A Simple Model for Studying the Correction of *In Utero* Hypothyroidism in the Rat

JOSEPH SACK, ARON WELLER, OREN RIGLER, AND AVIRAM ROZIN

Department of Pediatrics [J.S.], Sheba Medical Center, Tel Hashomer and Sackler School of Medicine, Tel Aviv University, and Department of Psychology [A.W., O.R., A.R.], Developmental Psychobiology Laboratory, Bar Ilan University, Ramat Gan, Israel

ABSTRACT

Although the placenta is only a limited barrier for the transfer of thyroxine (T_4) to the fetus, we have recently demonstrated that maternal T_4 does not suffice to prevent the effects of *in utero* hypothyroidism. The current study presents a convenient and minimally invasive animal model to study whether maternal-fetal transfer of T_4 administered to the pregnant rat corrects the newborn's *in utero* hypothyroidism. In this model pregnant rats receive the goitrogen methimazole in their drinking water from d 10 of gestation until birth, either alone or together with L- T_4 . Circulating T_4 levels in the dams and newborn pups are measured from blood spotted on filter paper by RIA. Using blood T_4 levels as the measure, in the present study we found similar

effects for orally and intraperitoneally administered T_4 . This rat model will allow future studies of whether maternal-fetal T_4 transfer can correct the detrimental neurobehavioral effects of intrauterine hypothyroidism. (*Pediatr Res* 37: 497–501, 1995)

Abbreviations

T_4 , thyroxine
 T_3 , 3,5,3'-triiodothyronine
MMI, methimazole
CV, coefficient of variance
i.p., intraperitoneal

The issue of placental transport of thyroid hormones has been debated for years. It was previously thought that there was minimal placental transport of these hormones in humans and in rats. This was attributed either to poor permeability of the fetal membranes and/or to active deiodinating mechanisms (1–4). Recent data, however, have shown that the rat placenta actively metabolizes T_4 to T_3 and that thyroid hormones do cross the placenta in both humans and rats, so that the placenta acts only as a limited barrier (5–8).

We have shown that maternal-fetal transport of T_4 in humans does not suffice to prevent *in utero* hypothyroidism (9). This conclusion is based on the observation that neonates with athyreotic congenital hypothyroidism are often born with more severe signs of hypothyroidism in the absence of epiphyseal ossification centers and with extremely high TSH levels at birth. It has been claimed that maternal transfer of T_4 could still provide the fetal brain with sufficient T_3 to avoid major irreversible CNS damage if the fetal thyroid is not functional (5). These authors suggested that the fetal brain might be protected by maternal T_4 , even if the transfer is not sufficient to protect other tissues, and that delayed skeletal maturation does not necessarily imply that the brain was T_3 deficient *in utero*.

However, the very high TSH levels evident already at birth suggest that the fetal pituitary lacks T_3 for negative feedback. Several studies have shown that congenitally hypothyroid newborns with the most retarded bone maturation and with the lowest T_4 levels at screening have the poorest prognosis for CNS development, despite prompt postnatal treatment with T_4 (10–13). These results imply that maternal T_4 transfer fails to provide adequate prevention of *in utero* CNS developmental delay.

Although several studies support the existence of maternal-fetal T_4 transfer in rats, T_4 was administered in these studies invasively, either intraperitoneally or through s.c. osmotic pumps. This could potentially produce a considerable degree of prenatal stress. Furthermore, none of these studies has followed the fetuses to birth. It would be of great value to have a rat model of *in utero* hypothyroidism in which the T_4 treatment is administered orally without traumatic manipulation of the pregnant dam and the postnatal development of the pups could be assessed. We have previously reported on the efficacy of using blood spotted on filter paper for T_4 measurement in the follow-up of children with congenital hypothyroidism (14). In the present study we applied this method, which requires only a drop of blood, to measure T_4 in pregnant, postpartum, and newborn rats. In addition, we considered it of interest to study whether T_4 administered to the dam reaches the fetus during gestation and can correct the fetal hypothyroxinemia.

Received June 10, 1994; accepted September 10, 1994.

Correspondence: Dr. Joseph Sack, Department of Pediatrics, Sheba Medical Center, Tel Hashomer, Israel.

A portion of this research was performed by Oren Rigler as partial fulfillment of the requirements for an M.A. degree in the Department of Psychology, Bar Ilan University.

METHODS

Animals and experimental design. Sprague-Dawley rats from the colony at the Developmental Psychobiology Laboratory in the Psychology Department at Bar-Ilan University were mated in polycarbonate cages ($38 \times 30 \times 27$ cm) with grid floors. Cages were checked daily, and the day in which a vaginal plug was secreted and detected was considered d 1 of gestation (G-1). Parturition occurred between G-21 and G-23. Pregnant rats were housed individually in polycarbonate cages ($38 \times 30 \times 27$ cm) with stainless steel wire lids and wood shavings as bedding material. Food and tap water were continuously available in the cage top. Lights in the colony were on from 0500 to 1900 h and temperature was maintained between 21 and 25°C. All conditions and treatments were in accordance with recommended humane conditions.

In experiment 1, the pregnant dams were divided into three experimental groups: MMI [1-methylimidazole-2-thiol, which crosses the placenta and blocks both maternal and fetal thyroid function (15); Sigma Chemical Co.] treated ($n = 9$), MMI + T_4 treated ($n = 10$), and controls ($n = 11$). Two of these groups were given 0.02% MMI (as in Ref. 16) as drinking water from G-10 to parturition. The third group (controls) received unaltered drinking water throughout pregnancy. From G-16 to term all rats received daily i.p. injections. One of the MMI-treated groups received 18 $\mu\text{g/kg}$ body weight levothyroxine sodium (Synthroid, Boots Pharmaceuticals, Inc., Lincolnshire, IL), whereas the other two groups (MMI and control) received 0.9% saline. Injection volume was 1 mL/kg body weight. In experiment 2, the dose of T_4 was increased to ensure sufficient delivery by the oral route, and the MMI dose was increased in an effort to maximize its effect. Pregnant rats drank tap water until d G-9. From G-10 until parturition they received either 1) tap water, 2) MMI (0.04%), or 3) MMI (0.04%) and $L-T_4$ (50 $\mu\text{g}/100$ mL solution, mean daily intake = 52.6 ± 1.6 $\mu\text{g/kg}$ body weight).

Tail blood spotted on filter paper was collected from the dams in experiment 1 on three occasions: before mating, on G-15, and within 1–16 h after parturition, and in experiment 2 on two occasions: before mating and after parturition. In both experiments within 1–16 h after parturition, trunk blood was collected after decapitation from two newborn pups (one male and one female) from each dam and spotted on filter paper (with special care not to include body fluids).

Measures and statistical analysis. After drying, all filter paper blood samples were kept in a cool, dark place. Samples from each experiment were assayed in duplicate, in a single assay in the National Screening Laboratory for Congenital Hypothyroidism, at the Sheba Medical Center. The Neonatal RIA kit for T_4 (DPC, Los Angeles, CA) was used (14). The kit's buffer contains a high concentration of T_4 displacing agents (from albumin and thyroid binding globulin, details not provided by the manufacturer). This, according to the manufacturer, together with the buffer's high ionic strength (which precludes red blood cells from interfering with the assay), allows measurement of T_4 in a variety of animal species with this kit. The assay's sensitivity is 0.05 $\mu\text{g/dL}$.

The assay's reliability for rat data were examined in a number of ways. First, intraassay reliability was studied by comparing the T_4 levels assessed from duplicates from the same blood spots examined in a single assay. The mean intraassay CV of the RIA was between 8 and 12% on separate runs. The results for experiment 1 showed $r = 0.88$, $p < 0.01$ for a sample of 17 postpartum rats and $r = 0.73$, $p < 0.01$ for a sample of 38 newborn rat pups, and in experiment 2 the correlation between the duplicates from 20 dams (almost all assessed twice at different times, $n = 39$ pairs of duplicates) was $r = 0.94$ ($p < 0.01$), and from 42 newborn pups $r = 0.89$ ($p < 0.01$). Second, interassay (or test-retest) reliability was assessed by reexamining the blood spots from 12 rats (four postpartum and eight newborn pups) 1 wk later. The results showed a $r = 0.90$ ($p < 0.01$; mean T_4 level = 1.11 $\mu\text{g/dL}$, mean CV = 14.44%; this CV is similar to those reported for interassay reliability of rat serum T_4 [mean CV = between 9.6 and 20.7%] assessed by the Coat-A-Count Total T_4 kit (DPC)). And third, T_4 levels assessed from serum and from filter paper were highly correlated ($r = 0.88$, $p < 0.001$; based on pups from 19 litters). The T_4 levels ranged from 0.53 to 3.16 $\mu\text{g/dL}$, which is around the absolute range of the (euthyretic) female Sprague-Dawley rat T_4 (0.8–4.8 $\mu\text{g/dL}$) assessed by the Coat-A-Count Total T_4 kit (DPC).

Maternal body weight during gestation and the newborn pups' weight, length, number, and sex ratio were recorded. All the results were analyzed by analysis of variance with *post hoc* contrasts and Duncan's multiple range tests when required (17).

RESULTS

Maternal T_4 Levels

Experiment 1. The blood T_4 level in the three groups did not differ before mating, but on G-16, both MMI-treated groups showed significantly lower levels than controls ($F(2,26) = 14.83$, $p < 0.001$). After parturition, T_4 levels in the three groups differed significantly from each other ($F(2,26) = 26.60$, $p < 0.001$). T_4 treatment significantly attenuated maternal T_4 , resulting in levels higher than in the MMI group but lower than in controls (Table 1).

Experiment 2. Tail blood T_4 concentrations were not significantly different in the three groups before mating (Table 1). However, after parturition, MMI-alone-treated rats had significantly lower T_4 levels than water-drinking and MMI + T_4 -drinking rats ($F(2,17) = 8.30$, $p < 0.01$). Paired *t* tests further showed that although the MMI-treated group showed a significant reduction in blood T_4 levels from the first to the second measurement ($t(8) = 8.22$, $p < 0.01$), the slight reductions evident in the control and MMI + T_4 groups were not significant ($p > 0.25$). Thus, $L-T_4$ in the drinking water corrected the rat dams' MMI-induced hypothyroxinemia.

Neonatal T_4 Levels

Experiment 1. The blood T_4 levels of newborn pups differed significantly between the two prenatal treatment groups ($F(2,54) = 11.61$, $p < 0.001$). Prenatal T_4 treatment resulted in

Table 1. T₄ levels [mean (SEM) µg/dL] of rat dams, before mating, on gestation d 16 and after parturition, and of the newborn pups

Group	T ₄ levels					
	Dams			Pups		
	<i>n</i>	Before	d 16	Birth	<i>n</i>	d 1
Experiment 1						
Water	10	2.35 (0.14)	2.05 (0.21)	2.79 (0.25)	19	1.08 (0.07)
MMI	9	2.23 (0.19)	0.74 (0.11)*	0.53 (0.17)*†	18	0.54 (0.08)*
MMI + T ₄	10	2.64 (0.30)	0.77 (0.23)*	1.47 (0.21)*‡	19	0.89 (0.08)
Experiment 2						
Water	6	2.77 (0.27)		2.68 (0.46)	13	1.14 (0.09)
MMI	8	3.35 (0.23)		0.90 (0.19)*†	19	0.89 (0.06)*†
MMI + T ₄	6	3.02 (0.40)		2.41 (0.41)‡	9	1.24 (0.11)

Treatments: water, tap water (controls); MMI, methimazole (0.02% in experiment 1; 0.04% in experiment 2) in drinking water from gestation d 10. MMI + T₄, MMI (as in previous group) with: 1) daily i.p. injections of L-T₄ (18 µg/kg) from gestation d 16 to parturition (experiment 1) or 2) L-T₄ (52 µg/kg) in the drinking water from gestation d 10 to parturition (experiment 2).

* Significantly lower than controls (water).

† Significantly lower than the MMI + T₄ group.

‡ Significantly higher than the MMI-alone group.

corrected neonatal T₄ levels: although the MMI group had significantly lower levels than controls, the MMI + T₄-treated group had levels that did not differ from controls (Table 1).

Experiment 2. Trunk blood T₄ levels in the pups born to dams that received MMI + T₄ or water alone (controls) were not significantly different; T₄ levels of both these groups were significantly higher than in the MMI-alone group ($F(2,38) = 5.41$, $p < 0.01$; Table 1). Thus, orally ingested L-T₄ in the MMI-treated pregnant rat reaches the fetus and significantly increases the newborn's blood T₄ levels, correcting the neonate's hypothyroxinemia.

Maternal Weight and Fertility

Experiment 1. At the start of the experiment (G-10), there was no significant difference between the body weights of the pregnant dams in the three groups (Table 2). After 6 d of drinking MMI, the mean increase in body weight was lesser for the MMI-treated rats than for controls, but the difference was not statistically significant ($p < 0.1$). After parturition, the MMI-only treated group weighed significantly less than the controls, although the weight of the MMI + T₄ postpartum dams did not differ from that in the other two groups ($F(2,28) = 3.99$, $p < 0.05$).

The mean number of pups delivered per litter (live and dead births), their sex ratio, and mean body or tail length were not significantly different in the three groups (Table 2). The mean body weight of the pups born in the control group was significantly greater than the weight of the pups treated with MMI. In contrast, the weight of the newborns in the MMI + T₄ group was not significantly reduced compared with the controls ($F(2,28) = 3.448$, $p < 0.05$). The pups of the three groups differed in their weight/length ratio ($F(2,28) = 4.58$, $p < 0.05$): the two MMI-treated groups were less obese than the control group.

Experiment 2. The body weight of pregnant dams did not differ significantly between the three groups on any of the days of pregnancy (Table 2). However, the groups showed a significantly different pattern of weight gain over this period. Spe-

cifically, between d G-10 and G-16, the two MMI-treated groups gained significantly less than did the controls ($F(2,16) = 5.15$, $p < 0.05$).

The three treatment groups did not differ significantly in mean number of pups born (live or dead), the pups' sex ratio, body weight, length, tail length, and weight/length obesity ratio.

DISCUSSION

In this study we measured T₄ from rat blood spotted on filter paper, using the human neonatal RIA kit. Compared with untreated pregnant rats, subjects that received MMI in their drinking water exhibited significantly reduced blood T₄ levels. The T₄ values of both groups were comparable to the values reported for female rats of the same strain by the manufacturer of the RIA kit. We also found high intraassay (duplicates), interassay, and intermethod (plasma-filter paper) correlations.

Daily i.p. administration of L-T₄ from G-16 until parturition significantly increased filter paper T₄ levels in MMI-drinking rats. This shows that the exogenous T₄ reached the maternal circulation. This increase was also observed in rats that received T₄ orally in the drinking water from G-10 until parturition.

The main goal of this study was to design a minimally invasive model for examining whether T₄ administered to the dam during gestation would reach the fetus and be detectable, in the newborns' circulation, by the simplest method. The results show that MMI-induced intrauterine hypothyroidism, at least as evidenced by low T₄ levels in the newborns' blood (hypothyroxinemia), can be corrected by prenatal treatment of the mother with T₄. A similar pattern of results was obtained by i.p. and oral routes of administration. These findings extend the report by Calvo *et al.* (5) that s.c. infusion of increasing doses of T₄ into MMI-treated pregnant rats preferentially protected the brain of the hypothyroid fetus from T₃ deficiency, whereas fetal plasma T₄ levels increased to a lesser degree. Taken together, these findings suggest the possibility that augmentation of the pregnant mother's circulating T₄ levels

Table 2. Maternal weight and fertility data

Measure*	Treatment group								
	Control			MMI			MMI + T ₄		
	n	Mean	SD	n	Mean	SD	n	Mean	SD
Experiment 1									
Weight (g)									
PG-10	11	312	64	9	270	48	11	277	43
PG-16	11	361	58	9	294	44	11	311	34
PG-21	8	397	53	9	334	39	9	352	36
After birth	11	325	56	9	257†	53	11	283	53
Weight gain (g)									
PG10-PG16	11	42	17	9	24	11	11	35	17
PG16-PG21	8	44	18	9	40	21	9	39	9.4
Newborn pups									
No. live	11	11.8	2.7	9	11.5	2.3	11	14.0	2.0
No. dead	11	0.1	0.3	9	0.2	0.4	11	0.9	1.8
Percent male	11	33	10	9	43	15	11	41	11
Mean body weight (g)	11	6.28	0.84	9	5.43†	0.72	11	5.77	0.58
Mean body length (cm)	11	6.61	0.38	9	6.40	0.35	11	6.65	0.19
Mean tail length (cm)	11	1.79	0.17	9	1.70	0.07	11	1.75	0.16
Mean "obesity" score‡	11	0.95	0.08	9	0.84†	0.08	11	0.87†	0.08
Experiment 2									
Weight (g)									
PG-10	6	349	47	8	341	25	6	362	53
PG-16	6	384	49	8	354	33	5	366	50
PG-21	6	438	59	8	385	38	5	404	51
Weight gain (g)									
PG10-PG16	6	35	10	8	13†	16	5	17†	10
PG16-PG21	6	54	23	8	31†	14	5	37	18
Newborn pups									
No. live	6	13.8	2.4	8	11.2	2.4	6	8.8	6.1
No. dead	6	0.2	0.4	8	0.6	1.7	6	1.3	2.0
Percent male	6	46	11	8	47	9	5	42	7
Mean body weight (g)	6	6.25	0.66	8	6.09	1.27	5	5.78	0.82
Mean body length (cm)	6	6.80	0.24	8	6.55	0.58	5	6.60	0.32
Mean tail length (cm)	6	1.82	0.10	8	1.87	0.32	5	1.65	0.20
Mean "obesity" score‡	6	0.92	0.06	8	0.93	0.23	5	0.88	0.11

* PG, day post gestation.

† Significantly lower than controls.

‡ Mean body weight/mean body length.

may also serve to prevent the detrimental developmental (physiologic, behavioral, and cognitive) outcomes of *in utero* hypothyroidism. Further work with animal models with goitrogens, such as MMI and propylthiouracil, is needed first. The current method provides a minimally invasive way to examine this issue.

A number of important limitations exist in the current study. First, one should be aware that the finding of significant maternal-fetal T₄ transfer was obtained in MMI-drinking pregnant rats. Although we do not have evidence, it is still possible that MMI alters the permeability of the placenta to T₄ and that the results would be different in rats treated with other goitrogens or, more importantly, in naturally hypothyreotic rats. This qualification holds equally for reports from other laboratories, on maternal-fetal T₄ transfer in MMI-drinking pregnant rats (e.g. see Refs. 5–7 and 16). Second, the doses of T₄ used in this study were not very low, and the efficacy of lower doses, in the 1 µg/kg range, should be examined in future studies. We note, however, that the current doses were identical to some cases and very close to others, in which T₄ replacement therapy was given prenatally (18) or postnatally (19). Furthermore, as

mentioned above, Calvo *et al.* (5) reported that the fetal brain apparently preferentially absorbs the T₄ transferred from the mother. It is therefore likely that higher doses are needed to correct the peripheral deficiency in T₄. It is relevant also that the doses of T₄ administered in the current study did not prevent body weight increase during the pregnancy of the MMI-treated dams, did not decrease litter size, and did not produce excessive blood T₄ levels in the dams. It is possible, even so, that lower doses of L-T₄ would have yielded similar results. Third, a portion of the effects of MMI in the current study may be attributed to prenatal "starvation" or undernutrition. After parturition, both the dams and the pups treated prenatally with MMI (and not "corrected" with T₄) weighed significantly less than controls. A prenatally "pair-fed" control group would have helped clarify the extent of the impact of this factor on the current results. And fourth, the validity of the rat (MMI) model for studying treatment of *in utero* hypothyroidism in humans should not be taken for granted.

The animal model provides a number of advantages that allow for further progress of research in this field. To our knowledge, this is the first study to examine maternal-fetal T₄

transfer in rats whose pregnancies are carried to term, and the impact of the transfer in the newborn pups assessed. In other studies on this issue pregnancy was terminated at various times during gestation. Furthermore, use of the blood spotted on filter paper technique in rats for this type of study (as first reported here) opens up the opportunity to conduct long-term, postnatal psychomotoric follow-up studies of offspring from treated (hypothyreotic and T₄-treated) pregnancies. This results from the ability to reliably assess blood T₄ levels from one drop of blood, thus allowing repeated examination of the mother and making it necessary to kill only one pup at a given time. This can allow the rest of the litter to develop, enabling the assessment of the behavioral and physiologic consequences of prenatal insult and therapy. Furthermore, this type of study need not be restricted to T₄: as in humans, blood spots on filter paper can be used in rats to assay levels of other hormones, *e.g.* TSH, 17-OH-progesterone, and cortisol (14, 20, 21).

In conclusion, we report a convenient and minimally invasive rat model that will enable future postnatal studies examining whether maternal-fetal T₄ transfer can correct the detrimental effects of *in utero* hypothyroidism.

Acknowledgments. The authors thank Avraham Goldstein for assistance in experiment 1, Ofra Schwartz for help in performing experiment 2, and Ora Amado and Yehudit Brook for performing the RIA.

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