Thyrotropin-releasing Hormone in the Systemic Circulation of the Neonatal Rat Is Derived from the Pancreas and Other Extraneural Tissues

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A B S T R A C T Thyrotropin-releasing hormone immunoreactivity (IR-TRH) has been detected in the circulation of the neonatal rat. This immunoreactivity was demonstrated in purified ethanol extracts of plasma, and was indistinguishable from synthetic TRH using radioimmunoassay and chromatographic criteria. To determine the source of the circulating IR-TRH, tissue concentrations of TRH were analyzed during maturation of the rat. These studies revealed that during the first 10 d of life, the pancreas contained the greatest concentration of IR-TRH of any organ (pancreas, 289 ± 35 pg/mg; hypothalamus, 13 ± 3 pg/mg, day 5). Thereafter, pancreatic IR-TRH concentrations declined progressively while hypothalamic concentrations gradually increased (pancreas, 1.2 ± 0.2 pg/mg; hypothalamus, 365 ± 54 pg/mg, adult rat). IR-TRH was also found throughout the gastrointestinal tract but was not detected in the liver, spleen, kidney, or heart. IR-TRH from the pancreas and gastrointestinal tract gave radioimmunoassay binding displacement curves that were parallel to a curve generated with synthetic TRH, and co-migrated with synthetic TRH on Sephadex G-10 and high performance liquid chromatography. In addition, IR-TRH from purified pancreatic extracts was biologically active in that it released thyrotropin and prolactin from rat adenohypophysial cells maintained in monolayer culture. When a total pancreatectomy was performed on the 5th d of life of the rat, mean plasma TRH concentrations were significantly decreased 3 h afterwards (84 ± 9 vs. 63 ± 7 pg/ml, $P < 0.05$). Neither the TRH concentrations in the brain, hypothalamus, or gastrointestinal tract, nor the pituitary-thyroid axis were affected by the pancreatectomy.

However, mean plasma TRH concentrations remained unaltered 3 h after removal of the hypothalamus and extrahypothalamic brain.

From these results we conclude the following: (*a*) the TRH immunoreactivity in the circulation, pancreas, and gastrointestinal tract of the neonatal rat is indistinguishable from synthetic TRH; (*b*) pancreatic secretion provides a significant contribution to the IR-TRH in plasma, and a proportion of the circulating IR-TRH is derived from other extraneural sites. These findings therefore imply that alterations in hypothalamic and extrahypothalamic brain secretion of TRH are not reflected by changes in levels of this tripeptide in the systemic circulation.

INTRODUCTION

Although considerable advances have been made in understanding the physiology of thyrotropin-releasing hormone (TRH)[1] in the central nervous system, relatively little is known about TRH in mammalian circulation. This neuropeptide was initially recognized as a hypothalamic hormone which was capable of stimulating the synthesis and release of pituitary thyrotropin (1, 2). Subsequently, TRH was found to be distributed in lower concentration throughout the mammalian nervous system (3–5), where it has a variety of electrophysiological and behavioral actions (6–12). More recently, Morley et al. (13) and Leppäluoto et al. (14) have reported the localization of TRH in the pancreas and gastrointestinal tract of the adult rat, and Martino et al. (15) have demonstrated that the pancreatic islets of Langerhans contain large quantities of this tripeptide.

Several investigators have demonstrated the presence of low levels of TRH in the blood and plasma of the adult rat (16–18), and more recently, higher concentrations have been found in the blood of the fetal and neonatal rat (19, 20). The reasons for the differences in the circulating levels of TRH at different periods of development of the rat have not been established. However, it has been shown that the degradation of this peptide is clearly different at varying stages of maturation of the rat. TRH is stable in the plasma of rats up to 16 d of age because of the absence of an active serum TRH-degrading system (21). With development, however, the serum-degrading activity matures (22, 23) so that TRH is rapidly degraded within the circulation of the adult rat (24–26).

Although the presence of TRH in the circulation of the neonatal and adult rat has been attributed to minute amounts entering the peripheral circulation from hypothalamic and extrahypothalamic brain sites (18, 20), this hypothesis has not been established. These studies were therefore undertaken to determine the source of the TRH immunoreactivity (IR-TRH) in the circulation of the neonatal rat. The neonatal period was chosen because ablation experiments can be readily accomplished in animals of this age, and because the stability of TRH within the circulation facilitates its measurement in plasma. As the studies progressed, it became apparent that the pancreas and gastrointestinal tract contained large amounts of IR-TRH, so experiments have been performed to assess the relative contribution of these extraneural sites and the central nervous system to the IR-TRH in the circulation. In addition, chromatographic and bioassay analysis of extracts of serum and extraneural tissue has been carried out to furnish independent proof of the identity of the IR-TRH.

METHODS

Pregnant female Sprague-Dawley rats between 15 and 18 d gestation were obtained from Charles River Breeding Laboratories, Wilmington, Mass. They were individually housed, and fed Charles River rat chow and tap water. The rooms were lighted from 7 a.m. to 5 p.m. and the ambient temperature was maintained at $21\pm1°C$. The animals were gently handled frequently to minimize the environmental stress on the day of the experiment.

Pancreatectomy in the neonatal rat. 5-d-old pups were removed from their mothers and kept warm under an artificial light. Hypothermia was induced by placing the neonates on ice; when all spontaneous movement had ceased, they were removed. The skin and peritoneum under the left costal margin were opened and the spleen and pancreas were lifted out of the abdomen and removed en bloc. The peritoneum and skin were closed in layers with 4-0 silk sutures, and the pups were slowly rewarmed. Several hours after the surgery, the neonates were returned to the dams where they began to feed. Sham-operated animals, which served as controls, were handled in the same manner. At allotted times, the pups were removed from their mothers and sacrificed by instant decapita-

tion; the trunk blood was collected for various hormone measurements as described below.

Encephalectomy in the neonatal rat. 5-d-old pups were anesthetized by hypothermia. A sagittal incision was made in the scalp and a circular piece of the cranium was removed. The entire forebrain and hypothalamus were then removed with a suction apparatus. Hemostasis was established, the skin was sewn with 4-0 silk, and the pups were rewarmed. 3 h later the pups were decapitated and the trunk blood was collected.

Extraction and purification of neonatal tissues. Tissues were placed in preweighed vials containing 1 N acetic acid at 0°C. The vials were then reweighed and the tissue weights were calculated. The tissues were then homogenized with a Polytron (Brinkmann Instruments Inc., Westbury, N. Y.), and heated for 5 min in a boiling water bath. After centrifugation (3,000 rpm, 15 min), the supernatant fluids were collected and lyophilized. The extent of purification required for each tissue was found to be dependent on the TRH concentrations and the fat content. The hypothalamic extracts from rats of all ages and pancreatic extracts from neonatal rats were simply reconstituted in 0.01 M phosphate buffer—0.15 M NaCl, pH 7.5, (PBS) just before use in the radioimmunoassay. Extracts of the remaining tissues when reconstituted in PBS were macroscopically lipemic, and were therefore purified further. These extracts were dissolved in 1 ml deionized water at 4°C, and an equal volume of acidified acetone was added (95 parts acetone, 5 parts 1 N acetic acid). After centrifugation, the supernatant fluids were collected and layered with 4 ml petroleum ether. The upper ether phase was removed, and the lower acetone phase was dried under an air stream at room temperature. The extrahypothalamic brain extracts were then redissolved in PBS for use in the radioimmunoassay. Extracts of liver, spleen, heart, and kidney were further purified by G-10 Sephadex chromatography (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.), while all the gastrointestinal extracts and the pancreatic extracts obtained from adult rats were purified by affinity chromatography before use in the assay. In recovery experiments in which synthetic TRH was added to tissue extracts, $88\pm2.5\%$ (mean$\pm$SE, $n = 10$) was recovered after the acetone-ether extraction. The recoveries after combined acetone-ether extraction and affinity chromatography were $83\pm3\%$ ($n = 6$).

Chromatography. Gel filtration of partially purified tissue and plasma extracts was performed on a Sephadex G-10 column, equilibrated, and eluted with 0.01 M phosphate buffer, pH 7.5. Fractions of 2 ml were collected, and aliquots of these were used directly in the radioimmunoassay. The elution patterns were compared with that obtained by the application of synthetic TRH (1 ng).

Affinity chromatography was performed using a TRH antiserum coupled to CH-Sepharose 4B (Pharmacia Fine Chemicals, Inc.) by reaction with 1-ethyl-3(3-dimethyl-amino-propyl)-carbodiimide HCl (E-7750, Sigma Chemical Co., St. Louis, Mo.). The method for the preparation of this affinity column has been described in detail elsewhere (27). When 50 ng of synthetic TRH was applied to the affinity column, >90% was bound to the antiserum. The specificity of the antiserum used has been described (3). The extracts were eluted from the column with 10 ml 1 N acetic acid, lyophilized, and resuspended in PBS just before use in the assay.

High pressure liquid chromatography (HPLC) of plasma and tissue extracts was performed on a reversed-phase uBondapak C_{18} column (4 mm × 30 cm) as described for neuronal tissues (28). After preliminary purification by affinity chromatography, the extracts were dissolved in 100–200 μl distilled water and eluted with 10 ml 10% ethanol in 0.2 M ammonium acetate, pH 4.6. 20 fractions (0.5 ml/fraction) were collected, lyophilized, and reconstituted in PBS for TRH

measurement. The elution profiles were compared with the pattern obtained by the application of 5 ng of synthetic TRH to the column.

Measurement of TRH in neonatal rat plasma. Studies were carried out to determine the stability of TRH in neonatal rat plasma. When synthetic TRH was added to the plasma of 10-d-old rats at either 0° or 22°C (room temperature), no degradation of TRH was observed over a period of 4 h. These studies confirmed the previously reported observation (21) that TRH is stable in the serum of rats during the first 2 wk of life, and indicated that it was feasible to use plasma rather than whole blood in the radioimmunoassay. Experiments were then performed to compare the efficiency of various organic solvents in extracting TRH from plasma. ^{125}I-TRH was added to adult rat plasma which had been heated to 56°C for 2 h to inactivate the TRH-degrading enzymes (20). Varying volumes of either absolute ethanol, acidified ethanol (95 parts ethanol, 5 parts 1 N acetic acid), or acidified acetone (95 parts acetone, 5 parts 1 N acetic acid) were then added at 0°C. This resulted in a white protein precipitate and a clear supernatant fraction. When aliquots of the supernatant fluids were counted, it was found that absolute ethanol (10 vol) was the most efficient in extracting ^{125}I-TRH from plasma. In separate experiments, various amounts of synthetic TRH were added to heat-inactivated adult rat serum and extracted with 10 vol of ethanol. When the supernatant fluid was air dried and reconstituted in PBS, a macroscopically lipemic solution resulted. When this extract was measured in several dilutions in the radioimmunoassay, parallelism with the standard curve was not obtained. This was attributed to the presence of interfering substances in the extract. Similar results were obtained when ethanol extracts of neonatal rat plasma were simply reconstituted in PBS and used in the assay. Therefore it became essential to purify the extract further. Ethanol extracts of plasma were reconstituted in 1 ml deionized water and an equal volume of acidified acetone. The mixture was centrifuged (3,000 rpm, 15 min), and the supernatant fluids were collected. These supernatant fluids were then layered with 4 ml of petroleum ether to extract the lipids. After centrifugation (3,000 rpm, 5 min), the upper ether layer was aspirated, and the procedure was repeated using an identical volume of petroleum ether. The lower acetone layer was then dried under an air stream at room temperature. When these extracts were reconstituted in PBS, parallelism with the standard curve was obtained, and the recovery of synthetic TRH (40–1,000 pg/ml) was $92\pm2\%$ ($n = 10$). The interassay coefficient of variation was 10% and the intraassay coefficient of variation was 6%.

Monolayer culture of dispersed rat adenohypophysial cells. The bioactivity of IR-TRH in pancreatic extracts was assessed in vitro using a monolayer culture of dispersed rat adenohypophysial cells. Anterior pituitaries were obtained from both male and female Sprague-Dawley rats (150–250 g) and dispersed using a modification (M. F. Scanlon and I. M. D. Jackson, manuscript in preparation) of the procedure of Vale et al. (29). The cells were cultured for 96 h in a 95% air and 5% CO_2 environment and at the end of this time, were washed with fresh medium. The cells were then exposed for 6 h to purified pancreatic extract and to synthetic TRH made up to known concentration with serum-free medium. The pancreatic extract had been purified by affinity chromatography before it was added to the monolayer cultures. Hormone concentrations were measured by specific radioimmunoassays with the hormone standards dissolved in the culture medium.

Hormone measurements. Thyrotropin (TSH) and prolactin were measured by radioimmunoassay with reagents distributed by the National Pituitary Agency, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Md. Triiodothyronine (T_3) and thyroxine (T_4) were measured by methods that have been described and which are currently in use in this laboratory (30).

Statistical evaluation of the data. Statistical analysis of the results was performed with Student's t test for paired samples (31).

RESULTS

Characterization of plasma IR-TRH. TRH immunoreactivity was detected in the plasma during the first 2 wk of life of the rat, after which time it became undetectable (<40 pg/ml plasma). IR-TRH in purified extracts of neonatal rat plasma gave binding inhibition curves that were parallel to those generated with synthetic TRH in the radioimmunoassay (Fig. 1). When plasma extracts were applied to a Sephadex G-10 column, IR-TRH eluted in a single peak corresponding to the elution pattern of synthetic TRH (Fig. 2). When plasma extracts, purified by Sephadex or affinity chromatography, were analyzed by HPLC, IR-TRH again eluted at the TRH position (Fig. 3).

Characterization of pancreatic IR-TRH. Immunoreactive TRH was easily detectable in 1 N acetic acid extracts of neonatal rat pancreas. This immunoreactivity gave binding inhibition curves that were parallel with those generated with synthetic TRH (Fig. 1), and was eluted in the TRH area on Sephadex G-10 gel chromatography (Fig. 2). The Sephadex column fractions containing the IR-TRH were collected and subjected to affinity chromatography. When applied to the HPLC, the IR-TRH obtained from this highly purified extract eluted at the TRH position (Fig. 3).

Characterization of IR-TRH in the gastrointestinal tract. IR-TRH was not detectable in 1 N acetic acid extracts of various portions of the gastrointestinal tract
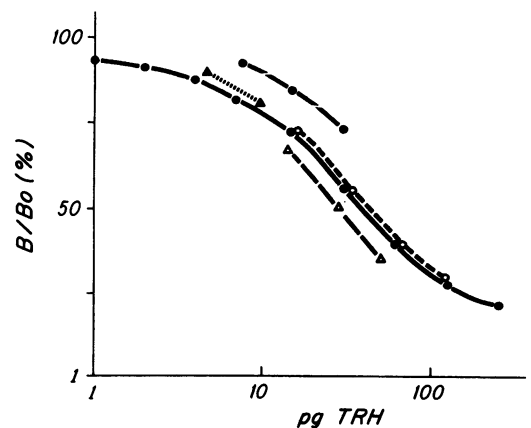

FIGURE 1 Comparison of the TRH standard curve with inhibition curves obtained by the assay of serial dilutions of extracts of plasma (●), pancreas (○), stomach (▲), and small intestine (△). The ^{125}I-TRH bound to the antiserum was assigned a value of 100, and all the other results have been expressed as a percentage.
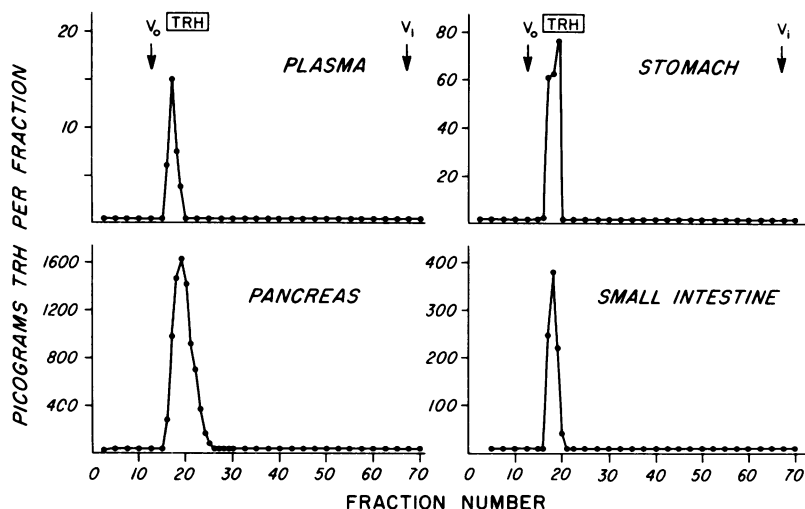
FIGURE 2   Elution patterns of TRH immunoreactivity after Sephadex G-10 chromatography of partially purified extracts of plasma, pancreas, stomach, and small intestine. A 1.2 × 55-cm column was eluted with a 0.01 M phosphate buffer, pH 7.5, and 2-ml fractions were collected. TRH in the fractions was determined by radioimmunoassay. Synthetic TRH eluted in fractions 16 to 22. The void volume (Vo) and the salt volume (Vi) are shown on the diagram.

purified by acetone and petroleum ether, but became readily measurable when these tissues were purified further by Sephadex G-10 or affinity chromatography. Gastrointestinal extracts purified in this manner had parallel binding inhibition curves with synthetic TRH in the radioimmunoassay (Fig. 1), and eluted at the TRH position after Sephadex G-10 chromatography (Fig. 2) and HPLC (Fig. 3).

Effect of pancreatic IR-TRH on the release of TSH and prolactin from cultured adenohypophysial cells.

At a concentration of 10 nM, synthetic TRH stimulated TSH release by $139\pm14\%$ ($P < 0.01$). The pancreatic extract, when applied in similar concentration, increased TSH release by $153\pm45\%$ when compared with the control cultures ($P < 0.05$). Synthetic TRH (10 nM) stimulated the release of prolactin by $291\pm27\%$ compared with the control cultures ($P < 0.01$), while this concentration of pancreatic IR-TRH increased the release of prolactin by $195\pm48\%$ ($P < 0.05$).
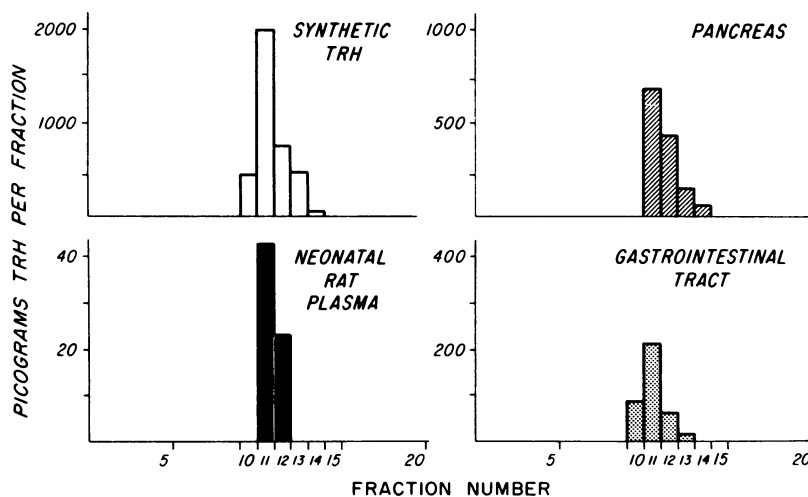
TRH concentrations in the hypothalamus, extra-

FIGURE 3   Characterization of the IR-TRH in plasma, pancreas, and gastrointestinal tract on HPLC after preliminary purification by affinity chromatography. The extracts were applied to a reversed phase uBONDAPAK column (4 mm × 30 cm), and the column was eluted with 10% ethanol in ammonium acetate, pH 4.6, at a flow rate of 1 ml/min. Fractions of 0.5 ml were collected, lyophilized, and resuspended in PBS for TRH measurement.

Thyrotropin-releasing Hormone in the Circulation and Pancreas of the Neonatal Rat   803

hypothalamic brain, pancreas, and gastrointestinal tract during maturation. High concentrations of IR-TRH were detected in pancreatic extracts during the first 2 wk of life (Fig. 4). 1 h after birth, the pancreatic content of TRH was 284±35 pg/mg, and rose to 630±40 pg/mg 18 h after birth. TRH concentrations declined sharply during the first week (113±8 pg/mg, day 8), and then more gradually, to reach very low levels in the adult rat (1.2±0.2 pg/mg).

Hypothalamic TRH content was low at birth (13±3 pg/mg), and gradually increased during the first week of life (Fig. 4). At day 14, the hypothalamus contained more than five times the concentration of TRH than did the pancreas (160±20 pg/mg), and by maturity, the concentration of TRH within the hypothalamus was 365±54 pg/mg.

The TRH content of the extrahypothalamic forebrain and brain-stem (Fig. 5) remained low throughout the first week of life (2.32±0.09 pg/mg, day 5), and gradually increased during maturation (11.0±0.9 pg/mg, adult rat).

TRH concentrations in the stomach were low throughout development and were generally <0.5 pg/mg (Fig. 5). In the upper small intestine, TRH concentrations were 11 pg/mg on the first postnatal day (Fig. 5), and declined to levels of <1 pg/mg during the second week, at which levels they remained throughout development. Lower concentrations were found in the lower small intestine and colon (Fig. 5).

TRH content of other viscera. When extracts of liver, spleen, kidney, and heart were purified by Sephadex G-10 gel chromatography, IR-TRH was not detectable in the column fractions. However, IR-TRH appeared to be present in very small concentrations (<0.5 pg/mg) in the lung of the neonatal rat, but was absent from this tissue in the adult rat.

Effect of pancreatectomy on plasma TRH. Removal of the pancreas in 5-d-old rats led to a decline in plasma TRH 3 h later (84±9 vs. 63±7 pg/ml, $P < 0.05$). A simi-

FIGURE 5 TRH concentrations in the extrahypothalamic brain, stomach, jejunum, and ileum during development of the rat. Shown are the mean±SE of five animals at each time point.

lar lowering of plasma TRH was observed at 24 h after the pancreatectomy (83±7 vs. 61±8 pg/ml, $P < 0.05$).

Effect of removal of the hypothalamus and extrahypothalamic forebrain on plasma TRH. The effect of removing the hypothalamus and extrahypothalamic forebrain on plsama TRH was studied in the 5-d-old rat. 3 h after the encephalectomy, plasma TRH was not significantly altered (87±8 vs. 81±9 pg/ml, P = NS).

Effect of administration of synthetic TRH on plasma TRH concentrations. The effect of administering synthetic TRH (20 ng, i.p.) was studied in the 5-d-old rat (Fig. 6). Plasma TRH concentrations increased from base-line levels of 78±7 pg/ml to 1,368±60 pg/ml 5 min
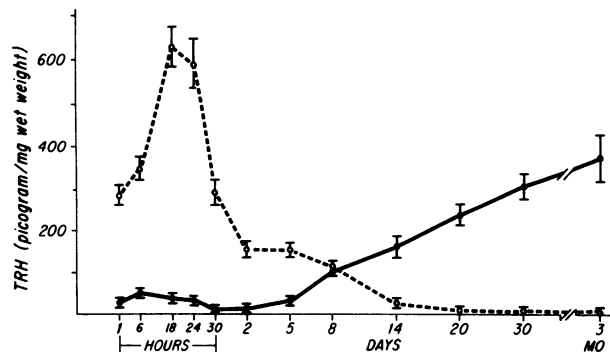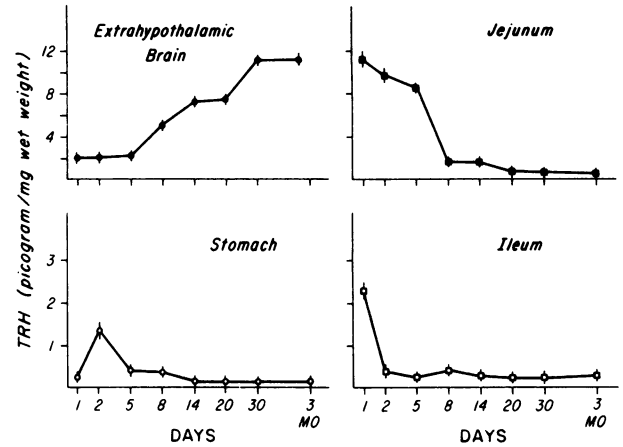
FIGURE 4 TRH concentrations in the pancreas (O) and hypothalamus (●) during maturation of the rat. Shown are the mean±SE of five animals at each time point.
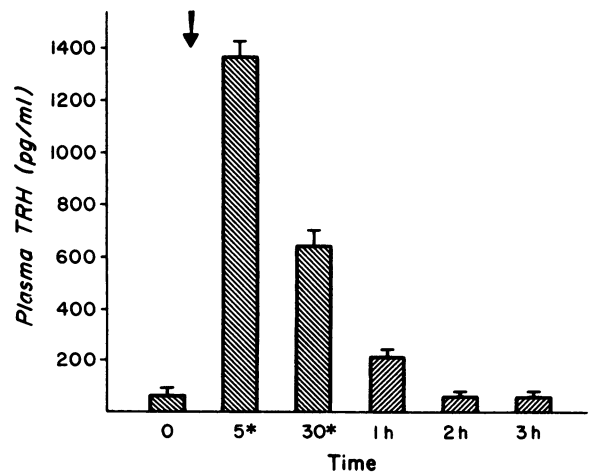
FIGURE 6 The effect of the administration of synthetic TRH (arrow) on plasma TRH concentrations. Animals were sacrificed at 5 min (*), 30 min (*), 1 h, 2 h, and 3 h after the injection. The animals sacrificed at time 0 represent a control group injected with normal saline alone. The bars represent the mean±SE of four animals per group.

after the injection. Thereafter, plasma TRH levels declined rapidly (644 ± 56 pg/ml at 30 min, 220 ± 27 pg/ml at 1 h) and reached base-line levels by 2 h after the injection.

Effect of pancreatectomy on TRH concentrations in the hypothalamus, brain, and gastrointestinal tract. When analyzed at 24 h after pancreatectomy, concentrations of TRH in the hypothalamus, brain, and gastrointestinal tract were not significantly altered when compared with the sham-operated animals (Table I).

Effect of pancreatectomy on serum TSH, T_4, and T_3 concentrations. At 24 h after pancreatectomy, when plasma TRH levels had significantly declined, there was no significant alteration in serum TSH, T_4, or T_3 (Table II).

DISCUSSION

This study has demonstrated that the TRH immunoreactivity in the plasma, pancreas, and gastrointestinal tract of the neonatal rat is indistinguishable from synthetic TRH. This conclusion is based on three kinds of evidence. First, IR-TRH obtained from purified extracts of plasma, pancreas, and gastrointestinal tract gave radioimmunoassay displacement curves that were parallel with those generated with synthetic TRH. Second, the IR-TRH obtained from these sites was eluted in the TRH area on Sephadex G-10 and HPLC, and third, the TRH in purified pancreatic extracts was biologically active as shown by its ability to release TSH and prolactin from the rat adenohypophysial cells maintained in monolayer culture. These findings agree with the studies of Leppäluoto et al. (14) and Spindel and Wurtman (32), which have provided chromatographic evidence to support the view that the IR-TRH in extraneural sites is indistinguishable from synthetic TRH. However, they are not in accord with the work of Youngblood et al. (33, 34), who have suggested that the IR-TRH in serum, extrahypothalamic brain, and in extraneural sites is not identical with synthetic TRH. These findings have not been replicated by Kreider et al. (35), nor by work from this laboratory (28).

TABLE II
Serum TSH, T_4, and T_3 Concentrations

	TSH	T_4	T_3
	ng/ml	µg/dl	ng/dl
Controls (5)‡	180 ± 80	1.41 ± 0.41	36 ± 7
Pancreatectomized (5)‡	270 ± 80§	1.31 ± 0.67§	31 ± 5§

Data expressed as mean±SE.
* 24 h after pancreatectomy in day 5 neonatal rats.
‡ Number of pools in parentheses.
§ P = NS.

The findings of one peak of TRH immunoreactivity in plasma on Sephadex chromatography reported here contrasts with the recent studies of Theodoropoulos et al. (36), who have reported that two peaks of TRH immunoreactivity appeared when methanol extracts of whole blood were applied to Sephadex columns. These workers have suggested that the first peak, which accounted for more than 90% of the immunoreactivity, represented a prohormone of TRH. These findings are probably due to the different methods of extraction of TRH used, and to the impure nature of the sample subsequently applied to the chromatography system. The procedure described here, that of ethanol extraction of plasma followed by further purification of the extract with organic solvents, essentially removes all the plasma proteins and lipids. Although methanol or ethanol efficiently extracts TRH from whole blood or plasma, it does not seem to adequately remove substances that interfere in the radioimmunoassay. The first peak of IR-TRH described by these workers may therefore represent TRH bound to either lipid or protein. In addition, the presence of lipids or proteins in the radioimmunoassay could interfere with the binding of iodinated TRH with its antiserum, and result in higher levels of TRH in blood compared with those reported here in plasma (19, 20).

During maturation, striking changes were observed in the concentrations of TRH in the pancreas and hypo-

TABLE I
TRH Concentrations in the Hypothalamus, Extrahypothalamic Brain, and Gastrointestinal Tract*

	Hypothalamus	Extrahypothalamic forebrain	Stomach	Upper small intestine	Lower small intestine	Colon
			pg/mg tissue			
Control (6)‡	108 ± 15	3.8 ± 0.4	0.48 ± 0.07	8.88 ± 1.74	0.35 ± 0.05	0.68 ± 0.15
Pancreatectomized‡ (6)	93 ± 12§	3.4 ± 0.1§	0.47 ± 0.10§	8.78 ± 1.82§	0.41 ± 0.08§	0.52 ± 0.17§

Data expressed as mean±SE.
* 24 h after pancreatectomy in day 5 neonatal rats.
‡ Number of pools given in parentheses.
§ P = NS.

thalamus. 1 h after birth, pancreatic TRH concentrations were the largest of any organ examined and indeed were 20 times higher than those in the hypothalamus. An extraordinary threefold increase in pancreatic TRH content occurred within the first 24 h of life, and over the next 10 d, a gradual decline was observed. By the day 10, hypothalamic TRH concentrations, which had increased during the first week of life, were greater than pancreatic TRH concentrations. During the next 3 mo, pancreatic TRH concentrations continued to decline, reaching very low levels in the mature rat. Conversely, TRH concentrations in the hypothalamus continued to increase, so that by maturity, the hypothalamus contained the largest concentrations of any tissue. The decline in pancreatic TRH concentrations with maturation described in this report confirms the recent findings of Koivusalo and Leppäluoto (37). The increase in hypothalamic TRH concentrations is similar to the findings of these authors and the earlier work of Dussault and Labrie (38). At present, the physiological significance of the developmental changes in pancreatic TRH concentrations remains unknown. However, it is noteworthy that during maturation of the rat, total pancreatic content of insulin, glucagon, and somatostatin gradually increase (39–41), whereas total TRH content decreases. In addition, the decline in pancreatic TRH concentrations in the first few days of life is paralleled by a very similar decrease in the concentration of somatostatin and number of somatostatin-containing cells in the thyroid (42). Whether a similar loss of TRH-containing elements may be simultaneously occurring in the pancreas is unknown.

The finding of high concentrations of TRH in the pancreas of the neonatal rat led to the hypothesis that this organ might be a source of the IR-TRH in the circulation. A near total removal of the pancreas in the 5-d-old rat resulted in a significant decline in plasma TRH at 3 h. These data, in the context of the high concentrations of TRH demonstrated within the pancreas, suggest that pancreatic secretion is a source of circulating TRH. The findings have not excluded the possibility that the islet cell hormones, insulin, glucagon, and somatostatin may modulate plasma TRH levels, and that their removal by pancreatectomy may have contributed to the observed decline in plasma TRH concentrations. The finding that circulating levels of TRH are only moderately reduced after removal of the pancreas suggests that other extraneural sites are also involved in secreting TRH into the blood. The gastrointestinal tract is likely to be a major source of circulating TRH; although the TRH concentration in the gut is low, the total content in this site is large. The approximate content of TRH in the hypothalamus of the 5-d-old rat is 0.6 ng, while the TRH content of the extrahypothalamic brain, pancreas, and gastrointestinal tract is 1 ng, 4 ng, and 3–4 ng, respectively. More recently, Pekary et al. (43) have reported that large amounts of TRH are present in the reproductive organs of adult male and female rats. These structures may therefore be a possible source of circulating TRH in the neonatal rat.

When the hypothalamus and extrahypothalamic forebrain were removed, plasma TRH levels were unaltered 3 h later. Stable levels of TRH 3 h after encephalectomy cannot be explained by a lack of degradation of the peptide, inasmuch as synthetic TRH, when administered parenterally, disappeared rapidly from the circulation with an apparent half-life of <30 min. Rather, these nondeclining levels of plasma TRH after encephalectomy suggest that the central nervous system makes little, if any, contribution to the TRH in the circulation.

The specificity of the anatomical localization of TRH to the central nervous system and the gastrointestinal tract of the neonatal rat is enhanced by the absence of demonstrable TRH in the heart, liver, spleen, and kidney, and a similar distribution of TRH has also been described in the adult rat (14). However, in contrast to the adult rat in which TRH is absent from the lung, very low concentrations of IR-TRH were present in the lung of the neonatal rat. A similar sequence of events, namely a disappearance of immunoreactivity from the lung during maturation, has also been described for bombesin (44).

When plasma TRH concentrations were reduced by pancreatectomy, the concentrations of TRH in the hypothalamus, extrahypothalamic brain, and gastrointestinal tract remained unaltered. There are at least two possible interpretations of these findings. The first is that these organs have the capacity to take up TRH from the circulation and to maintain constant tissue levels of the peptide despite large changes in circulating levels. Second, and more likely, is that the TRH present within these sites is synthesized locally. These results are reminiscent of the studies of Havrankova et al. (45) and Rosenzweig et al. (46) which have demonstrated that insulin concentrations in the brain and extrapancreatic tissues of the rat are unchanged when pancreatic and plasma insulin levels are reduced by streptozotocin.

The pituitary-thyroid axis, as assessed by the measurement of serum TSH, T_4, and T_3 concentrations, was unaltered 24 h after plasma TRH concentrations had been lowered by pancreatectomy. These findings add complementary evidence to the experiments of Theodoropoulos et al. (20), and are consistent with the view that in the neonatal period of the rat, maintenance of the functional integrity of the pituitary-thyroid axis is not dependent on the presence of TRH in the circulation.

Although the concept of a pancreatic and gastroin-

testinal source of circulating TRH may seem surprising at first glance, support for this proposal is derived from the observation that these sites are the major sources of circulating insulin, glucagon, somatostatin, vasoactive intestinal peptide, bombesinlike immunoreactivity, and pancreatic polypeptide (47–54). All of these peptides share with TRH a dual distribution, not only in the pancreas and gut, but also in the central nervous system (55–57). Although this study has not defined the cellular origin of TRH within the pancreas and gastrointestinal tract, there is a similarity between the distribution of this peptide and those belonging to the Diffuse Neuroendocrine System. This is constituted by cells that have arisen either in the embryonic epiblast itself or in one of its descendents, and that migrate to the endoderm during embryonic development (58). The central division of the Diffuse Neuroendocrine System contains the neuroendocrine and endocrine cells of the hypothalamic-pituitary axis and the pineal gland, whereas the peripheral division contains all the amine-precursor uptake and decarboxylation cells outside these sites. The majority of the latter are situated in the gastrointestinal tract and pancreas and, particularly in the fetus, are also distributed widely in the respiratory and urogenital tracts, the thyroid and parathyroid glands, the thymus, adrenal medulla, and the sympathetic nervous system.

In summary, this study has demonstrated that the TRH circulating in the blood of the neonatal rat is derived from the pancreas, the gastrointestinal tract, and possibly other extraneural sites, and is not of hypothalamic or extrahypothalamic brain origin. Its functions in the circulation remain to be determined.

ACKNOWLEDGMENTS

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