Ontogeny of Thyrotropin Releasing Hormone and Precursor Peptide in the Rat

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ABSTRACT. Thyrotropin releasing hormone (TRH) and its precursor peptide pGlu-His-Pro-Gly (TRH-Gly) were measured in serum and in a variety of tissues of developing rats using specific RIA. TRH and TRH-Gly immunoreactivities were detected in most tissues. TRH concentrations were highest in pancreas, in which mean ($\pm$ SEM) TRH concentrations were 138 ± 20 pmol/g wet tissue 2 d before birth and 644 ± 80 and 586 ± 86 pmol/g, respectively, 2 and 5 d after birth. Hypothalamic TRH levels gradually increased from 4 d before birth (12 ± 2.5 pmol/g) to 77 d of postnatal age (348 ± 33 pmol/g). Hypothalamic concentrations were lower than levels in pancreas until 13 d of age. The mean serum TRH level at 2 d was 80 ± 20 pmol/L and fell to the adult range by 21 d.

TRH-Gly concentrations were highest in small gut (371 ± 64 pmol/g) during the neonatal period, falling gradually to adult levels (33 ± 4.8 pmol/g) by 35 d. Mean hypothalamic TRH-Gly concentrations increased to a peak of 62 ± 4.5 pmol/g at 13 d, falling thereafter. High TRH-Gly concentrations (>100 pmol/g) also were observed in pancreas (at d 2), kidney, and pituitary gland (at d 21). Serum TRH-Gly concentrations were highest (mean 417 ± 26 pmol/L) on the 2nd postnatal day and gradually decreased to the adult level by 35 d. Changes in the TRH-Gly/TRH ratio were inversely correlated with tissue TRH concentrations in hypothalamus, pancreas, and liver. Our results suggest that 1) tissue levels of TRH-Gly and TRH are developmentally regulated, 2) the conversion of TRH-Gly to TRH varies among tissues, and 3) the major tissue site of TRH biosynthesis changes during development from liver during fetal life to pancreas in the neonatal period and to hypothalamus in the adult. The consistently high TRH-Gly/TRH ratio in serum suggests that the circulating peptides are derived from multiple and predominantly non-hypothalamic tissue sources. (*Pediatr Res* 30: 28-33, 1991)

Abbreviations

TRH, thyrotropin releasing hormone, pGlu-His-Pro-NH₂
TRH-Gly, pGlu-His-Pro-Gly
prepro, precursor protein

TRH (pGlu-His-Pro-NH₂) is derived from a large prepro-TRH protein (1). Rat hypothalamic proTRH is a 255-amino acid protein containing five copies of the TRH progenitor sequence, Gln-His-Pro-Gly. ProTRH is sequentially cleaved to a variety of

precursor fragments by trypsin-like enzymes (2). A C-terminal glycine extended form of TRH (TRH-Gly) is the immediate TRH precursor and is processed to TRH via amidation of the proline residue using the C-terminal glycine residue as the amide donor (3) after cyclization of the N-terminal glutamine (4). Colocalization of TRH and TRH-Gly has been reported in both neural and extraneural tissues of adult rats (5).

In the perinatal period, important changes in TRH metabolism have been reported. The serum TRH concentration is relatively high in neonatal rats (6) at least in part because of low levels of TRH degrading activity in newborn serum (7). The TRH contents of pancreas and other gut tissues exceed hypothalamic levels and circulating TRH seems largely derived from these gut tissues (8, 9). There are several recent ontogenic studies of other TRH precursor peptides in rat pancreas (10, 11), but little data regarding the ontogenesis of tissue TRH-Gly concentrations in other tissue sites. In the present study, we measured TRH and TRH-Gly concentrations in a variety of fetal and neonatal rat tissues to further characterize the developmental changes in TRH synthesis and metabolism.

MATERIALS AND METHODS

Subjects and Sampling. Adult and timed pregnant Wistar rats, obtained from Harlan Sprague Dawley Inc. (Indianapolis, IN), were maintained in an air conditioned environment (at 21°C, 55% humidity) with rat food and water available *ad libitum*. Lights were on from 0600 to 1800 h. Adult animals were housed one to a cage and pups were housed with their mothers. The day vaginal plugs and sperm were found was taken as d 0 of pregnancy. The day of parturition was the 1st postnatal day of age. Animals were studied from 6 d before birth to 35 d after birth, and body weights ranged from 0.5 to 145 g (Table 1). Because individual organs could not be studied in the early fetal period, organs from several littermates were pooled and studied collectively. The number of fetal littermates combined for each pool as well as the number of pools studied are indicated in Table 1.

Between 0900 and 1200 h, adult rats were killed by decapitation and trunk blood was collected in precooled glass tubes. Neonatal rats were decapitated immediately after collecting blood by heart puncture. Fetuses were removed after hysterotomy, blotted dry, weighed, and then decapitated. Organs were excised and blotted to remove excess blood. Samples were weighed and placed in cold 1 M acetic acid (20% wt/vol, minimum 1 mL). Except in the youngest fetuses, the whole brain was divided into cerebrum, hypothalamic regions, and cerebellum with brain stem. The hypothalamic fragment was delimited in the sagittal plane by the posterior margin of the optic chiasm and the anterior portion of the mammillary body; the lateral margins were the hypothalamic sulci. The dorsal extent of the cut was 2 mm. Digestive organs were divided into stomach, duodenum, and small and large intestine; contents were washed out with chilled distilled water. Intestinal tissues were not able to be separated into duodenum, and small and large bowel earlier

Received August 13, 1990; accepted February 22, 1991.

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Supported by Grants HD 04270-21 and HD-00746-03.

Table 1. Age and body weight of animals*

Age (d)†	Body wt (g)	n‡
-6	0.52 ± 0.01	5 (6)
-4	1.1 ± 0.1	5 (5)
-2	3.7 ± 0.1	5 (4)
2	7.3 ± 0.2	9
5	9.4 ± 0.2	6
13	26 ± 1.2	5
21	47 ± 3.4	7
35	145 ± 3.1	6

* Values are mean ± SEM.

† Birth = d 1.

‡ Number of pools or individual animals used for statistical analysis. Numbers in parentheses indicate number of fetuses in each pooled group.

than 2 d before birth, and values for these animals are reported for the whole gut.

Extraction of TRH and TRH-Gly from Tissues. Tissues were heated at 97°C for 15 min to inactivate endogenous TRH degrading enzymes and then processed using a Polytron tissue

grinder and an Econogrind homogenizer (Randoti Glass Technology Inc., Los Angeles, CA). The homogenates were dried completely with a SVC200H Speed Vac concentrator (Savant Instruments, Hicksville, NY) and extracted twice with 2 mL of methanol. The methanol extracts were pooled, dried, and stored at -20°C. The dried extract was reconstituted in 0.5 to 2.0 mL of assay buffer just before assay.

Blood was placed on ice for 1 h to facilitate clotting. Serum separated by centrifugation was applied to a SEP-PAK C18 cartridge (Waters Associates Inc., Milford, MA) after addition of 2 N acetic acid (10% vol). The column was washed with 20 mM triethylamine, then eluted with an elution solvent (20 parts 20 mM triethylamine, pH 4.0, 80 parts 100% methanol), and the sample was dried and stored at -20°C.

TRH and TRH-Gly immunoreactivities were determined in the same tissue extracts. Recovery, calculated as percent of recovered synthetic peptide added to original tissue homogenates, was greater than 85% for TRH and 98% for TRH-Gly. Recovery of TRH and TRH-Gly from blood averaged 87% for TRH and 98% for TRH-Gly when synthetic peptides were added just before extraction. Serum degradation of these peptides was not

Table 2. Tissue and serum TRH concentrations during development in rat*

	Age (d)							
	-6	-4	-2	2	5	13	21	35
Whole brain	3.0 (0.3)							
Cerebrum		2.5 (0.3)	<4	0.8 (0.3)	1.7 (0.3)	1.9 (0.3)	3.6 (1.1)	8.6 (0.8)
Hypothalamus		12 (2.5)	28 (3.3)	64 (15)	91 (17)	97 (13)	149 (22)	202 (24)
Cerebellum		4.7 (0.8)	5.2 (0.8)	2.5 (0.1)	6.9 (0.6)	8.0 (0.8)	5.8 (0.8)	12 (1.1)
Pituitary			<36	47 (12)	77 (9)	130 (44)	105 (12)	61 (10)
Whole gut	8.3 (1.9)	7.7 (3.0)						
Stomach			<6	5.2 (0.8)	3.0 (0.8)	<2	3.3 (0.6)	1.9 (0.3)
Duodenum			<18	58 (9.4)	4.1 (0.8)	3.0 (0.06)	0.3 (0.03)	1.4 (0.3)
Small gut			<4	15 (0.3)	5.5 (0.8)	6.9 (0.6)	3.3 (0.6)	3.3 (0.6)
Large gut			<8	23 (5.8)	6.9 (1.4)	20 (7.7)	3.6 (0.8)	0.8 (0.3)
Pancreas		<69	138 (20)	644 (80)	586 (86)	47 (6.6)	6.4 (0.6)	3.3 (0.3)
Lung	<15	12 (4.1)	14 (2.2)	12 (3.3)	50 (7.7)	55 (9.9)	1.7 (0.8)	8.6 (2.8)
Heart	<30	17 (3.9)	20 (3.9)	8.8 (3.9)	58 (4.7)		12 (4.4)	<3
Liver	23 (3.9)	21 (3.6)	17 (5.8)	13 (1.1)	1.4 (0.3)	3.3 (0.6)	1.9 (0.3)	1.7 (0.3)
Kidney			<6	<2	5.2 (1.9)	1.4 (0.3)	3.0 (0.6)	4.1 (0.8)
Spleen				55 (11)	9.4 (1.9)		1.1 (0.3)	0.6 (0.08)
Adrenal						12 (0.03)	10.4 (0.1)	13 (0.6)
Testis					9.4 (4.4)	2 (0.08)		4.7 (0.8)
Ovary					<4	2.5 (0.3)		5.8 (1.1)
Serum				80 (20)	55 (9.7)	36 (7.2)	30 (6.6)	30 (7.5)
Placenta	<20	<10	5.8 (0.8)					

* Values are mean (SEM) expressed as pmol/g wet tissue or pmol/L serum.

Fig. 1. Developmental changes in TRH concentrations in pancreas and neural tissues. Values are means $\pm$ SEM. See text for details. Adult (77-d) values from reference 12 are shown for comparison.

determined. No corrections were made for recovery in the calculation of tissue peptide concentrations.

RIA for TRH and TRH-Gly. TRH. TRH was determined using our previously described methods (12). 125 I-labeled TRH was prepared as described by Butler *et al.* (13). The TRH antiserum was obtained from rabbits after immunization with synthetic TRH conjugated with BSA (14). The TRH RIA system showed little or no cross-reaction with TRH-Gly or TRH-COOH (each $<0.01\%$). Duplicate determinations were carried out for each sample. Tubes were incubated at 4°C for 72 h. The smallest detectable amount of ligand in the assay system was 20 fmol/assay tube. Within-assay and across-assay coefficients of variation were 2.2–8.7 and 8.4–14.9%, respectively.

TRH-Gly. The TRH-Gly RIA was similar to that for TRH except for substitution of TRH-Gly standards, 125 I-labeled TRH-Gly tracer, and rabbit anti-TRH-Gly antibody as previously described (12). There was no significant cross-reaction with TRH, TRH-Lys-Arg, TRH-Gly-NH₂, Arg-Glu-His-Pro-Gly, prepro-TRH (115–151) or cyclo (His-Pro) (12). The average detection limit for the assay was 26 fmol/assay tube. The within-assay and across-assay coefficients of variation were 3.7–5.9 and 5.7–15.4%, respectively.

Synthetic TRH and TRH-Gly were obtained from Peninsula Laboratories (San Carlos, CA). Goat antirabbit precipitating antibody was purchased from Antibodies Incorporated (Davis, CA).

Statistical Analysis. TRH and TRH-Gly concentrations were expressed as pmol/g wet tissue or pmol/L serum. When samples contained undetectable radioimmunoactivity, the value for the sensitivity threshold of the assay was assigned to calculate mean values. Results were analyzed using one-way analysis of variance. A probability value of less than 0.05 was used as the limit of significance.

RESULTS

Developmental changes in tissue TRH concentrations. Tissue and serum TRH concentrations are shown in Table 2 and selected values are graphed in Figure 1. Concentrations of TRH in neural tissues, including cerebrum, cerebellum, and hypothalamus, were relatively low during the neonatal period and increased thereafter to adult levels. During the fetal period, relatively high TRH levels were observed in extraneural tissues including pancreas, liver, heart, and lung. TRH concentrations in liver gradually decreased by the 5th d after birth, whereas in lung, heart, gut tissues, spleen, and serum, a peak of TRH

concentration was observed in the early neonatal period. In kidney, adrenal, testis, and ovary, TRH immunoactivities comparable to adult levels were detectable after birth, and levels were relatively stable during the study period.

TRH concentrations in brain and pancreas during development are depicted in Figure 1. In pancreas, TRH immunoreactivity approximated 138 ± 20 pmol/g tissue 2 d before birth, then sharply increased to levels of 580–650 pmol/g tissue between 2 and 5 d after birth. These values were 7 to 10-fold higher than those in hypothalamus at the same time during development. After the 13th postnatal day, pancreatic TRH levels decreased and were <10 pmol/g tissue at 21 and 35 d. Hypothalamic TRH levels gradually increased from 12 ± 2.5 pmol/g tissue 4 d before birth to 202 ± 24 pmol/g tissue at 35 d of age. In cerebrum, TRH levels remained low (<4 pmol/g tissue) through 13 d of age and increased thereafter to 8.6 ± 0.8 pmol/g at 35 d. Levels in cerebellum (not shown) ranged from 2.5 to 8.0 pmol/g tissue through 21 d and increased to 12 pmol/g at 35 d. Pituitary TRH was quantifiable on the 2nd postnatal day (47 ± 12 pmol/g tissue). Levels progressively increased, peaked at 130 ± 44 pmol/g tissue on the 13th d, and thereafter decreased to 61 ± 10 pmol/g tissue at 35 d.

Developmental changes in tissue TRH-Gly concentrations. Tissue and serum TRH-Gly concentrations are shown in Table 3 and selected values are plotted in Figures 2 and 3. TRH-Gly was detected in most tissues studied; concentrations varied with age with peak concentrations usually observed between 5 and 21 postnatal days. TRH-Gly was demonstrable in placenta, whole brain and liver as early as 6 d before birth. During the fetal period, the highest level (36 ± 5.5 pmol/g tissue) was detected in pancreas, with significant concentrations (14–18 pmol/g) observed in hypothalamus, duodenum, and heart.

Figure 2 displays the developmental changes of TRH-Gly concentrations in selected extraneural tissues (pancreas, small gut, and kidney) and serum. The mean serum TRH-Gly concentration was highest (417 ± 26 pmol/L) on the 2nd postnatal day and gradually decreased to the adult level by the 35th d of age (202 ± 29 pmol/L). The mean pancreatic TRH-Gly concentration was highest (117 ± 15 pmol/g tissue) on the 2nd postnatal day and decreased to the 23–33 pmol/g range at 5 and 13 d and to the 3–11 pmol/g range at 21–35 d. TRH-Gly levels in small gut increased from the 2nd postnatal day to a peak on the 5th postnatal day, when the mean value was 371 ± 64 pmol/g tissue. Levels then fell progressively to a mean value of 33 ± 4.8 at 35 d. The peak TRH-Gly concentration in kidney (129 ± 21 pmol/g tissue) occurred on the 5th postnatal day, then decreased to 83 ± 18 pmol/g tissue by the 21st d and 31 ± 5.0 pmol/g at 35 d.

Figure 3 summarizes the changes in TRH-Gly concentrations for selected neural tissues. TRH-Gly levels in hypothalamus, cerebrum, and cerebellum increased steadily during the neonatal period, peaking between 13 and 21 d of age. The mean peak values (24 to 62 pmol/g) were lower than concentrations in kidney or small gut. The mean pituitary TRH-Gly concentration reached a peak value of 112 ± 18 pmol/g tissue at 21 d. This value was the highest among all tissues except serum at this age.

In other tissues (stomach, duodenum, large gut, lung, heart, liver, spleen, adrenal, testis, and ovary) TRH-Gly concentrations manifested a peak in the late neonatal period. Generally, tissue TRH-Gly levels in the perinatal period were higher than adult levels (except in pituitary, spleen, adrenal, testis, and ovary).

TRH-Gly/TRH ratios in tissues and serum. The mean values for the TRH-Gly/TRH ratios in various tissues of developing rats are summarized in Table 4. The tissues can be roughly divided into two groups on the basis of low (<0.80) and high (>0.80) ratios. The tissues with relatively low ratios during the perinatal period (tissue group 1, Table 4) include liver, lung, pancreas, heart, and hypothalamus. High ratio tissues (tissue group 2, Table 4) include placenta, brain, and gut. The TRH-Gly/TRH ratio in serum is relatively high from 2 d through 35 d. Serum was not obtained from late gestation fetuses. The mean

Table 3. Tissue and serum TRH-Gly concentrations during development in rat

	Age (d)							
	-6	-4	-2	2	5	13	21	35
Whole brain	6.9 (1.9)							
Cerebrum		3.3 (0.5)	5.0 (0.5)	10 (1.2)	12 (0.7)	21 (2.1)	24 (3.3)	2.1 (0.5)
Hypothalamus		13 (1.9)	18 (3.6)	19 (2.6)	22 (3.6)	62 (4.5)	8.8 (0.7)	9.3 (1.4)
Cerebellum		<4	<5	4.3 (0.5)	8.6 (0.5)	31 (3.3)	36 (3.6)	12 (2.1)
Pituitary					<33	<43	112 (18)	21 (2.1)
Whole gut	<4	6.2 (1.0)						
Stomach			7.6 (1.7)	10 (1.7)	13 (1.2)	33 (1.7)	67 (10)	18 (2.4)
Duodenum			14 (2.1)	23 (3.8)	43 (5.7)	57 (7.6)	36 (4.5)	13 (2.1)
Small gut			10 (1.1)	52 (7.9)	371 (64)	157 (40)	67 (9.1)	33 (4.8)
Large gut			9 (0.8)	18 (2.4)	24 (4.5)	33 (9.5)	22 (4.1)	6.2 (1.7)
Pancreas		36 (5.5)	22 (2.4)	117 (15)	23 (6.4)	33 (3.3)	11 (1.4)	3.1 (0.7)
Lung		<4	5.0 (0.7)	10 (1.7)	10 (0.5)	33 (6.4)	50 (3.6)	5.7 (1.9)
Heart	<20	15 (1.2)	7.9 (1.7)	5.7 (0.5)	7.6 (1.4)	19 (5.0)	15 (2.9)	7.6 (0.5)
Liver	5.2 (1.2)	6.4 (1.0)	5.7 (0.5)	9.8 (1.4)	20 (2.4)	40 (3.1)	24 (3.1)	29 (5.7)
Kidney		<19		26 (6.7)	129 (21)	33 (9.1)	83 (18)	31 (5.0)
Spleen			<12	9.5 (1.7)	9.8 (2.1)	29 (1.7)	31 (3.3)	20 (3.1)
Adrenal							26 (4.5)	8.1 (0.5)
Testis					<8		16 (0.7)	
Ovary							11 (1.2)	3.6 (0.7)
Serum				417 (26)		352 (12)	293 (38)	202 (29)
Placenta	9.3 (0.7)	8.6 (1.0)	6.0 (0.5)					

* Values are mean (SEM) expressed as pmol/g tissue or pmol/L serum.

TRH-Gly/TRH ratios in liver, pancreas, and hypothalamus are summarized in Figure 4 with the corresponding TRH concentrations. In liver, the TRH-Gly/TRH ratio was low during the period from 6 d before birth to 2 d after birth (0.23 to 0.75), increased markedly to values in the 12 to 17 range between 5 and 35 d, and then fell to the adult level of 3.1. In pancreas, the TRH-Gly/TRH ratio decreased to a nadir value of 0.05 at 5 d, increased to a peak value of 2 at 21 d, and then fell to the adult level of 0.8. The TRH-Gly/TRH ratio in hypothalamic tissue was 1.1 at 4 d before birth, remained in the 0.2 to 0.7 range between -2 and 13 d of life, and decreased progressively thereafter to the adult level of 0.03. The changes in the TRH-Gly/TRH ratio in these tissues were generally inversely correlated with the respective tissue TRH-Gly concentrations.

DISCUSSION

The TRH and TRH-Gly RIA systems used in our study were quite specific (12). Moreover, by HPLC analysis, tissue and serum TRH and TRH-Gly immunoreactivities coeluted with synthetic peptide standards (12). In our study we detected TRH

and TRH-Gly immunoreactivities in most rat tissues. It is possible that blood contamination of the tissue extracts contributed to the measured tissue levels of TRH and TRH-Gly, inasmuch as high concentrations of the peptides were observed in serum during the postnatal period. Although there are no data regarding the amount of serum in developing rat tissues and we did not measure Hb concentrations in the extracts, we calculated the possible contribution of the blood peptide levels to the measured tissue concentrations of TRH and TRH-Gly using the data of D'Ercole *et al.* (15); these authors estimated blood contamination of adult rat tissues using measurements of tissue Hb levels. Using these estimates of blood contamination of tissues, the estimated concentration of TRH-Gly derived from blood in brain, heart, lung, liver, and kidney ranged from 0.2 to 0.002% of the total measured peptide. The range for TRH was 0.02 to 0.003%. Even considering that the blood content of fetal and neonatal tissues might be greater than that in adult tissues, these data suggest that the contribution of sequestered blood peptide to the tissue levels of TRH and TRH-Gly in our study was insignificant.

TRH immunoreactivity in our study was characterized from late fetal life to 35 postnatal days. At this time measured values

Fig. 2. Developmental changes of TRH-Gly concentration in serum, pancreas, kidney, and small gut. Values are means $\pm$ SEM. See text for details. Adult (77-d) values from reference 12 are shown for comparison.

Fig. 3. Developmental changes of TRH-Gly concentration in neural tissues. Values are means $\pm$ SEM. See text for details. Adult (77-d) values from reference 12 are shown for comparison.

were very similar to concentrations in adult animals in our laboratory (12). TRH immunoreactivity was quantifiable in whole brain 6 d before birth (15 gestational days). In hypothalamus, TRH was detected 4 d before birth (17 gestational days), but levels remained low during the early weeks of life. In earlier studies, TRH was detectable by RIA in whole brain between 12 and 17 fetal days (16–19). The first neurons containing TRH mRNA appear in the lateral hypothalamus on the 14th d of gestation (20). Our results are in agreement with these reports as well as other data (21–24).

Relatively high tissue TRH levels, exceeding adult values, were observed in several developing tissues, including pancreas, liver, lung, heart, and large and small gut, and in serum (12). Highest levels were observed in pancreas where we detected TRH immunoreactivity 2 d before birth (19 gestational days). TRH levels in pancreas rapidly increased to a peak between 2 and 5 postnatal days, when concentrations exceeded those in hypothalamus nearly 10-fold (Table 2, Fig. 1). Levels decreased thereafter to values comparable to those in adult rats (12). This developmental change in pancreatic TRH is consistent with previous reports (9, 19). TRH mRNA and other TRH precursor peptides have been described in the pancreas of the perinatal rat. Pancreatic TRH

Table 4. TRH-Gly/TRH concentration ratios in various tissues

	Age (d)							
	-6	-4	-2	2	5	13	21	35
Tissue group 1								
Liver	0.23	0.30	0.34	0.75	14.3	12.1	12.6	17.0
Lung		0.33	0.36	0.83	0.20	0.60	29.4	0.66
Pancreas	<0.52	0.16	0.18	0.04	0.70	1.72	0.94	
Heart	<0.67	0.88	0.40	0.65	0.13	1.25	>2.55	
Hypothalamus		1.08	0.64	0.30	0.24	0.64	0.06	0.05
Tissue group 2								
Placenta		>0.86	1.03					
Whole brain	2.3							
Cerebrum		1.3	>1.25	5.56	7.06	11.1	6.67	0.24
Cerebellum		<0.85	<0.96	1.72	1.25	3.88	6.21	1.00
Whole gut		<0.48	0.80					
Stomach			1.3	1.92	4.33	11.5	20.3	9.47
Duodenum			>0.78	2.52	10.5	19.0	120.0	9.28
Small gut				3.47	67.5	22.8	20.3	10.0
Large gut				0.78	3.48	1.65	6.11	7.75
Serum				5.21		6.40	9.76	6.73

mRNA has been detected at 19 gestational days with highest expression at birth and lower levels at 14 postnatal days (25). Prepro-TRH peptides have been characterized in the β -cells of islets of Langerhans at d 21 of gestation (10). The concentrations of these peptides increased rapidly after birth, reaching maximum levels at 2–4 postnatal days (11). Pancreatic α -amidating monooxygenase activity also parallels the prepro-TRH and TRH peptide levels (26). Proposed physiologic roles of pancreatic TRH in the perinatal period are 1) to inhibit the action of somatostatin (27), 2) to modulate islet development, or 3) to modulate exocrine function (10).

TRH-Gly was also widely distributed in developing rat tissues. The pattern of distribution was comparable to that previously reported in adult rats (5, 12, 28). In general, levels of both TRH and TRH-Gly were elevated in tissues obtained during the perinatal period. The tissues with relatively high TRH-Gly levels (14–36 pmol/g tissue) in the fetal period were hypothalamus, pancreas, gut tissues, and heart. After birth, peaks of TRH-Gly concentrations (>100 pmol/g tissue or pmol/L serum) were observed in serum and pancreas (at d 2); small gut, kidney, and hypothalamus (at d 5); and pituitary gland (at d 21). Among these tissues, changes of TRH-Gly levels paralleled those of TRH in serum, pancreas, and small gut. The significance of high serum TRH-Gly concentrations but low TRH levels in developing rat tissues also remains unclear. However, such dramatic changes in peptide concentrations suggest that TRH-Gly may exert specific functions via endocrine or paracrine routes. Biologic activities of TRH-Gly have been reported recently in adult rats: synthetic TRH-Gly injected intracisternally stimulated gastric acid secretion (29), as well as TSH release (30). A biologic role for TRH-Gly in developing animals has not been shown. TRH-Gly has been shown to increase prolactin secretion in anorectic women; this response was not observed in matched control females or in patients with uremia, acromegaly, or prolactinoma (31).

Conversion of TRH-Gly to TRH is mediated by an α -amidating enzyme (peptidyl glycine α -amidating monooxygenase) (3). The inverse relationship between the TRH-Gly/TRH ratio and TRH concentration observed in hypothalamus, pancreas, and liver (Fig. 4) suggests that the activity of this enzyme may be a rate-limiting step in TRH biosynthesis, and the dramatic changes in the TRH-Gly/TRH ratio during development in these tissues suggest developmental regulation of this enzyme system.

On the basis of these observations, we conclude that tissue levels of TRH and TRH-Gly are developmentally regulated. Assuming that TRH-Gly is a marker for tissue TRH synthesis, our results suggest a progressive change during development in

Fig. 4. Changes in mean TRH-Gly/TRH-ratios and TRH concentrations in liver (upper panel), pancreas (middle panel), and hypothalamus (lower panel). Values are mean $\pm$ SEM. TRH-Gly/TRH ratios are plotted as open circles and dashed lines; TRH values are plotted as closed circles and solid lines. Adult (77-d) values from reference 12 are shown for comparison.

the major tissue site of TRH biosynthesis. Given its relatively large mass, relatively high TRH concentration, and low TRH-Gly/TRH ratio, liver seems to be the major site of TRH synthesis in the fetus. During the neonatal period, the pancreas and other gut tissues are major TRH-producing tissues (Table 2, Fig. 4). After 3 wk, the major site of TRH biosynthesis is the hypothalamus (Table 2, Fig. 4). Serum levels of TRH and TRH-Gly seem to reflect multiple tissue sources, and the predominant circulating species is TRH-Gly (Table 4). This is also true in adult animals, where the high TRH-Gly/TRH ratio reflects a predominantly nonhypothalamic tissue source of peptide (12).

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