Maternal–Fetal Endocrine Interrelations: Demonstration of TSH Release from the Fetal Hypophysis in Pregnant Rats Administered Synthetic TRH[1] (35538)

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Intensive physiologic (1, 2) and chemical (3, 4) researches during the last decade have recently culminated in the demonstration that the thyrotropin releasing hormone (TRH) of the hypothalamus in pigs, cattle, and sheep is a tripeptide, consisting of three amino acids, glutamic acid–histidine–proline (5–7) in that sequence. Bowers and associates (8) have shown further that TRH isolated from the hypothalami of pigs, is biologically identical with tripeptide, L-(pyro)-Glu–L-His–L-Pro–NH_2, recently synthesized by Folkers *et al.* (5, 6). The synthetic TRH is highly potent in effecting TSH release from the adenohypophysis. It is active *in vivo* at 1 ng in mice, stimulates TSH release from rat anterior pituitary glands *in vitro* and elevates plasma TSH levels in hypophysectomized rats bearing renal pituitary transplants (8).

The chemical characterization, synthesis, and now, commercial availability of TRH permit investigation into certain problems of hypothalamo-hypophyseal interaction hitherto not feasible. Although the functional development of the rat fetal pituitary–thyroid axis has been studied often (9, 10), little attention has been given to the possible role of the hypothalamus in the functional maturation of this system. The precise time in either pre- or postnatal development when TRH begins to influence pituitary TSH secretion in the rat has not been established. The present study was undertaken to determine whether the hypophysis of the rat can respond to TRH during the perinatal period. The *in vivo* and *in vitro* results described below demonstrate clearly that synthetic TRH stimulates TSH release from the pre- and early postnatal pituitary.

Materials and Methods. Timed-pregnant, Sprague Dawley rats (Charles River Breeding Laboratories, Wilmington, Mass.) were maintained in separate cages under controlled conditions of temperature (23 ± 1°) and artificial illumination (7 a.m. to 7 p.m.). Tap water and Purina Rat Chow were allowed *ad libitum*. On day 20 (a.m.) of gestation, each pregnant rat was injected ip with 8 μCi of ^{131}I to label iodothyronines in maternal and fetal thyroids. On the next day, pregnant rats were administered subcutaneously 250–500 μg of TRH,[2] dissolved in 1 ml of saline, every 2 hr until spontaneous delivery occurred. Rats which had not delivered by 3 p.m. (after 3 injections) were subjected to cesarean section. Control pregnant rats received saline injections alone. Thyroidal radioiodine uptakes were determined in mothers and newborn 1–2 hr after the last injection of TRH and 24–28 hr after the labeling dose was administered. The thyroids were homogenized in vials containing 2% NaOH and radioactivity was determined in a well-type scintillation counter. Blood from neonates was collected by decapitation and thoracic incision several minutes after a heparin injection (0.05 ml). Blood was obtained from mothers under light ether anesthesia by direct cardiac puncture. Radioactivity of the whole blood (0.2-ml samples) was measured immediately after collection.

The thyroid and pituitary glands were removed from mothers and newborn (under

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[2] The synthetic TRH was prepared by Dr. Karl Folkers.

TABLE I. Effects of Synthetic TRH on the Pituitary–Thyroid System of Maternal and Neonatal Rats.

Treatment (no. of rats)	Body wt (g ± SE)	Thyroid wt (mg/100 g of body ± SE)	% ^{131}I uptake × 10^{-2}	
			Thyroid (mg ± SE)	Blood (ml ± SE)
Mothers				
Normal, spon. del. (3)	253 ± 15	6.52 ± 1.0	21.4 ± 0.9	1.6 ± 0.1
TRH, spon. del. (4)	263 ± 5	6.31 ± 0.5	19.2 ± 0.5 ($p < .100$)	1.9 ± 0.7
Normal, cesarean (2)	340	4.23	28.9	2.3
TRH, cesarean (1)	408	3.25	13.2	3.9
Newborn				
Normal, spon. del. (29)	5.9 ± 0.08	35.1 ± 1.8	9.3 ± 0.4	2.2 ± 0.2
TRH, spon. del. (44)	6.1 ± 0.05	34.3 ± 1.5	6.6 ± 0.4 ($p < .001$)	6.0 ± 0.5 ($p < .001$)
Normal, cesarean (18)	5.8 ± 0.13	27.6 ± 0.8	11.3 ± 1.5	4.7 ± 0.7
TRH, cesarean (13)	5.6 ± 0.06	29.4 ± 1.4	3.4 ± 0.3 ($p < .001$)	6.3 ± 0.4 ($p < .05$)

dissecting microscope), weighed on a microtorsion balance to 0.1 mg and prepared for histological or chemical study. TSH content of pooled plasma and acid saline extracts of hypophyses were measured by bioassay in the stasis tadpole (11). TSH potency and 95% confidence limits were determined by analysis of variance (12). For the *in vitro* studies, two to four whole pituitaries were removed from immature rats by decapitation with a guillotine and were added to each 10-ml Teflon beaker containing 1 ml of Krebs–Ringer–bicarbonate solution. Incubations were made as previously described (8) except that the same pituitaries served as both control and experimental. After two preincubation periods of 1 hr each, TRH was added and incubation continued another hour. After incubation, equal aliquots of medium were diluted with 1% bovine serum albumin in 0.9% NaCl solution and assayed for TSH in T_3-TSH assay mice (8). ^{125}I blood levels were measured before and 2 hr after an intravenous injection of samples of unknown or standard bovine TSH. The difference in radioiodine levels, recorded as the ^{125}I Δcpm, was proportional to the amount of TSH administered. Statistical significance of the observed values was ascertained by the Wilcoxin method.

TABLE II. TRH Administration and TSH Levels in Mother and Neonate.

Treatment	TSH assay	
	Plasma (mU/100 ml)	Pituitary (mU/mg)
Mother		
Normal	119 (86–167)[a]	65.5 (46.6–92.1)
TRH	121 (91–173)	33.2 (23.7–46.1)
Newborn		
Normal	65 (54–93)	3.9 (2.8–5.7)[b]
TRH	188 (125–254)	2.7 (1.9–3.9)

[a] In both columns, mean and (in parentheses) 95% confidence limits.

[b] Values for adenohypophysis in mothers but for whole pituitary in neonates.

Results. Results of the *in vivo* experiments indicate that administration of TRH to pregnant rats on the last day of gestation induces substantial change in levels of radioactivity of blood and thyroid glands in the newborn (Table I). Significant release of radioiodine from the thyroid, with commensurate increase in blood radioactivity occurred in spontaneously delivered neonates, as well as in those delivered by cesarean section. Comparison of radioiodine levels with those of control offspring indicated that the decreases in thyroidal ^{131}I and corresponding increases in blood ranged from 30 to 70% and 40 to 170%, respectively. Maternal thyroidal ^{131}I uptake declined only slightly (10%); and radioactivity in blood remained essentially normal.

The bioassay studies clearly demonstrate that TRH stimulates *in vivo* release of TSH from the fetal hypophysis (Table II). There was a significant rise (3 fold) in plasma TSH concentration of offspring delivered spontaneously by TRH-treated mothers. Mean

TABLE III. Effect of TRH on the *in Vitro* Release of TSH from the Pituitary Gland of Rats of Different Ages.

Age of rats (days)	^{125}I Δcpm blood levels of TSH assay mice ± SE			
	Preincubation, before TRH		Incubation, after TRH	
	1	2	1′	*p* value 2 vs 1′
—20[a]	2500 ± 140	—123 ± 28	1583 ± 170	<.001
	1186 ± 110	—20 ± 15	1320 ± 152	<.001
+1	—	836 ± 90	1426 ± 150	<.05
	—114 ± 25	67 ± 10	426 ± 80	<.05
	1560 ± 135	616 ± 45	1147 ± 150	<.001
+5	—	374 ± 50	2410 ± 205	<.001
	1177 ± 155	906 ± 102	3547 ± 450	<.001
	332 ± 25	—30 ± 5	995 ± 85	<.001
+10	1757 ± 195	836 ± 70	1426 ± 110	<.02
	83 ± 10	735 ± 56	1700 ± 150	<.01
	2530 ± 350	390 ± 48	1830 ± 195	<.001
+15	1923 ± 250	1314 ± 150	2597 ± 210	<.01
	2270 ± 195	704 ± 50	4433 ± 525	<.001
	2350 ± 280	583 ± 65	4287 ± 505	<.001
+20	3227 ± 295	2060 ± 210	2793 ± 325	ns
	3217 ± 301	2656 ± 245	3830 ± 303	<.05
	3337 ± 350	1756 ± 205	3703 ± 325	<.001
	5227 ± 650	1880 ± 195	4485 ± 540	<.001

[a] Day of gestation. Each value recorded is the mean results obtained from 5 mice. 50 ng of synthetic TRH was added/1.0 ml of incubation medium.

TSH content of the fetal hypophysis decreased about 30% (TSH assays in newborn delivered by cesarean section are now in progress). Change in maternal pituitary and plasma TSH levels was less marked. A decrease in pituitary TSH stores (50%) of mothers receiving TRH was not associated with significant change in plasma TSH titers; and plasma hormone levels were actually higher in their newborn.

The data from the *in vitro* experiments (Table III) also indicate that TRH effects release of TSH from the early postnatal, as well as the fetal pituitary (day 20). As has been found for adult hypophysis, TSH release decreased during the second preincubation period in the absence of TRH. Addition of TRH after the second preincubation period re-initiated the increase in TSH release (Table III, 2 versus 1′). The amount of TSH released in the first and third hours of incubation was of the same order of magnitude. Since a relatively large amount (50 ng) of synthetic TRH was added, it was not possible to conclude whether the sensitivity of the pituitary to TRH varied with age of the rat. Also, no attempt was made to ascertain a sex difference in pituitary response to TRH for either the *in vivo* or *in vitro* experiments.

Discussion. It is evident from the *in vivo* and *in vitro* results of this study that the rat adenohypophysis during the perinatal period has the capacity to release TSH in response to synthetic TRH. D'Angelo and Wall (10, 13) have already demonstrated that the fetal hypophysis of the rat, although containing relatively small amounts of TSH can enhance TSH secretion after administration of goitrogens to the mother. The 3-fold rise in fetal plasma TSH levels after TRH administration appears to be maximal and of the same order of magnitude as that found in goitrous newborn. Whether or not endogenous TRH exists in the fetal hypothalamus and, if so, whether it actually influences functional ma-

turation of the pituitary–thyroid system has not been established. There are some indications that the perinatal period of the rodent is characterized by significant functional change in the pituitary–thyroid axis. Radioiodine uptake by the rat (14) and guinea pig (15) thyroid is unusually high at birth, even though maternal TSH secretion is previously suppressed with thyroxine (15), and thyroidal uptake declines sharply during the first postnatal week (14, 15). It is not clear whether these early postnatal changes in thyroid activity reflect previous hormonal events of late prenatal stages or simply represent new conditions induced by the external environment.

The fact that synthetic TRH crosses the placenta to release TSH from the fetal pituitary raises the question as to the possibility of a transplacental effect of a maternal hypothalamic hormone upon TSH secretion in the fetus. Florsheim *et al.* (16) demonstrated that lesions in the thyrotropin area of the mother's hypothalamus did not prevent fetal goitrogenesis when propylthiouracil was given. The question of endogenous TRH activity in the perinatal period also remains open. Convincing proof that a fully developed hypothalamo–hypophyseal portal system is present in the rat neonate has yet to be given. Observations on blood flow, however, suggest that the vascular system is present and probably functional by postnatal day 4 (17). Encephalectomy of the rat fetus, however, results in impairment of ACTH secretion while thyrotropin function is largely maintained (18). Experiments on the effects of TRH in the hypophysectomized-pregnant rat and on fetuses *in utero* are in progress.

Summary. Synthetic TRH (L-(pyro)-Glu–L-His-L-Pro–NH_2), administered to the pregnant rat on the last day of gestation, stimulates release of TSH from the fetal hypophysis, as evidenced by a 3-fold elevation of plasma TSH level and significant change in the ratios of thyroid and blood radioactivity of the newborn. The stimulatory effect of TRH on the *in vitro* release of TSH from the postnatal pituitary of rats 1–20 days old was also demonstrated. The capacity of the perinatal pituitary to respond to TRH suggests that functional maturation of the hypothalamo–hypophysial–thyroid system may occur in this period.

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