AUTORADIOGRAPHIC LOCALIZATION OF THYROTROPIN-RELEASING HORMONE RECEPTORS IN THE RAT CENTRAL NERVOUS SYSTEM[1]

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Abstract

We employed quantitative autoradiography to examine the distribution of thyrotropin-releasing hormone (TRH) receptors in the rat CNS. The binding of [³H]3-methyl-histidine-TRH ([³H]MeTRH) to TRH receptors in frozen rat brain sections was saturable, of a high affinity (K_d = 5 nM), and specific for TRH analogs. Autoradiograms of [³H]MeTRH binding showed highest concentrations of TRH receptors in the rhinencephalon, including accessory olfactory bulb, nuclei of the amygdala, and the ventral dentate gyrus and subiculum of the hippocampus. Moderate TRH receptor concentrations were found within the thalamus and hypothalamus, in most regions of the rhombencephalon, such as the cranial nerve nuclei, and in the substantia gelatinosa of the spinal cord. Neocortex and basal ganglia contained low densities of TRH receptors. This distribution correlates well with the sensitivity of brain regions to the known effects of TRH, and suggests that TRH receptors may mediate the actions of TRH in the rat CNS.

Thyrotropin-releasing hormone (TRH) was initially isolated and characterized on the basis of its hypophysiotropic actions (Boler et al., 1969; Burgus et al., 1969). Sequencing revealed its tripeptide structure as pyroglutamyl-histidyl-prolyl-amide. The development of a radioimmunoassay for TRH (Bassiri and Utiger, 1972) led to studies of its distribution throughout the central nervous system. In addition to being highly concentrated in the hypothalamus, TRH was demonstrated to be present in numerous sites throughout the brain (Brownstein et al., 1974; Jackson and Reichlin, 1974a; Oliver et al., 1974; Winokur and Utiger, 1974). To date, TRH has also been demonstrated within olfactory bulb (Kreider et al., 1981; Stanton et al., 1982; Dluzen and Ramirez, 1983), and spinal cord (Kardon et al., 1977; Leppaluoto et al., 1978). A wide variety of species have been shown to contain substantial amounts of TRH, including rat (Leppaluoto et al., 1978; Spindel and Wurtman, 1980), frog (Jackson and Reichlin, 1974b; Taurog et al., 1974), ground squirrel (Stanton et al., 1982), salamander (Taurog et al., 1974), chicken, snake, and salmon (Jackson and

Reichlin, 1974b), and human (Okon and Koch, 1976; Kubek et al., 1977; Parker and Porter, 1983).

More specific regional localization of TRH has been evaluated by means of the nuclei-punch technique of Palkovits (1973). TRH is distributed in rat hypothalamic and forebrain nuclei (Brownstein et al., 1974), brainstem nuclei (Kubek et al., 1983) and grey matter of the spinal cord (Kardon et al., 1977). Immunocytochemical studies (Hokfelt et al., 1975a, 1975b) revealed TRH-containing cell bodies within the hypothalamus and brainstem, and TRH-containing fibers within the septal region of the rat.

Burt and Snyder (1975) first demonstrated receptor binding of tritiated TRH to membrane homogenates of rat brain tissue. Gross dissection of rat (Burt and Snyder, 1975), monkey (Ogawa et al., 1981), and sheep (Burt and Taylor, 1980) brains to investigate the regional distribution of TRH receptors showed high receptor concentrations in membrane homogenates from the hypothalamus, amygdala, hippocampus, midbrain, and cerebral cortex. However, the results of these studies were complicated by binding of the ligand to filter blanks, and by the presence of a low affinity binding site in the homogenates which alone could account for up to two-thirds of the specific binding seen (Burt and Snyder, 1975). These technical difficulties have been overcome by using tritiated (3-Me-His²)-TRH (MeTRH), an analog known to have up to 10-fold higher potency than TRH in both effecting hormonal release (Vale et al., 1971; Sowers et al., 1976a, 1976b; Dannies and Markell, 1980) and binding to pituitary membrane receptors (Grant et al., 1973; Hinkle et al., 1974). Studies of MeTRH binding to membrane homogenates from brain regions obtained by gross

dissection from rat (Simasko and Horita, 1982), sheep (Taylor and Burt, 1981), chicken (Thompson et al., 1981), guinea pig, rabbit, cat, dog, cow, and pig (Taylor and Burt, 1982), mouse, and fish (Burt and Ajah, 1984) have all yielded consistent results. In addition, Sharif and Burt (1983) have demonstrated MeTRH binding throughout the rabbit spinal cord. However, binding assays of membrane homogenates obtained by crude dissection of brain regions cannot provide the same degree of resolution and discrete localization of receptors that can be obtained with quantitative autoradiography.

Several groups have demonstrated the feasibility of TRH receptor autoradiography in rat brain (Palacios, 1983; Rostene et al., 1983; Pilotte et al., 1984), guinea pig brain (Palacios, 1983), and rabbit spinal cord (Sharif and Burt, 1983). We report here the use of quantitative autoradiography to localize TRH receptors throughout the rat CNS, and describe in detail the kinetic, biochemical, and competitive binding characteristics of [³H]MeTRH to frozen slices of rat brain.

Materials and Methods

Animals. We assayed TRH receptor levels in brain and spinal cord tissue from male Sprague-Dawley rats (180–200 gm; Ace Animals, Inc., Boyertown, PA). The rats were housed under a 14:10-hr light-dark cycle, and maintained with Purina rat chow and water *ad libitum* until sacrifice.

Chemicals and TRH analogs. MeTRH was obtained from Bachem (Torrance, CA). TRH-OH (free acid) was purchased from Cambridge Research Biochemicals (Atlantic Beach, NY). Both Glu-His-Pro-NH₂ and pGlu-His-Pro-Gly-NH₂ were obtained from Peninsula Laboratories (Belmont, CA). [³H]MeTRH (specific activity, 76.7 Ci/mmol) was purchased from New England Nuclear, Boston, MA. TRH, cyclo(-His-Pro-), Pro-NH₂, Tris-HCl, MgCl₂·6H₂O, bovine serum albumin, and Bacitracin were all obtained from Sigma Chemical Co. (St. Louis, MO).

Tissue preparation. Animals were decapitated; their brains or spinal cords rapidly were removed, mounted on cryostat chucks, and frozen in dry ice. Coronal sections 32-μm thick were cut at −18°C and thaw-mounted onto chromate/gelatin-subbed slides. Sections were then stored with desiccant at −70°C. For characterization of [³H]MeTRH binding by scintillation counting, consecutive coronal sections were cut through the amygdala at Paxinos and Watson (1982) levels 17 to 26 and mounted two per slide. For calculation of Scatchard and inhibition data by quantitative densitometry, one section was mounted per slide. For regional localization by quantitative densitometry, four consecutive sections at 500-μm intervals were mounted, two per slide for total and nonspecific binding determinations.

Binding procedures. Slides were warmed to room temperature, and preincubated for 10 min in slide jars filled with 25°C buffer (50 mM Tris-HCl/5 mM MgCl₂/0.2% bovine serum albumin, pH 7.4). After preincubation, sections were immediately chilled to 4°C, and allowed to air dry. Once dry, slides were incubated with 300 μl of ice-cold buffer containing 20 μM Bacitracin and nanomolar concentrations of [³H] MeTRH. This volume was sufficient to cover thoroughly both sections on a slide. Concentrations of [³H]MeTRH used were 10 nM for regional localization and from 0.3 to 22.9 nM for Scatchard analysis. For inhibition and kinetic analyses, a 2 nM solution was used. Nine concentrations of TRH analogs, ranging over 5 orders of magnitude, were sometimes included in the incubation for inhibition analysis. Nonspecific binding was defined as the binding of [³H]MeTRH in the presence of 10 μM TRH. At 10 nM [³H]MeTRH, specific binding was 80 to 90% of total binding (1500 to 2000 cpm).

The sections were incubated at 4°C for 2 hr. After incubation, the slides were washed with ice-cold buffer four times for 30 sec each. Preliminary experiments established that fewer washes after incubation resulted in a greater proportion of nonspecific binding (40 to 60% of total binding). For scintillation counting, the sections were wiped off the slides with Whatman glass fiber filter discs, placed in vials with 5 ml of Liquiscint (National Diagnostics, Somerville, NJ), and counted at 35% efficiency. For autoradiography, the slides were dipped in ice-cold distilled water to remove buffer salts and then rapidly dried on a 60 to 70°C slide drier.

Kinetic analysis. To study the rate of [³H]MeTRH binding, sections were labeled for varying lengths of time up to 4 hr. The rate of dissociation was examined by incubating for 2 hr, and then plunging

slides into ice-cold buffer with 10 μM TRH (to prevent reassociation of dissociated radioligand) for time intervals up to 4 hr. After labeling or labeling and dissociation, sections were washed four times and wiped off for scintillation counting as described above.

Quantitative densitometry. Quantitative autoradiograms of [³H] MeTRH binding was performed as described previously (Rainbow et al., 1982). Slides were affixed to cardboard Kodak x-ray exposure holders, and LKB Ultrofilm (LKB, Inc., Gaithersburg, MD) was apposed to the slides. Film cassettes were shelved and weighted to ensure steady, uniform apposition of film and tissue. After 2 months of exposure at room temperature, the LKB film was developed in Kodak GBX developer for 3 min followed by 6 min in Kodak rapid fixer.

Densitometric analysis of [³H]MeTRH binding was carried out according to methods described previously (Unnerstall et al., 1982; Rainbow et al., 1982; Rainbow et al., 1984). Slide-mounted autoradiograms were magnified 10 times and the image was maneuvered over a photocell 100 μm in diameter. This photocell was connected to a microcomputer capable of converting density values into femtomoles/mg of protein using tritium film standards prepared from brain mash mixed with known quantities of [³H]leucine (Unnerstall et al., 1982; Rainbow et al., 1984). These standards are calibrated for the tritium content in gray matter structures (Rainbow et al., 1984). Therefore, we have analyzed only grey matter structures to avoid the problem of tritium quench by white matter (Alexander et al., 1981). Values for total and nonspecific binding of [³H]MeTRH were obtained for each region by averaging four to eight readings for each brain structure on an individual autoradiogram. Equal numbers of readings were taken bilaterally and averaged together.

Results

Scatchard analysis of [³H]MeTRH binding to frozen brain sections by liquid scintillation counting. Experiments showed specific [³H]MeTRH binding to sections through rat amygdala began to saturate at ligand concentrations greater than 10 nM. Linear Scatchard curves were generated with seven ligand concentrations ranging from 0.3 to 22.9 nM. We obtained a K_d of 5.80 ± 0.64 nM, which compares well with previous reports (Taylor and Burt, 1981; Simasko and Horita, 1982; Thompson et al., 1981; Burt and Ajah, 1984). The B_{max} was 43.92 ± 4.71 fmol/mg of protein. Our data also provide no evidence of cooperativity or multiple binding sites, with a Hill coefficient (1.16 ± 0.09) not significantly different from 1.0 ($t(3) = 1.778$; $p = NS$).

Kinetic analysis of [³H]MeTRH binding to frozen brain sections by liquid scintillation counting. Examination of the time course of [³H]MeTRH binding revealed a rate constant (k_{-1}) of 0.00576 ± 0.00027 min⁻¹ for the dissociation reaction. For the association reaction, k_{obs} was determined to be 0.00802 ± 0.00114 min⁻¹, and equilibrium was reached at approximately 90 min. The calculated K_d of 5.10 nM provided close agreement with the results of our Scatchard analysis of [³H]MeTRH binding to rat brain slices. The calculated K_d also closely agree with Simasko and Horita (1982) and Taylor and Burt (1981), who reported calculated K_d values of 1.6 and 5.4 nM, respectively.

Inhibition analysis of [³H]MeTRH binding to frozen brain sections by liquid scintillation counting. TRH analogs were added to the incubation fluid to determine their ability to inhibit the binding of [³H]MeTRH. The data are presented in Table I. MeTRH, TRH, and TRH-Gly-NH₂ all inhibited the binding of [³H]MeTRH by 50% with nanomolar potency. TRH-OH and Glu-His-Pro-NH₂ were only moderately effective, with potencies in the micromolar range. Pro-NH₂ and cyclo(-His-Pro-) were the least effective compounds at inhibiting the binding of [³H]MeTRH with millimolar IC₅₀ values. The rank order potencies of these compounds correlate closely with those seen in brain homogenates from sheep (Taylor and Burt, 1981; Spearman rank order correlation coefficient $r_s = 0.96$; $p = 0.02$).

Densitometric characterization of [³H]MeTRH binding to frozen brain sections. Frozen brain sections were incubated with varying concentrations of [³H]MeTRH, or incubated with 2 nM

TABLE I

Relative potency of TRH analogs in displacing [³H]MeTRH binding to rat brain sections

Several concentrations of TRH analogs were included in the incubation with 2 nM [³H]MeTRH. Binding was assayed by liquid scintillation counting as described in the text. Values are means and standard errors for three to seven experiments.

Compound	IC$_{50}$ (Mean ± SEM)
MeTRH	2.10 ± 1.30 nM
TRH	41.40 ± 16.00 nM
TRH—Gly—NH$_2$	143.90 ± 26.70 nM
TRH—OH	1.99 ± 0.54 μM
Glu—His—Pro—NH$_2$	3.03 ± 1.44 μM
cyclo(—His—Pro—)	1.11 ± 0.57 mM
Pro—NH$_2$	13.87 ± 4.83 mM

[³H]MeTRH in the presence of varying concentrations of TRH or MeTRH. Autoradiograms were thus generated and quantified by densitometry for Scatchard and inhibition analyses. The data for five representative limbic nuclei are presented in Table II. The K_d and IC$_{50}$ values for each nucleus corresponded well to the values obtained by liquid scintillation counting of sections labeled with [³H]MeTRH (Table I). Each B_{max} was 5 to 10 times greater than the B_{max} determined by Scatchard analysis of [³H]MeTRH binding to brain slices. This reflected dilution of TRH receptors in Scatchard analysis of pooled brain regions seen with brain slices containing heterogeneous TRH receptor concentrations. The rank orders of TRH receptor concentrations in these nuclei determined by densitometric Scatchard analysis and densitometric mapping studies were identical (see Table III).

Densitometric quantification of regional binding. We observed marked heterogeneity in the pattern of [³H]MeTRH binding to TRH receptors (Fig. 1; Tables III and IV). Receptors were discretely localized throughout the CNS, with highest concentrations present in rhinencephalic structures. Areas of the olfactory apparatus, the entire amygdala and adjacent regions, and discrete sites within the hippocampus and septum contained moderately high (100 to 200 fmol/mg of protein) to very high (200 to 400 fmol/mg of protein) concentrations of TRH receptors. Moderate densities (50 to 100 fmol/mg of protein) of TRH receptors were observed in the brainstem, spinal cord, hypothalamus, and thalamus. Finally, the basal ganglia, neocortex, and cerebellum possessed low levels (less than 50 fmol/mg of protein) of TRH receptors. Throughout the CNS, nonspecific binding did not exceed film background measured by densitometry, and was visually equivalent to film background. Although regions may appear homogeneously gray (e.g., cerebellum in Fig. 1*J*), they nonetheless contain specific TRH receptors, albeit in low concentrations.

Within the rhinencephalon, the accessory olfactory bulb contained the highest concentration of TRH receptors (363.1

± 18.6 fmol/mg of protein) seen on the neuraxis. The granular layer of the accessory olfactory bulb also possessed a very high density of TRH receptors, exceeding 200 fmol/mg of protein.

Caudally within the rhinencephalon, all the nuclei of the amygdala contained high concentrations of TRH receptors. Labeling by [³H]MeTRH in the posterior cortical, lateral, medial, and basolateral nuclei was very high, whereas moderately high levels of TRH receptors were contained in the central and anterior cortical nuclei. The adjacent endopiriform nucleus contained a moderately high density of TRH receptors (110.7 ± 10.5 fmol/mg of protein), and the amygdalohippocampal area was very heavily labeled with TRH receptors (250.4 ± 74.6 fmol/mg of protein).

In the hippocampus, very high densities of TRH receptors were concentrated in the ventral regions of the subiculum and the dentate gyrus. However, only low concentrations of TRH receptors, never exceeding 50 fmol/mg of protein, were seen in the dorsal subfields of the hippocampus. This clear dorsal/ventral variation in the hippocampus was manifested by a 10-fold greater concentration of TRH receptors in the ventral dentate gyrus than in the dorsal dentate gyrus.

Rhinencephalic cortical structures, overlying the amygdala and hippocampus, possessed moderately high levels of TRH receptors. The concentration within entorhinal cortex exceeded 100 fmol/mg of protein. In the cortex delineating the rhinal fissure, corresponding to area no. 13 in the scheme of Krieg (1946), the TRH receptor concentration was over 180 fmol/mg of protein. This large region of cortex was concentrated in TRH receptors, extending from levels 13 to 30 in the atlas of Paxinos and Watson (1982). However, the discrete pattern of [³H]MeTRH binding was very consistent, the points of origin and termination of the labeling pattern each varying no more than 500-μm between rats. In addition, no significant anteroposterior variation in TRH receptor concentrations through the rhinal cortex was noted.

A discrete pattern of labeling was noted within the septal area of the rhinencephalon. The bed nucleus of the stria terminalis, the septohypothalamic nucleus, and the islands of Calleja Major all contained high concentrations of TRH receptors, as did both the vertical and the horizontal limbs of the diagonal band of Broca. The taenia tecta, medial septum, septohippocampal nucleus, and columns of the fornix each possessed more moderate densities of TRH receptors. Low levels of TRH receptors were found in the lateral septum and the olfactory tubercle.

In contrast to the rhinencephalon, the concentration of TRH receptors in the mesencephalon and the rhombencephalon were moderate, generally ranging from 50 to 100 fmol/mg of protein. Within the mesencephalon, the interpeduncular nucleus and the inferior colliculus contained concentrations of TRH receptors approaching 70 fmol/mg of protein. Exceptions to the general pattern of moderate TRH receptor concentrations within the mesencephalon included the superior colliculus, with

TABLE II

Densitometric characterization of [³H]MeTRH binding in representative limbic nuclei of the rat

For Scatchard analysis, several concentrations of [³H]MeTRH were included in the incubation. For inhibition analysis, several concentrations of TRH or MeTRH were included in the incubation with 2 nM [³H]MeTRH. Sections were then dried and apposed to LKB Ultrofilm for 2 months to generate autoradiograms, and binding in specific nuclei was quantified by densitometry as described in the text. Nonspecific binding was defined as binding in the presence of 10 μM TRH. Values are means and standard errors of three or four experiments.

Structure	K_d	B_{max}	TRH IC$_{50}$	MeTRH IC$_{50}$
	nM	*fmol/mg protein*	*nM*	*n$_m$*
Medial nucleus, amygdala	3.49 ± 0.68	493.9 ± 80.8	19.8 ± 6.7	1.0 ± 0.5
Basolateral nucleus, amygdala	2.78 ± 0.38	373.1 ± 79.8	25.7 ± 11.5	1.3 ± 0.5
Central nucleus, amygdala	5.17 ± 2.18	333.4 ± 67.6	40.7 ± 25.0	1.7 ± 0.7
Endopiriform nucleus	2.68 ± 0.40	316.0 ± 59.2	10.7 ± 3.4	0.8 ± 0.1
Anterior cortical nucleus, amygdala	2.84 ± 0.58	250.4 ± 40.5	24.9 ± 16.3	0.5 ± 0.2

TABLE III

Concentration of TRH receptors in rat brain determined by quantitative autoradiography

Frozen 32-μm thick brain sections were labeled as described in the text with 10 nM [³H]MeTRH, and apposed against LKB Ultrofilm for 2 months to generate autoradiograms. Brain sections were taken at 500-μm intervals corresponding to coronal levels of the atlas of Paxinos and Watson (1982). Serially cut sections at each level were used for determination of total and nonspecific binding. Four to eight readings were taken for each brain structure on an individual autoradiogram. Densitometric readings were converted into molar levels of [³H]MeTRH binding by reference to a tritium brain-mash standard curve (Unnerstall et al., 1982; Rainbow et al., 1984). Nonspecific binding, defined as binding in the presence of 10 μM TRH, did not exceed film background. All layers of the cerebral cortex were labeled homogeneously, as were all the nuclei and lamina of the cerebellum. Values are means and standard errors of femtomoles of [³H]MeTRH specifically bound per mg of protein, from readings taken from four rats.

Structure	Mean ± SEM
Olfactory apparatus	
Olfactory bulb	22.2 ± 7.3
Granular layer, accessory olfactory bulb	262.5 ± 47.5
Accessory bulb	363.1 ± 18.6
Basal ganglia	
Accumbens nucleus	21.4 ± 8.4
Caudate putamen	17.7 ± 10.2
Limbic forebrain	
Taenia tecta	98.7 ± 17.5
Islands of Calleja Major	131.0 ± 21.8
Olfactory tubercle	3.9 ± 1.5
Medial septum	70.2 ± 5.7
Lateral septum, intermediate	38.4 ± 18.0
Lateral septum, dorsal	14.3 ± 4.6
Lateral septum, ventral	35.5 ± 18.1
Diagonal band of Broca, vertical limb	134.2 ± 13.2
Diagonal band of Broca, horizontal limb	107.1 ± 16.5
Bed nucleus of the stria terminalis	161.7 ± 15.9
Septohippocampal nucleus	57.4 ± 9.0
Septohypothalamic nucleus	108.4 ± 26.3
Ventral palladium	28.3 ± 8.9
Fornix	56.9 ± 19.5
Cortex	
Rhinal cortex	182.7 ± 10.6
Frontal cortex	26.4 ± 3.7
Primary olfactory cortex	34.0 ± 7.4
Anterior cingulate cortex	45.3 ± 9.8
Posterior cingulate cortex	63.7 ± 7.4
Entorhinal cortex	126.8 ± 36.2
Amygdala	
Central nucleus	137.7 ± 18.6
Medial nucleus	215.3 ± 53.9
Lateral nucleus	231.0 ± 61.6
Basolateral nucleus	207.4 ± 36.1
Anterior cortical nucleus	129.3 ± 24.5
Posterior cortical nucleus	243.8 ± 22.8
Endopiriform nucleus	110.7 ± 10.5
Amygdalohippocampal area	250.4 ± 74.6
Thalamus	
Paraventricular nucleus	135.1 ± 10.4
Centromedial nucleus	43.5 ± 7.4
Gelatinosus nucleus	36.0 ± 6.9
Intermediodorsal nucleus	51.9 ± 8.7
Reuniens	77.0 ± 13.0
Lateral posterior nucleus	32.3 ± 8.3
Habenular nuclei	33.4 ± 8.8

TABLE III—*Continued*

Structure	Mean ± SEM
Hypothalamus	
Lateral preoptic area	79.2 ± 19.2
Medial preoptic area	91.6 ± 8.3
Suprachiasmatic nucleus	126.9 ± 8.8
Lateral hypothalamic area	48.0 ± 6.6
Anterior hypothalamic area	71.6 ± 16.0
Ventromedial nucleus	78.4 ± 17.1
Paraventricular nucleus	90.9 ± 6.5
Periventricular nucleus	93.8 ± 22.7
Arcuate nucleus	68.9 ± 9.9
Median eminence	36.0 ± 9.1
Dorsomedial nucleus	95.2 ± 14.8
Posterior nucleus	106.2 ± 16.4
Zona incerta	67.0 ± 12.5
Mammillary nuclei	96.2 ± 22.2
Supramammillary nuclei	94.6 ± 14.4
Hippocampus	
Dorsal	
CA1	25.7 ± 4.3
CA2	31.2 ± 7.5
CA3	25.0 ± 8.0
Dentate gyrus	23.7 ± 8.1
Ventral	
CA3	97.0 ± 32.9
CA4	28.5 ± 3.6
Dentate gyrus	272.4 ± 57.8
Subiculum	314.9 ± 35.8
Midbrain	
Interpeduncular nucleus	65.7 ± 31.0
Substantia nigra	30.7 ± 8.4
Central gray	75.6 ± 13.5
Superior colliculus	125.8 ± 21.9
Inferior colliculus	68.6 ± 17.0
Cranial nerve nuclei	
Motor nucleus (5)	55.6 ± 7.9
Principal sensory nucleus (5)	56.8 ± 12.3
Spinal nucleus (5)	30.0 ± 6.9
Facial nucleus (7)	77.3 ± 9.6
Medial vestibular nucleus (8)	90.0 ± 22.9
Lateral vestibular nucleus (8)	40.7 ± 3.5
Superior vestibular nucleus (8)	34.0 ± 5.5
Solitary nucleus and tract (10)	108.0 ± 4.3
Dorsal motor nucleus (10)	150.6 ± 15.7
Hypoglossal nucleus (12)	140.6 ± 14.5
Brainstem	
Paragigantocellular nucleus	64.4 ± 16.3
Parvocellular reticular nucleus	51.6 ± 5.1
Pontine caudal reticular nucleus	50.2 ± 16.5
Lateral reticular nucleus	62.9 ± 9.7
Medullary reticular nucleus	39.8 ± 11.4
Superior olive	46.4 ± 16.1
Inferior olive	46.7 ± 22.7
Dorsal medial raphe	64.6 ± 6.3
Locus ceruleus	74.1 ± 12.7
Cerebellum	14.5 ± 4.1

a moderately high density of TRH receptors, and the interpeduncular nucleus, with a low density of TRH receptors. The central gray, moderately dense with TRH receptors (75.6 ± 13.5 fmol/mg of protein), possessed homogeneous labeling throughout the mesencephalon and extending into the pontine region of the rhombencephalon.

Moderate concentrations of TRH receptors were observed throughout the rhombencephalon. Several nuclei of the reticular formation, the dorsal medial raphe, and the locus ceruleus all contained TRH receptors in concentrations of 50 to 75 fmol/
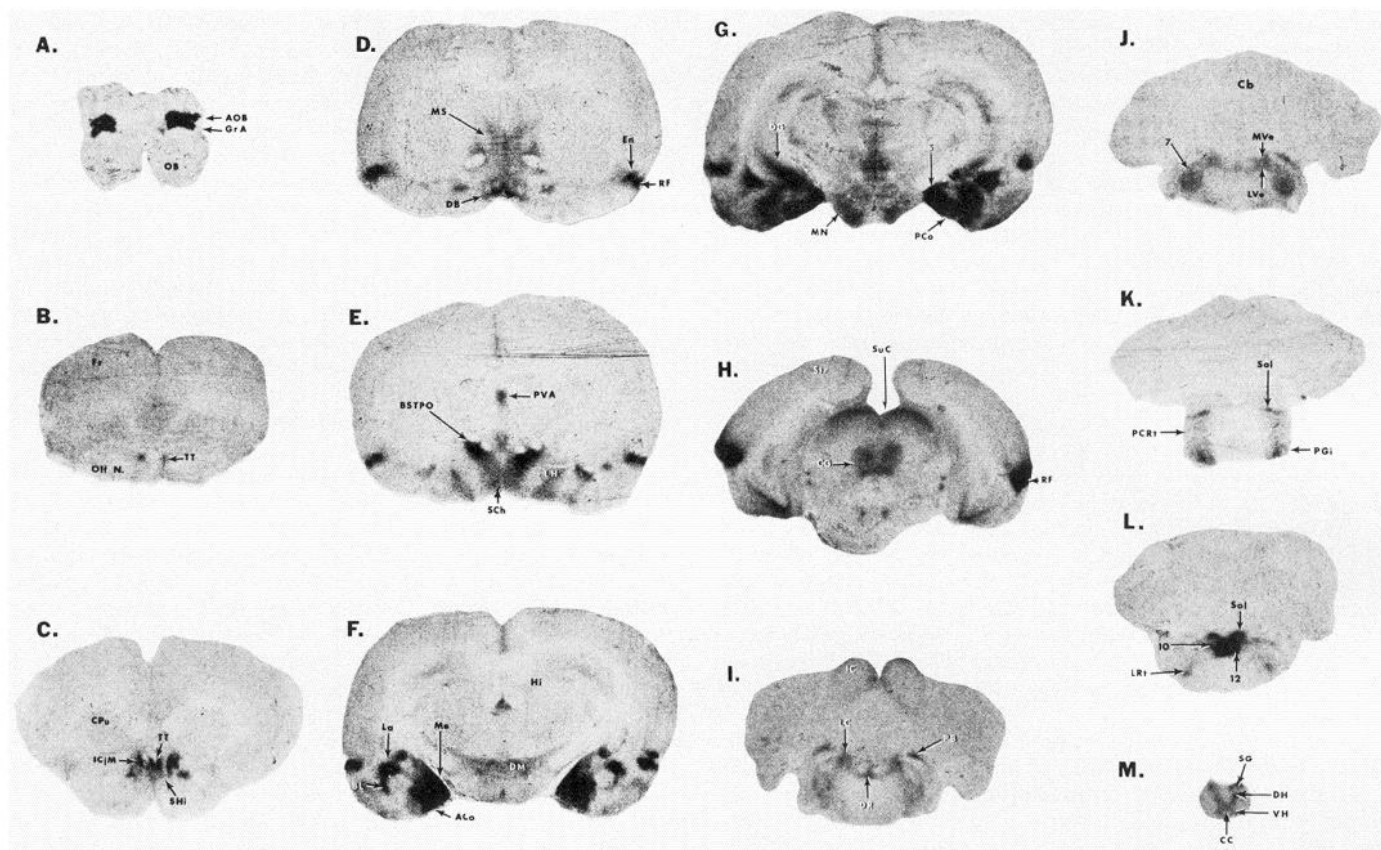
Figure 1. Distribution of TRH receptors in rat brain and spinal cord determined by quantitative autoradiography. Frozen 32-μm thick sections of rat brain and spinal cord were labeled as described in the text with 10 nM [³H]MeTRH, and apposed to LKB Ultrofilm for 2 months to generate autoradiograms. The visual contribution of nonspecific binding was equivalent to the binding over white matter. Each of the frames *A* to *M* represents a coronal level from the atlas of Paxinos and Watson (1982). *A*, level 4: *AOB*, accessory olfactory bulb; *GrA*, granular layer of the accessory olfactory bulb; *OB*, olfactory bulb. *B*, level 7: *Fr*, frontal cortex; *TT*, taenia tecta; *Olf N.*, anterior olfactory nuclei. *C*, level 11: *CPu*, caudate putamen; *TT*, taenia tecta; *ICjM*, islands of Calleja Major; *SHi*, septohippocampal nucleus. *D*, level 14: *MS*, medial septum; *DB*, diagonal band of Broca; *En*, endopiriform nucleus; *RF*, rhinal cortex. *E*, level 17: *PVA*, paraventricular thalamic nucleus; *BSTPO*, preoptic bed nucleus of the stria terminalis; *LH*, lateral hypothalamus; *SCh*, suprachiasmatic nucleus. *F*, level 20: *Hi*, dorsal hippocampus; *DM*, dorsomedial hypothalamus; *Me*, medial nucleus, amygdala; *ACo*, anterior cortical nucleus, amygdala; *La*, lateral nucleus, amygdala; *BL*, basolateral nucleus, amygdala. *G*, level 24: *DG*, ventral dentate gyrus; *S*, subiculum; *MN*, mammillary nuclei; *PCo*, posterior cortical nucleus. *H*, level 29: *SuC*, superior colliculus; *Str*, striate cortex; *CG*, central gray; *RF*, rhinal cortex. *I*, level 33: *IC*, inferior colliculus; *LC*, locus ceruleus; *DR*, dorsal raphe; *PB*, parabrachial nucleus. *J*, level 35: *Cb*, cerebellum; *7*, facial nucleus; *MVe*, medial vestibular nucleus; *LVe*, lateral vestibular nucleus. *K*, level 38: *Sol*, solitary nucleus and tract; *PCRt*, parvocellular reticular nucleus; *PGi*, paragigantocellular reticular nucleus. *L*, level 41: *Sol*, solitary nucleus and tract; *12*, hypoglossal nucleus; *10*, dorsal motor nucleus; *LRt*, lateral reticular nucleus. *M*, level 68: *SG*, substantia gelatinosa; *DH*, dorsal horn; *VH*, ventral horn; *CC*, central canal.

TABLE IV

Concentration of TRH receptors in rat spinal cord

Frozen 32-μm thick spinal cord sections were labeled as described in the text with 10 nM [³H]MeTRH, and apposed against LKB Ultrofilm for 2 months to generate autoradiograms. Spinal cord sections were taken from cervical, thoracic, lumbar, and sacral levels. Serially cut sections at each level were used for determination of total and nonspecific binding. Four to eight readings were taken for each spinal cord structure on an individual autoradiogram. Values are means and standard errors of femtomoles of [³H]MeTRH specifically bound per mg of protein, from readings taken from four to seven rats.

Level	Substantia Gelatinosa	Dorsal Gray	Ventral Gray	Central Canal	Intermediolateral Column
Cervical	106.2 ± 9.0	55.5 ± 6.1	71.9 ± 3.9	84.6 ± 10.1	
Thoracic	92.5 ± 18.6	48.8 ± 9.8	63.1 ± 9.6	82.8 ± 13.1	68.5 ± 13.5
Lumbar	109.3 ± 4.7	62.4 ± 3.6	56.5 ± 3.9	88.9 ± 6.4	
Sacral	107.9 ± 20.0	63.0 ± 11.3	61.2 ± 10.1	86.1 ± 7.7	

mg of protein. The cranial nerve nuclei, however, displayed a highly discrete distribution, with TRH receptor concentrations appearing denser in more dorsal nuclei. The motor and principal sensory nuclei of the trigeminal nerve contained about 55 fmol/mg of protein of TRH receptors, while the spinal trigeminal nucleus contained little more than half that amount (30.0 ± 6.9 fmol/mg of protein). The lateral and superior vestibular nuclei possessed less than half the density of TRH receptors

seen in the medial vestibular nucleus, which possessed 90 fmol/ mg of protein of TRH receptors. Finally, the dorsal motor nucleus of the vagus and the hypoglossal nucleus, the most dorsal of the cranial nerve nuclei, as well as the solitary tract and nucleus, all contained over 100 fmol/mg of protein of TRH receptors.

In contrast to the cranial nerve nuclei, no rostrocaudal variation was seen in the moderate levels of TRH receptors present

throughout the length of the spinal cord (Fig. 1; Table 4). The TRH receptor concentration in the substantia gelatinosa (98.0 ± 9.5 fmol/mg of protein) was the highest of any spinal cord region. The intermediolateral column of the thoracic cord contained a moderate density of TRH receptors. The dorsal and ventral horns and the central canal possessed moderate densities of TRH receptors ranging from 50 to 80 fmol/mg of protein.

The diencephalon was also moderately dense in TRH receptors. Within the hypothalamus, TRH receptor concentrations ranged from 50 to 100 fmol/mg of protein in many nuclei, including the periventricular, paraventricular, arcuate, ventromedial, and dorsomedial nuclei. Regions such as the lateral and medial preoptic areas and the lateral hypothalamus also contained moderate densities of TRH receptors. However, the posterior and the suprachiasmatic nuclei contained high concentrations of TRH receptors, exceeding 100 fmol/mg of protein each. The median eminence, containing only 36 fmol/mg of protein, was also an exception to the moderate levels of TRH receptors seen in the hypothalamus.

The thalamic diencephalon contained moderate to low levels of TRH receptors. Within the thalamus, we observed low levels of TRH receptors (habenular, centromedial, gelatinosus, and lateral posterior nuclei) and moderate levels of TRH receptors (intermediodorsal and reuniens nuclei). The single thalamic structure containing a high concentration of TRH receptors was the paraventricular nucleus (135.1 ± 10.4 fmol/mg of protein).

In more recently evolved regions of brain, including neocortex and basal ganglia, we observed low levels of TRH receptors. This labeling, though of low magnitude, clearly exceeded background binding levels. The accumbens nucleus, caudate putamen, and ventral palladium each possessed less than 30 fmol/mg of protein. Neocortical regions, such as striate and cingulate cortex, also possessed low levels of TRH receptors, with all cortical layers homogeneously labeled.

[^{3}H]MeTRH labeled the cerebellum homogeneously and in low levels (14.5 ± 4.1 fmol/mg of protein). No differences in regions of cerebellar cortex or nuclei were detectable.

Discussion

We have shown, by quantitative autoradiography, the distribution of TRH receptors throughout the rat CNS (Fig. 1; Tables 3 and 4). Highest concentrations of TRH receptors were localized to the rhinencephalon, including the accessory olfactory bulb, ventral hippocampus, and amygdala. Moderate TRH receptor densities were found in the diencephalon, rhombencephalon, and spinal cord. Recently evolved regions of brain, such as neocortex and basal ganglia, contained low levels of TRH receptors.

Our results agreed with the localization of TRH receptors described by membrane homogenate techniques of other groups (Burt and Snyder, 1975; Simasko and Horita, 1982; Taylor and Burt, 1982). All groups showed high labeling in the amygdala, hypothalamus, and rhinal cortex, with more moderate labeling in diencephalic, mesencephalic, and rhombencephalic structures. All found little receptor labeling in the neocortex, basal ganglia, and cerebellum. Few important differences were observed between studies, and the differences noted were probably due to variations in technique. For example, Taylor and Burt (1982) reported relatively high concentrations in the nucleus accumbens, which we found to be extremely low in TRH receptors. However, the gross dissection technique used to obtain nucleus accumbens tissue may have included regions of the taenia tecta, islands of Calleja Major, bed nucleus of the stria terminalis, diagonal band of Broca, or medial septum which contained significant concentrations of TRH receptors.

The distribution of TRH receptors in brain regions observed in the present study differed substantially from the pattern of localization of TRH seen in previous reports (Brownstein et al., 1974; Eskay et al., 1983; Kubek et al., 1983). For example, very high levels of TRH receptors are found in the nuclei of the amygdala and ventral hippocampus, regions reported to contain low concentrations of endogenous TRH. Conversely, high TRH concentrations have been observed in median eminence and olfactory bulb, yet these areas contain low levels of TRH receptors. However, only limited information is available on the quantitative distribution of TRH in nuclei and subregions of rat brain. Moreover, a direct comparison of the regional distributions of TRH and TRH receptors should optimally be carried out in the same study. If such a comparison substantiated the lack of correspondence between TRH and its receptors, it would represent another example of the inconsistent relationship previously reported for the distributions of many other neurotransmitters and neuromodulators and their respective receptors (Kuhar, 1981).

While there is a lack of correspondence between the distribution of TRH and its receptors, a good correspondence exists between the distribution of TRH receptors and the effects of exogenously applied TRH. Microiontophoretically applied TRH lowers neuronal firing rates in the preoptic area, the septum, and the hypothalamus, but not in the cerebral cortex (Dyer and Dyball, 1974; Renaud and Martin, 1975; Renaud et al., 1975; Winokur and Beckman, 1978). The medial and lateral preoptic areas, numerous structures within the septal area, and all the hypothalamic nuclei contain greater TRH receptor concentrations than the frontal cortex. In microinjection studies, regions which show the greatest response to TRH are areas containing moderate to high densities of TRH receptors. Wei et al. (1975) elicited shaking behaviors after microinjecting TRH into the central gray, medial thalamus, medial preoptic area, and the medial hypothalamus. Boschi and Rips (1981) elicited a hyperthermia after microinjection of TRH into the diagonal band of Broca, the medial and lateral preoptic areas, and the anterior and lateral hypothalamus. Kalivas and Horita (1979) reversed pentobarbital narcosis with microinjection of TRH into the septum, medial preoptic area, medial thalamus, locus ceruleus, and mesencephalic periventricular gray. Additionally, Kalivas and Horita (1980) demonstrated the diagonal band of Broca and the medial septal nucleus to be more sensitive to the effect of TRH in reversing pentobarbital narcosis than the lateral septal nuclei. These regions of physiologic sensitivity to TRH administration correspond closely to the relative distribution of TRH receptors in the septum.

Other studies indicate possible effects of exogenously applied TRH in regions where we find low levels of TRH receptors. Miyamota and Nagawa (1977) and Heal and Green (1979) microinjected TRH into the nucleus accumbens of rats to elicit locomotion, along with several other associated behaviors. We, unlike Taylor and Burt (1982), failed to see significant levels of TRH receptors in the nucleus accumbens. Rather, the effect may be secondary to local spread of TRH to immediately adjacent regions, such as the diagonal band of Broca and the islands of Calleja Major, that were highly concentrated with TRH receptors. Stanton et al. (1980) microinjected TRH into the dorsal hippocampus of the ground squirrel, producing arousal from hibernation, and elevation of body temperature and metabolic rate. However, greater densities of TRH receptors were seen ventrally within the hippocampus. It is possible that regional differences in TRH receptor density in the hippocampus existed as a function of species differences.

While iontophoretic and microinjection studies with TRH suggest that some TRH receptors are postsynaptic on neurons, it is conceivable that some TRH receptors are presynaptic. For example, the distribution of TRH receptors in the rat CNS resembled the distribution of norepinephrine terminals. The accessory olfactory bulb (Fallon and Moore, 1978), the bed

nucleus of the stria terminalis and diagonal band of Broca (Lindvall and Stenevi, 1978; Moore, 1978), the paraventricular thalamic nucleus (Lindvall et al., 1974), and the amygdala (Fallon et al., 1978) all contain high levels of norepinephrine terminals. These regions also contain high to very high levels of TRH receptors (Table 3). The locus ceruleus contains the cell bodies whose noradrenergic fibers project throughout the CNS (Carlsson et al., 1962; Fuxe, 1965; Swanson and Hartman, 1975). The locus ceruleus also possessed a moderate density of TRH receptors. Other interactions between TRH and norepinephrine have been demonstrated in TRH microiontophoresis studies (Winokur and Beckman, 1978), norepinephrine turnover studies (Horst and Spirt, 1974; Keller et al., 1974), and 6-hydroxydopamine lesion studies (Winokur et al., 1978). These data suggest that some TRH receptors are presynaptic on norepinephrine terminals.

Just as the cellular localization of TRH receptors remains unknown, so does the biologic function of these receptors. However, on the basis of our results it is now possible to propose functions for TRH receptors in specific brain regions. For example, the suprachiasmatic nucleus contains a moderately high concentration of TRH receptors (126.9 ± 8.8 fmol/mg of protein). This nucleus also plays a role in the cyclic levels of several neurotransmitter receptors (Kafka et al., 1983). TRH levels within the rat amygdala (Collu et al., 1977), mediobasal hypothalamus (Mannisto et al., 1978; Kerdelhue et al., 1981), preoptic area (Kerdelhue et al., 1981), and retina (Schaeffer et al., 1977) have been shown to undergo circadian variation. Additionally, TRH levels in the preoptic area and olfactory bulb of the ground squirrel demonstrated seasonal variation (Stanton et al., 1982). These data may suggest a role for the suprachiasmatic nucleus in regulating the cyclicity of TRH concentrations and possibly TRH receptor concentrations in the brain.

While we can propose functions for some of the receptors for TRH localized by quantitative autoradiography, the significance of these receptors in most brain regions remains unknown. TRH occurs in extrahypothalamic neural tissues from a wide variety of lower vertebrates (Jackson and Reichlin, 1974b; Taurog et al., 1974; Stanton et al., 1982). The observation that TRH receptors are primarily localized in phylogenetically ancient brain regions may suggest that TRH and TRH receptors function in the regulation of basic biologic processes within the central nervous system.

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