PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 187, 360–365 (1988)

Developmental Changes in Rat Thyroid Responsiveness to Thyrotropin Administered by the Subcutaneous and Peroral Route[1] (42677)

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Abstract. It has been demonstrated that orally administered thyrotropin (bovine, bTSH) evokes an increase in circulating T_4 and T_3 levels in 15-day-old suckling rat pups, but not in weaned animals. Because the feedback mechanisms of the hypothalamo–pituitary–thyroid axis change dramatically during the neonatal period, we chose to examine the efficacy of exogenous bTSH in eliciting a thyrostimulatory response via the subcutaneous (sc) or peroral (po) route in rat pups at 5, 8, 12, and 15 days postpartum. Suckling pups were divided into four groups and received one of the following: (i) 2 IU bTSH/100 g body wt administered sc; (ii) distilled H_2O (dH_2O) sc; (iii) 2 IU bTSH/100 g body wt given po; (iv) dH_2O po. Animals were sacrificed at Time 0 and 1, 2, and 3 hr post-treatment, and the collected serum was analyzed for T_4 and T_3 by RIA. Maximum serum T_4 levels were attained at 2–3 hr post-treatment, and the T_4 response to sc-bTSH was significantly greater than that of the po-bTSH groups at all ages examined. This difference became progressively greater with increasing age, due to a persistent decline in T_4 responsiveness in animals receiving po-bTSH. No significant differences in T_4 or T_3 levels attained were observed in 8-day-old rat pups treated with rat vs bovine TSH, either sc or po. Percentage T_4 response (vs basal levels) steadily declined between Days 5 and 15 postpartum, in both sc- and po-bTSH treatment groups. Percentage T_3 responsiveness to sc-bTSH also declined between 5 and 12 days postpartum, after which time T_3 generation increased. Our results suggest that the neonatal rat is highly responsive to exogenous TSH late in the first week of life, and that the permeability of the gut at this stage of development further facilitates the impact of orally ingested TSH in the suckling.

Both human (1) and rat (2) milk contain the pituitary glycoprotein hormone thyrotropin (TSH). It has been demonstrated previously that perorally administered bovine thyrotropin (bTSH) is absorbed by the 15-day suckling rat gut in a biologically active form, as evidenced by elevated serum T_4 and T_3 levels (3, 4). This response is absent in weaned (30-day-old) rats. As the maturing gut becomes increasingly less "permissive" to the transfer of macromolecules over the course of development from birth to weaning (5, 6), the hypothalamo–pituitary–thyroid axis is also undergoing profound changes in its negative feedback control "set point" (7–10). The extent to which the neonate is capable of deriving benefit from milk-borne TSH at any given developmental stage is thus contingent not only upon the amount of hormone present in the milk but also upon the degree to which it survives the digestive tract in a physiologically active form, and the responsiveness of the suckling thyroid gland to that hormone. Since relatively little is known regarding the developmental aspects of thyroid responsiveness to TSH in the neonate, we undertook an investigation of the ontogeny of neonatal thyroxinemia in response to both subcutaneous and peroral TSH administration.

Materials and Methods. Virgin female Sprague–Dawley rats (Charles River Breeders, Wilmington, MA) were mated in our own animal facilities. Dams and their litters were housed in individual cages and given rat laboratory chow and tap water *ad libitum*. Litters were adjusted to eight pups each on Day 2 postpartum. Groups of 40–64 animals

[1] This work was supported by NIH Research Grant RO1 21227 from the National Institute of Child Health and Human Development. We gratefully acknowledge the receipt of rat TSH from the National Institute of Diabetes, Digestive, and Kidney Diseases, and the National Hormone and Pituitary Program, University of Maryland School of Medicine.

0037-9727/88 $1.50

each were studied at 5, 8, 12, or 15 days of life in a given experiment and randomly assigned across litters for weight and sex to one of four treatment groups: (i) 2 IU bTSH (Sigma Chemical Co., St. Louis, MO)/100 g body wt administered subcutaneously (sc) in a volume of 0.1 ml dH_2O; (ii) 0.1 ml distilled H_2O sc; (iii) 2 IU bTSH/100 g body wt as above administered perorally (po); or (iv) 0.1 ml dH_2O po. In order to determine whether species-specific thyrotropin (rTSH) would influence degree of thyroidal response, an additional experiment was performed in which 8-day-old rats were assigned to four groups: (i) as above; (ii) 2 IU rat TSH/100 g body wt sc; (iii) as above; and (iv) 2 IU rTSH/100 g body wt po. Peroral dosing was accomplished using newborn animal feeding tubes (curved, 22 gauge × 1.5 in., Popper & Sons, New Hyde Park, NY) inserted into the gastric lumen. All animals were fasted for 2 hr prior to commencing the experimental procedure.

Following injection/feeding, rat pups were kept in polycarbonate cages with bedding; floor temperature was maintained at a range of 26–32°C by placing half of each cage on an electric heating pad. At Hours 0, 1, 2, and 3 post-treatment, rats were sacrificed by decapitation and trunk blood was collected directly into polypropylene microcentrifuge tubes. After clotting, samples were centrifuged and the serum (120–400 μl) was quantitatively removed and frozen at −50°C for subsequent radioimmunoassay. Dams were likewise sacrificed by decapitation at the end of each experimental day.

Serum concentrations of T_4 and T_3 for a given experiment were measured using commercially available RIA kits (^{125}I-T_4 and ^{125}I-T_3 Quanticoat, Kallestad Laboratories, Chaska, MN). The inter- and intraassay coefficients of variation were 9.5 and 6.4%, respectively, for the T_4 RIA and 9.8 and 5.9% for the T_3 RIA. Data were analyzed by analysis of variance and Student's t test using Microstat software (Ecosoft, Inc., Indianapolis, IN) on an IBM personal computer.

Results. Administration of 2 IU bTSH/100 g body wt by either the subcutaneous or the peroral route to neonatal rats at 5, 8, 12, and 15 days postpartum resulted in significant increases in circulating T_4 levels relative to H_2O-treated controls (Figs. 1a–d). Maximum serum T_4 levels in TSH-treated rats were achieved at 2–3 hr postinjection/feed in all cases. T_4 concentrations of the control animals at Hours 1, 2, and 3 post-treatment did not differ significantly from basal levels at Hour 0 (1.02 ± 0.13, Day 5; 2.68 ± 0.11, Day 8; 3.49 ± 0.17, Day 12; and 4.82 ± 0.22

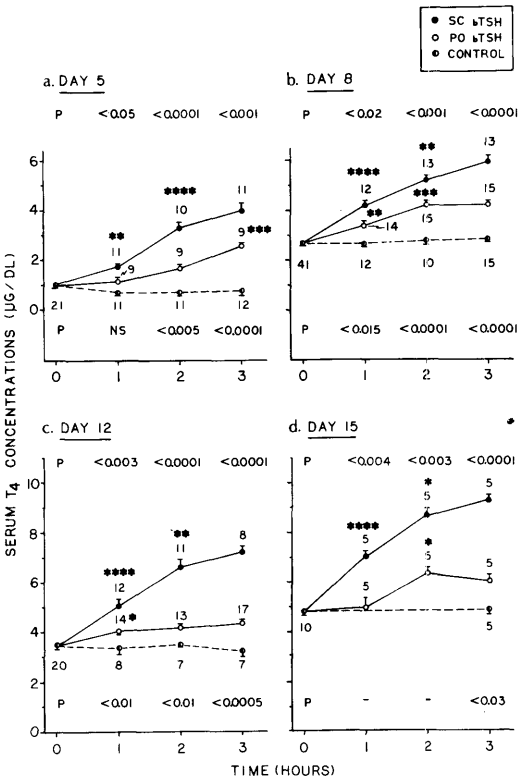

FIG. 1. Changes in serum T_4 concentrations after subcutaneous or peroral administration of bTSH to suckling rat pups on Days 5 (a), 8 (b), 12 (c), and 15 (d) postpartum. Each point represents the mean ± SE of n individual determinations as noted. (●) sc-TSH; (○) po-TSH; (◐) po + sc H_2O (controls). The latter H_2O-treated controls were combined because no significant differences in T_4 levels at any time point examined were observed. Asterisks indicate a significant change from the previous time point on the curve. (****) $P < 0.0002$; (***) $P < 0.001$; (**) $P < 0.015$; (*) $P < 0.03$. P values at the top of each graph indicate level of significance between sc-TSH and po-TSH groups at a given time post-treatment. P values at the bottom of each graph indicate level of significance between po-TSH and the control group. T_4 levels in the sc-TSH groups were in all cases significantly higher than those of the controls ($P < 0.0002$).

μg/dl, Day 15); no significant differences between po-H_2O and sc-H_2O groups were observed throughout the protocol.

For all age groups and at all time periods examined, sc-TSH evoked a T_4 response significantly higher than that observed in animals given po-TSH. This difference became progressively greater as the animal matured. Relative to H_2O-treated controls, the magnitude of the increases in serum T_4 concentrations resulting from po-TSH administration was higher in the 5 (po-TSH 3.07 ± 0.12 vs H_2O 1.02 ± 0.13 μg/dl)- and 8 (4.19 ± 0.14 vs 2.68 ± 0.11 μg/dl)-day-old animals than in the 12- (4.34 ± 0.13 vs 3.49 ± 0.17 μg/dl)- and 15 (5.95 ± 0.26 vs 4.82 ± 0.22 μg/dl)-day-old pups.

The pattern of serum T_3 responsiveness to sc-TSH administration (Fig. 2) reflected that seen for T_4, with highly significant increases

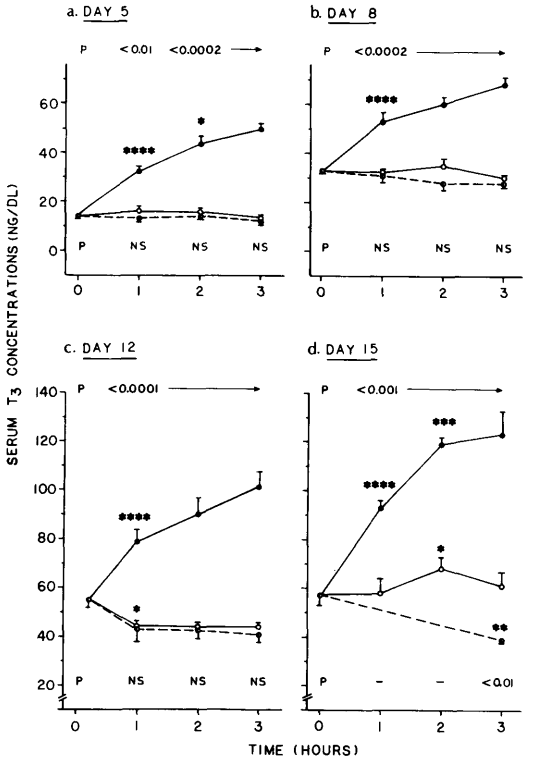

FIG. 2. Changes in serum T_3 concentrations after subcutaneous or peroral administration of bTSH to suckling rat pups on Days 5 (a), 8 (b), 12 (c), and 15 (d) postpartum. Legend as for Fig. 1. T_3 levels in the sc-TSH groups were in all cases significantly higher than those of the controls ($P < 0.0002$).

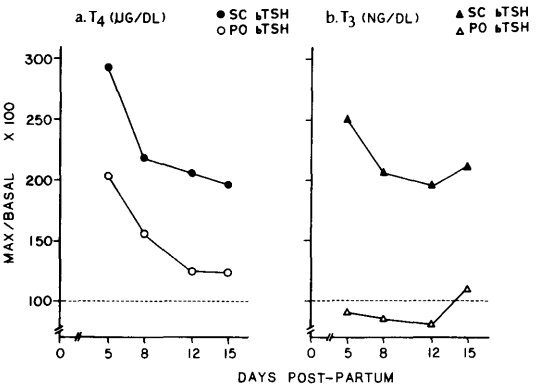

FIG. 3. Changes in serum T_4 (a) and T_3 (b) concentrations relative to basal hormone levels (Hour 0) following subcutaneous or peroral bTSH administration in suckling rats. Maximum T_4 and T_3 levels attained were divided by respective hormone levels at Hour 0 and multiplied by 100 to yield percentage of basal levels achieved by each age group for a given treatment regimen. (a) T_4 response to (●) sc-TSH; (○) po-TSH. (b) T_3 response to (▲) sc-TSH; (△) po-TSH. Dashed line denotes no change from Hour 0 levels.

at Hour 1 over basal T_3 levels at Hour 0 (12 ± 1, Day 5; 33 ± 1, Day 8; 55 ± 3, Day 12; and 57 ± 4 ng/dl, Day 15) (Figs. 2a–d). In contrast, we observed no significant changes in serum T_3 concentrations after po-TSH administration in 5-, 8-, or 12-day-old rat pups; only at 15 days postpartum was a small increase in circulating T_3 levels evident. T_3 levels of the control animals on postpartum Days 5 and 8 did not change significantly over the course of the experiment, but tended to decline modestly; 12- and 15-day-old control rats displayed a significant depression of serum T_3 levels during this same period, however (to 41 ± 3 and 39 ± 2 ng/dl, respectively, at Hour 3). T_4 and T_3 responses of 8-day-old pups at Hour 3 to po (T_4: 4.04 ± 0.26 μg/dl; T_3: 34 ± 3 ng/dl, $n = 8$) or sc (T_4: 5.41 ± 0.25 μg/dl; T_3: 54 ± 4 ng/dl, $n = 7$) administration of *rat* TSH did not differ significantly from those of the bTSH-treated animals.

Relative changes in T_4 and T_3 were calculated (as maximum T_4 or T_3 level attained/basal value at Hour 0) for each treatment and age group (Fig. 3) in order to assess further the maturational aspects of thyroid hormone response observed. A relative decline in the responsiveness of circulating T_4 levels to

sc-bTSH administration between 5 and 15 days postpartum was evident (Fig. 3a). T_4 responsiveness to po-bTSH exhibited a similar decline with increasing age.

Relative T_3 responsiveness to sc-bTSH injection of 5-day-old rats was elevated (2.5 fold greater than basal levels) vis-à-vis that of 8-, 12-, and 15-day-old sucklings (Fig. 3b). As previously noted, serum T_3 levels in all but 15-day postpartum rats were unresponsive to po-TSH administration.

Discussion. Postnatal developmental patterns of the rat hypothalamo–pituitary–thyroid axis have been widely studied as models of human fetal and premature neonatal thyroid development. It is well-established that dramatic changes in T_4, T_3, reverse T_3 (7, 8, 11–13,17), TSH (15), and free T_4 and T_3 (10) occur between birth and weaning. There is no consensus, however, regarding what effects maternal hormones, ingested by the neonate via breast milk, have upon these changing patterns.

The presence of TSH in rat (2) and human (1) milks has been reported. Levels of 167 ng TSH/ml rat milk (vs 163 ng/ml serum) were demonstrated by Krulich *et al.* (2) in euthyroid dams 14 days postpartum. Given an average milk consumption at 14-days postpartum of approximately 6 ml and circulating TSH levels of approximately 500–600 ng/ml in 14-day-old rat pups (10), it is reasonable to hypothesize that TSH concentrations in rat milk contribute to the suckling's thyroid status, as has been suggested by a decline in thyroid activity followed by progressive activation of the thyroid gland accompanying premature weaning (15). Krulich's group likewise observed enhanced TSH transfer into the milk of thyroidectomized dams. In light of these data, earlier observations that the thyroid status of rat pups suckling thyroidectomized dams differs little from that of their counterparts suckling euthyroid dams (16) may be interpreted as reflecting the enhanced secretion of TSH in the milk of thyroidectomized dams, rather than that concentrations of thyroid hormones in dam's milk are irrelevant to the suckling's thyroxinemia (17).

TSH has been shown previously to survive the gastrointestinal tract in a biologically active form in the 15-day-old rat pup, with some loss of potency due either to partial destruction by the gastrointestinal tract or to absorptive delay; the efficacy of perorally administered bTSH is completely abolished in the 30-day-old weanling, however (3). Our present studies confirm those data in 15-day-old rat pups and demonstrate further that neonatal responsiveness to both subcutaneous and peroral bTSH administration is altered over the course of development. While absolute increases in T_4 levels are greater with increasing age in response to sc-bTSH administration, they decline with age in response to po-bTSH administration. These data are consistent with a reduced permeability of the gut to macromolecules as maturation proceeds; a gradual decline in pinocytotic activity during this period has been demonstrated (5). Our data further indicate that, at least in the 8-day-old suckling, bTSH is as effective as rTSH in stimulating the thyroid, whether administered po or sc. This dose equivalency persists at lower concentrations (1.0 and 0.5 IU/100 g body wt) as well (unpublished observations).

Calculations of percentage change in serum T_4 concentrations over basal levels may suggest that the youngest (5-day-old) animals are most responsive to TSH administration regardless of route of administration. In fact, on a relative basis, 2 IU/100 g body wt bTSH administered po in the 5-day-old rat pup evokes the same (twofold) increase in T_4 levels as that of a 12-day-old pup receiving TSH by sc injection.

The sensitivity of the 5-day-old rat thyroid to TSH stimulation is supported by the data of Kojima and Hershman (18), who administered TRH intraperitoneally to 1- to 7-day-old rat pups and then enumerated formation of colloid droplets in thyroid cells as an index of thyroid hormone secretion induced by TSH. Colloid droplet formation was unchanged in the thyroids of 1- to 2-day-old pups, but was markedly increased in those animals 3 days or older. Highest levels were achieved at Days 5–6 postpartum. Hence, the period of greatest potential thyroid responsiveness to TSH may be, not in the second week of life as previously suggested (9), but rather around Day 5 postpartum. Our findings likely differ from those of Stolc and Knopp (9) for several reasons. Their experi-

mental procedure examined suckling rats at Days 2, 9, 10, and 15 postpartum (as well as older animals); hence, the period late in the first week of life was not studied. TSH dosing was accomplished by repeated injection (6 doses of 0.5 U/100 g body wt) over a 36-hr period prior to sacrificing the animals; our studies, in contrast, employed a single injection or feed. Lastly, their studies focused upon *intrathyroidal* changes in thyroid hormone levels and their metabolites at 4 and 22 hr after the last TSH dose, whereas our experiments were designed to assess the acute responsiveness of the suckling rat pup to sc- or po-TSH by tracking *circulating* T_3 and T_4 levels at hourly intervals after treatment. Maximum thyroid responsiveness to *exogenous* TSH would be expected to occur at a time when *endogenous* TSH is low. If relative increments in thyroid function are indicative of enhanced thyroid responsiveness, then our findings in 5-day-old rat pups are consistent with the data of Walker *et al.* (10), in which lowest *endogenous* TSH levels occurred in 5-day-old pups, increased to their highest levels on Day 12, and declined thereafter. It should be noted, however, that the decrease in relative increments of T_4 and T_3 we have observed may merely reflect the progressive increase in basal levels of the circulating hormone. Our present data do not, in addition, exclude the possibility of a further change in thyroid responsiveness during and immediately following the weaning period (21–26 days postpartum), when further resetting of the hypothalamo–pituitary–thyroid axis may occur related to the stress of weaning (10).

The developmental changes in circulating T_3 levels on Days 5 and 8 in response to sc-bTSH administration are consistent with the pattern of T_4 responsiveness to sc-bTSH. Between Days 12 and 15, however, T_3 generation increases relative to that of T_4. Little alteration in hepatic T_4-5′-monodeiodinase activity occurs during this period (13, 19) which might otherwise explain such a shift in metabolite generation. Suzuki *et al.* (19), however, have suggested that a profound increase in renal T_3 generation beyond Day 14 postpartum may contribute to the elevation in serum T_3 levels which persists at least through Days 26–28, at a time when serum T_4 levels have essentially reached a plateau. Thus, our data may reflect this additional source of T_3 generation. The absence of a T_3 response to po-bTSH administration in 5- to 12-day-old pups, in contrast, was unexpected. Alteration of the thyrotropin molecule by the gut may result in qualitative as well as quantitative differences in thyroidal response; this hypothesis does not, however, explain why circulating T_3 levels increased in response to po-bTSH in 15-day-old pups, and this phenomenon requires further investigation.

Lastly, it should be emphasized that in addition to T_4 and T_3 generated via TSH stimulation of the thyroid, extrathyroidal sources of these hormones (such as from breast milk) may likewise influence the neonatal thyroxinemia. Secondary effects mediated by the TSH-stimulated thyroid such as enhanced rate of milk digestion (and hence facilitated absorption of milk thyroid hormones (20) as well as iodine (21) from the gut) may likewise result. Such considerations emphasize the complicated nature of iodine and thyroid hormone homeostasis in early postnatal life.

In summary, our data indicate that glycoprotein hormones such as TSH survive the digestive tract of the suckling rat between 5 and 15 days of life, but evoke an age-dependent attenuated response relative to an equivalent dose administered subcutaneously. The extent to which the thyroxinemia of the suckling will be altered in response to peroral administration of TSH is dependent both upon the permissiveness of the gut and upon the responsiveness of the thyroid at any given point in maturation. Our data suggest that in the suckling rat pup, the greatest potential for impact by milk-borne TSH exists between 5 and 8 days postpartum, because both permissiveness of the gut and thyroid responsiveness are favorable at this time.

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Received September 9, 1987 P.S.E.B.M. 1988, Vol. 187.
Accepted November 16, 1987.