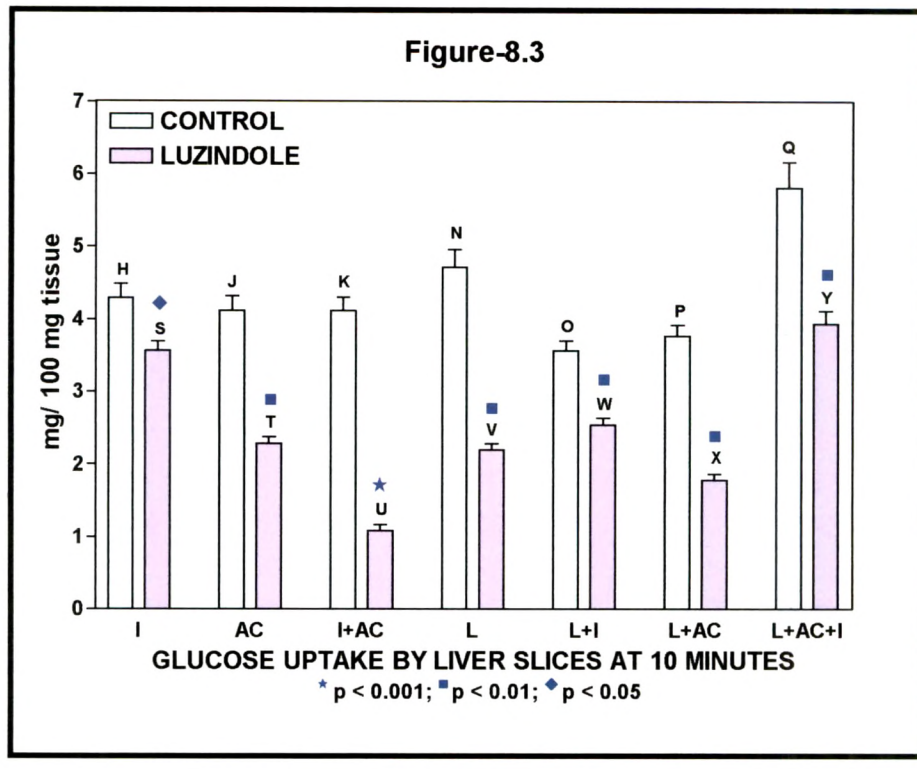

Figure 8.3: Glucose uptake at 10 minutes by liver slices of adult rats on 60th day with combinations of insulin, acetylcholine and luzindole subjected to neonatal luzindole treatment:

	I	AC	I+AC	L	L+I	L+AC	L+AC+I
CONTROL	4.29 (H) ±0.20	4.12 (J) ±0.19	4.11 (K) ±0.19	4.71 (N) ±0.25	3.57 (O) ±0.13	3.77 (P) ±0.15	5.81 (Q) ±0.36
LUZINDOLE	◆3.56 (S) ±0.13	■2.28 (T) ±0.09	*1.08 (U) ±0.08	■2.20 (V) ±0.09	■2.54 (W) ±0.093	■1.78 (X) ±0.085	◆3.94 (Y) ±0.17

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

Table 8.4

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	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	✶	✶	✶
	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<0.05; NSNon Significant

Figure 8.4: Glucose uptake at 90 minutes by liver slices of adult rats on 60th day with combinations of insulin, acetylcholine and luzindole subjected to neonatal luzindole treatment:

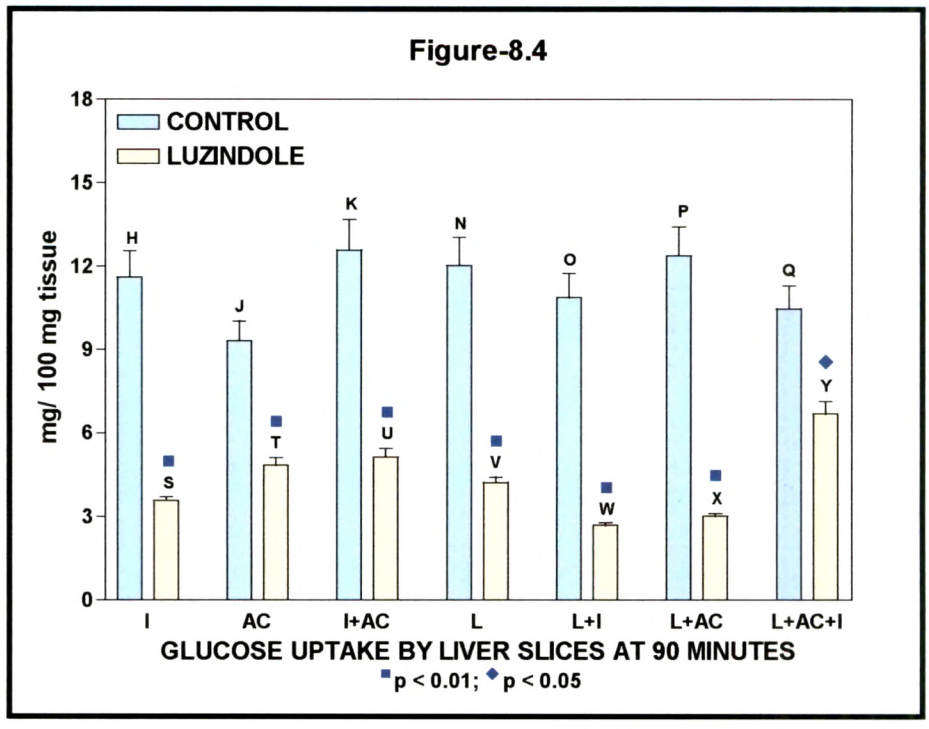

Table 8.4: Glucose uptake at 90 minutes by liver slices of adult rats on 60th day with combinations of insulin, acetylcholine and luzindole subjected to neonatal luzindole treatment:

	I	AC	I+AC	L	L+I	L+AC	L+AC+I
CONTROL	11.61(H) ±0.94	9.32(J) ±0.71	12.59(K) ±1.09	12.03(N) ±1.01	10.88(O) ±0.86	12.38(P) ±1.03	10.47(Q) ±0.82
LUZINDOLE	■3.57(S) ±0.13	■4.84(T) ±0.26	■5.14(U) ±0.30	■4.22(V) ±0.20	■2.68(W) ±0.094	■3.01(X) ±0.10	◆6.69(Y) ±0.44

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

Table 8.5

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	⊙	✶
	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	✶	■

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	✶	⊙	✶	✶
	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 8.5: Glucose uptake at 10 minutes by liver slices of adult rats on 60th day with combinations of insulin, acetylcholine, melatonin and luzindole subjected to neonatal luzindole treatment:

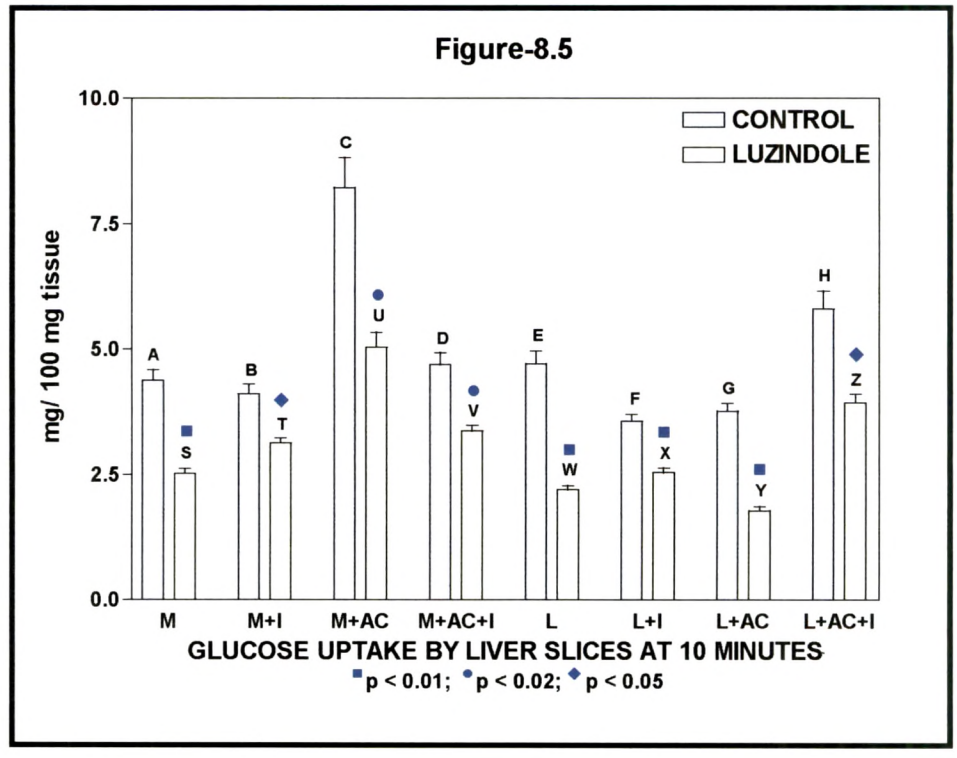

Table 8.5: Glucose uptake at 10 minutes by liver slices of adult rats on 60th day with combinations of insulin, acetylcholine, melatonin and luzindole subjected to neonatal luzindole treatment:

	M	M+I	M+AC	M+AC+I	L	L+I	L+AC	L+AC+I
CONTROL	4.38(A) ±0.21	4.11(B) ±0.19	8.22(C) ±0.60	4.69(D) ±0.24	4.71(E) ±0.25	3.57(F) ±0.13	3.77(G) ±0.15	5.81(H) ±0.36
LUZINDOLE	■2.52(S) ±0.093	◆3.13(T) ±0.10	●5.04(U) ±0.30	●3.37(V) ±0.11	■2.20(W) ±0.09	■2.54(X) ±0.093	■1.78(Y) ±0.085	◆3.94(Z) ±0.17

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

Table 8.6

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	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	✶	✶
	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	✶	✶

*p<0.001; ⊙P<0.05; NSNon Significant

Figure 8.6: Glucose uptake at 90 minutes by liver slices of adult rats on 60th day with combinations of insulin, acetylcholine, melatonin and luzindole subjected to neonatal luzindole treatment:

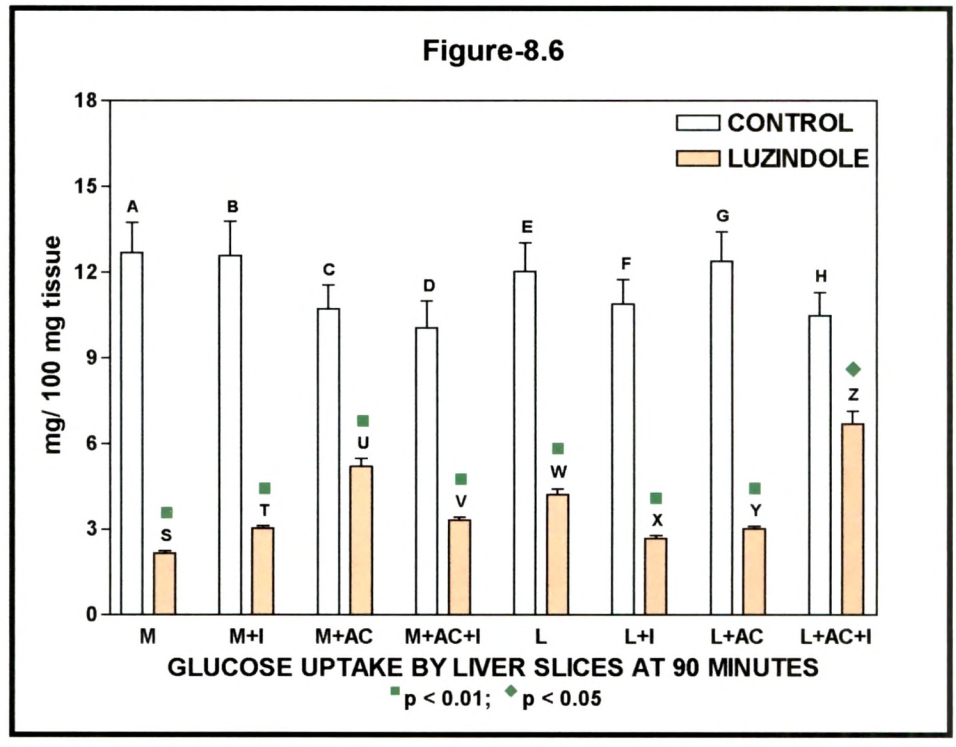

Table 8.6: Glucose uptake at 90 minutes by liver slices of adult rats on 60th day with combinations of insulin, acetylcholine, melatonin and luzindole subjected to neonatal luzindole treatment:

	M	M+I	M+AC	M+AC+I	L	L+I	L+AC	L+AC+I
CONTROL	12.70$^{(A)}$ ±1.03	12.59$^{(B)}$ ±1.20	10.71$^{(C)}$ ±0.85	10.06$^{(D)}$ ±0.94	12.03$^{(E)}$ ±1.01	10.88$^{(F)}$ ±0.86	12.38$^{(G)}$ ±1.03	10.47$^{(H)}$ ±0.82
LUZINDOLE	■2.15$^{(S)}$ ±0.09	■3.03$^{(T)}$ ±0.10	■5.19$^{(U)}$ ±0.30	■3.31$^{(V)}$ ±0.11	■4.22$^{(W)}$ ±0.20	■2.68$^{(X)}$ ±0.094	■3.01$^{(Y)}$ ±0.10	◆6.69$^{(Z)}$ ±0.44

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

Table and Figure: 8.7

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	■
	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	■	

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	

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

Figure 8.7: Glucose uptake at 10 minutes by muscle slices of adult rats on 60th day with insulin, acetylcholine and melatonin subjected to neonatal luzindole treatment:

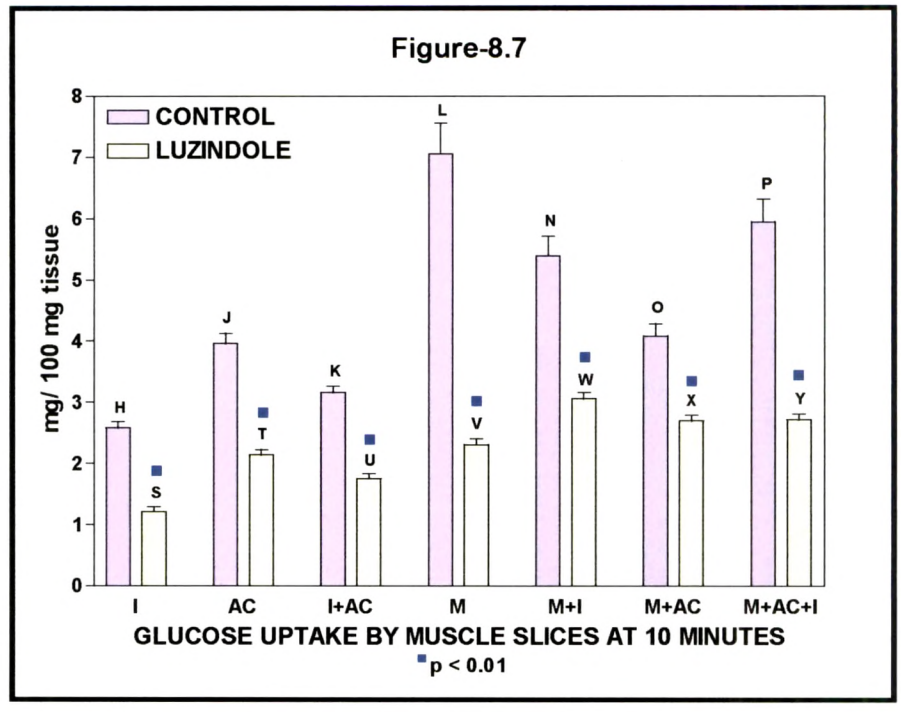

Figure 8.7: Glucose uptake at 10 minutes by muscle slices of adult rats on 60th day with combinations of insulin, acetylcholine and melatonin subjected to neonatal luzindole treatment:

	I	AC	I+AC	M	M+I	M+AC	M+AC+I
CONTROL	2.58(H) ±0.099	3.96(J) ±0.17	3.16(K) ±0.10	7.06(L) ±0.50	5.40(N) ±0.32	4.09(O) ±0.20	5.95(P) ±0.37
LUZINDOLE	■1.21(S) ±0.08	■2.14(T) ±0.09	■1.78(U) ±0.085	■2.31(V) ±0.091	■3.06(W) ±0.10	■2.70(X) ±0.095	■2.72(Y) ±0.095

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

Table and Figure: 8.8

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	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	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	*	

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

Figure 8.8: Glucose uptake at 90 minutes by muscle slices of adult rats on 60th day with combinations of insulin, acetylcholine and melatonin subjected to neonatal luzindole treatment:

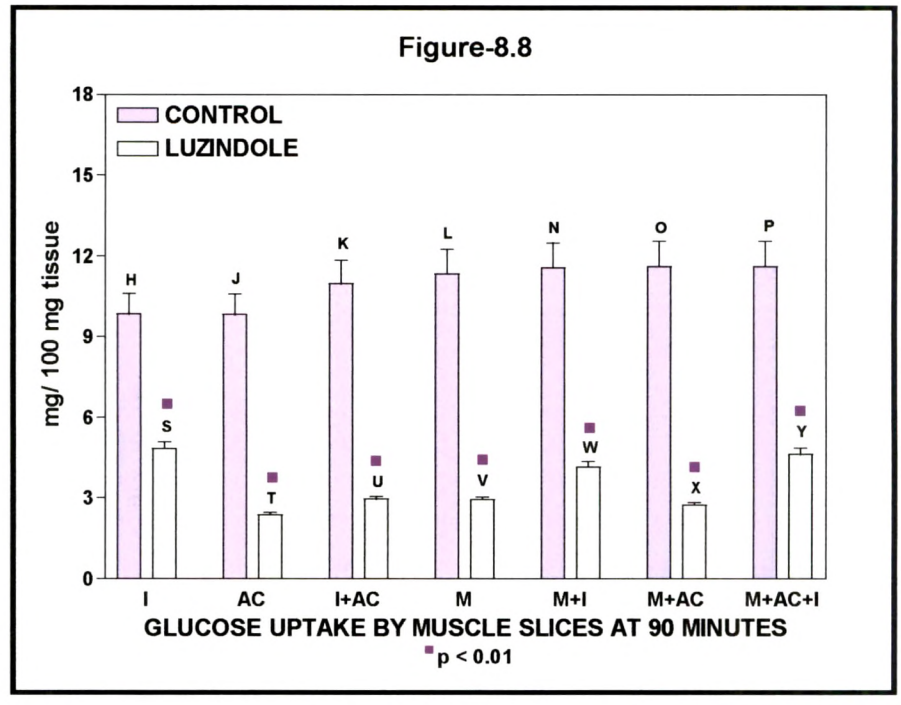

Figure 8.8: Glucose uptake at 90 minutes by muscle slices of adult rats on 60th day with combinations of insulin, acetylcholine and melatonin subjected to neonatal luzindole treatment:

	I	AC	I+AC	M	M+I	M+AC	M+AC+I
CONTROL	9.85 (H) ±0.76	9.82 (J) ±0.76	10.97 (K) ±0.87	11.35 (L) ±0.91	11.56 (N) ±0.93	11.61 (O) ±0.94	11.61 (P) ±0.94
LUZINDOLE	▪4.83 (S) ±0.26	▪2.37 (T) ±0.091	▪2.96 (U) ±0.097	▪2.94 (V) ±0.097	▪4.16 (W) ±0.20	▪2.74 (X) ±0.095	▪4.63 (Y) ±0.24

Values are expressed as mean ± SEM, ▪p < 0.01

Table 8.9

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	*	■	*	*	*	*	*
	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	

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	

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

Figure 8.9: Glucose uptake at 10 minutes by muscle slices of adult rats on 60th day with combinations of insulin, acetylcholine and luzindole subjected to neonatal luzindole treatment:

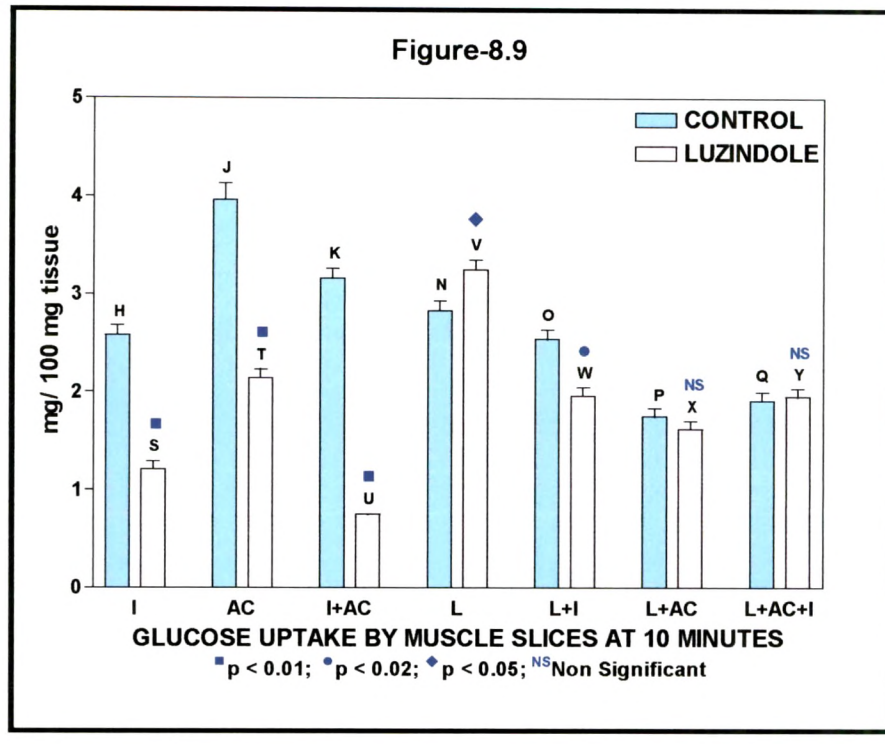

Table 8.9: Glucose uptake at 10 minutes by muscle slices of adult rats on 60th day with combinations of insulin, acetylcholine and luzindole subjected to neonatal luzindole treatment:

	I	AC	I+AC	L	L+I	L+AC	L+AC+I
CONTROL	2.58 (H) ±0.099	3.96 (J) ±0.17	3.16 (K) ±0.10	2.83 (N) ±0.096	2.54 (O) ±0.093	1.75 (P) ±0.085	1.91 (Q) ±0.087
LUZINDOLE	■1.21 (S) ±0.08	■2.14 (T) ±0.09	■0.75 (U) ±0.005	◆3.25 (V) ±0.10	•1.96 (W) ±0.087	NS1.62 (X) ±0.084	NS1.95 (Y) ±0.087

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

Table 8.10

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	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	✶
	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	✶	

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

Figure 8.10: Glucose uptake at 90 minutes by muscle slices of adult rats on 60th day with combinations of insulin, acetylcholine and luzindole subjected to neonatal luzindole treatment:

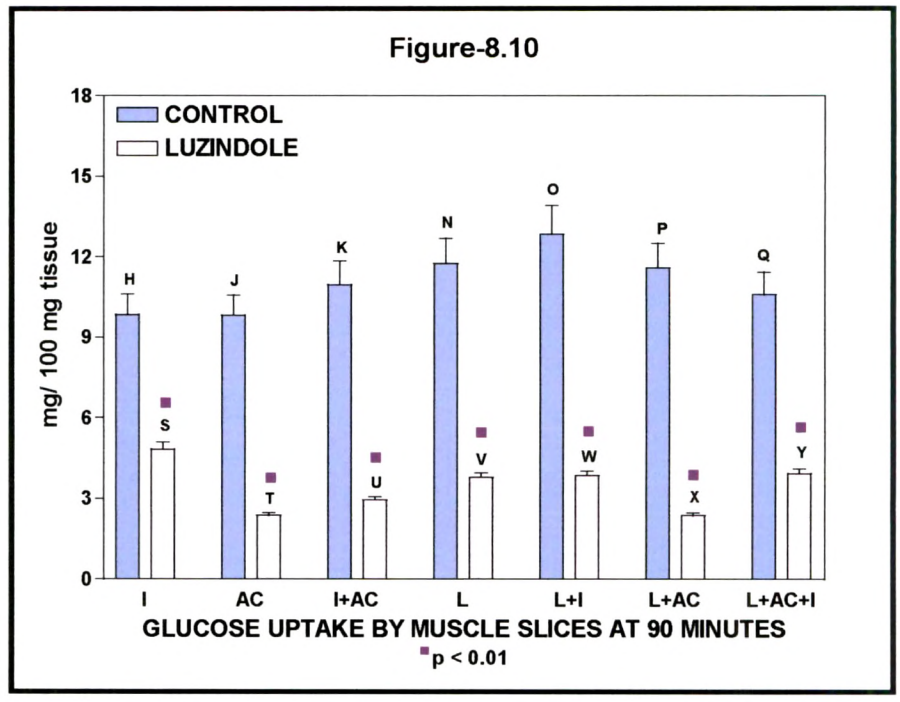

Table 8.10: Glucose uptake at 90 minutes by muscle slices of adult rats on 60th day with combinations of insulin, acetylcholine and luzindole subjected to neonatal luzindole treatment:

	I	AC	I+AC	L	L+I	L+AC	L+AC+I
CONTROL	9.85(H) ±0.76	9.82(J) ±0.76	10.97(K) ±0.87	11.75(N) ±0.95	12.85(O) ±1.06	11.59(P) ±0.93	10.61(Q) ±0.84
LUZINDOLE	■4.83(S) ±0.26	■2.37(T) ±0.091	■2.96(U) ±0.097	■3.80(V) ±0.16	■3.87(W) ±0.16	■2.37(X) ±0.091	■3.94(Y) ±0.17

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

Table 8.11

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	*	*	*	*	■
	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

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
	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

*p<0.001; ⊙P<0.05; NSNon Significant

Figure 8.11: Glucose uptake at 10 minutes by muscle slices of adult rats on 60th day with combinations of insulin, acetylcholine, melatonin and luzindole subjected to neonatal luzindole treatment:

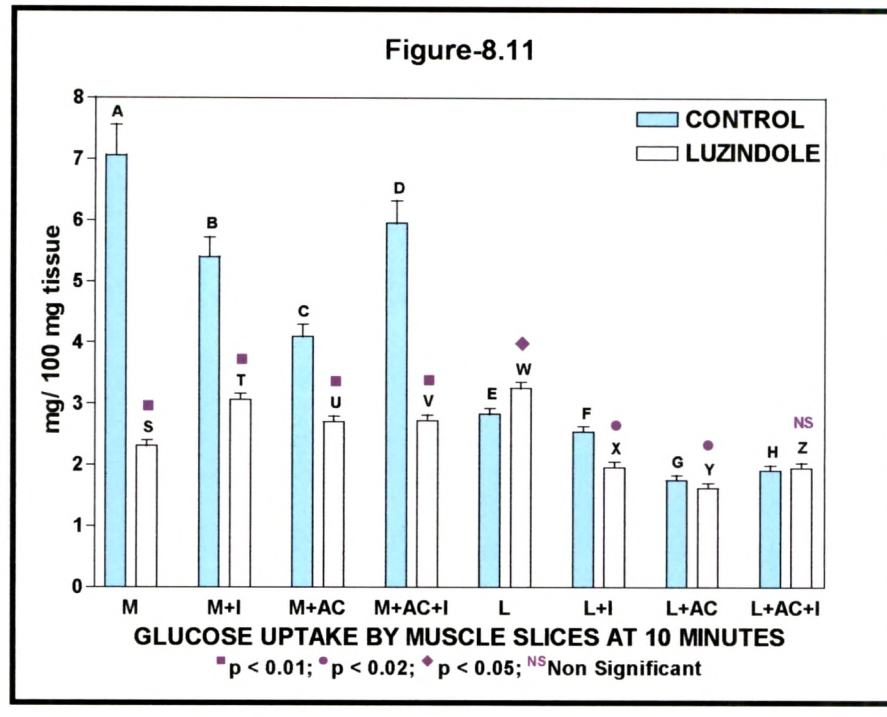

Table 8.11: Glucose uptake at 10 minutes by muscle slices of adult rats on 60th day with combinations of insulin, acetylcholine, melatonin and luzindole subjected to neonatal luzindole treatment:

	M	M+I	M+AC	M+AC+I	L	L+I	L+AC	L+AC+I
CONTROL	7.06$^{(A)}$ ±0.50	5.40$^{(B)}$ ±0.32	4.09$^{(C)}$ ±0.20	5.95$^{(D)}$ ±0.37	2.83$^{(E)}$ ±0.096	2.54$^{(F)}$ ±0.093	1.75$^{(G)}$ ±0.085	1.91$^{(H)}$ ±0.087
LUZINDOLE	■2.31$^{(S)}$ ±0.091	■3.06$^{(T)}$ ±0.10	■2.70$^{(U)}$ ±0.095	■2.72$^{(V)}$ ±0.095	◆3.25$^{(W)}$ ±0.10	●1.96$^{(X)}$ ±0.087	●1.62$^{(Y)}$ ±0.084	NS1.95$^{(Z)}$ ±0.087

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

Table 8.12

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	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	*
	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 8.12: Glucose uptake at 90 minutes by muscle slices of adult rats on 60th day with combinations of insulin, acetylcholine, melatonin and luzindole subjected to neonatal luzindole treatment:

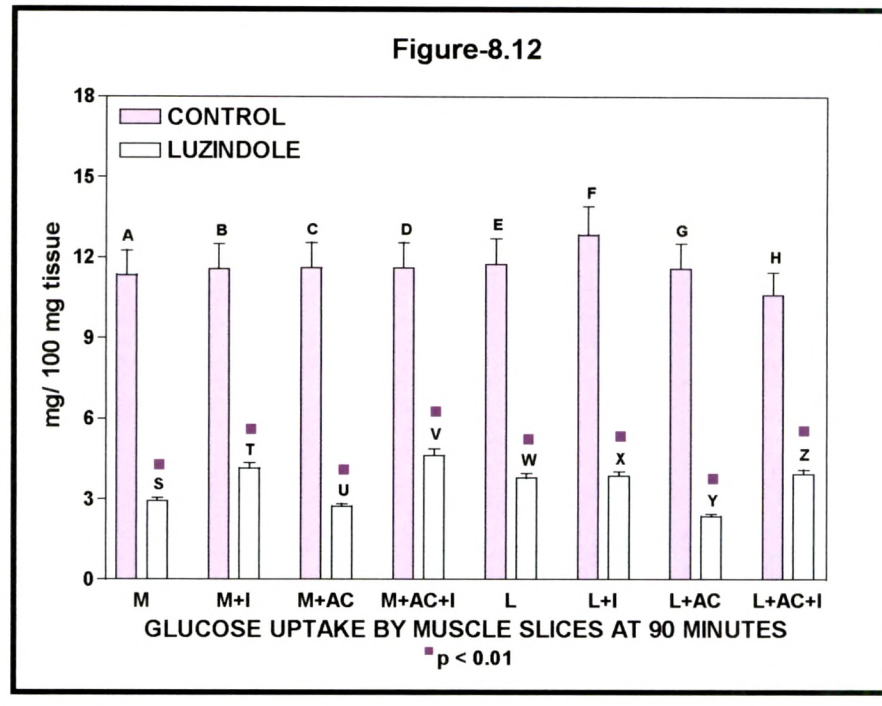

Table 8.12: Glucose uptake at 90 minutes by muscle slices of adult rats on 60th day with combinations of insulin, acetylcholine, melatonin and luzindole subjected to neonatal luzindole treatment:

	M	M+I	M+AC	M+AC+I	L	L+I	L+AC	L+AC+I
CONTROL	11.35$^{(A)}$ ±0.91	11.56$^{(B)}$ ±0.93	11.61$^{(C)}$ ±0.94	11.61$^{(D)}$ ±0.94	11.75$^{(E)}$ ±0.95	12.85$^{(F)}$ ±1.06	11.59$^{(G)}$ ±0.93	10.61$^{(H)}$ ±0.84
LUZINDOLE	$^{\blacksquare}$2.94$^{(S)}$ ±0.092	$^{\blacksquare}$4.16$^{(T)}$ ±0.20	$^{\blacksquare}$2.74$^{(U)}$ ±0.095	$^{\blacksquare}$4.63$^{(V)}$ ±0.24	$^{\blacksquare}$3.80$^{(W)}$ ±0.16	$^{\blacksquare}$3.87$^{(X)}$ ±0.16	$^{\blacksquare}$2.37$^{(Y)}$ ±0.091	$^{\blacksquare}$3.94$^{(Z)}$ ±0.17

Values are expressed as mean ± SEM, $^{\blacksquare}p < 0.01$

The liver slices of luzindole treated rats showed significantly decreased glucose uptake as compared to that of control rats. Whereas the uptake promoted by luzindole was similar to that of acetylcholine alone and less than that by insulin alone, the uptake induced by the combination of L+Ac+I was maximum as compared to any other combination or any of the agents individually in the liver slices of luzindole treated rats. However L+I and L+Ac induced glucose uptake was significantly less than that by insulin alone in the liver slices of luzindole treated rats (Figure and Table; 8.3, 8.4).

Muscle Slices:

- **Uptake in presence of Insulin, Acetylcholine and Melatonin:** The uptake induced by melatonin in the liver slices of control rats was significantly higher while that by insulin was significantly lower. However the control muscle slices showed significantly higher glucose uptake in presence of either of the agents as compared to the experimental rat muscle slices. In the experimental rats also the uptake induced by melatonin was higher than that by insulin. The acetylcholine induced uptake was higher than that by insulin but still lower as compared to that by melatonin alone in the muscle slices of experimental rats (Figure and Table; 8.7, 8.8)
- **Uptake by combinations of Insulin, Acetylcholine and Melatonin:** In the muscle slices of control rats the uptake induced by M+Ac+I was maximum as compared to other

combinations but was significantly lower as compared to that of melatonin alone. However the uptake promoted by other combinations was in the order of I+Ac<M+Ac<M+I and was lower than that by melatonin alone in the muscle slices of control rats. The uptake promoted by M+I was significantly higher as compared to any other combination in the muscle slices of experimental rats. However the uptake promoted by M+Ac and M+Ac+I was similar that, of I+Ac was reduced in the muscle slices of experimental rats. Glucose uptake by the muscle slices of experimental rats decreased significantly as compared to that of control rats in presence of any of the agent or there combinations (Figure and Table; 8.7, 8.8).

- **Uptake by combinations of Insulin, Acetylcholine and Luzindole:** The uptake promoted by luzindole alone was significantly higher than that by insulin alone in the muscle slices of control rats. However the uptake induced by the combinations of luzindole was in the order L+Ac<L+Ac+I<L+I and was significantly reduced as compared to I+Ac. The uptake promoted by luzindole in the muscle slices of experimental rats increased significantly as compared to insulin or acetylcholine alone in the experimental rats and to that of control slices in presence of luzindole alone. However the combinations of insulin and acetylcholine in presence of luzindole showed uptake in the order of L+Ac<L+Ac+I<L+I and were significantly higher as compared to that by insulin alone in the experimental

rat muscle slices and reduced as compared to control slices except for L+Ac+I (Figure and Table; 8.9, 8.10).

DISCUSSION:

A Significant decrease in glucose uptake was shown in the pubertal period related to the weaning period in a previous study (Chapter 2 & 5). However, neonatal hypomelatonemic rat tissues were shown to have significantly higher glucose uptake in the pubertal period compared to controls, suggesting a better glucose uptake capacity due to neonatal melatonin antagonism. A closer examination however reveals that the glucose uptake by the tissues of neonatal hypomelatonemic rats does not show any change from the weaning period and hence the apparently observed increase in the pubertal period is essentially due to a reduced glucose uptake potentiated by the tissues of control animals (Chapter 5). In the present study the liver slices of control rats show a significantly increased glucose uptake related to the pubertal period, nevertheless lesser than that in the weaning period (Chapter 2). But the liver slices of experimental rats show a unchanged glucose uptake potential from the pubertal period. Apparently the glucose uptake potential of liver of neonatal hypomelatonemic rats is consistently down regulated from weaning through pubertal to adult stage (Fig. and Tab.; 8.1, 8.2, 8.3, 8.4). Relatively lesser hepatic glycogen recorded in the experimental rats compared to control rats attests to the presently noted glucose uptake potential (Chapter 7). The decreased potential of hypomelatonemic liver is further confirmed by the observed no change in the degree of

glucose uptake from 10 to 90 minutes while the control liver slices show a significant further increment in uptake from 10 to 90 minutes (Fig. and Tab.; 8.1-8.6). Interestingly, though insulin, acetylcholine and melatonin showed similar ability to support glucose uptake, a combination of M+Ac seems to have a significant additive influence in promoting glucose uptake in control rats. This potentiating effect of M+Ac combination is clearly indicated by the relatively higher glucose uptake promoted by this combination even in hypomelatonemic rat liver slices, though relatively lesser than in controls (Fig. and Tab.; 8.1, 8.2). Obviously a possibility of an age related increase in cholinergic sensitivity which can be potentiated by melatonin need to be ascertained. Another novel observation is the ability of luzindole and combinations of luzindole with insulin and acetylcholine to promote relatively greater, more significantly at 90 minutes than by melatonin as seen on the control liver slices (Fig. and Tab.; 8.3, 8.4). This ability though attenuated is still observable in hypomelatonemic slices. Though the ability of luzindole to promote glucose uptake is understandable in the context of it being an analog of melatonin, its ability to promote higher uptake than melatonin in adult rats needs further evaluations.

The muscle slices of hypomelatonemic rats show a significantly reduced sensitivity to uptake promoting agents and reduced glucose uptake compared to control muscle slices (Fig. and Tab. 8.7-8.12). Though this decreased sensitivity shown by hypomelatonemic muscle is similar to that shown by liver, nevertheless there is a better

sensitivity to insulin as seen by the increased uptake at 90 minutes by insulin as well as combinations containing insulin, which was not seen in the case of liver. Though luzindole alone promotes relatively greater uptake even more than by melatonin in the muscle of hypomelatonemic rats, unlike melatonin which could not antagonize the action of insulin, luzindole apparently antagonizes the action of insulin and decreases insulin induced uptake (Fig. and Tab.; 8.9, 8.10). With regard to increasing insulin sensitivity and resistance and decreased uptake by liver and peripheral tissues with increasing age, it has been suggested that increasing fat mass/fat stores and serum free fatty acid levels are the causes for the same (Seraphim *et al.*, 1997; Lima et al., 1994). However in the present study this level does not seem to hold good as hypomelatonemic rats have relatively lesser tissue fat load and serum free fatty acids (Chapter 9) and yet there is increased muscle lipid and glycogen contents in hypomelatonemic rats (Chapter 7 & 9), it is conceivable that there is an impairment in normal metabolic homeostasis in the form of differential partitioning of carbohydrates among tissues and channelisation into intracellular pathways and this is manifested as extra hepatic, extra adipose tissue deposition in muscle. These inferences as well as the mechanisms contributing to these changes need to be studied in detail to decipher the long term changes in metabolism induced by neonatal melatonin antagonism.

SUMMARY:

Recent studies have shown that pinealectomy causes glucose intolerance, insulin resistance and decreased adipose cell

responsiveness to insulin. The present study in this backdrop has been designed to evaluate the effects of neonatal melatonin antagonism on *in* vitro tissue glucose uptake in the adult rats. 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 60^{th} day. The liver slices of luzindole treated rats showed significantly decreased uptake with insulin(I), acetylcholine(Ac), melatonin(M) as well as with their combinations at both 10 and 90 minutes as compared to the control liver slices. Also, luzindole(L) as well as its combinations with I and Ac induced significantly decreased glucose uptake at both 10 and 90 minutes by the liver slices of experimental rats. The muscle slices of the experimental rats showed significantly decreased glucose uptake at both 10 and 90 minutes with I, Ac, M as well as their combinations whereas, the uptake induced by L and its combinations did not show any significant alteration as compared to control muscle slices. It is conceivable from the present study that, there is an impairment in normal metabolic homeostasis in the form of differential partitioning of carbohydrates among tissues and channelisation into intracellular pathways and, this is manifested as extra hepatic, extra adipose tissue deposition in muscle. These inferences as well as the mechanisms contributing to these changes need to be studied in detail to decipher the long term changes in metabolism induced by neonatal melatonin antagonism.