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High concentration of thyrotropin-releasing hormone in pancreatic islets

(somatostatin/streptozotocin diabetes/neurohumoral regulation/hypothalamus)

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ABSTRACT **The concentration of thyrotropin-releasing hormone (TRH, thyroliberin) in rat islets of Langerhans is 30-fold higher than in whole rat pancreas, indicating that the islets are the main source of pancreatic TRH. The TRH extracted from islets is indistinguishable from synthetic TRH in its immunological and biological properties and in its inactivation by human serum. The physiologic function of islet TRH is unknown. However, because TRH is antagonistic to somatostatin in other systems, and somatostatin also is concentrated in islets in high concentrations, it is possible that islet TRH may serve a similar antagonistic function in the regulation of islet cell secretory activity.**

A rather ubiquitous distribution of thyrotropin-releasing hormone (TRH, thyroliberin) has been demonstrated through the use of a sensitive and specific radioimmunoassay. Significant amounts of TRH have been found in extra hypothalamic areas of the human brain (1–3) as well as in the central nervous system of several mammalian and submammalian species (4–6). The presence of TRH has also been reported in human placenta (7), rat retina (8), and frog skin (9). For this reason, it has been postulated that TRH may not only play a role in the regulation of pituitary thyrotropin and prolactin secretion but may also act as a neurotransmitter or modulator of synaptic function (5, 10). Recently Morley *et al.* (11) reported that TRH is present throughout the rat gastrointestinal tract as well as in the pancreas. This report confirms the latter finding and localizes TRH to the islets of Langerhans.

MATERIALS AND METHODS

Preparation of Animals. All rats were males weighing 200–250 g. Strains used were CD (Charles River Breeding Laboratories, Waltham, MA), Lewis (Microbiological Associates, Bethesda, MD), and Sprague–Dawley (Laboratory Supply, Indianapolis, IN). Purina rat chow was continuously available except during 48-hr fast. The animals were housed in screen-floor cages and were given tap water ad lib. Hypothyroid rats were prepared by surgical thyroidectomy, followed by intraperitoneal injection of 80 μCi of ^{131}I per rat and maintained on low-iodine diet (Teklad Mills, Madison, WI). They were used two months after the induction of hypothyroidism. Thyroid hormone-replaced rats were hypothyroid rats treated with 15 μg of triiodothyronine intraperitoneally 24 hr before the tissues were obtained. Rats of the Lewis and Sprague–Dawley strains were made diabetic by a single intravenous injection of streptozotocin (65 mg/kg of body weight) and used 7 and 10 days later.

Isolation of Whole Pancreas and Islets. Rats were killed by decapitation and the pancreases were immediately excised. Fat and lymph nodes were trimmed away, and the residual tissue was weighed and frozen on dry ice. For isolation of pancreatic islets, rats were injected intraperitoneally with 0.25 ml of 4% pilocarpine·HCl 2 hr prior to decapitation. A group of rats was similarly pretreated prior to the isolation of the whole pancreas to check whether pilocarpine had any effect on the concentration of total pancreatic TRH. The pancreas was dissected as described above and isolated islets were prepared from pools of 8–10 animals as previously described (12). Briefly, the steps included collagenase digestion, several washes by centrifugation in Hanks' medium, Ficoll gradient centrifugation followed by additional washes, and individual selection of about 1500–2000 islets under a stereomicroscope. The final islet preparation was washed, pelleted by centrifugation, and weighed prior to freezing on dry ice.

Sample Preparation for TRH Measurements. TRH was extracted from frozen tissue with 90% methanol. After homogenization in a glass–glass homogenizer the residue was pelleted by centrifugation and the supernatant was dried in a water bath at 37°C under a stream of air. The dried extract was diluted in 0.5 ml of phosphate-buffered saline (0.01 M sodium phosphate/0.15 M NaCl, pH 7.6). The efficiency of the extraction procedure, as assessed by recovery of ^{125}I-labeled TRH (^{125}I-TRH), was invariably above 90%. Thus, the data were not corrected for extraction losses.

Analytical Methods. Concentration of TRH in tissue extracts was measured by a double antibody radioimmunoassay as previously described (13). The sensitivity of tissue TRH to proteolytic degradation by fresh human serum was compared to that of synthetic TRH, and to TRH extracted from rat hypothalami. Incubation in the presence of serum was carried out at 37°C for 3 hr and the residual immunoassayable TRH was measured. The biological activity of TRH extracted from isolated islets and quantitated by radioimmunoassay was compared to that of synthetic TRH by using an *in vitro* bioassay based on the ability of TRH to release pituitary thyrotropin. In this bioassay, fresh rat pituitaries were divided into four sections. Individual assays consisted of two pituitary quarters incubated in 2 ml of Krebs–Ringer bicarbonate buffer, pH 7.4, for 90 min at 37°C in the presence of 5% CO_2/95% O_2 and 100% humidity (14). TRH or tissue extract in appropriate dilutions made in phosphate-buffered saline was added to the medium in a volume of 100 μl. The release of thyrotropin in the medium was measured by a specific radioimmunoassay (15). The results are expressed as changes in the medium thyrotropin concentration in percent of control, corrected for pituitary protein content measured by the method of Lowry *et al.* (16).

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Abbreviation: TRH, thyrotropin-releasing hormone (thyroliberin).

Table 1. Concentrations of TRH in whole pancreas and in isolated islet cells

Rat	No. of rats*	TRH, pg/mg wet weight*	P†
	Whole pancreas		
Charles River			
Normal	3	6.3 ± 1.5	
Hypothyroid	3	9.3 ± 1.3	NS
Hypothyroid + triiodothyronine	3	7.1 ± 0.8	NS
Sprague–Dawley			
Fed	6	2.9 ± 1.2	
Pilocarpine	6	2.2 ± 0.9	NS
Starved	6	4.2 ± 1.0	<0.05
	Isolated islet		
Lewis	8	157.0	
	8	158.0	
Sprague–Dawley	8	76.2	
	8	89.8	

* Data from whole pancreas represent duplicate determinations in individual animals. Isolated cells from the entire group of animals were pooled in a single preparation and values given are the mean of duplicate determinations. Results are given as mean ± SD.

† *P*, probability that sample is same as the control. NS, difference not significant.

RESULTS

The concentrations of immunoreactive TRH in whole pancreas and in isolated islets of Langerhans are shown in Table 1. A marked difference among strains was observed. Hypothyroidism and replacement with triiodothyronine had no significant effect on the TRH concentration in whole pancreas. In contrast, 48-hr starvation produced a significant increase. Treatment with pilocarpine did not affect the TRH concentration in whole pancreas. The concentration of TRH in isolated pancreatic islets was considerably higher and again showed strain variation. Compared to the whole pancreas in animals of the same strain, concentration of TRH in pancreatic islets was about 30-fold higher.

The TRH immunoreactivity of materials extracted from whole pancreas and from islets was compared to that of synthetic TRH by the ability of the materials, in various dilutions, to displace ^{125}I-TRH bound to a specific antiserum. A parallelism between the three curves is shown in Fig. 1. Data presented in Table 2 show the ability of fresh serum to completely inactivate the immunoreactivity of synthetic TRH as well as TRH extracted from rat hypothalami, total pancreas, and isolated islets. The biologic activity of immunoreactive TRH extracted from isolated islets is shown in Fig. 2. Two hundred and 500 pg of immunoreactive TRH extracted from the islets had a biologic potencies similar to equal amounts of synthetic TRH.

Table 2. Sensitivity of immunoreactive TRH to inactivation by fresh human serum

Sample	TRH, pg Before	TRH, pg After
Synthetic TRH	500	<2
Hypothalamic extract	250	<2
Total pancreas extract	250	<2
Islet extract	100	<2

Rats rendered diabetic with streptozotocin showed a marked reduction in the concentration of TRH in whole pancreas and in isolated islets (Table 3). This observation was reproduced in two different strains of rats (data not shown). No significant changes in the concentration of hypothalamic TRH resulted from this treatment.

DISCUSSION

Morley *et al.* (11) have recently reported the presence of significant amounts of TRH in the rat gastrointestinal tract, including the pancreas, indicating that these tissues represent an additional source of extrahypothalamic TRH. The present report localizes the source of pancreatic TRH to the islets of Langerhans. Although significant variations in the level of pancreatic TRH were observed among various species of rats, islet TRH concentration, in terms of wet tissue weight, was approximately 30-fold higher than that found in corresponding whole pancreas. The concentration in islets was ⅓ to ½ that found in the hypothalamus.

TRH extracted from pancreatic islets, as well as from whole pancreas, was immunologically identical to synthetic TRH as

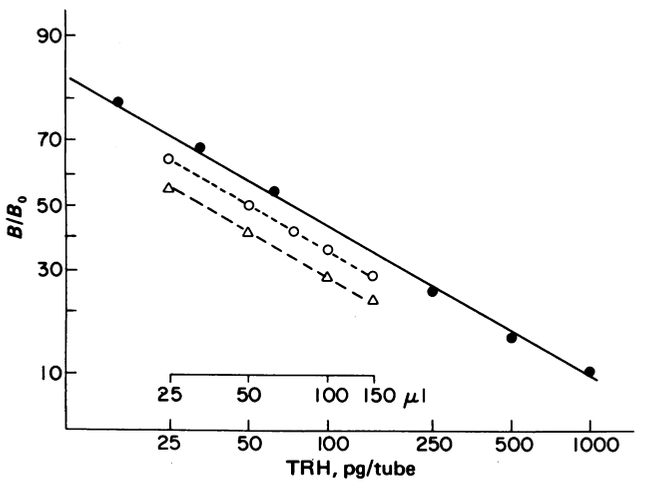

FIG. 1. Displacement of ^{125}I-TRH from a specific antibody by synthetic TRH (●) and by material extracted with methanol from isolated rat islets (○) and total pancreas (△). Note complete parallelism of the curves. B/B_0, amount of ^{125}I-TRH bound divided by maximum binding in the absence of unlabeled TRH, expressed in percent.

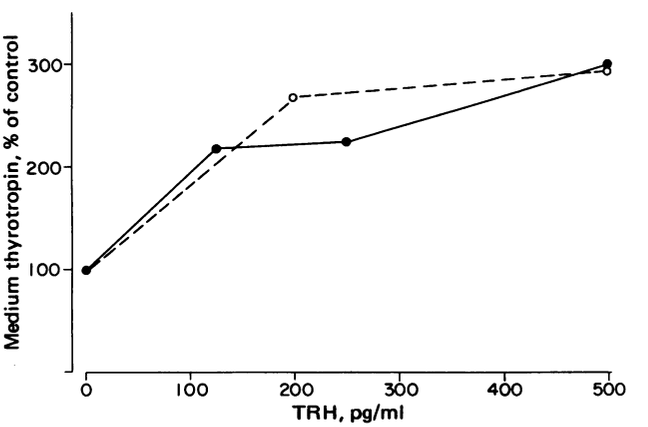

FIG. 2. Biologic activity of immunoreactive TRH extracted from isolated rat pancreatic islets (○) as compared to that of synthetic TRH (●). The release of thyrotropin from rat pituitaries incubated *in vitro* was measured. Results are expressed as percent change in thyrotropin released in the absence of added TRH or islet cell extract.

Table 3. Effect of streptozotocin on islet TRH

Sample	TRH, pg/mg of wet weight	
	Normal rats	Streptozotocin-treated rats
Whole pancreas	2.47 ± 0.88 (6)	0.43 ± 0.31 (4)
Islet	89.8 (8)	8.3 (10)
Hypothalamus	291 ± 89 (6)	220 ± 50 (13)

Results are expressed as in Table 1. Numbers of the Sprague–Dawley rats are in parentheses.

shown by their equal abilities to displace a radioiodinated TRH tracer from a specific antiserum. Exposure of islet TRH to serum resulted in complete inactivation indistinguishable from that of hypothalamic or synthetic TRH. Furthermore, the ability of immunoassayable islet TRH to stimulate release of thyrotropin from rat pituitaries *in vitro* was quantitatively identical to that of synthetic TRH.

Changes in thyroid function did not alter the concentration of pancreatic TRH. Similar results have been observed in measurements of hypothalamic TRH (17, 18) despite the known role of TRH in the control of thyroid gland function and hormone synthesis (19, 20). In contrast, induction of diabetes profoundly depressed the concentration of TRH in the pancreatic islets.

The latter observation gives rise to a number of speculations concerning the origin and possible physiologic role of pancreatic islet TRH. The marked depression in islet TRH content in diabetic animals suggests a cellular origin, associated in some way with the beta cells. However, if this were the case, their content of TRH would be approximately 1/10000th that of their content of insulin. Alternatively, the islet TRH may arise from a small subpopulation of streptozotocin-sensitive cells that are distinct from the majority of beta cells, or from nerve endings, closely associated with beta cells, that undergo parallel atrophy after streptozotocin treatment. The recent demonstration of the hypothalamic peptide somatostatin in the islets of Langerhans (21–23) and its relatively increased content in streptozotocin-diabetic rats (24) are also of interest, especially in view of the generally antagonistic effects of TRH and somatostatin on the secretory control of a number of peptide hormones (25–34). Thus, while TRH stimulates the secretion of growth hormone (25, 27), prolactin (29, 32), and thyrotropin (28, 31), somatostatin inhibits the basal and/or stimulated secretion of these same hormones (25–30). Similarly, TRH potentiates glucagon secretion from the perfused pancreas under certain conditions (35). In islets of Langerhans, somatostatin has been localized to the D cells (21–23) and, as in the case of TRH, the concentration found in pancreatic islets is high and does not differ much from that found in the hypothalamus (24). Because somatostatin has been shown to inhibit insulin and glucagon release (32–34), a possible function of islet TRH may be to oppose these effects of somatostatin and thus provide a balanced system in the neurohumoral regulation of islet cell function.

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