Ind. J. Physiol. Pharmac., Volume 33, Number 4, 1989

ALTERED MATERNAL THYROID FUNCTION : FETAL AND NEONATAL DEVELOPMENT OF RAT

RATAN KUMAR* AND B. N. CHAUDHURI

Department of Endocrinology and Metabolism,
Defence Institute of Physiology and Allied Sciences, Delhi Cantt. - 110 010

(Received on October 3, 1989)

Abstract : Influence of maternal thyroid status on fetal and neonatal development of rats has been studied. Maternal hypothyroidism resulted impaired reproduction and intrauterine growth retardation of offsprings as revealed by their reduced body weight, heart weight, body length and tail length. Offsprings born to hypothyroid mothers showed very high rate of mortality and none of them survived beyond eight days. Maternal hyperthyrodism did not cause any abnormality on reproduction. Hyperthyroid mothers showed increased rate in body weight gain during pregnancy which was associated with increased weight of body and heart of fetuses born to hyperthyroid mothers. Plasma thyroxine was not measurable in fetus from hypothyroid mothers till 21st day of gestation. The results of the present study showed that maternal thyroid status plays an important role in fetal and neonatal growth and development.

Key words : maternal thyroid fetal neonatal development

INTRODUCTION

Thyroid hormones exert influence on various physiological functions. The necessity of thyroid hormones for early fetal life is not clearly understood. Observations have been made that growth of body mass inutero appears to be totally or almost independent of thyroid hormones (1). On the other hand Verma et al (2) observed that thyroid hormones influence fetal growth.

Inspite of the extensive work done on the aetiology of malformations induced by hyper and hypothyroidism, the contributions of thyroid hormones in development are not clear. These considerations necessated to initiate the present study on the effect of maternal thyroid hormones in fetal and neonatal development.

* Corresponding Author

METHODS

Colony bred sprague Dawley rats (Weighing approximately 140-160 g) were used in this study. All animals were maintained at comfortable temperature (24°C±1°C) and had free access to water and food in pellet form (Hindustan Lever Ltd, Bombay, India). The rats were divided into three groups. Group-I female rats served as control Mothers and no treatment was given. Group II female rats were rendered hypothyroid by iujecting 1 mci ^{131}I (Bhabha Atomic Research Centre, Bombay, India), i p. to each rat. These rats were kept separate for 6-8 weeks before they were used for mating purpose. Group III female rats were rendered hyperthyroid by injecting 70 ugm L-Thyroxine (Sigma, Ltd. USA) per rat per day i.p. Thyroxine injections were started two weeks prior to the rats were used for mating and continued during

pregnancy and lactation period. Normal male rats of same strain and age were used for mating female of all three groups. The gestational age was determined by vaginal smear technique. The sperm positive day of vaginal smear was taken as the day of conception (0 day of pregnancy). Deliveries occurred in all the three groups on 22nd day±12 hrs. Age groups studied were day 19 and 21 of fetal age and 0 day (day of birth), 7 days, 14 days, 21 days and 28 days neonatal age.

Parameters studied : (i) Growth rate of female rats after conception (ii) Reproduction and its associated changes were assessed by observing percentage of occurrence of pregnancy, resorption and survival of offsprings. (iii) Maternal, fetal and neonatal thyroid status was assessed by determining plasma Thyroxine (T_4) Concentration in heparinized blood using thyroxine radio immunoassay kits obtained from Bhaba Atomic Research Centre, Bombay, India. (iv) On 19 and 21 day of gestation pregnant rats were anesthetized with ip administration of nembutal (40 mg/kg body weight) and live litters were removed surgically. Body weights were recorded. Fetal heart was weighed immediately on removal. Body weight and heart weight of neonates of different age groups understudy were also recorded. (v) Placental weight of 19 and 21 day of gestation, body length and tail length of fetuses and neonates at different age gronps understudy were recorded for a better assessment of their growth.

Statistical analysis of data : Data was analyed using student 't' test.

RESULTS

Growth rate of mothers after conception : The Table I shows results of maternal body weight gain during pregnancy. Hyperthyroid mothers showed significant increase in body weight gain almost at all the tested points of gestational periods. In contrast, hypothyroid moteers showed reduced body weight gain during the entire gestational period.

TABLE I : Gain in maternal body weight during pregnancy.

Groups	*Gestational age (days)*									
	3	*5*	*7*	*9*	*11*	*13*	*15*	*17*	*19*	*21*
Control	6.9±0.4	11.0±0.6	14.7±0.7	19.4±0.6	23.3±0.9	28 3±1.0	34.4±1.6	43.0±2.4	53.3±2.4	71.2±2.3
Hyperthyroid	6.2±0.5	12.4±0.9	17.5±0.9	21.7±1.1	32.7±1.4	37.3±0.9	47.2±1.5	61.6±2.3	77.0±1.9	83.6±1.9
	NS	NS	P<0.05	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
Hypothyroid	0.5±0.1	2.1±0.3	3.9±0.4	6.4±0.5	9.6±0.9	13.7±1.2	19.7±1.4	27.2±2.1	32.5±1.9	37.3±1.6
	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

Values have been expressed in g. All the values are mean±SEM of 10 samples in each group.

Reproduction and survial of offsprings : The results on influence of maternal thyroid status on reproduction and other associated changes have been given in Table II. Litter size from hypothyroid mothers was low. Percentage of conception was very low in hypothyroid mothers, out of which a significant number of cases showed resorptions and vaginal bleeding. The life span of neonates born to hypothyroid mothers was approximately 8 days, hence in the present study all the parameters could be studied only upto 7 days neonatal age in case of offsprings born to hypothyroid mothers.

Maternal, fetal and neonatal plasma thyroxine levels : The results of maternal and fetal and neonatal plasma thyroxine levels are given in Table III. Pleasma thyroxine levels were significantly high in hyperthyroid mothers at all age groups while the reverse has been observed in hypothyroid mothers (Table-III). Fetuses and neonates born to hyperthyroid mothers showed higher plasma thyroxine levels while in case of offsprings born to hypothyroid mothers it was low (Table III).

TABLE II : Reproduction and survival of offsprings.

Groups	*Letter size*	*% Pregnancies*	*Out of 100% Pregnancies*		*Out of total number of offsprings % of mortality*							
			% Resorptions	*% Vaginal bleeding*	*Days after birth*							
					1	*2*	*3*	*4*	*5*	*6*	*7*	*Total*
Control	7.7±0.4	86.0	ND	ND	4.3	6.5	2.6	—	1 3	1.3	—	16%
Hyperthyroid	7.8±0 6	79.0	ND	ND	7.7	7.7	2.6	2.6	3.8	2.6	—	27%
Hypothyroid	5.4±0.4 P<0.001	40.0	27.0	10.8	50.0	8.5	7.4	5.6	6.6	7.6	8.4	94%

All the results are of 10 samples in each group. Values have been expressed as % for pregnancies occurred and mortality but are mean±SEM for litter size. ND — Not detectable.

TABLE III : Plasma thyroxine (T_4) concentration of mothers and their offsprings (Fetuses/neonates) during gestational and lactational period.

Groups		*Gestational age/fetal age (days)*		*Day of birth*	*Lactational age/neonatal age (days)*			
		19	*21*	*0*	*7*	*14*	*21*	*28*
Control	Mother	3 5±0.1	3 3±0.1	3.4±0.2	5.4±0.2	3 9±0.3	5.2±0.2	5.4±0.3
	Offspring	0.4±0.1	0.6±0.1	0.6±0.1	1.5±0.2	2.3±0.2	2.9±0.3	3.6±0.2
Hyperthyroid	Mother	7.4±0.2 P<0.001	6.3±0.5 P<0.001	5.2±0.4 P<0.01	8.2±0.1 P<0.001	7.9±0.1 P<0.001	8.4±0.2 P<0.001	9.2±0.2 P<0.001
	Offspring	0.6±0.1 NS	0.9±0.1 P<0.02	1.2±0.1 P<0.001	1.8±0.1 NS	3.2±0.2 P<0.01	4.0±0.1 P<0.01	3.8±0.2 NS
Hypothyroid	Mother	0.8±0.1 P<0.001	0.7±0.1 P<0.001	0.9±0.1 P<0.001	0.7±0.1 P<0.001	—	—	—
	Offspring	ND	0.3±0.05 P<0.001	0.3±0.04 P<0.02	0.5±0.1 P<0.001	—	—	—

Results have been expressed as plasma thyroxine μg/dl and all the values are mean±SEM of 5 samples in each group. ND — Not detectable

Fetal and neonatal heart weight and body weight : The results of fetal and neonatal body weight, heart weight and heart weight (mg)/body weight (g) ratio have been given in Table IV. Fetuses born to hyperthyroid mothers showed significant increase in body weight and heart weight at 21 day fetal age. The neonatal heart weight was higher at 7 days neonatal age Consequently heart weight/body ratio showed decreases and increases depending upon changes in body weight and heart weight. In contrast, offsprings born to hypothyroid mothers showed decreased body weight and heart weight in fetal and neonatal age groups. According changes in heart weight/body weight were recorded (Table IV).

Body length, tail length and placental weight : The results of body length, tail length and placental weights have been given in Table V. Offsprings born to hyperthyroid mothers showed increase in body length at 19 day of fetal and 7 days neonatal age, while their tail length showed significant increase at all age except 28 day of neonatal age. Placental weight also showed increase in hyperthyroid state. In offsprings born to hypothyroid mothers body length and tail length showed decrease at all age groups. No significant change was observed in placental weight of hypothyroid mothers

TABLE IV : Fetal and neonatal body weight, heart weight and heart weight/body weight ratio.

Groups		*Fetal age (days)*		*Day of birth*	*Neonatal age (days)*			
		19	*21*	*0*	*7*	*14*	*21*	*28*
Control	Body weight (g)	2.07±0.04	4.42±0.16	5.29±0.02	10.80±0.25	15.25±0.20	20.25±0.34	39.30±0.37
	Heart weight (mg)	15.20±0.33	20.40±0.70	28.50±0.40	67.00±1.08	99.80±2.22	116.90±4.50	164.70±2.40
	Heart wt (mg)/ Body wt (g)	7.35±0.08	4.62±0.07	5.38±0.05	6.22±0.43	6.50±0.07	5.76±0.13	4.19±0.04
	Body weignt (g)	2.45±0.04 P<0.001	5.02±0.02 P<0.01	5.28±0.04 NS	11.30±0.15 NS	17.10±0.28 P<0.001	20.20±0.33 NS	38.20±0.44 NS
Hyperthyroid	Heart weight (mg)	16.00±0.21 NS	28.80±0.38 P<0.001	29.10±0.43 NS	86.50±0.87 P<0.001	96.80±0.81 NS	116.80±2.35 NS	167.70±3.92 NS
	Heart wt (mg)/ Body wt (g)	6.54±0.07 P<0.001	5.74±0.05 P<0.001	5.58±0.14 NS	7.63±0.06 P<0.001	5.67±0.06 P<0.001	5.77±0.04 NS	4.39±0 13 NS
Hypothyroid	Body weight (g)	1.91±0.02 P<0.01	3 06±0.03 P<0.001	4.12±0.09 P<0.001	5.99±0.03 P<0.001	—	—	—
	Heart weight (mg)	10.30±0.21 P<0.001	17.40±0.52 P<0.01	25.00±0.42 P<0.001	38.80±0.55 P<0.001	—	—	—
	Heart wt (mg)/ Body wt (g)	5.42±0.07 P<0.001	5.67±0.12 P<0.001	6.07±0.07 P<0.001	6.48±0.62 NS	—	—	—

Results have been expressed as mean±SEM of 10 samples in each group.

TABLE V : Placental weight, body length and tail length of fetuses and neonates.

Groups		Fetal age (days)		Day of birth	Neonatal age (days)			
		19	21	0	7	14	21	28
Control	Placental weight (mg)	479±4	535±10	—	—	—	—	—
	Body length (mm)	27.0±0.3	37.0±0.4	40.9±0.4	49.5±0.3	60.1±0.6	67.6±0.9	76.7±0.5
	Tail length (mm)	10.4±0.2	13.2±0.2	16.9±0.3	29.2±0.3	38.0±0.2	46.1±0.5	56.7±0.6
Hyperthyroid	Placental weight (mg)	552±5 P<0.001	611±9 P<0.001	—	—	—	—	—
	Body length (mm)	29.8±0.5 P<0.02	37.8±0.4 NS	41.4±0.4 NS	51.2±0.3 P<0.05	60.9±0.7 NS	68.6±0.8 NS	76.5±0.5 NS
	Tail length (mm)	12.3±0.1 P<0.001	14.2±0.2 P<0.02	17.8±0.2 P<0.05	31.9±0.1 P<0.01	41.8±0.3 P<0.001	51.9±0.5 P<0.001	57.0±0.5 NS
Hypothyroid	Placental weight (mg)	465±7 NS	541±9 NS	—	—	—	—	—
	Body length (mm)	24.0±0.3 P<0.001	28.5±0.2 P<0.001	31.4±0.3 P<0.001	39.7±0.4 P<0.001	—	—	—
	Tail length (mm)	9.0±0.2 P<0.01	11.2±0.3 P<0.001	13.4±0.2 P<0.001	21.3±0.2 P<0.001	—	—	—

Results are mean±SEM of 10 samples in each group.

DISCUSSION

Hyperthyroidism is associated with high cardiac output (3) which might have resulted in better uteroplacental blood flow and supply of nutrients, causing better fetal growth. In hypothyroidism cardiac output and blood volume is low (4) which might have resulted in reduced placental blood flow. Hence, the decreased availability of nutrients could therefore be responsible in retarded fetal growth which in turn decreased gain in maternal body weights of hypothyroid mothers in the present study. The altered cardiovascular effects have been considered in addition to direct effect of maternal thyroid hormones on fetal growth, if any. The observed reproductive dysfunctions may be due to altered endocrine support of pregnancy (5). In the offsprings born to hypothyroid mothers had a very high rate of mortality after birth, no offspring survived after 8 days of birth. All the pups were small, with loose skin, cool body and showed poor attachment with their mothers for suckling. Middlesworth (6) also observed similar findings with severe mortality of offsprings born to hypothyroid mothers. High mortility of the offsprings born to hypothyroid mothers might have caused by low plasma T_4 resulting in hypothermia (7) and reduced birth weights (8). There was no measurable plasma T_4 in the offsprings born to hypothyroid mothers at day 19 of gestation (Table-III). Perhaps due to low maternal plasma T_4 levels of hypothyroid mothers no plasma T_4 was detected in fetuses upto 19 day of gestation and

afterwards detected plasma T_4 levels may be due to thyroxine synthesized by fetal thyroid gland itself, because fetal pituitary thyroid axis is independent from maternal thyroid status in rat (2).

The observed increases in body weight and heart weight in offsprings born to hypothyroid mothers and its reversal in offsprings of hypothyroid mothers can be attributed to altered availability of thyroid hormones either through placenta (9) or through milk from lactating mothers. The changes observed in heart weight in hyperthyroid state are classical signs of thyroid hormones excess (10). Body length and tail length have been considered to be a sensitive parameter with respect to thyroid hormones (11). The present findings on tail length and body length with altered maternal thyroid function are in good agreement with the reported literature (11). The present study showed that maternal thyroid status plays an important role in fetal and neonatal growth and development.

REFERENCES

1. Hamburg M. An analysis of the action of thyroid hornone on development based in vivo and in vitro studies. *Gen Comp Endocrinol* 1968: 10 : 198-213.
2. Verma SK, Murray R, Stanbury JB. Effect of maternal hypothyroidism and T_3 on the fetus and newborn in rats. *Endocrinology* 1978; 102 : 24-30.
3. Lorijin RHW, Nelson JC, Lawrence DL. Induced fetal hyperthyroidism : Cardiac output and O_2 consumption. *Am J Physiol* 1980: 239 : H 302 307.
4. Balourdas TA, Croll MN, Scott JC. Coronary blood flow and haemodynamic factors in hypothyroidism. *Fed Proc* 1959; 18 : 7-13.
5. Baksi SN. Effect of thyroparathyroidectomy on implantation and early gestation in rats. *J Reprod Fert* 1973; 32 : 513-516.
6. Middlesworth LV. Poor survival of offsprings from female rats previously exposed to perinatal propylthiouracil. *Horm Metab Res* 1984; 16 : 452-3.
7. Blake HH, Henning SJ. Weaning in the rat : a study of hormonal influence. *Am J Physiol* 1983; 244 : R 537-43.
8. Mc Cormic MC. The contribution of low birth weight to infant mortality and childhood morbidity. *New Eng J Med* 1985; 312 : 82-90.
9. Dussault JH, Morrissett J, Laberge C. Blood T_4 level is lower in low birth weight infants. *Clin Chem* 1979; 25 : 2047-9.
10. Beznak M, Korecky B, Thomas G. Regression of cardiac hypertrophies of various origins. *Can J Physiol Pharmacol* 1969; 47 : 579-86.
11. Degroot CA. Tail growth in the thyroxine treated hypophysectomized rat, a sensitive criterion for growth hormone activity. *Acta Endocr* 1963; 42 : 423-31.