THYROTROPIN-RELEASING HORMONE IN THE GASTROINTESTINAL TRACT

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Received September 20,1977

SUMMARY

TRH immunoreactivity has been shown to occur throughout the rat gastrointestinal tract. This immunoreactivity demonstrates parallelism with TRH, is destroyed by fresh human serum, and co-chromatographs with TRH on a Sephadex G-10 column and on a SP Sephadex C-25 column. In addition pancreatic extracts showed bioactivity in a mouse bioassay for TRH.

Several hormonal peptides have been shown to occur both in the brain and the gastrointestinal tract (1). Pearse (2) has postulated that the cells producing endocrine peptides are all derived from specialized ectoderm or, more specifically, from the endocrine epiblast and are programmed for neuroendocrine function. Thus, it should no longer be regarded as surprising to find "brain peptides" produced by endocrine cells in sites far removed from the central nervous system. The majority of human subjects injected with synthetic TRH complain of mild nausea and urinary urgency (3). A preliminary study showed that TRH caused tonic contraction in the guinea pig ileum and that TRH potentiated the contraction of the human bladder induced by acetylcholine *in vitro* (4). We report here evidence for TRH immunoreactivity and TRH biologic activity in the gastrointestinal tract and the pancreas.

MATERIALS AND METHODS

Fresh samples of rat gastrointestinal tract and other organs were rapidly removed after decapitation and boiled for 10 minutes in distilled water to destroy any TRH degrading enzyme activity that may be present. The tissues were weighed and homogenized. The homogenate was extracted with 5 ml methanol and the supernatants dried in a water bath at 60°C under a stream of air. The distilled water in which the tissues had been boiled was lyophilized. The dried and lyophilized extracts were each diluted in 0.5 ml phosphate-buffered saline (0.15 M NaCl, 0.01 M sodium phosphate, pH 7.5)(PBS) and 100 µl aliquots were assayed in duplicate for TRH by radioimmunoassay as described by Bassiri and Utiger (5). Antiserum to TRH was kindly supplied by Dr. Robert D. Utiger, University of Pennsylvania School of Medicine. Total amounts of TRH were calculated by combining the quantities in the water and methanol extracts. Aliquots of the water and methanol extracts were radioimmunoassayed in multiple

ISSN 0006-291X

TABLE 1. TRH CONTENT OF GASTROINTESTINAL AND OTHER TISSUES.

ORGAN	TOTAL TRH ng/g wet weight*	WATER EXTRACT %
Stomach-Fundus	2.6 ± 1.1	84.3
Antrum	0.4 ± 0.02	37.6
Duodenum	1.6 ± 0.1	68.2
Jejunum	1.2 ± 0.1	69.1
Ileum	2.3 ± 0.8	63.6
Caecum	3.3 ± 0.9	69.8
Colon	2.1 ± 0.1	63.6
Rectum	1.0 ± 0.2	78.4
Pancreas	3.4 ± 0.9	78.4
Lung	1.4 ± 0.4	61.4
Kidney	0.8 ± 0.3	28.3
Spleen	1.0 ± 0.2	25.7
Heart	0.7 ± 0.1	42.5
Liver	0.6 ± 0.1	19.7

*Mean ± SEM,
3 observations per organ

dilutions and the curves thus obtained were compared to the standard curve for TRH by the t-test for parallelism (6). In addition, 100 µl aliquots of 2 extracts each of pancreas and duodenum were incubated with 100 µl of fresh human serum in a water bath at 37°C for 2 hours. The mixture was once again extracted with methanol and the dried supernatant was diluted in 250 µl of PBS and assayed for TRH-immunoreactivity. The values obtained were compared to controls of 100 µl extract and 100 µl PBS (rather than serum) which were treated in the same manner. Aliquots of the methanol extracts were chromatographed on a 1 x 43 cm Sephadex G-10 (Pharmacia) column and eluted with PBS. One pancreatic extract was fractionated by ion exchange on a 1 x 43 cm SP Sephadex C-25 column and eluted with 0.2 M acetate buffer (pH 6.2). Boiled water extract of pancreas which had been purified on a Sephadex G-10 column was bioassayed for TRH activity in a mouse bioassay (7).

RESULTS

The immunoreactive TRH content of rat gastrointestinal tissues and other organs is shown in Table 1. The largest amounts were found in the

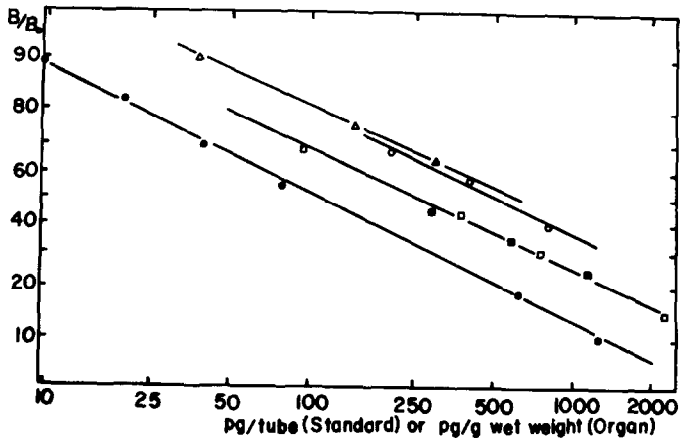

Fig. 1 Demonstrating parallelism of pancreatic (2 specimens, □-□ and ■-■), antral (Δ-Δ) and duodenal (o-o) extract to the TRH standard curve (●-●) in TRH radioimmunoassay.

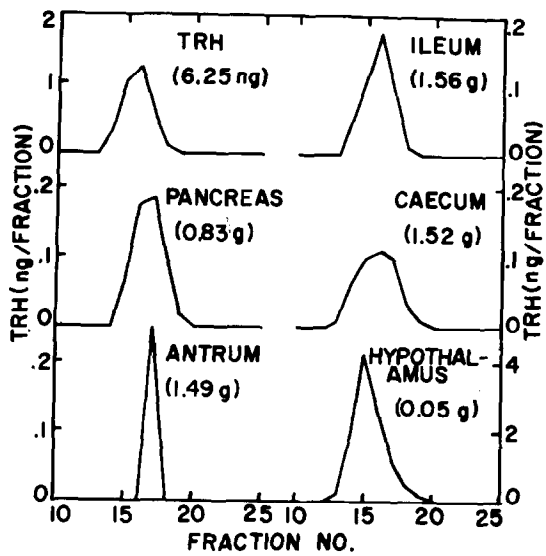

Fig. 2 G-10 Sephadex chromatography of gastrointestinal organs compared to TRH and to hypothalamic extract.

large bowel and the pancreas. Serial dilutions of pancreatic, antral and duodenal specimens demonstrated that the slopes of their dose-response lines were parallel to the standard curve for TRH (Fig. 1). After incubation

TABLE 2. TRH BIOACTIVITY IN MOUSE BIOASSAY: EFFECT OF PANCREATIC EXTRACT; 6 MICE/SUBSTANCE.

	CONTROL	TRH 8 ng/mouse	*PANCREATIC WATER EXTRACT
†C_2/C_0	0.90 ± 0.06	5.20 ± 2.23	6.05 ± 2.91

*TRH content by immunoassay equivalent to 15.4 ng/mouse
†Blood ^{125}I 2 hours after injection compared to blood ^{125}I prior to injection, mean ± SD.

for 2 hours in human serum, the duodenal and pancreatic extracts failed to show any TRH-immunoreactivity as compared to the control samples, all of which had measurable TRH-immunoreactivity. Extracts of pancreas, ileum, antrum and caecum co-chromatographed with TRH and with TRH-immunoreactivity of a hypothalamic extract on the Sephadex G-10 column (Fig. 2). Pancreatic extract co-chromatographed with TRH on the SP Sephadex C-25 column. Pancreatic extracts with immunoreactivity of 15.4 ng showed bioactivity greater than that seen with an 8 ng TRH standard (Table 2).

DISCUSSION

We have demonstrated that TRH-immunoreactivity occurs throughout the gastrointestinal tract and in a number of other organs. The quantities of TRH found were smaller than those previously reported for somatostatin (8) and enkephalin (9) in the gastrointestinal tract and smaller than the concentration of TRH reported to occur in the hypothalamus and other areas of the brain (10). TRH has also been found in large quantities in frog skin (11,12) and the placenta has been reported to be capable of synthesising TRH *in vitro* (13). TRH has been localized to the synaptosomes and has many chemical characteristics typical of a neurotransmitter; it has a low molecular weight; it is water soluble; and it is rapidly inactivated by tissue and blood. In view of its distribution throughout the body, it would appear that TRH is an

ubiquitous substance which the pituitary has co-opted as a TSH-regulatory hormone.

At the present time we do not know if TRH plays a physiological role in the control of gastrointestinal function. The report (14) of its effect on guinea pig ileum suggests that it may play a role in the control of gut motility. Moltz et al (15) have recently shown that a substance released from ventromedial hypothalamic segments in vitro inhibited insulin release and stimulated glucagon release from the islets of Langerhans; appreciable quantities of TRH are present in the ventromedial nucleus (16). Preliminary studies in our laboratory have shown TRH to be present in the portal effluent of an isolated gut preparation. Gastrointestinal physiology appears to be partly regulated by locally acting hormones, and TRH may be one of these hormones.

ACKNOWLEDGMENTS

We thank Nancy Meyer for her help with the bioassay. This work was supported by the Medical Research Service of the Veterans Administration and USPHS Research Grant HD-7181.

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