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A PROLACTIN INHIBITORY FACTOR WITH IMMUNOCHARACTERISTICS SIMILAR TO THYROTROPIN RELEASING FACTOR (TRH) IS PRESENT IN RAT PITUITARY TUMORS (GH3 AND W5), TESTICULAR TISSUE AND A PLANT MATERIAL, ALFALFA

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SUMMARY

Thyrotropin releasing hormone (TRH) is distributed throughout the extrahypothalamic central nervous system and the gastrointestinal tract. We report here evidence for a naturally occurring substance with TRH-like immunoreactivity which inhibits prolactin release in pituitary cultures. This substance co-elutes with synthetic TRH on a G-10 Sephadex column but shows non-identity with TRH on cation exchange chromatography (SP-Sephadex C-25). We have demonstrated the presence of this substance in rat pituitary tumors, rat testicular tissue, and in a vegetable material, alfalfa.

INTRODUCTION

Thyrotropin releasing hormone (TRH) immunoreactivity is found in the extrahypothalamic central nervous system (1), the retina (2), the pancreas and gastrointestinal tract (3,4), the placenta (5), in amniotic fluid (6), in breast milk (7), and in frog skin (8). Phylogenetic studies of TRH distribution have shown that TRH occurs in species such as lamprey which lacks thyrotropin, and in amphioxus and snail, species without pituitaries (9,10). Evidence that this immunoreactive TRH is identical to hypothalamic TRH has been provided by the demonstration that extrahypothalamic TRH co-chromatographs with both synthetic and hypothalamic TRH on a number of chromatographic systems and that TRH-like bioactivity has been found to be present in all tissues so far tested (11). Additionally a substance obtained from the skin of Bombina orientalis was identified as TRH based upon its amino acid composition and thin-layer chromatographic properties in two solvent systems (12). Because of the widespread distribution of TRH, its rapid degradation in

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blood and tissue (13), its concentration in synaptosomes (14) and its demonstrated action on isolated neurons (15), TRH is probably an ubiquitous neurotransmitter that has been co-opted by the pituitary as a releasing factor.

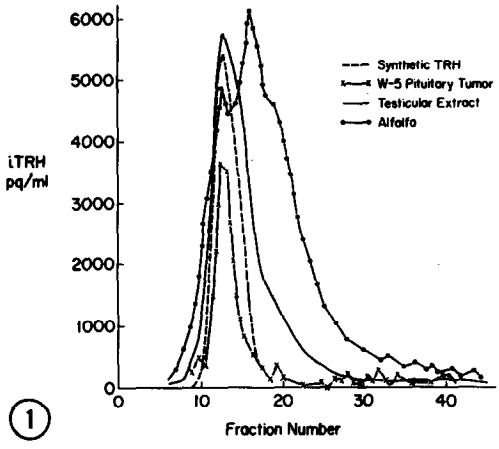
We report here evidence for a naturally occurring substance with TRH-like immunoreactivity which shows non-identity with synthetic TRH on cation exchange chromatography and inhibits prolactin release from acute pituitary cultures. We have demonstrated the presence of this substance in rat testicular tissue, rat pituitary tumors and in a vegetable material, alfalfa.

MATERIALS AND METHODS

Two transplantable rat pituitary tumors were studied. The GH3 pituitary clonal cell line was obtained from American Type Cell Culture (Rockville, Maryland), and originally cultured as monolayers in plastic culture plates. When adequate growth was established, the monolayer cultures were injected into the flank of female Wistar-Furth rats, and the tumors were maintained for 3 passages in these animals. The tumors were demonstrated to produce growth hormone and prolactin, both initially and after the animal passages. The second tumor studied was the Mst/W5 which had been originally obtained from the Mason Research Institute, Worcester, Massachusetts and maintained in our laboratory carried in female Wistar-Furth rats by serial subcutaneous transplantation. Fresh samples of the tumor tissue and testicular tissue from 100 g Sprague-Dawley rats were rapidly removed after the rats were killed by decapitation. The tissue was immediately frozen on dry ice and rapidly weighed. The tissues were then homogenized in 3% acetic acid, and after centrifugation (2000 rpm for 20 min. at 4°C), the supernatants were dried in a water bath at 80°C under a stream of air. Alfalfa, commercially obtained from Paramount Cubing Company, Paramount, California, was also homogenized in 3% acetic acid and treated similarly to the tissue specimens. Recovery of synthetic TRH with this system varies from 92 to 103%. Aliquots of the extracts were chromatographed on 1 x 20 cm Sephadex G-10 (Pharmacia) column eluted with 0.5 M phosphate-buffered saline (0.15 M NaCl, 0.01 M sodium phosphate, pH 7.5) and on 1 x 39 cm SP-Sephadex C-25 column eluted with 0.2 M acetic buffer (pH 6.2). Concentrations of TRH-like immunoreactivity were determined by radioimmunoassay as previously described by us (16). Histidyl-proline diketopiperazine, a TRH breakdown product known to inhibit prolactin release (17), did not cross-react in the assay up to a concentration of 1 µg/ml. Column fractions in which TRH-like immunoreactivity was present were lyophilized, and then tested in an acute pituitary quarters explant culture system (18). The TSH and prolactin release into the medium was measured in radioimmunoassays using reagents supplied by NIAMDD.

RESULTS

Immunoreactive TRH was present in the GH3 pituitary tumor (1.9 ng/g), the W-5 tumor (2.8 ng/g), testicular tissue (7.3 ng/g) and alfalfa (1.8 ng/g). Serial dilutions of the various extracts demonstrated that the slopes of their dose-response lines were parallel to the standard curve for synthetic TRH. Extracts of the W-5 tumor and testicular tissue co-chromatographed with synthetic TRH on the Sephadex



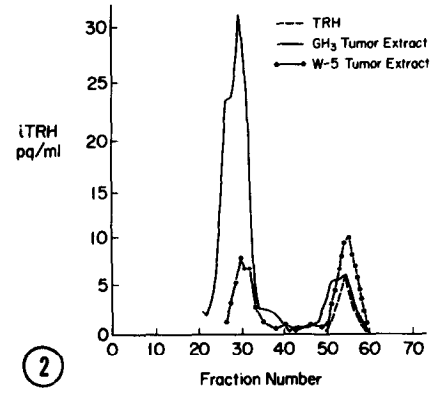


Figure 1. Chromatographic profiles of synthetic TRH, W-5 pituitary tumor, testicular tissue and alfalfa on Sephadex G-10.

Figure 2. Chromatographic profiles of tumor extracts (W-5 and GH3) on SP-Sephadex C-25.

G-10 column (Fig. 1). Alfalfa extracts demonstrated 2 peaks, the first of which co-chromatographed with synthetic TRH (Fig. 1).

On the SP Sephadex C-25 cation exchange column the GH3 extracts produced two TRH immunoreactive peaks (Fig. 2). The smaller second peak co-eluted with synthetic

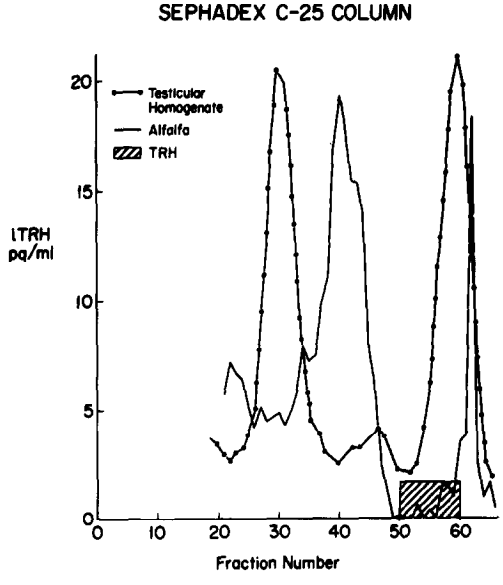


Figure 3. Chromatographic profiles of testicular homogenates, alfalfa and synthetic TRH on SP-Sephadex C-25.

Table 1

Effect of Alfalfa Extract and GH3 Pituitary Tumor Extract on Prolactin and TSH Release from Pituitary Explant Cultures

	n	PROLACTIN (ng/ml/ng)	TSH (μU/ml/mg)
CONTROLS	6	971 ± 49	52 ± 9
TRH (5 ng/ml)	4	2206 ± 512*	194 ± 47*
GH3 PEAK I (6 ng/ml)	6	615 ± 62*	45 ± 9
ALFALFA (5 ng/ml)	6	501 ± 48*	37 ± 9

Amount of TRH-like activity was ascertained by radioimmunoassay of the extracts. (All results expressed as mean ± S.D. *$p < 0.01$; Peaks labelled sequentially from cation exchange column).

TRH. A similar profile was observed for the W-5 tumor extract except that in this case the peak co-eluting with synthetic TRH was slightly larger than the first peak (Fig. 2). A similar elution profile was obtained for testicular tissue (Fig. 3). Alfalfa extract produced multiple immunoreactive peaks, none of which were coincident with the elution pattern for synthetic TRH (Fig. 3).

In the acute pituitary explant cultures, the fractions from the first peak of the GH3 tumor extract markedly inhibited prolactin release ($p < 0.001$) and increased TSH release (Table I and Table II). The second peak fractions increased TSH release without altering prolactin release. Similarly the first immunoreactive area of the alfalfa extract on the cation exchange column inhibited prolactin release from the pituitary explants without altering TSH release (Table I). Both peaks I and II of the testicular extract inhibited prolactin release. Neither peak altered TSH release (Table II).

DISCUSSION

This preliminary communication demonstrates the occurrence of a naturally occurring prolactin inhibitory factor (PIF) that cross-reacts with an antibody to TRH. The PIF-like substance is widely distributed in nature being present in transplanted pituitary tumors, normal testicular tissue and in the plant material, alfalfa. The

Table 2

Effect of GH3 Pituitary Tumors and Testicular Extracts on Prolactin and TSH Release from Pituitary Explant Cultures

	n	PROLACTIN (ng/ml/mg)	TSH (μU/ml/mg)
CONTROLS	15	1028 ± 199	102 ± 41
GH3 PEAK I (10 ng/ml)	12	727 ± 146**	197 ± 78*
GH3 PEAK II (5 ng/ml)	12	1123 ± 432	237 ± 54**
TESTIS PEAK I (5 ng/ml)	5	303 ± 127**	100 ± 28
TESTIS PEAK II (5 ng/ml)	6	342 ± 100**	82 ± 26
TRH (5 ng/ml)	3	1498 ± 294*	335 ± 118**

(All results expressed as mean ± S.D. *$p < 0.01$, ** $p < 0.001$; Peaks labelled sequentially from cation exchange column.)

substance co-eluted with synthetic TRH on Sephadex G-10, suggesting it is of similar molecular weight to TRH. Failure of this material to co-elute with synthetic TRH on cation exchange chromatography (a method which is capable of separating TRH from pyroglutamyl-N^{3im}-methyl-histidyl prolineamide (19)) demonstrates that the substance is not identical to TRH. Both the tumors and testicular tissue also contained a substance which co-migrated with synthetic TRH on SP Sephadex C-25 and which therefore appears to be identical with TRH.

The existence of a hypothalamic prolactin inhibitory factor has been well established in most species including man (20). Most studies suggest that dopamine is the main PIF (21) although there is evidence for the existence of a peptide with prolactin inhibitory properties (20). Dopamine is readily inactivated by oxidation, and the methods we used to handle the tissue exclude the possibility that our PIF was dopamine. We have been unable to demonstrate any substances in the hypothalamus that have TRH immunoreactivity that do not co-elute with synthetic TRH on cation exchange chromatography (19). This suggests that, if this PIF-like substance can be demonstrated to occur in normal pituitaries as well, it may be the result of modifications of the TRH molecule occuring after synthesis in the hypothalamus.

Jackson (22) detected TRH in alfalfa, a plant consituent of commercial rat food, and found TSH releasing activity of rat food extracts in vivo. We did not observe TSH releasing activity in pituitary explants, suggesting that the TSH releasing activity in vivo may be mediated through the hypothalamus rather than directly on the pituitary. Like Jackson, we found two peaks of TRH-like immunoreactivity on Sephadex G-10 chromatography, but on cation exchange chromatography none of the TRH-like immunoreactivity co-eluted with synthetic TRH. Thus there is good evidence for non-identity of alfalfa TRH-like immunoreactivity with synthetic TRH.

We have also found a PIF-like substance with TRH-like immunocharacteristics in crudely purified extracts of the sperm attractants of 2 hydrozoa species, Tubularia and Aequoria (Miller, Morley and Hershman; unpublished observations). Together with the observations reported here, this suggests that the TRH-like substance with prolactin inhibitory activity is widely distributed in both the animal and vegetable kingdoms. In the past decade there has been an enormous increase in the number of known neurotransmitters. It is possible that the substance described in this communication represents yet another ubiquitous neurotransmitter.

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