CHAPTER 10

NEONATAL PINEALECTOMY NEGATES THE EFFECT OF HYPERTHYROIDISM AND ADVANCES THE ONSET OF ADULT TYPE CARBOHYDRATE HOMEOSTASIS IN THE MALE RATS

Carbohydrate metabolism is important in the overall metabolic strategy of the adult testis. Current state of understanding, is in consonance with the Sertoli cells supplying adequate amounts of lactate to the advanced germ cells (much needed for their survival), by active glucose catabolism (see, Grootegoed and Den Boer, 1990). Though blood glucose is the principal source for Sertoli cells in the adult condition, previous studies have shown the importance of testis store of glycogen in the initial wave of spermatogenesis. The control rats as well as the experimental rats depicted significant and sudden depletion of glycogen to low adult levels coinciding with the establishment of active spermiogenesis for the first time (chapters 6-9). Since the postnatal changes in carbohydrate metabolism occurring in the testis are related with the overall systemic changes, an analysis of blood glucose levels and hepatic carbohydrate metabolism carried out concurrently, revealed alterations signifying the transition from immature to mature stages (chapters 6-9).

Previous studies showed that neonatal hyperthyroidism and Px, induced characteristic changes in systemic and testis carbohydrate metabolism due to an altered endocrine milieu and

in relation to the effects on testis function (chapters 2, 4, 7 and 9). In this behest , an evaluation of the possible effects of a combined experimental paradigm of neonatal hyperthyroidism and Px on systemic and testis carbohydrate metabolism gained curiosity; more so with the observed interesting effects on testis functions (chapter 5).

RESULTS

BLOOD GLUCOSE (Table 10.1; Fig. 1)

Control: The blood glucose level was significantly high at 35 days which then decreased to a significantly low level at 45 days. The glycemic level then increased significantly by 60 days (though significantly less than the 35 day level) and this level was maintained thereafter.

HPRT + Px: The HPRT + Px animals showed significant hypoglycemic status at 35 days. Thereafter, the blood glucose level increased at 45 days, then decreased at 60 days and again increased to significant hyperglycemic level at 90 days.

HEPATIC GLUCOSE-6-PHOSPHATASE (Table 10.3; Fig. 3)

Control: The G-6-Pase activity showed a continuous increase from 35 to 60 days to attain the highest level of activity at this period. Thereafter, the activity decreased significantly at 90 days.

HPRT + Px: The G-6-Pase activity was significantly higher in the HPRT + Px rats at 35 days. Thereafter, there was a significant and consistent decrease in the enzyme activity through 45 days to reach a low level at 60 days. However, at 90 days, the enzyme activity increased significantly.

HEPATIC GLYCOGEN (Table 10.3; Fig. 4)

Control: The glycogen content was more or less steady between 35 and 60 days in control animals with a slight decrement at 45 days. But at 90 days there was a significant increase in hepatic glycogen content.

Table. 10.1 Chronological alterations in Blood Glucose (mg/dL) level in intact and hyperthyroid rats subjected to pinealectomy (HPRT + Px)

Treatment	Age in Days			
	35	45	60	90
Control	122.55 ± 10.87@	89.25 ± 7.48	108.77 ± 10.34	103.40 ± 14.06
HPRT + Px	115.99 ± 13.14^{ns}	145.61 ± 13.42^{d}	120.57 ± 10.28^{ns}	134.33 ± 12.13^{c}

@ Values expressed as Mean ± SD of five experiments

^{c} $p < 0.01$; ^{d} $p < 0.001$; ^{ns} Not Significant

Table. 10.2 Chronological alterations in Testis Glycogen (μg/100 mg) and Phosphorylase activity (μmoles of P released/mg protein/10 min.) in intact and hyperthyroid rats subjected to pinealectomy (HPRT + Px)

Treatment	GLYCOGEN				PHOSPHORYLASE			
	Age in Days				Age in Days			
	35	45	60	90	35	45	60	90
Control	5.10 ± 1.20@	24.90 ± 2.00	9.60 ± 2.50	9.40 ± 0.30	1198.96 ± 49.59	94.07 ± 8.94	72.79 ± 6.34	239.54 ± 17.08
HPRT + Px	45.70 ± 6.70^{d}	38.20 ± 5.00^{d}	14.30 ± 3.70^{b}	18.30 ± 2.50^{d}	185.71 ± 13.47^{d}	77.02 ± 7.06^{c}	634.89 ± 12.11^{d}	241.84 ± 10.73^{ns}

@ Values expressed as Mean ± SD of five experiments

^{b} $p < 0.025$; ^{c} $p < 0.01$; ^{d} $p < 0.001$; ^{ns} Not Significant

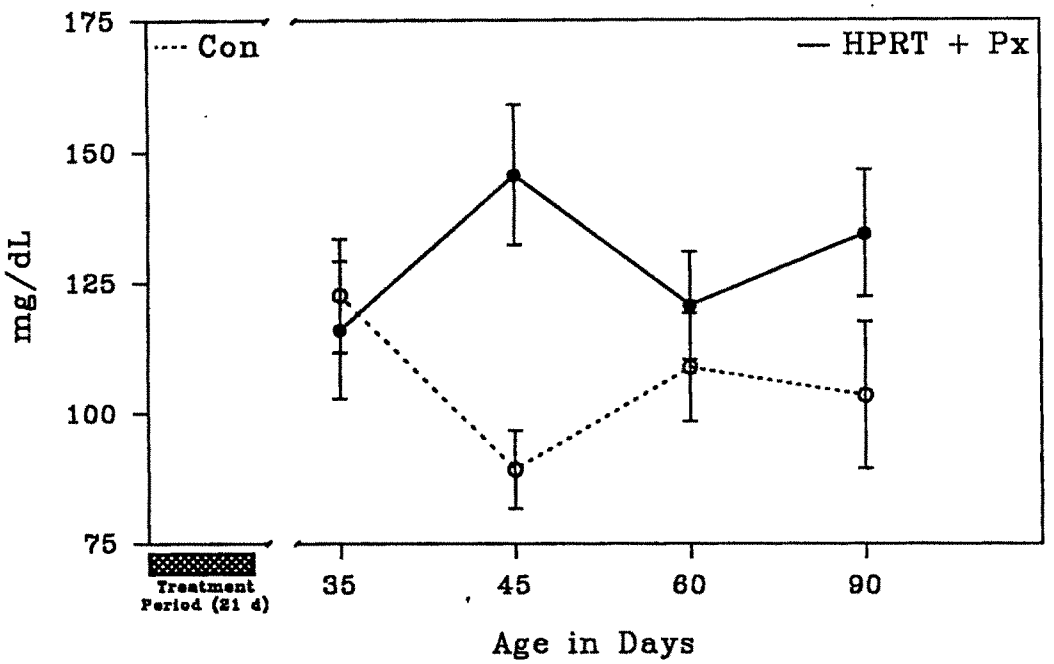

Fig. 1 Chronological alterations in blood glucose level in hyperthyroid rats subjected to pineal ectomy (HPRT + Px)

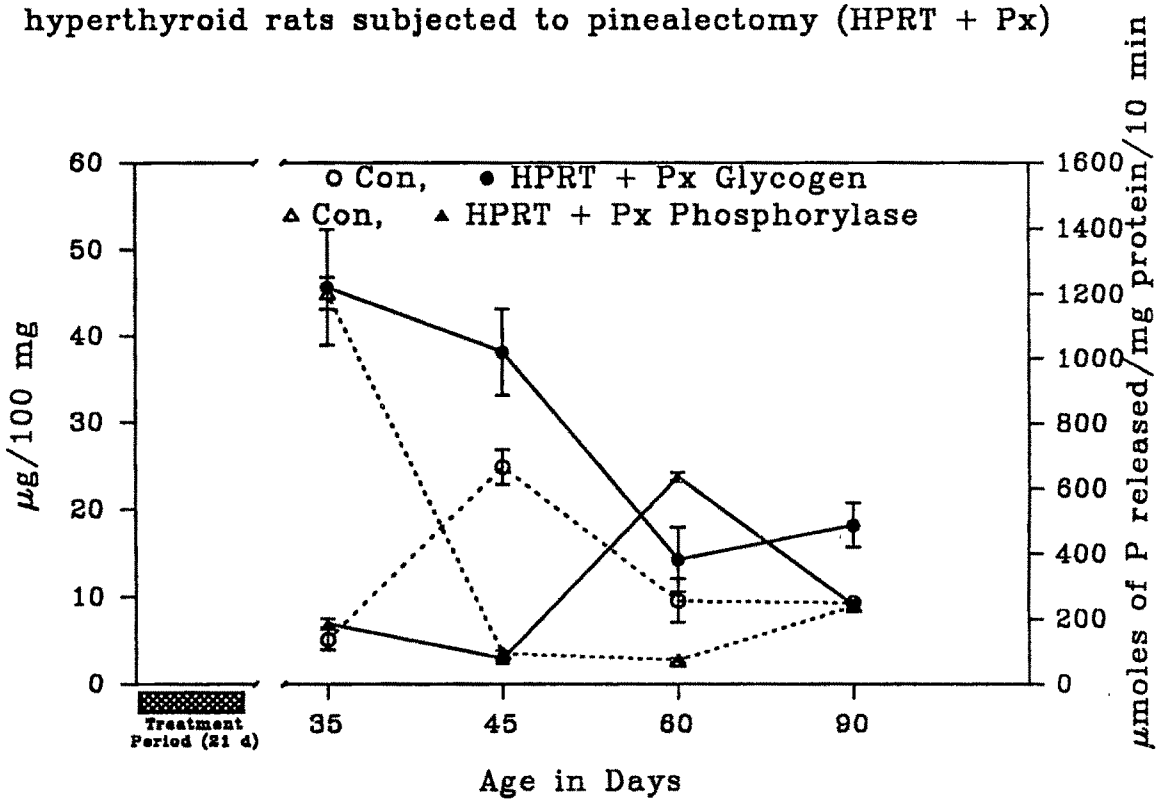

Fig. 2 Chronological alterations in testis glycogen and phosphorylase activity in intact and HPRT + Px rats

Table 10.3 Chronological alterations in Hepatic Glycogen (mg/100 mg), Phosphorylase (μmoles of P relased/mg protein/10 min.) and G-6-Pase (μmoles of PO_4 relased/mg protein/10 min.) activities in intact and hyperthyroid rats subjected to pinealectomy (HPRT + Px)

Treatment	GLYCOGEN				PHOSPHORYLASE				G-6-PASE			
	35	45	60	90	35	45	60	90	35	45	60	90
Control	1.49 ± 0.09@	1.09 ± 0.13	1.38 ± 0.02	4.10 ± 0.43	690.88 ± 47.70	61.54 ± 1.97	230.61 ± 10.62	198.88 ± 8.56	14.96 ± 0.89	16.08 ± 2.70	17.96 ± 4.35	13.94 ± 2.70
HPRT + Px	3.45 ± 0.38^{d}	1.74 ± 0.19^{d}	1.44 ± 0.24^{ns}	3.38 ± 0.34^{c}	130.44 ± 13.25^{d}	85.50 ± 7.15^{d}	47.95 ± 2.99^{d}	256.29 ± 23.59^{d}	18.76 ± 1.85^{c}	14.45 ± 2.47^{ns}	7.11 ± 1.90^{d}	16.70 ± 2.79^{ns}

@ Values expressed as Mean ± SD of five experiments

^{c} $p < 0.01$; ^{d} $p < 0.001$; ^{ns} Not Significant

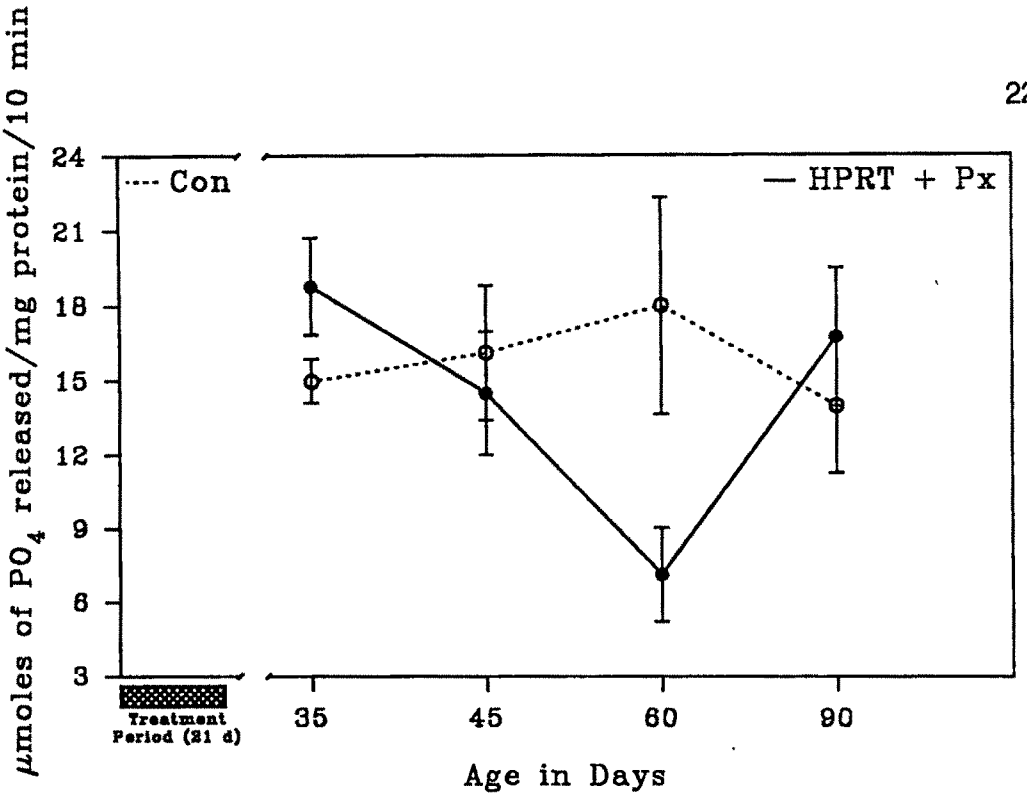

Fig. 3 Chronological alterations in liver G-6-Pase activity in hyperthyroid rats subjected to pinealectomy (HPRT + Px)

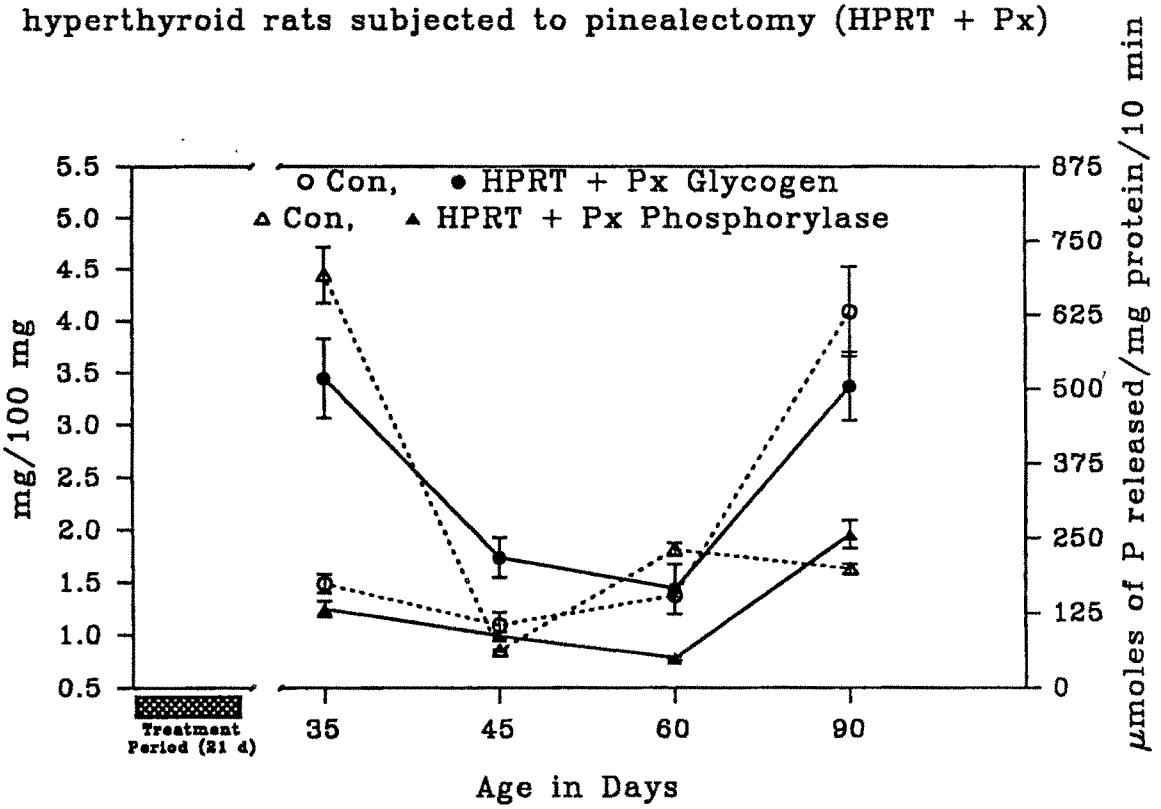

Fig. 4 Chronological alterations in hepatic glycogen and phosphorylase activity in intact and HPRT + Px rats

HPRT + Px: The glycogen content was significantly higher at 35 days. There was significant depletion in the glycogen content at 45 days which further decreased at 60 days but thereafter it increased to the characteristic adult level by 90 days.

HEPATIC PHOSPHORYLASE (Table 10.3; Fig. 4)

Control: The phosphorylase activity was significantly high at 35 days which thereafter showed a drastic decline at 45 days. The enzyme activity later on increased to attain the adult levels through 60 to 90 days.

HPRT + Px: The enzyme activity was significantly reduced at 35 days. Thereafter, it further decreased at 45 days to reach a steady level at 60 days. The enzyme activity then increased significantly at 90 days.

TESTIS GLYCOGEN (Table 10.2; Fig. 2)

Control: The glycogen content was low at 35 days, but then increased significantly by 45 days However, at 60 days, there was significant depletion and, this level was maintained thereafter.

HPRT + Px: The HPRT + Px rats had significantly higher glycogen content at 35 days. Thereafter, they showed a steady decline in the glycogen content through 45 days to reach the lowest level at 60 days. More or less the same level was maintained even at 90 days.

TESTIS PHOSPHORYLASE (Table 10.2; Fig. 2)

Control: There was significantly higher phosphorylase activity at 35 days in the control animals. The enzyme activity showed significant decrease thereafter and reached the lowest level at 60 days. At 90 days the enzyme activity reached to an adult level.

HPRT + Px: The enzyme activity was significantly decreased at 35 days which continued to do so to reach the lowest level at 45 days. Thereafter, the enzyme activity showed significant increase at 60 days and then decreased to an adult level at 90 days.

DISCUSSION

The present investigation shows an overall hyperglycemic state and early increment in hepatic glycogen content in HPRT + Px rats. The glycogen content at 35 days is identical to the level characteristic of control rats at 90 days, though there was reduced contents at 45 and 60 days. The early attainment of adult level hepatic glycogen store is indicative of a temporally augmented setting in of increased insulin to glucagon molar ratio, known to occur during the transition from immature to the adult stage (Margolis, 1983). Such an augmented attainment was postulated to occur even in other experimental paradigms like, neonatal hypothyroidism, Px, hypothyroidism along with Px and neonatal hyperthyroidism (chapter 6-9). A common feature in all the above experimental animals was the state of hypothyroidism in the postweanling periods. This was taken to suggest that, thyroid hormone resists the attainment of adult level insulin to glucagon molar ratio and that, transient hypothyroidism in the immediate postweanling periods could consequentially result in the early attainment of increased insulin:glucagon ratio. In the present experimental condition also, hypothyroidism was manifested in the postweanling period (as seen at 35 days), which is reflected well in the recorded hepatic glycogen content. Reduced glycogen content at 45 and 60 days could be due to other exigences, one of which could be body growth as, significantly increased growth rate was seen between 45 and 60 days. The increased glycogen content at 35 days could also be influenced by increased insulin sensitivity due to the previously reported neonatal hyperthyroidism induced decrease in GH secretion (chapter 4), which was also shown to manifest to a lesser degree in HPRT + Px animals (chapter 5).

Despite the inferred increased insulin:glucagon ratio and insulin sensitivity, paradoxically the HPRT + Px rats, exhibit significant hyperglycemia. In the earlier studies, hyperthyroidism was

shown to cause hypoglycemia and Px hyperglycemia, these were related to reduced G-6-Pase activity and corticosterone action and increased G-6-Pase activity and corticosterone action respectively (chapters 7 and 9). However, in the present study, the HPRT + Px rats despite registering significantly reduced G-6-Pase activity, depicted significant hyperglycemia, even greater than in the Px animals. Apparently, neonatal hyperthyroidism and Px exert individual as well as combined effects leading to altered hormonal balances and sensitivities; the balance of interactions of which results in incoherent and inexplicable metabolic alterations. This is confirmed by the presently recorded unrelated changes in hepatic glycogen content, phosphorylase and G-6-Pase activities and blood glucose level, as well as the unpublished observations on hepatic and testis lipid and protein contents and various enzymes. Neonatal hyperthyroidism was shown to lower the set-point of hypothalamo-pituitary-thyroid axis, resulting in hypothyroidism in the adult stage, while, neonatal Px was shown to delay the maturation of the hypothalamic-pituitary-thyroid axis (chapters 2 and 4). Presently, the HPRT + Px rats did not show a hypothyroidic state in the adult condition. Inferably, Px nullifies the action of hyperthyroidism on the hypothalamic-pituitary-thyroid axis. It is apparent that the action of thyroid hormone in lowering the set-point of hypothalamic-pituitary-thyroid axis is manifested only in a pineal intact animal suggesting an interaction between pineal hormone and thyroxine on the hypothalamic-pituitary-thyroid axis. Since a robust pineal rhythm commences only after 10 days, the presumed interaction between these two hormones should therefore be occurring in the second neonatal week. The observed hypothyroidism in the postweanling period is a consequence of delayed maturation of the hypothalamic-pituitary-thyroid axis is due to Px and, viewed in this context, the action of thyroid hormone seems to occur during a critical window of maturation of hypothalamic-pituitary-thyroid axis which, coincides with the establishment of pineal rhythmicity.

The increased testis glycogen content in relation to the control at 35 days is in keeping with the prevailing increased insulin action. The significant depletion in the testis glycogen

content between 45 and 60 days, coincides with the observed initial wave of spermatogenesis (chapter 5). Such a depletion in testis glycogen, coinciding with progression of spermiogenesis and appearance of sperms, was evident in control animals, as well as in the previous experimental groups (chapters 6-9). Advanced germ cells from pachytene spermatocytes onwards, especially spermatids, are known to be dependant on an effective supply of lactate moieties for their survival and maturation (Leiderman and Mancini, 1969; Fouquet and Guha, 1969: Gunaga *et al.*, 1972; Grootegoed and Den Boer, 1990). The continued supply of lactate has been shown to be a function of Sertoli cells for which they are dependant on blood glucose in the adult condition (Grootegoed and Den Boer, 1990). Based on the consistent observations of increasing testis glycogen content in the prepubertal period and its sudden depletion coinciding with the first wave of spermatogenesis (essentially during the formation of spermatids and the progression of spermiogenesis), in all the previous studies, it was inferred that the testis glycogen store is the metabolite of choice during the first wave of spermatogenesis (chapters 6-9). A rational explanation extended to this contention was the relative inadequacy/immaturity of the glucose transporting and concentrating mechanisms in the Sertoli cells and also the possible increased energy demand during the first wave of spermatogenesis (chapter 6). The present observations on the depletion of testis glycogen in HPRT + Px animals coinciding with the period of establishment of spermiogenesis fosters the above alluded functional significance of glycogen. Another notable observation is the significantly higher testis glycogen content in the adult stage in the HPRT + Px rats compared to the controls. This may find correlation with the earlier reported increased number of Sertoli and germ cells (chapter 5). In this context, spermatogonia and Sertoli cells have been shown to be glycogen rich cells in the testis (Fouquet and Guha, 1969, Leiderman and Mancini, 1969).

Finally, based on the present observations, the following conclusions can be drawn:

- Neonatal hyperthyroidism in pinealectomised animals can temporally advance the attainment of insulin:glucagon molar ratio.
- Pinealectomy can nullify the influence of thyroid hormone in the neonatal stage on the hypothalamic-pituitary-thyroid axis.
- Neonatal hyperthyroidism and Px can create ill-understood alterations in hormonal secretions and interactions leading to altered metabolic profile.
- Testis glycogen store is an important energy source during the first wave of spermatogenesis.