BINDING OF THYROTROPIN RELEASING HORMONE BY THYROTROPIN-SECRETING CELLS

Lynne J. Eddy, Jerome M. Hershman, R. E. Taylor, Jr. and S. B. Barker

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Summary - In order to localize and identify receptor structures, the binding of radiolabeled thyrotropin releasing hormone (TRH) to thyrotropin (TSH)-secreting cells from rat and bovine anterior pituitaries and from a mouse TSH-secreting tumor was studied in vitro. The binding of TRH to rat anterior pituitaries increased linearly with the log of TRH concentration in the incubation medium. Plasma membranes were the only subcellular fractions isolated after incubation from bovine anterior pituitary and the TSH tumor which bound detectable quantities of TRH.

INTRODUCTION

Since the identification of the structure of thyrotropin releasing hormone (TRH) in several species as (pyro)glutamyl-histidyl-prolineamide (1-3), much work has been directed toward delineating the mechanism of action of this hypothalamic hormone. The biological activity of synthetic TRH is identical to the native hormone, causing both synthesis and release of thyrotropin in the anterior pituitary (4). These effects are presumably consequent to binding of the hormone by specific receptor sites within the responsive cells or on their membranes.

In order to determine whether or not specific cellular TRH receptor sites existed, we studied the binding of TRH in vitro by rat and bovine anterior pituitaries and by a mouse TSH-secreting tumor.

MATERIALS AND METHODS

Synthetic TRH (100 μg/ml) was supplied by Dr. Michael Anderson and ^{14}C-TRH, 256 Ci/mole, by Dr. George Flouret, Abbott Laboratories. ^{3}H-TRH, 40 Ci/mmole, was purchased from New England Nuclear Corp. The TSH-secreting tumor, originally obtained from Dr. Jacob Furth, was grown in radiothyroidectomized LAF, mice (Jackson Laboratories).

Tissue incubations were for 30 min at 37 C in Kreb's Ringer bicarbonate (KRB). Tissue homogenizations, zonal centrifugations, and gel filtrations

were all carried out at 4 C in a 10 mM Tris-HCl buffer, pH 7.4, containing 1 mM $MgCl_2$. Bound and unbound TRH were separated on a 1 x 30 cm Sephadex G-50 column, eluted with the Tris buffer.

Rat anterior pituitaries were incubated with radiolabeled TRH at concentrations of 25 pmoles ^{3}H-TRH/ml, 137 pmoles ^{14}C-TRH/ml, and 2700 pmoles either ^{3}H- or ^{14}C-TRH/ml. After incubation the pituitaries were homogenized and centrifuged at 500 x g for 8 min. The supernatant was filtered on a Sephadex G-50 column. The rat anterior pituitaries did not yield sufficient quantities of tissue for separation of subcellular fractions by zonal centrifugation. Therefore, the next series of experiments was done with bovine anterior pituitaries and a mouse TSH tumor, both of which were obtainable in quantities more suitable for zonal centrifugation.

Bovine anterior pituitaries were either minced with scissors or sliced with a Stadie-Riggs tissue slicer before incubation with 12.5 pmoles ^{3}H-TRH/ml. Mouse tumor slices were incubated with 6.25 pmoles ^{3}H-TRH/ml. After incubation the tissue was homogenized and centrifuged at 500 x g for 8 min. The supernatant was layered in a B-XIV zonal centrifuge rotor on a discontinuous sucrose gradient (9-55%) and centrifuged at 30,000 rpm for 35 min in a Spinco ultracentrifuge, L2-65B. The absorbance at 278 mμ was monitored during unloading of the rotor. Weight percent of sucrose was measured refractometrically on each 10 ml fraction and ^{3}H-TRH distribution among the fractions was determined. Fractions containing both protein and ^{3}H-TRH were filtered on a Sephadex G-50 column to separate bound from unbound TRH. After gel filtration the fractions containing both protein and ^{3}H-TRH were incubated at 37 C for 30 min both in the presence and absence of a 1000- to 2000-fold excess of unlabeled TRH. These samples were then filtered on a Sephadex G-50 column to check stability of binding and displacement of the labeled hormone.

RESULTS AND DISCUSSION

Gel filtration of the rat anterior pituitary homogenate showed a small

Table 1

Incubation of rat anterior pituitaries with radiolabeled TRH. The equivalent of 2 pituitaries was incubated with the radiolabeled TRH. After incubation the tissue was homogenized, centrifuged, and the 500 x g supernatant was filtered on Sephadex G-50. Radioactivity and protein were determined on the hormone-bound fraction and the results expressed as fmoles bound per mg protein.

Incubation conc. of TRH (pmoles/ml)	No. experiments	Radiolabel	fmoles TRH bound per protein ($\bar{x}$ ± S.D.)
25	4	^{3}H	15 ± 1.5
137	2	^{14}C	292 ± 13.5
2700	4	^{3}H	814 ± 134
2700	1	^{14}C	1190

amount of the TRH bound to a macromolecule. The amount of TRH complexed to the macromolecule increased with increasing incubation concentrations of TRH (Table 1). The results at the highest concentration of TRH, 2700 pmoles/ml, showed that both the ^{14}C-TRH and the ^{3}H-TRH were bound to the same extent. TSH release has been demonstrated to be linearly related to the log of the TRH dose both *in vivo* (5) and *in vitro* (6). The experiments reported here for rat anterior pituitaries show that, within a wide range of TRH concentrations, the binding of TRH increased linearly with the log of the TRH concentration (Figure 1). Cerebral cortex pieces used as controls showed no detectable bound TRH.

After zonal centrifugation of the bovine anterior pituitary most of the ^{3}H-TRH remained at the lighter end of the gradient, but some migrated with two protein peaks. Gel filtration of these fractions showed that the TRH was bound to a macromolecule. Both fractions contained plasma membranes, identified by the presence of 5'-AMPase activity.

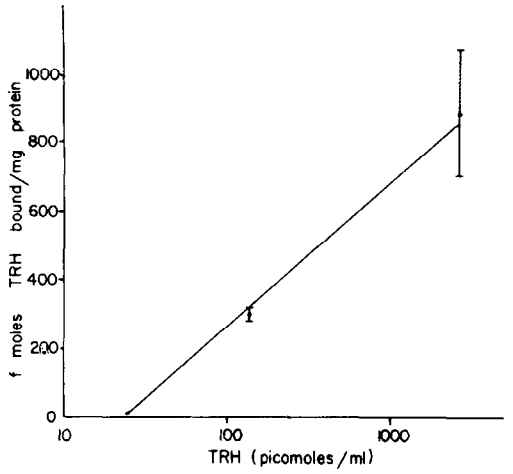

Fig. 1.

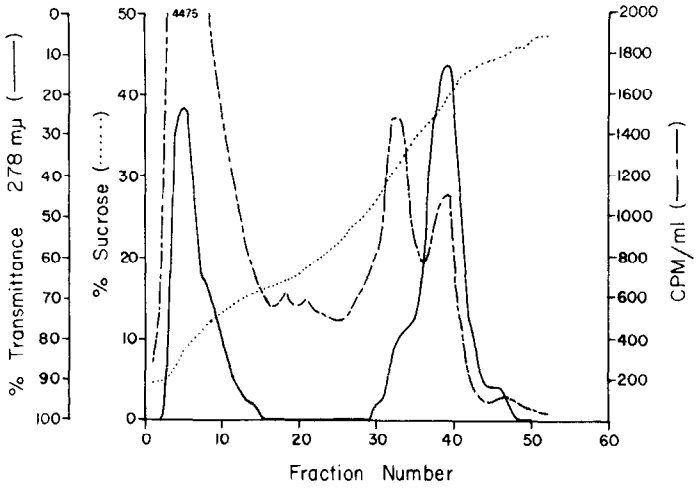

Fig. 2.

The bovine anterior pituitary containing few thyrotrophs was considered inappropriate for detailed studies. The TSH tumor, while not normal tissue in many respects, was better for detailed studies since the major anterior pituitary cell type was the thyrotroph.

Zonal centrifugation of the tumor homogenate showed similar results as the bovine anterior pituitary, with most of the TRH remaining at the lighter end of the gradient, but some migrating with two protein peaks (Figure 2). Gel filtration of these fractions demonstrated that the TRH

Table 2

^{3}H-TRH binding to plasma membranes of TSH tumor. TSH tumor slices were incubated with ^{3}H-TRH, homogenized and centrifuged at 500 x g. The supernatant was subjected to zonal centrifugation. The results are of individual fractionations and show the difference in specific binding between the membrane fractions at sucrose densities 1.14 and 1.16.

Experiment number	fmoles TRH/mg protein	
	1.14 sucrose density	1.16 sucrose density
1	100	60
2	280	100
3	450	58
4	280	130
Mean	278	87

at 33 and 37 percent sucrose was bound to a macromolecule. Although there was considerable variability in the amount of TRG bound per mg protein among the experiments, there was always greater binding to the macromolecule complex at 33 percent sucrose than at 37 percent sucrose (Table 2).

Incubation of these fractions in the presence of excess unlabeled TRH demonstrated that about 60% of the bound TRH was displaceable by a 1200- to 2000-fold excess of unlabeled TRH.

Attempts to measure 5'-AMPase activity in the TRH tumor homogenate or zonal fractions were unsuccessful. Electron microscopy was used to further delineate the subcellular species responsible for TRH binding. Both fractions revealed mostly membranous vesicles, probably derived from plasma membranes, with few mitochondria and no rough endoplasmic reticulum.

TSH secretory granules were attached to the membranes in many instances.

Mouse liver slices were treated identically to the tumor slices and after zonal centrifugation all the ^{3}H-TRH remained at the lighter end of the gradient. There was no migration of TRH with any particulate fraction.

The early reports that TRH stimulated adenyl cyclase in anterior pituitaries (7) and that dibutyryl cyclic AMP stimulated TSH (8) are evidence that TRH action is mediated through the adenyl cyclase system. Since adenyl cyclase is a plasma membrane enzyme, it was proposed that the specific TRH receptor was on the plasma membrane. Labrie et al (9) and Grant et al (10) recently reported binding of TRH to plasma membranes after isolation from TRH responsive cells.

The results reported here show that TRH binding is specific for plasma membranes of thyrotropic cells and this binding is a physiological phenomenon in that TRH will bind to the plasma membranes of intact cells. These experiments support the hypothesis that TRH binds to a specific receptor located on the plasma membranes of TRH responsive cells. The nature of the binding is not known; however, the displacement of labeled TRH by unlabeled TRH indicates that the binding is not covalent.

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