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Thyrotropin-Releasing Hormone in Specific Nuclei of Rat Brain

Abstract. *The regional distribution of thyrotropin-releasing hormone (TRH) in rat brain was studied. The greatest concentration of TRH was found in the median eminence. High concentrations were also found in several hypothalamic nuclei. Outside the hypothalamus, relatively large amounts of TRH were found in the septal and preoptic areas.*

In 1947 Green and Harris (*1*) proposed that neurons located in the hypothalamus might secrete into the portal circulation certain substances which are essential for the normal function of the pituitary (*2*). In the late 1950's and early 1960's hypothalamic extracts were prepared which could stimulate thyrotropin secretion (*3*). The active principle in such extracts, which is called thyrotropin-releasing hormone (TRH), was ultimately isolated, and its structure was determined to be pyroglutamyl-histidyl-prolineamide (*4*). Synthetic TRH has been produced, and a highly sensitive and specific radioimmunoassay has been developed using the synthetic hormone (*5*).

Recently a technique has been described which allows discrete nuclei to be dissected from the brain of the rat (*6*). This technique involves punching small pellets of tissue from frozen sections of the brain under stereomicroscopic control. The method has been used to study the distribution of several putative transmitters among the hypothalamic nuclei of the rat (*7–10*). We report here the localization of TRH in hypothalamic and extrahypothalamic brain nuclei.

Female rats (Osborne-Mendel, NIH strain) (average weight, 150 g) were used. The animals, which were housed in diurnal lighting conditions (light 6 a.m. to 6 p.m., dark 6 p.m. to 6 a.m.) with free access to food and water, were killed by decapitation at 9:00 a.m. Their brains were removed quickly, frozen on a block of Dry Ice, and sectioned with a cryostat-microtome. Sections 300 μm thick were cut through the brain in the frontal plane. In initial experiments relatively large regions of the brain (for example, septum, amygdala, and mammillary body) were cut or punched from the sections. The tissues obtained from two rats were pooled and homogenized in 200 μl of 2.0*N* acetic acid; 5 μl of each homogenate was removed for protein determination according to the method of Lowry *et al.* (*11*). Bovine serum albumin (BSA) served as the standard. The homogenates were centrifuged and the supernatants were lyophilized. Subsequently, the samples were dissolved in a solution consisting of 0.25 percent BSA, 0.01*M* phosphate, and 0.15*M* NaCl (*p*H 7.5), and their TRH contents were determined by radioimmunoassay (*5*). Results were expressed as nanograms of TRH per milligram of protein. Of exogenous TRH (1 ng) added to brain homogenates and carried through the entire assay procedures, 96.2 ± 5.6 percent (mean ± standard error of the mean) was recovered.

In the preliminary experiments described above, the protein content of each sample was in the range 0.2 to 1 mg. No TRH was detected in samples from the following regions (*12*): pineal gland, habenula, central gray, reticular formation, tegmentum, cerebellar cortex, amygdala, hippocampus, cingulate cortex, parietal cortex, olfactory tubercle, caudate nucleus, and anterior pituitary. When larger samples than those used in this study were assayed, TRH was found in the thalamus, brain-

Table 1. Distribution of TRH in the hypothalamus, preoptic area, and septum. The abbreviations are as used in Fig. 1; *N*, number of samples assayed; ng/mg, nanograms per milligram of protein (mean ± standard error of the mean).

Brain region	Abbreviation	*N*	TRH content	
			ng/mg	Nanograms per nucleus
Hypothalamus			1.3*	
Nucleus periventricularis†	NPE	4	4.25 ± 0.69	0.22
Nucleus suprachiasmaticus	NSC	4	1.79 ± 0.18	0.05
Nucleus supraopticus	NSO	4	0.85 ± 0.17	0.02
Nucleus anterior	NHA	4	0.82 ± 0.28	0.15
Nucleus hypothalamicus lateralis anterior	MFB	4	0.70 ± 0.23	
Nucleus paraventricularis	NPV	4	2.60 ± 0.74	0.15
Nucleus arcuatus	NA	6	3.92 ± 0.90	0.41
Nucleus ventromedialis pars medialis	NVMm	4	9.02 ± 3.3	0.80
Nucleus ventromedialis pars lateralis	NVMl	4	2.95 ± 0.59	0.16
Nucleus dorsomedialis	NDM	4	3.95 ± 0.75	0.29
Nucleus perifornicalis	NPF	4	2.03 ± 0.71	0.06
Area hypothalamica lateralis posterior	MFB	4	1.15 ± 0.49	
Nucleus posterior	NHP	4	1.79 ± 0.20	0.10
Nucleus premammillaris dorsalis	NPMD	4	1.48 ± 0.16	0.03
Nucleus premammillaris ventralis	NPMV	4	1.34 ± 0.27	0.02
Median eminence	ME	4	38.38 ± 8.27	0.89
Mammillary body	MB	6	<0.32‡	
Preoptic area		6	1.09 ± 0.08	
Nucleus preopticus medialis	NPOm	4	1.95 ± 0.13	0.20
Septum		6	0.72 ± 0.09	
Nucleus medialis	Sm	3	0.35 ± 0.07	
Nucleus dorsalis	Sd	3	1.86 ± 0.38	
Nucleus dorsalis pars intermedia	Si	3	0.47 ± 0.11	
Nucleus fimbrialis		3	0.62 ± 0.24	
Nucleus triangularis		3	0.51 ± 0.04	
Nucleus lateralis	Sl	3	2.97 ± 0.34	

* This is based on the data of Winokur and Utiger (*13*), who found 0.129 ± 0.007 ng of TRH per milligram of tissue (wet weight) ($N = 30$) in hypothalamic fragments with an average weight of 32.6 mg. † The periventricular nucleus was removed from brain sections with a small knife. The nucleus consists of a fine line of cells and was contaminated by tissue lying lateral to it in our samples. Therefore the concentration of TRH in the periventricular nucleus may be greater than reported. ‡ TRH could be measured in only three of the six samples assayed. The mean TRH concentration in these samples was 0.32 ng/mg.

stem, and cerebral cortex (see *13, 14*).

As expected, TRH was found in high concentrations in the hypothalamus (Table 1). There were also relatively large amounts of TRH in the preoptic and septal areas, and a small amount was found in the mammillary body (Table 1). Therefore, the distribution of TRH among discrete nuclei of the hypothalamus, septum, and preoptic area was studied. Sixteen hypothalamic nuclei, the median eminence, the medial preoptic nucleus, and six septal nuclei could be dissected from the brain of each rat (*7*). These nuclei were removed from frozen brain sections 300 μm thick with small punches (*6*). The inner diameter of the punch was 300 or 500 μm and never exceeded the smallest cross-sectional diameter of the nucleus that was being removed. For each nucleus two to four pellets of tissue were removed from each brain. Nuclei from five rats were pooled and homogenized in 200 μl of 2.0*N* acetic acid in microhomogenizers (Micrometric Instrument Co.). The homogenates were extracted and assayed as described above.

The concentrations of TRH in isolated nuclei of rat brain are listed in Table 1. Since the volumes of the hypothalamic nuclei (except for the lateral anterior and lateral posterior nuclei), the medial preoptic nucleus, and the median eminence were known (*15*) the amount of TRH in these regions could be calculated. These amounts also appear in Table 1.

The median eminence contained 1.04 ng of TRH; this is about 25 percent of the TRH found in a 30-mg fragment of hypothalamus (*13, 14*). The concentration of TRH in the median eminence was four times greater than that in the medial part of the ventromedial nucleus, the nucleus with the highest TRH concentration. Of the TRH located within the hypothalamic nuclei, approximately 32 percent was found in the medial part of the ventromedial nucleus, 12 percent in the dorsomedial nucleus, 16 percent in the arcuate nucleus, 7 percent in the lateral part of the ventromedial nucleus, 9 percent in the periventricular nucleus, and 23 percent in the eleven remaining nuclei (*16*). The three subdivisions of the medial part of the ventromedial nucleus were separated and assayed for TRH. The amount of TRH per subdivision and the contribution of each subdivision to the total TRH in the medial part of the ventromedial nucleus were determined. The medial posterior subdivision contributed 51 percent to the total, the medial anterior subdivision 46 percent, and the ventromedial anterior nucleus 3 percent.

The medial preoptic nucleus, which comprises one-half of the preoptic area, contained most of the TRH in that area (Table 1). The dorsal and lateral septal nuclei had much higher concentrations of the peptide than did the other four septal nuclei (Table 1).

These results show that TRH is scattered among several hypothalamic nuclei and that it is present in extrahypothalamic sites. Figure 1 shows the distribution of TRH. It was found in highest concentrations adjacent to the third ventricle in the hypothalamus and adjacent to the lateral ventricles in the septum.

In all likelihood, the TRH found in the median eminence is present in axons and nerve endings which terminate on or near the portal vessels. It is not clear, however, whether the TRH measured elsewhere is present in cell bodies, axons, or nerve terminals. Cell bodies that produce TRH may reside solely in one, two, or three nuclei which send their axons to the median eminence and to other areas of the brain. Thus, the peptide might act as a releasing hormone at the anterior pituitary and as a transmitter in other regions. The development of an immunohistochemical technique for visualizing TRH in nervous tissue should help to resolve these questions.

A variety of techniques have been used to study the neural control of thyrotropin secretion. The earliest studies involved examination of the effect of brain lesions on the function of the thyroid (*17*). Greer (*18*) used this technique to delineate a "thyrotrophic area" of the brain. This area is found in the midline between the paraventricular nuclei and the median eminence. Elec-

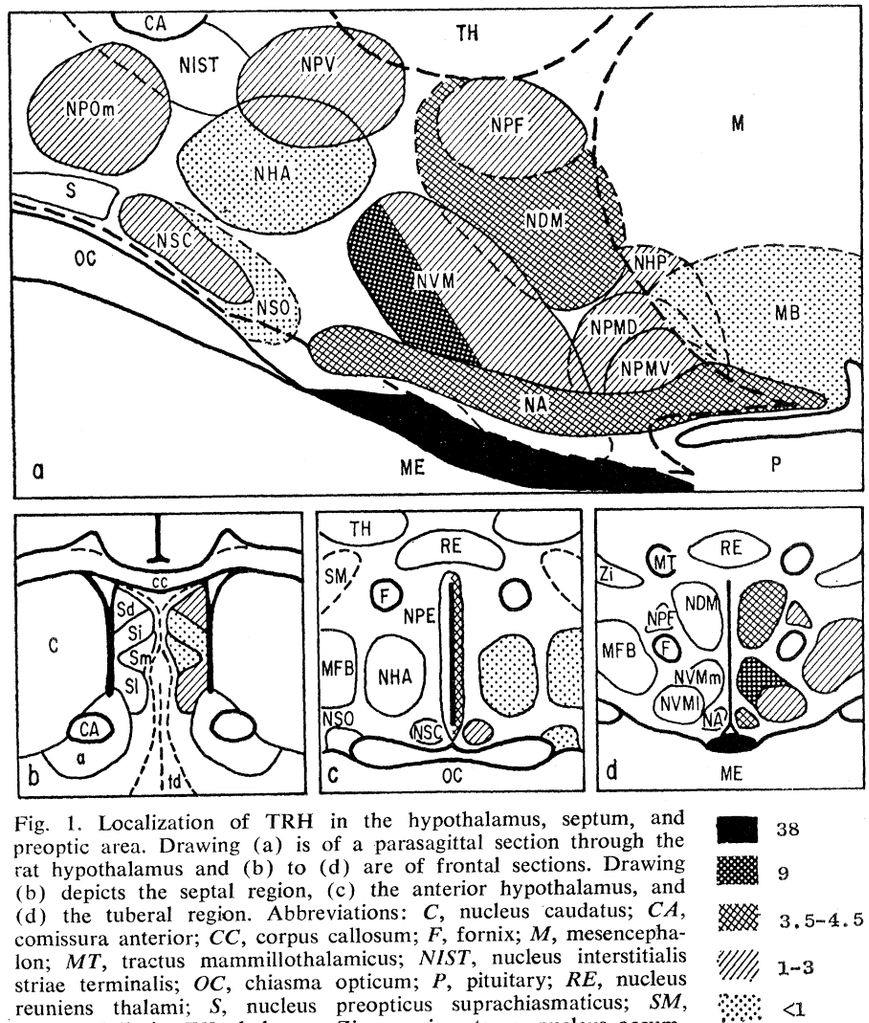

Fig. 1. Localization of TRH in the hypothalamus, septum, and preoptic area. Drawing (a) is of a parasagittal section through the rat hypothalamus and (b) to (d) are of frontal sections. Drawing (b) depicts the septal region, (c) the anterior hypothalamus, and (d) the tuberal region. Abbreviations: *C*, nucleus caudatus; *CA*, comissura anterior; *CC*, corpus callosum; *F*, fornix; *M*, mesencephalon; *MT*, tractus mammillothalamicus; *NIST*, nucleus interstitialis striae terminalis; *OC*, chiasma opticum; *P*, pituitary; *RE*, nucleus reuniens thalami; *S*, nucleus preopticus suprachiasmaticus; *SM*, stria medullaris; *TH*, thalamus; *Zi*, zona incerta; *a*, nucleus accumbens; and *td*, nucleus tractus diagonalis. The remainder of the abbreviations appear in Table 1. Key: nanograms of TRH per milligram of protein.

trical stimulation of this region of the hypothalamus produced an increase in plasma thyrotropin concentration (*19*), and pituitary implants in this area show thyrotrope differentiation and stimulate thyroid function (*20*). Measurements of TRH remaining in the hypothalamus after lesions were also compatible with its being localized in the thyrotrophic area (*21*). In our study TRH was found in highest concentrations within nuclei of the thyrotrophic area, but was found outside this area as well.

The distribution of TRH differs from that of the luteinizing hormone releasing hormone, which is found almost exclusively in the arcuate nucleus and median eminence (*22*). The fact that TRH is distributed among several nuclei suggests that more detailed anatomical, physiological, and pharmacological studies of these nuclei are needed to ascertain their individual roles in generating the neuroendocrine (*4, 23*) and behavioral (*24*) effects that have been attributed to TRH. In addition, it has recently proved possible to measure norepinephrine (*7*), dopamine (*7*), serotonin (*8*), histamine (*9*), and choline acetyltransferase (*10*) in isolated hypothalamic nuclei. It is hoped that the part played by one or more of the biogenic amines in the control of TRH release can be established.

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Lectins: A Possible Basis for Specificity in the Rhizobium–Legume Root Nodule Symbiosis

Abstract. *Soybean lectin labeled with fluorescein isothiocyanate combined specifically with all but 3 of 25 strains of the soybean-nodulating bacterium* Rhizobium japonicum. *The lectin did not bind to any of 23 other strains representative of rhizobia that do not nodulate soybeans. The evidence suggests that an interaction between legume lectins and* Rhizobium *cells may account for the specificity expressed between rhizobia and host plant in the initiation of the nitrogen-fixing symbiosis.*

Roots of many legume plants form symbiotic nitrogen-fixing relationships with soil bacteria of the genus *Rhizobium*. A considerable degree of specificity often is manifest between bacteria and legume host. Rhizobia that initiate the symbiotic root nodule structure in soybeans, for example, are incapable of forming root nodules with clover, alfalfa, garden beans, or others. Such specificity is the basis for cross-inoculation classification in legume production and for species differentiation in the genus *Rhizobium*. Management of the legume root–*Rhizobium* interaction provides the main means by which biological nitrogen fixation is harnessed for agricultural production (*1*).

A major unresolved problem related to *Rhizobium*-legume specificity is the mechanism whereby this specificity is expressed. Hamblin and Kent (*2*) explored the hypothesis that lectin may bind the bacteria to the roots. They worked with a single strain of *R. phaseoli* and found that lectin-treated bacteria were capable of agglutinating erythrocytes, but they did not address the question of specificity. Our investigations were based on a similar hypothesis and led to techniques whereby reactions between soybean lectin and soybean rhizobia (*R. japonicum*) could be observed by direct microscopy. We sought to examine the specificity of the lectin-*Rhizobium* interaction.

Lectins were prepared from ground and defatted seeds and roots of soybean (variety 'Chippewa') according to the procedures of Liener and Pallansch (*3*) leading to their fraction II. The seed lectin had a protein content of 12 mg/ml (*4*) and a hemagglutination titer of 6400 (*5*), as compared to 1.5 mg/ml and 128 for the soybean root lectin. The low titer of the soybean root preparation nonetheless reflected substantial lectin activity since controls gave no hemagglutination.

Soybean seed lectin was conjugated (*6*) with the fluorochrome fluorescein isothiocyanate for use as a stain in the microscopic examination of rhizobia. Bacteria were observed by fluorescence microscopy (*7*) after staining with the fluorochrome-labeled lectin to detect the