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THYROTROPIN-RELEASING HORMONE (TRH) RECEPTORS

Localization by Light Microscopic Autoradiography in Rat Brain Using [^{3}H][3-Me-His^2]TRH as the Radioligand[1]

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Abstract

Thyrotropin releasing hormone (TRH) is a putative neurotransmitter in both the central and peripheral nervous system. In the present report, we have used autoradiography coupled with densitometric analysis of tritium-sensitive film to investigate the distribution of [^{3}H][3-Me-His^2]TRH ([^{3}H]MeTRH)-binding sizes in the rat brain. Previous pharmacological reports have established that many of these [^{3}H]MeTRH-binding sites have a structure-activity profile consistent with being a physiological TRH receptor. A high level of TRH receptors were observed in the accessory olfactory bulb, lateral nucleus of the amygdala, dentate gyrus, and entorhinal cortex. Moderate levels of TRH receptors were observed in the rhinal cortex, hypothalamus, superior colliculus, several brainstem motor nuclei, and lamina I of the spinal trigeminal nucleus pars candalis, while low concentrations of receptors are present in the cerebral cortex, striatum and ventral horn of the spinal cord. Very low levels of receptors were observed in the globus pallidus and in most nuclei of the dorsal thalamus. Comparisons of the distribution of TRH receptors to TRH-immunoreactive content indicates that, while in some areas of the brain there is a rough correlation between levels of TRH peptide and its receptor, in most brain areas there is little obvious correlation between the two. While such a discrepancy has been observed for other peptides and their receptors, the extensive distribution of TRH receptors in the central nervous system does provide an explanation for the variety of behavioral effects observed when TRH is infused into the central nervous system.

The tripeptide thyrotropin-releasing hormone (TRH, pGlu-His-Pro-NH_2) was originally isolated from the hypothalamus of pigs and sheep and shown to act upon the anterior pituitary to evoke release of thyrotropin-stimulating hormone (Guillemin, 1978; Schally, 1978). Since this original isolation, it has become apparent that thyrotropin release is only one of the many actions of TRH. Using radioimmunoassay (Brownstein et al., 1974; Jackson and Reichlin, 1974; Winokur and Utiger, 1974; Kardon et al., 1977) and immunohistochemistry (Hokfelt et al., 1975a, 1975b), TRH (Jackson, 1980) has been shown to be present in both the CNS and in a variety of peripheral tissues including the pancreas and digestive system (Morley, 1979). Interestingly, the distribution of TRH receptors has been shown to be even more ubiquitous than the peptide TRH. Using homogenate tissue binding, it was shown that TRH receptors are also present in the forebrain, brainstem (Burt and Snyder, 1975; Ogawa et al. 1981; Taylor and Burt, 1982; Simasko and Horita, 1982), retina (Burt, 1979), spinal cord (Sharif and Burt, 1983; Sharif et al., 1983), and some peripheral tissues (Burt and Snyder, 1975; Taylor and Burt, 1982). That these TRH receptors are functional is suggested by electrophysiological studies which have demonstrated that neurons responsive to TRH are found in cerebral cortex (Renaud and Martin, 1975; Braitman et al., 1980), septum (Winokur and Beckman, 1978), hypothalamus (Renaud and Martin, 1975; Winokur and Beckman, 1978), cerebellum (Renaud and Martin, 1975), and the spinal cord (Nicoll, 1977). Behavioral data also supports the hypothesis that TRH interacts with its receptors outside the hypothalamo-pituitary axis. Thus, TRH infusion in the CNS has been shown to produce a pressor response (Beale et al., 1977), tachycardia (Hine et al., 1973), hyperthermia (Metcalf, 1974), satiety (Vijayan and McCann, 1979; Morley and Levine, 1980), locomotor activity (Vogel et al., 1979), arousal (Stanton et al., 1980), and to antagonize the effects of barbiturates and alcohol in the CNS (Kalivas and Horita, 1980). Other more controversial reports have suggested that TRH administration may be of therapeutic benefit for patients afflicted with depression (Kastin et al., 1972; Prange et al., 1972; however, see Coppen et al., 1974, and Montjoy et al., 1974), schizophrenia (Inanaga et al., 1978), amyotrophic lateral sclerosis (Engel et al., 1983), or spinal trauma (Faden et al., 1981).

In the present study, we have examined the distribution of TRH receptors in the rat CNS using a modification of the Young and Kuhar (1979) autoradiographic binding technique. Previous *in vitro* binding studies using rat brain homogenates have established that the ligand we have used [^{3}H][^{3}-methyl-

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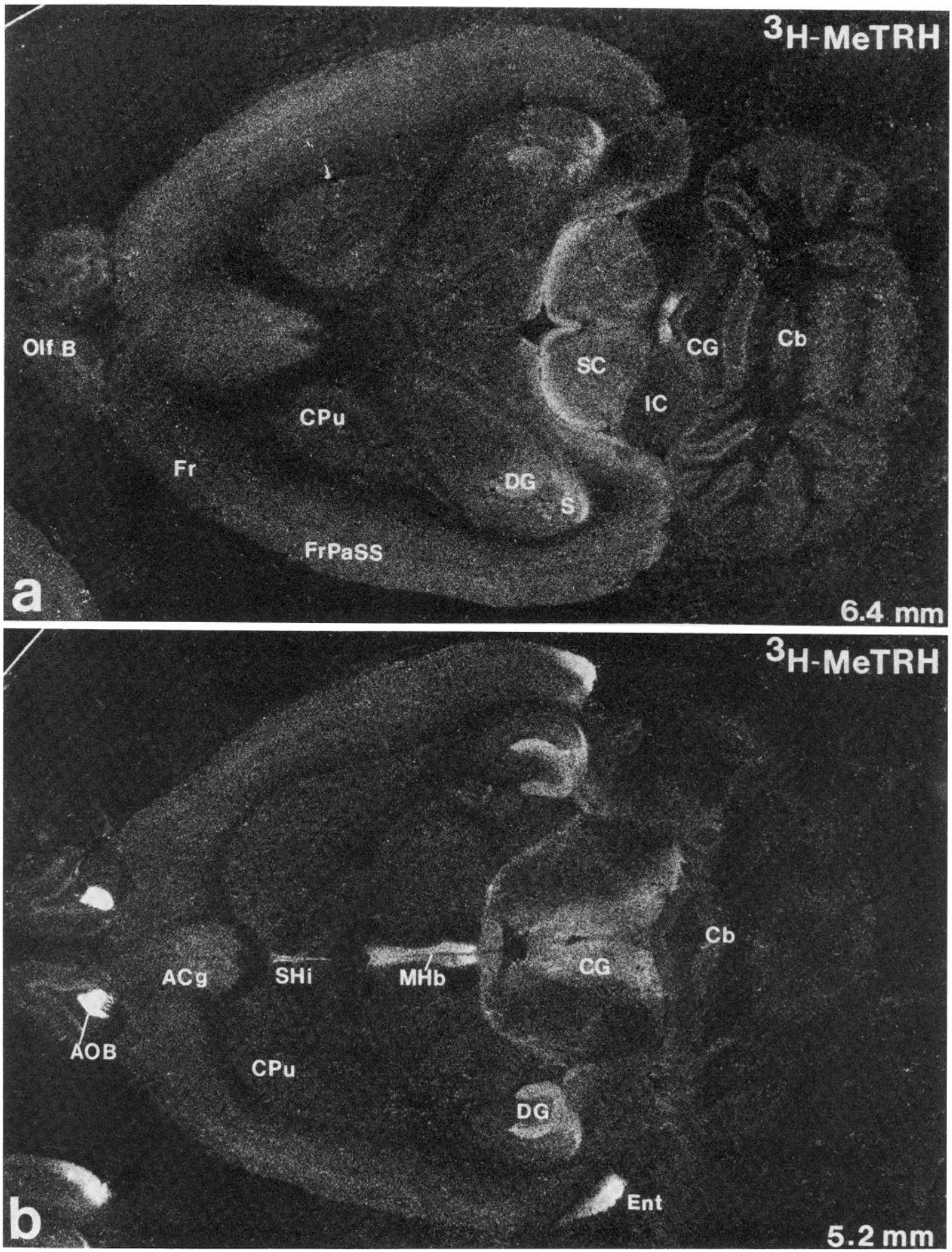
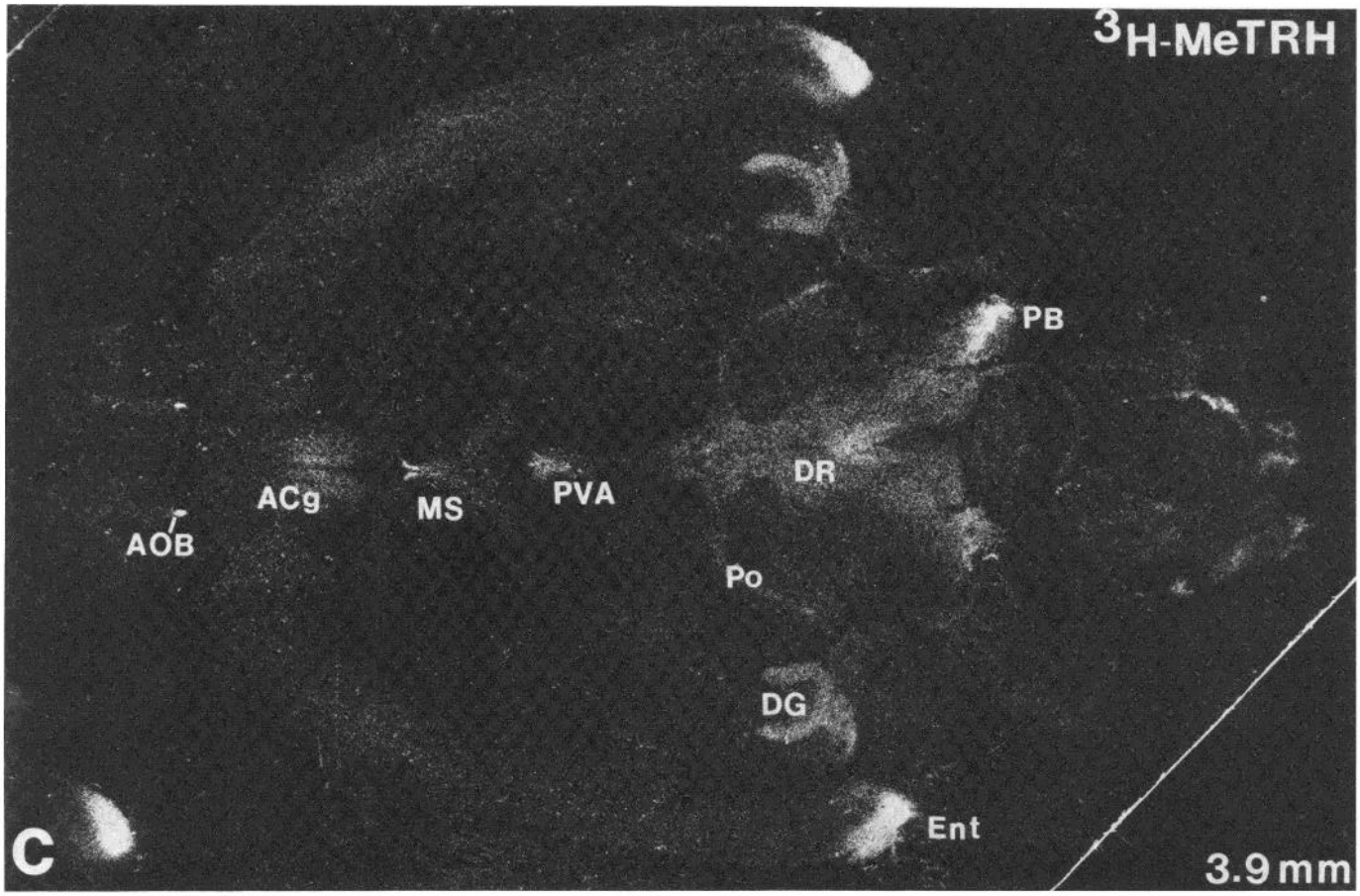


Figure 1. a and b



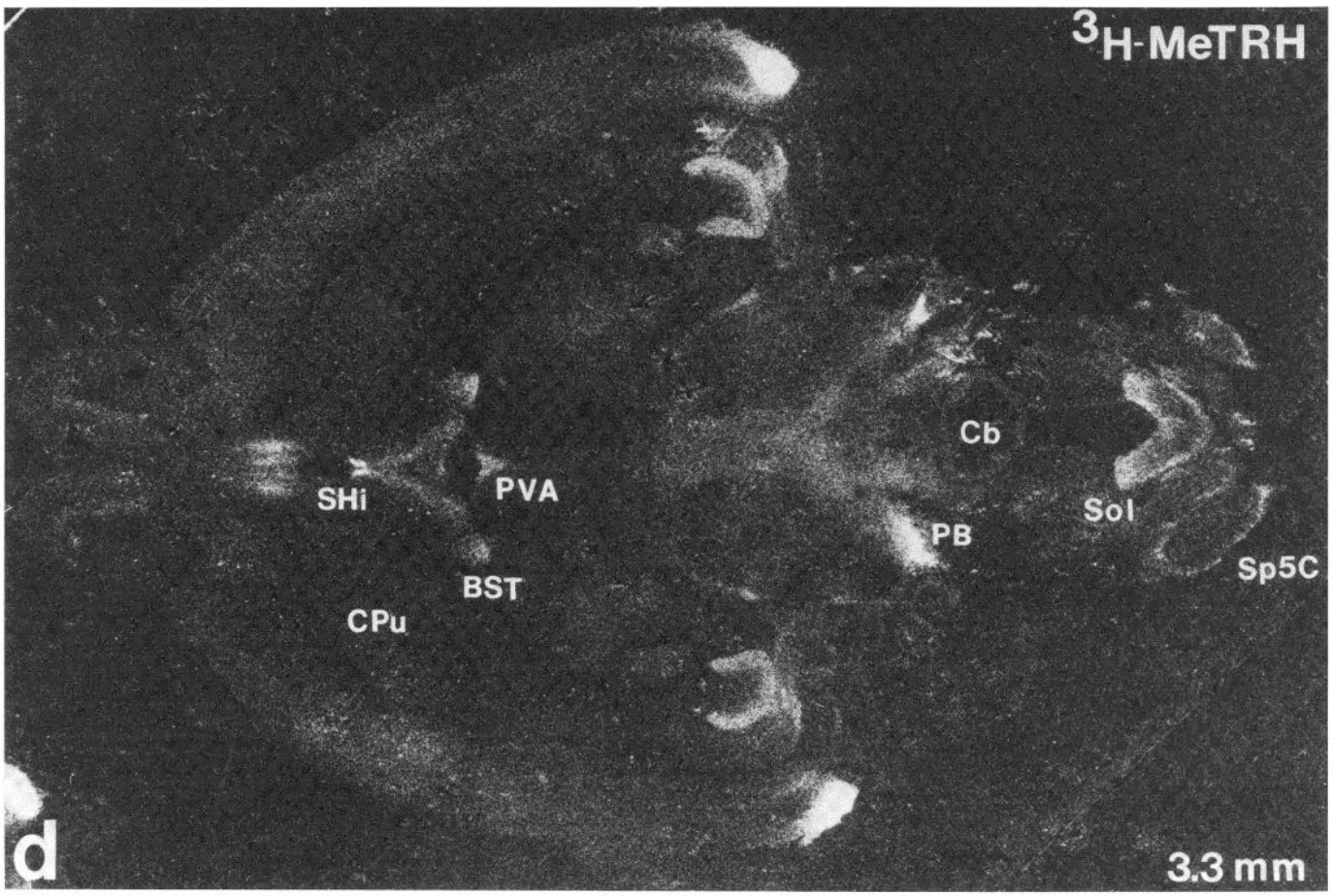


Figure 1. c and d

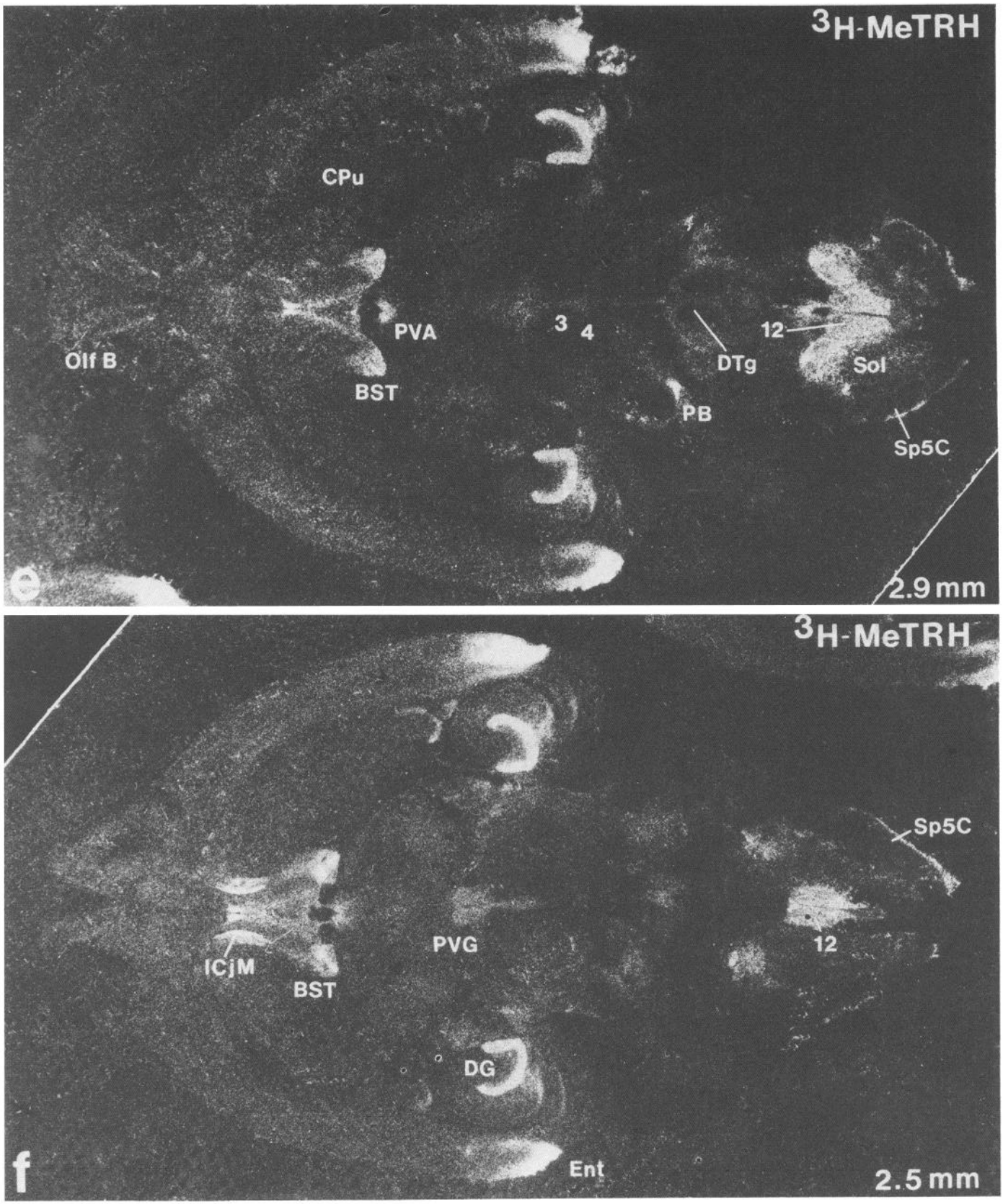


Figure 1. e and f

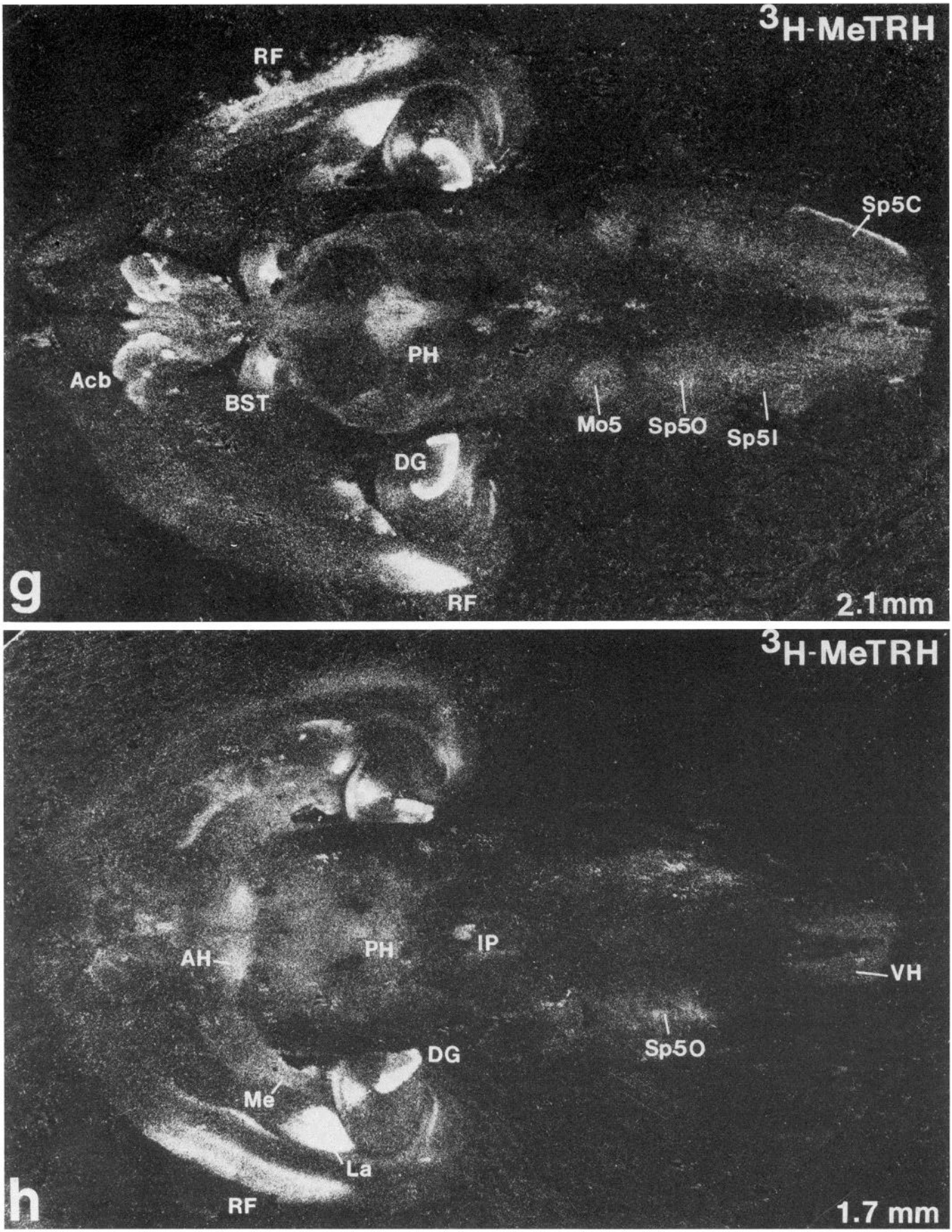
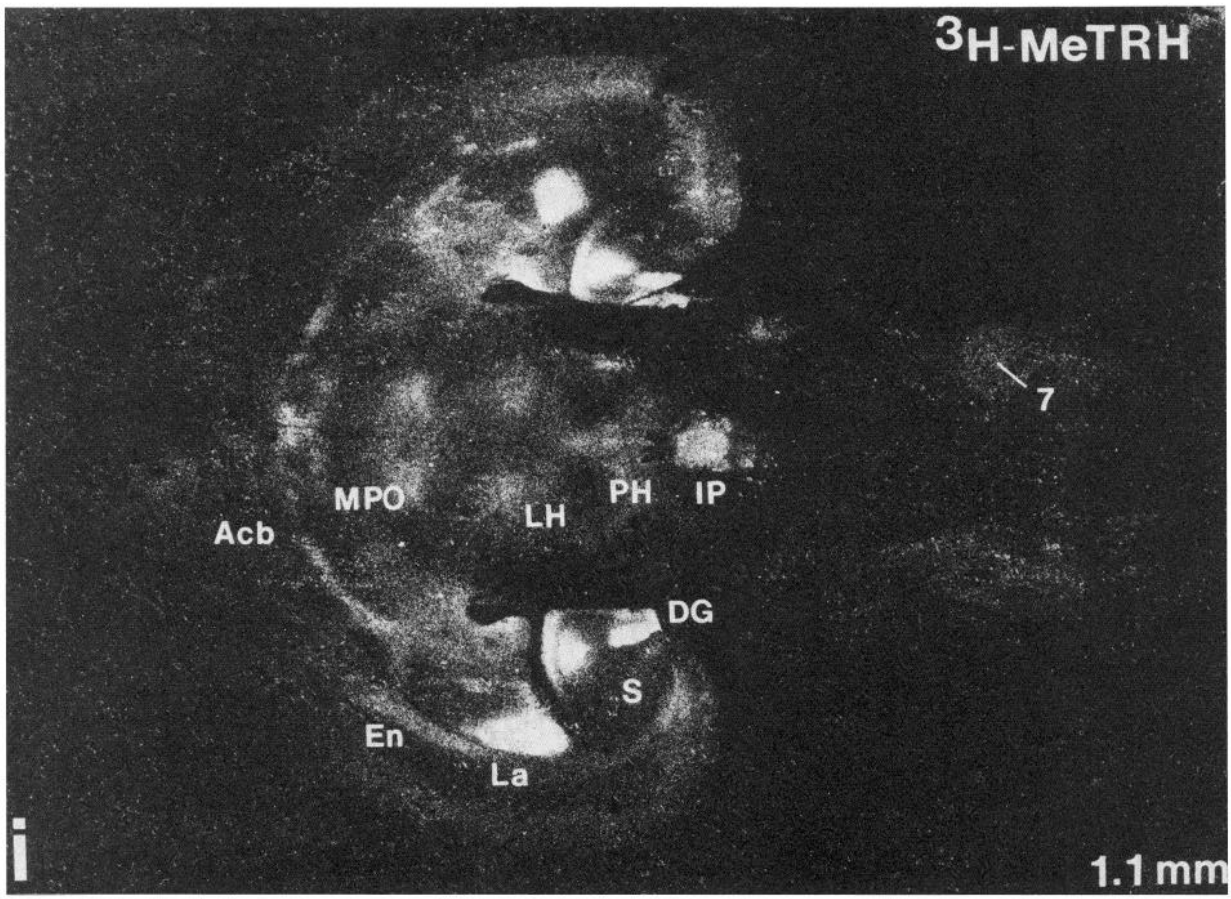


Figure 1. g and h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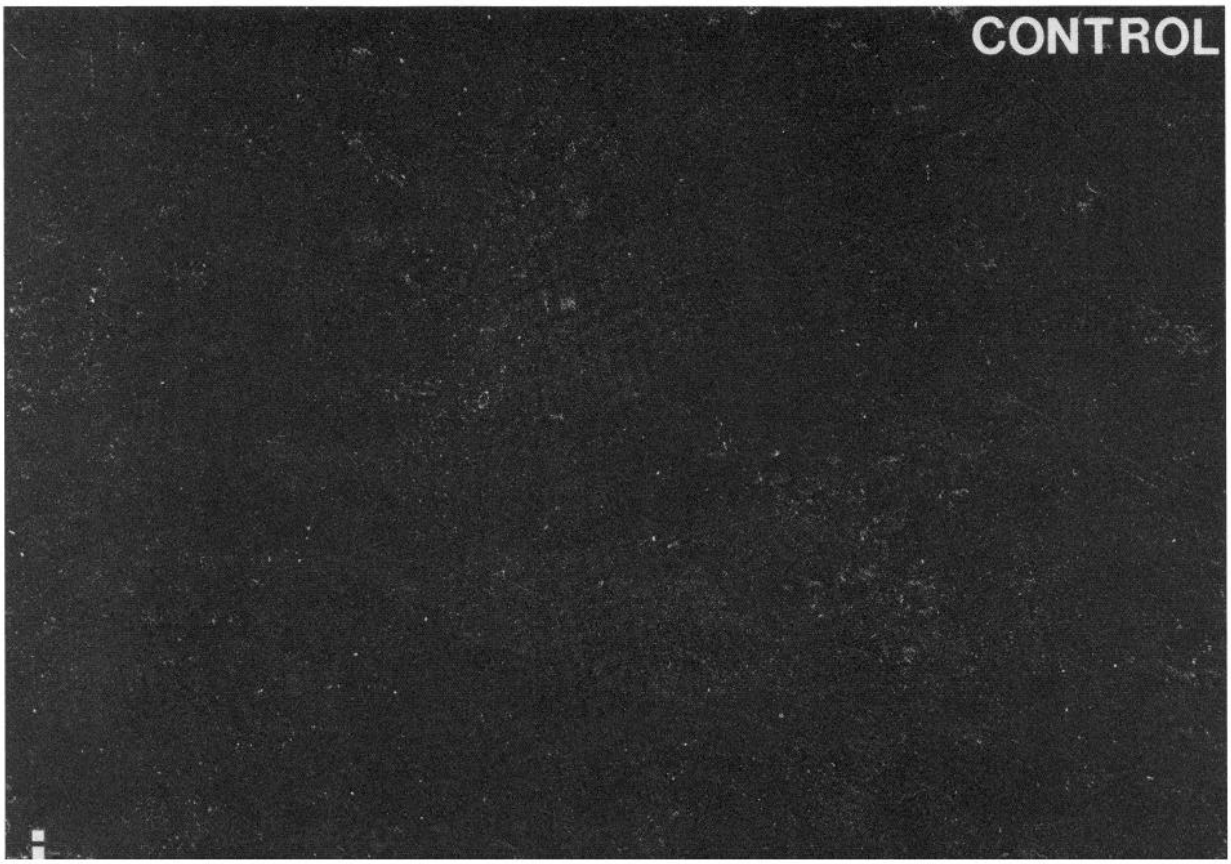
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His^2]thyrotropin-releasing hormone ([^{3}H]MeTRH) displays a similar pharmacological structure-activity profile as TRH itself (Taylor and Burt, 1981b; Simasko and Horita, 1982). Thus, we have used this radiolabeled analogue to visualize the location of TRH receptors in the CNS.

Materials and Methods

Male Sprague-Dawley rats (150–250 gm) were killed by decapitation; the brains rapidly were removed, blocked in the horizontal plane, placed on brass microtome chucks, and frozen in dry ice. The brains were then serially sectioned (20 μm) and thaw-mounted on gelatin-coated microscope slides and stored at −20°C in boxes of desiccant for 48 hr.

Previous brain homogenate studies have extensively defined the optimal incubation conditions necessary to achieve the highest specific/nonspecific binding ratios using [^{3}H]MeTRH as the radioligand (Taylor and Burt, 1981b; Simasko and Horita, 1982). In the present study, we have used a nearly identical set of incubation conditions as previously described except that we have performed the incubations on slide-mounted tissue sections rather than brain homogenates.

The slide mounted 20-μm tissue sections were allowed to come to room temperature and then placed in the incubation medium (0°C on ice for 2.5 hr in a cold room 4°C) of 0.1 M sodium phosphate buffer (pH 7.4) containing 5 nM [^{3}H]MeTRH [L-histidyl-4-^{3}H], L-prolyl-3,4-^{3}H] (New England Nuclear; specific activity, 76.7 Ci/mmol) in the absence (total binding) or presence (nonspecific binding) of 10 μM TRH. After this incubation period, the slide-mounted tissue sections were rinsed with three washes of ice-cold 0.1 M sodium phosphate buffer (pH 7.4, 0°C on ice, 5 min each in the cold room at 4°C) and three washes of dH$_2$O (0°C on ice, 5 sec each in the cold room at 4°C) and then quickly dried in the cold room using a stream of cold air. Sections were left a further 3 hr to dry in the cold room and then stored in desiccant-filled boxes overnight at −20°C. The slides were then placed in apposition to LKB tritium-sensitive Ultrofilm. After 6 to 8 weeks, the film was developed in D-19 developer, washed, and fixed. Sections were then placed in Carnoys fixative for 15 min, Nissl stained, and mounted with Depex. Darkfield photomicrographs were then taken using the exposed Ultrofilm and compared with the corresponding lightfield photomicrographs of the Nissl-stained sections. Controls for chemographic artifacts were made by performing the binding exactly as described above except that [^{3}H]MeTRH was omitted from the incubation medium.

To measure the ratio of specific to nonspecific [^{3}H]MeTRH binding to the tissue, several pairs of tissue sections (one incubated in 5 nM [^{3}H]MeTRH and its serial pair in 5 nM [^{3}H]MeTRH + 10 μM TRH as described above) were scraped from the slide with a razor blade, weighed, and then placed in tubes containing 10 ml of scintillation fluid (Aquasol-2, New England Nuclear) and shaken overnight at room temperature, and the radioactivity was measured using scintillation spectrometry.

To estimate semiquantitatively the density of [^{3}H]MeTRH-binding sites, microdensitometry combined with tritium-sensitive film was performed as previously described (Palacios et al., 1981; Hunt and Mantyh, 1984). The exposed film was placed onto the stage of a Leitz Orthoplan microscope equipped with a Vario-Orthomat camera system which incorporates a back-projected exposure guide. A small and variable sized spot of light indicates the area of tissue or negative from which exposure is being read. Direct reading of the voltage content from the photosensitive cell served as a density reading, being directly proportional to the optical density of the film. Previous calibration experiments determined at which point given density was increasing in a linear fashion with increasing concentrations of isotope. Nonlinearity occurs close to saturation of the film and this region was avoided. Background labeling was defined as the 5 nM [^{3}H]MeTRH binding that was not displaced in the presence of 10 μM TRH.

Results

The results of the autoradiographic studies are shown in Fig. 1, *a* to *j* and quantified in Table 1. In general, nearly all (95%) of the TRH receptors were located in the gray matter with very low levels being observed in most white matter tracts (i.e., corpus callosum, internal and external capsule). In experiments testing for chemography artifacts, no positive or negative chemography was observed in any area of the brain. Experiments to determine the ratio of specific to nonspecific binding showed that the specific binding represented approximately 75% of [^{3}H]MeTRH binding to the slide.

In presenting the autoradiographic data, we used horizontal brain sections spaced at approximately 0.5-mm intervals in the dorsal-ventral plane so as to provide an atlas of TRH receptors in the rat brain. In describing the distribution of TRH receptors a very low density of receptors will correspond to those areas having an optical density (OD) of 0.1: low, an OD of 0.2 to 1.0; moderate, OD of 1.1 to 2.0; heavy, OD 2.1 to 3.0; and very dense OD, greater than 3.1.

Very dense levels of TRH receptors were observed in the accessory olfactory bulb (Fig. 1, *b* and *c*) which appeared to have the highest concentration of TRH receptors in the entire rat brain. Other areas of the olfactory bulb such as the external plexiform layer and granule cell layer had low levels of receptors while the inner plexiform layer and mitral cell layer had a moderate to low density of TRH receptors (Fig. 1*b*).

In the cerebral cortex, the frontal, anterior cingulate, somatosensory, and visual cortex have a low density of TRH receptors (Fig. 1, *a* to *f*). In these cortical areas, TRH receptors were distributed homogenously except for layer III which had a slightly reduced density of receptors as compared to the other cortical layers. In contrast, the posterior-lateral tip of the entorhinal cortex had a very dense concentration of receptors while the rhinal cortex had a heavy concentration of receptors

Figure 1. *a* and *b*, Two darkfield photomicrographs of LKB film autoradiographs which show horizontal rat brain sections incubated in 5 nM [^{3}H]MeTRH. In these and the following photomicrographs (*c* to *i*) the white grains indicate [^{3}H]MeTRH-binding sites. Given the large number of discrete anatomical structures that are resolvable in these sections, we have opted to quantitate the concentration of receptors in Table 1 and to label only a few structures for orientation purposes in the photomicrographs. For those desiring a more detailed labeling of these brain sections, we have provided the numbers in the *lower right-hand corner* which indicate the distance (in millimeters) that each horizontal section lies above the interaural line as given in the atlas of Paxinos and Watson (1982). *c* and *d*, Two darkfield photomicrographs of LKB film autoradiographs which show horizontal sections incubated in 5 nM [^{3}H]MeTRH. *e* and *f*, Two darkfield photomicrographs of LKB film autoradiographs which show horizontal sections incubated in 5 nM [^{3}H]MeTRH. *g* and *h*, Two darkfield photomicrographs of LKB film autoradiographs which show horizontal sections incubated in 5 nM [^{3}H]MeTRH. *i* and *j*, Darkfield photomicrograph of LKB film autoradiographs showing horizontal rat sections incubated in 5 nM [^{3}H]MeTRH in the absence (*i*) and presence of (*j*) 10 μM TRH. *i* and *j* are serial pairs. As in the previous darkfield photomicrographs, [^{3}H]MeTRH binding appears as white silver grains. Since nonspecific binding is defined as that [^{3}H]MeTRH binding which remains in the presence of 10 μM TRH, *j* is an image of the nonspecific binding. Thus, the specific binding in *i* is obtained by subtracting the nonspecific binding shown in *j*. *3,* principal oculomotor nucleus; *4,* trochlear nucleus; *7,* facial nucleus; *12,* hypoglossal nucleus; *Acb,* accumbens nucleus; *ACg,* anterior cingulate cortex; *AH,* anterior hypothalamic area; *AOB,* accessory olfactory bulb; *BST,* bed nucleus of the stria terminalis; *Cb,* cerebellum; *CG,* central gray; *CPu,* caudate-putamen (striatum); *DG,* dentate gyrus; *DR,* dorsal raphe; *DTg,* dorsal tegmental nucleus (Gudden); *En,* endopiriform nucleus; *Ent,* entorhinal cortex; *Fr,* frontal cortex; *FrPaSS,* frontoparietal cortex, somatosensory area; *IC,* inferior colliculus; *ICjM,* islands of Calleja, major island; *IP,* interpeduncular nucleus; *La,* lateral amygdaloid nucleus; *Me,* medial amygdaloid nucleus; *MHb,* medial habenula nucleus; *Mo5,* motor trigeminal nucleus; *MPO,* medial preoptic area; *MS,* medial septal nucleus; *Olf B,* olfactory bulb, *PB,* parabrachial nucleus; *PH,* posterior hypothalamic nucleus; *Po,* posterior thalamic nuclear group; *PVA,* paraventricular thalamic nucleus; *PVG,* periventricular gray; *RF,* rhinal fissure; *S,* subiculum; *SC,* superior colliculus; *Shi,* septohippocampal nucleus; *Sol,* nucleus of the solitary tract; *Sp5C,* nucleus of the spinal tract of the trigeminal nerve, caudal part; *Sp5I,* nucleus of the spinal tract of the trigeminal nerve, interpositus part; *Sp50,* nucleus of the spinal tract of the trigeminal nerve, oral part; *VH,* ventral horn or the spinal cord.

TABLE I

Optical density of tritium-sensitive film exposed to rat horizontal brain sections with [^{3}H]MmeTRH-labeled receptors

Using microdensitometry produced errors of less than 5% in the values below. Background labeling on the film has an optical density of 0 while the accessory olfactory bulb has the highest optical density value of 5.0. Each value is the mean of seven determinations from at least two different tissue sections.

Brain Region	Optical Density
Cerebral cortex	
Frontal	0.5
Anterior cingulate	0.8
Rhinal fissure	2.2
Entorhinal	3.4
Somatosensory	0.5
Olfactory bulb	0.5
Accessory olfactory bulb	5.0
Bed nucleus of the stria terminalis	2.3
Endopyriform nucleus	1.6
Accumbens	1.5
Septum medial	1.0
Septum lateral	0.7
Septohippocampal nucleus	2.0
Caudate-putamen	0.4
Globus pallidus	0.3
Amygdala	
Basal nucleus	0.7
Central nucleus	0.9
Medial nucleus	1.7
Lateral nucleus	4.0
Hippocampus	
CA1-CA4	0.5
Dentate gyrus	3.0
Subiculum	
Dorsal	0.6
Ventral	1.7
Dorsal thalamus	
Anterior nucleus	0.2
Medial dorsal nucleus	0.1
Periventricular gray	1.1
Pretectal nucleus	1.2
Ventrobasal nucleus	0.1
Hypothalamus	
Anterior nucleus	1.9
Lateral nucleus	1.5
Paraventricular nucleus	0.4
Posterior nucleus	1.4
Preoptic nucleus	0.7
Ventromedial nucleus	0.2
Ventral thalamus	
Reticular thalamic nucleus	0.2
Epithalamus	
Habenular nucleus	
Medial	1.6
Lateral	0.2
Paraventricular thalamic nucleus	1.7
Mesencephalon	
Interpeduncular nucleus	2.3
Periaqueductal grey	0.9
Substantia nigra	0.1
Superior colliculus	
Superficial layer	1.7
Intermediate layer	1.0
Deep layer	0.7
Third nucleus	0.1
Pons	
Cerebellum	0.3
Cuneiformis nucleus	0.1
Dorsal raphe	1.0
Dorsal tegmental nucleus	0.2
Fourth nucleus	0.1
Inferior colliculus	0.2
Lateral dorsal tegmental nucleus	0.6
Motor trigeminal nucleus	0.7
Parabrachial nucleus	2.7
Principal nucleus of 5	0.1
Medulla	
Abducens nucleus	0.1
Dorsal motor nucleus of the vagus	1.5
Facial nucleus	0.9
Hypoglossal nucleus	2.3
Inferior olive	0.1
Solitary nucleus	1.7
Trigeminal nucleus	
Oralis	0.4
Interpolaris	0.5
Candalis	
Lamina I	1.7
Lamina II–V	0.5
Ventral horn (C1-C2)	0.6

with layer III again having slightly lower levels of TRH receptors (Fig. 1, *g* and *h*).

In the corpus striatum, low levels of receptors were observed in the caudate-putamen and very low levels in the adjacent globus pallidus (Fig. 1, *a* to *f*).

In the hippocampus, the dentate gyrus had a heavy concentration of receptors (Fig. 1, *b* to *i*) but the concentration of receptors appeared to be heavier in the more ventral aspects of this brain area (compare Fig. 1*b* to 1*g*). A similar dorsal-ventral gradient in receptor density was also apparent in the subiculum while other areas such as the CA1-CA4 region were uniformly low (Fig. 1, *b* to *i*).

A low to moderate concentration of receptors was observed in both the medial and lateral septum (Fig. 1, *c* to *g*) while the nucleus accumbens had a moderate concentration of receptors (Fig. 1*g*). In the amygdala, low to moderate concentrations of receptors were observed in the basal and central nucleus, while moderate and very dense concentration of receptors was present in the medial and lateral nuclei respectively (Fig. 1, *g* to *i*).

In the dorsal thalamus, moderate levels of receptors were present in the paraventricular nucleus and the caudal periventricular gray region which merges caudally with the midbrain central gray (Fig. 1, *b* to *g*). The ventrobasal and lateral posterior nucleus had a low level of receptors.

In the hypothalamus, moderate levels of receptors were observed in the anterior, lateral, and posterior nucleus while only low levels were present in the paraventricular, preoptic, and ventromedial nucleus (Fig. 1, *g* to *i*).

In the mesencephalon, a heavy concentration of receptors was present in the interpeduncular nucleus (Fig. 1*i*) while the central gray had a moderate density of receptors (Fig. 1*b*). In the superior colliculus, the superficial layer had a moderate number of receptors (Fig. 1*a*) while the intermediate and deep layers had slightly lower levels of receptors (Fig. 1*a*). The substantia nigra (Fig. 1*H*) and third nucleus (Fig. 1*e*) both had very low levels of receptors.

In the pons, the lateral parabrachial nucleus (Fig. 1, *c* and *d*) had a heavy density of receptors while the medial aspect of the dorsal raphe (Fig. 1*c*) had a low to moderate number of recep-

tors. In the motor trigeminal nucleus (Fig. 1*g*) and the dorsal tegmental nucleus (Fig. 1*e*), a low density of receptors was observed while the fourth nucleus, principal nucleus of five, and cuneiformis had a very low density of receptors.

In the medulla the highest density of receptors was located in the hypoglossal nucleus (Fig. 1, *e* and *f*) while several other motor nuclei such as the dorsal motor nucleus of the vagus and the facial nucleus (Fig. 1*i*), had a moderate to low concentration of receptors. Several sensory nuclei such as the solitary nucleus (Fig. 1, *d* and *e*) and all three divisions of the nucleus of the spinal tract of the trigeminal nerve (Fig. 1*g*) had low yet significant concentrations of receptors. Surprisingly, the superficial laminae of the upper spinal cord (C1-C2) had a moderate number of receptors (Fig. 1, *f* and *g*) while at the same level (C1-C2) the ventral horn had only a low concentration of receptors (Fig. 1*h*).

Discussion

Nine years after the original isolation of TRH, Burt and Snyder (1975) described some of the properties of brain TRH receptors using [^{3}H]TRH as the radioligand. In this initial study, the authors observed that both high (K_D = 50 nM) and low (K_D = 5 μM) affinity TRH-binding sites were ubiquitously present throughout the rat CNS. While this study was critical in establishing that TRH receptors exist outside the pituitary, these results were complicated by a high concentration of low affinity binding sites and a high degree of nonspecific binding. Recently, a new TRH analogue, [^{3}H]MTRH (Taylor and Burt, 1981a), has become commercially available which offers several advantages over [^{3}H]TRH. These include: a 7-fold higher affinity for the receptor than TRH (Taylor and Burt, 1981b), a lower degree of nonspecific binding (Taylor and Burt, 1981b), a higher behavioral potency (Wei et al., 1976), and a 1000-fold decrease in affinity for the low affinity binding sites (Taylor and Burt, 1981b). Using this analogue, several groups have shown that [^{3}H]MeTRH demonstrates many of the same properties as [^{3}H]TRH including a parallel distribution of binding sites in the CNS and a nearly identical ability to be displaced by TRH analogues (Taylor and Burt, 1981b; Simasko and Horita, 1982). Together these results have led the authors to conclude that [^{3}H]MeTRH is labeling TRH receptors.

In the present report, we have used this analogue, [^{3}H] MeTRH, to further define the distribution of TRH receptors in the CNS using receptor-binding autoradiography. The present results are in good agreement with previous homogenate receptor-binding studies which showed that, while as a region the amygdala had the highest concentration of TRH receptors and the cerebellum has the lowest, nearly every gray matter area in the brain has at least a very low but detectable level of TRH receptors (Burt and Snyder, 1975; Ogawa et al., 1981; Shimasko and Horita, 1982; Taylor and Burt, 1982). Whether this distribution of receptors implies that TRH serves as both a neurotransmitter involved in specific brain functions, in those areas where high concentrations of receptors are confined to discrete nuclei, and as a general "neuromodulator," via the receptors diffusely distributed throughout the CNS, is not clear. However, what is apparent is that this "diffuse/concentrated" receptor distribution pattern is not confined to TRH receptors, since neurotensin receptors have similar distribution patterns in the rat brain (Young and Kuhar, 1981; Goedert et al., 1984b).

Previous behavioral studies have described several CNS areas where infusion of TRH will elicit a stereotypic behavior. Thus, microinjection into the septum causes an analeptic response (Kalivas and Horita, 1980), injection into the nucleus accumbens stimulates locomotion (Miyamoto and Nagawa, 1977; Heal and Green, 1979), hypothalamic injection produces hyperthermia (Kalivas and Horita, 1980; Metcalf, 1982), hippocampal injection in hibernating ground squirrels causes arousal from hibernation (Stanton et al., 1980), and intraventricular injection produces satiety (Vijayan and McCann, 1979; Vogel et al., 1979; Morley and Levine, 1980). In the present study, we have shown that nearly all of these CNS areas have a significant number of TRH receptors which presumably interact with the peptide TRH to give rise to the response. The present data are also in good agreement with previous electrophysiological data which have shown TRH-responsive neurons in the cerebral cortex (Renaud and Martin, 1975; Braitman et al., 1980), septum, hypothalamus (Dyer and Dyball, 1974; Renaud and Martin, 1975; Winokur and Beckman, 1978), cerebellum (Renaud and Martin, 1975), and the ventral horn (Nicoll, 1977). As with the behavioral data, all the neuronal areas which have been reported to be responsive to TRH application also appeared to have significant concentrations of TRH receptors as visualized autoradiographically in the present study. While the exact CNS site(s) of the TRH satiety effect is unknown, high concentrations of TRH receptors are localized in the solitary and parabrachial nucleus, both of which are near the ventricles and both of which have been implicated in conveying visceral and taste information within the CNS (Mantyh and Hunt, 1984).

Recently, several clinical reports have suggested that TRH may be of therapeutic value in the treatment of depression (Kastin et al., 1972; Prange et al., 1972) (however, see Coppen et al., 1974; Mountjoy et al., 1974), schizophrenia (Inanaga et al., 1978), amyotrophic lateral sclerosis (Engel et al., 1983), and spinal trauma (Faden et al., 1981). While the site of TRHs action in producing any of these effects is unknown, the TRH receptors present in all area of the cerebral cortex and amygdala may be important in explaining the effect of TRH in depression and schizophrenia. Most authors have focused on spinal sites of action in attempting to explain the effect of TRH in amyotrophic lateral sclerosis and spinal trauma. Thus, in patients with amyotrophic lateral sclerosis, TRH is throught to interact with motor neurons in the ventral horn of the spinal cord (Engel et al., 1983) whereas the improvement in spinal trauma is thought to involve the blocking of vascular edema in the spinal cord (Faden et al., 1981). The present report would appear to be consistent with both of these hypothesized sites of TRH action since significant numbers of TRH receptors are present in both the ventral and dorsal horns of the spinal cord.

It is clear from both the present and previous studies that the distribution of TRH receptors is not well correlated with TRH itself (Taylor and Burt, 1982; Palacios, 1983; Rostene et al., 1983; Sharif et al., 1983; Pilotte et al., 1984). Thus, there are examples of brain areas which are low in the TRH peptide (Hokfelt et al., 1975a, 1975b) but high in TRH receptor levels (dentate gyrus, amygdala, dorsal horn of the spinal cord) whereas other regions of the brain which have a relatively high concentration of TRH peptide (hypothalamus, striatum, ventral horn of the spinal cord) have only low to moderate levels of TRH receptors. Such a discrepancy has been noted for other neurotransmitters and their receptors including substance P (Mantyh et al., 1984), neurotensin (Young and Kuhar, 1979; Goedert et al., 1984a), opiates (Atweh and Kuhar, 1977; Simantov et al., 1977), and noradrenaline (Alexander et al., 1975). As we have discussed previously (Mantyh et al., 1984), there are several possible explanations for these discrepancies including multiple molecular forms of the ligand, only some of which are recognized by immunohistochemistry or radioimmunoassay, differential distribution of ligand-degrading enzymes, receptor subtypes which only bind under an appropriate set of conditions, different rates of "neurotransmitter" turnover in different brain areas, and that neurotransmitters might diffuse over long distances to interact with the receptor. Several of these possibilities are particularly relevant in comparing the distribution of TRH to its receptors.

In a study which examined the breakdown of TRH in the brain, pituitary, and a variety of peripheral tissues, it was found that TRH-degrading activity varied widely depending on the tissue examined (Prasad and Peterkofsky, 1976). Most striking was the finding that while the hypothalamus had relatively high levels of TRH-degrading activity, one of the major sites of action of TRH, the pituitary, had virtually no TRH-degrading activity. This finding raises the possibility that the levels of TRH degrading activity may also vary between different brain areas. If such differences were found in the brain, then a simple correlation between TRH concentration and TRH receptor concentration would not be expected since the amount of peptide action would also be dependent on TRH-degrading activity. That levels of TRH-degrading enzyme may indeed vary in the brain is suggested by the finding that the distribution of one of the metabolites of TRH, histidyl-proline diketopiperazine (cyclo-[His-Pro]) is not correlated with the distribution of TRH immunoreactivity (Mori et al., 1982). Furthermore cyclo-[His-Pro] also appears to be pharmacologically active (Mori et al., 1981; Morley et al., 1981) but does not compete at micromolar concentrations for either [^{3}H]TRH- or [^{3}H]MeTRH-binding sites (Burt and Taylor, 1980). Such results suggest that not only are there regional differences in TRH-degrading enzymes but also that cyclo-[His-Pro] may have either its own set of receptors or may bind at a different site on the TRH receptor than TRH itself. Thus, while there is a poor correlation between the concentration of TRH immunoreactivity and the concentration of TRH receptors, it is clear that TRH and its receptor should provide an excellent system to investigate the interaction between neuropeptides and their receptors.

In summary, the present results have demonstrated that TRH receptors are ubiquitously distributed in the CNS and that there are marked differences in receptor concentration in various regions and nuclei of the brain. These large differences in TRH receptor concentration in the brain may partially explain the wide variety of behavioral, physiological, and clinical actions TRH is known to have in the CNS. Hopefully, future studies will elucidate whether specific populations of TRH receptors participate in specific brain functions and also what regulates the expression of TRH and its receptor.

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