Distribution of Thyrotropin-Releasing Hormone (TRH)-Containing Cells and Fibers in the Human Hypothalamus

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ABSTRACT

In the present study, we describe for the first time the distribution of thyrotropin-releasing hormone (TRH)-containing cells and fibers in the human hypothalamus using brain material obtained with a short postmortem delay. Following fixation in paraformaldehyde, glutaraldehyde and picric acid, excellent staining was obtained with two different TRH antisera. Many TRH-containing neurons were present in the paraventricular nucleus (PVN), especially in the dorsocaudal part of this nucleus. They were mostly parvicellular, but a few magnocellular TRH-positive neurons were observed as well. The PVN also contained a dense network of TRH fibers. The supraoptic nucleus (SON) did not show any TRH immunoreactivity, excluding the possibility of cross-reactivity of the antiserum with neurohypophysial hormones or their precursors. In addition, TRH cells were found in the suprachiasmatic nucleus (SCN), which is the circadian clock of the brain, in the sexually dimorphic nucleus (SDN) and dorsomedially of the SON. We observed small numbers of TRH cells throughout the hypothalamic gray in all subjects studied.

A high density of TRH-containing fibers was seen not only in the median eminence but also in other hypothalamic areas, e.g., in the ventromedial nucleus (VM) and in the perifornical area. The results generally agree with earlier data in the rat, with the exception of the absence of TRH cells in the SON. The large number of sites of TRH-containing fiber terminations on neurons suggests important physiological functions of this neuropeptide as a neurotransmitter or neuromodulator in the human brain, in addition to its role as a neurohormone in pituitary secretion of thyroid-stimulating hormone (TSH). © 1994 Wiley-Liss, Inc.

Key words: sexually dimorphic nucleus, paraventricular nucleus, suprachiasmatic nucleus, thyroid, Alzheimer

The tripeptide thyrotropin-releasing hormone (TRH) was the first hypothalamic hormone to be isolated and structurally characterized (Bøler et al., 1969; Burgus et al., 1969). Apart from preliminary data on pro-TRH-positive cells (Germak et al., 1989), no immunocytochemical studies of the hypothalamic distribution of TRH cells have been performed in the human brain. Hökfelt et al. (1975) confirmed earlier radioimmunological data (e.g., Brownstein et al., 1974) on the presence of TRH in the rat hypothalamus using an indirect immunofluorescence technique. After improvements of fixation and tissue treatment, TRH-containing neurons were demonstrated in the dorsomedial nucleus (DM), parvicellular portion of the paraven-

tricular nucleus (PVN), suprachiasmatic nucleus (SCN) and perifornical region of the rat (Lechan and Jackson, 1982). Using an antiserum raised against a peptide sequence present in the precursor for TRH, Lechan et al. (1986) showed pro-TRH-immunoreactive neurons in portions of the rat telencephalon and brainstem where TRH had not been described previously. In colchicine-treated rats, numerous extrahypothalamic TRH-containing neu-

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TABLE 1. Brain Material

Subject no.	Side of hypothalamus	Sex	Age (years)	Clock time of death	Date of death	Postmortem delay (hours)	pH ¹	Clinical diagnosis
92.071	R	F	77	15.00	10-09-92	4	8.3	Major depression Chronic obstructive pulmonary disease Moderate renal insufficiency
93.003	L	M	76	00.30	10-01-93	4	6.6	Alzheimer's disease Mitral stenosis
93.004	L	F	72	09.40	12-01-93	4	6.5	Alzheimer's disease Angina pectoris
93.005	L	M	72	10.30	12-01-93	4.5	6.8	Acute heart failure Long-standing cardiovascular disease Carcinoma of the bladder
93.015	R	M	75	11.15	22-01-93	4.5	7.2	Chronic obstructive pulmonary disease Pneumonia
93.045	R	F	83	17.30	25-03-93	5	6.5	Respiratory failure Alzheimer's disease Cardiovascular disease
93.047	L	M	70	15.20	27-03-93	4.5	6.4	Urinary tract infection Alzheimer's disease Urinary tract infection Ileus
93.048	R	F	92	23.45	27-03-93	4	6.7	Ischemic heart disease Alzheimer's disease
93.049	R	M	84	04.15	29-03-93	4	6.6	Chronic obstructive pulmonary disease Dementia with extrapyramidal disorder
93.075	R	M	72	09.30	16-06-93	9	6.7	Chronic obstructive pulmonary disease Dementia and cerebrovascular disease
93.103	L	M	83	05.00	27-08-93	8	— ²	Malignant tumor right lung
93.110	R	M	73	03.30	15-09-93	9	6.1	Malignant tumor right lung Carcinoma of the bladder
93.112	L	M	67	03.40	17-09-93	9	7.2	Cardiovascular disease Aortic and mitral valve replacement Pneumonia and mediastinitis
93.139	L	F	78	08.00	11-11-93	5.5	6.5	Myocardial infarction Metastatic pulmonary carcinoma Gastritis, osteoporosis Chronic heart failure

¹pH of cerebrospinal fluid at autopsy.
²Data not present.

rons were observed, e.g., in the olfactory bulb, hippocampus, periaqueductal gray, medulla oblongata and the dorsal horn of the spinal cord (Tsuruo et al., 1987), suggesting physiological functions of the peptide as a neurotransmitter or neuromodulator, in addition to its role as a neurohormone in the control of anterior pituitary secretion of thyroid-stimulating hormone (TSH). Subsequent double

staining showed hypothalamic TRH immunoreactivity to be present in the same neurons as oxytocin (OXT)-like immunoreactivity in the supraoptic nucleus (SON) and enkephalin-like immunoreactivity in the perifornical area of the rat brain (Tsuruo et al., 1988).

The results of immunocytochemical studies involving thyroid hormone-deficient rats showed that only the TRH-containing neurons in the parvicellular portion of the PVN were involved in the hypothalamic-hypophyseal-thyroid axis (Nishiyama et al., 1985). Indeed, Segerson et al. (1987) found an increase in pro-TRH messenger RNA (pro-TRH mRNA) only in the PVN of hypothyroid rats, as revealed by quantitative Northern blot analysis. In addition, this response occurred exclusively in parvicellular neurons of the PVN as revealed by in situ hybridization. A recent study in rats by Greer et al. (1993) reinforced the idea that TRH from the PVN plays a role in set-point regulation of the pituitary thyrotroph.

In view of the high prevalence of disorders of the hypothalamic-hypophyseal-thyroid axis in the general population (e.g., Petersen et al., 1991) and the wide use of TRH for diagnostic purposes in the clinic, it is surprising that no immunocytochemical data are available on the neuroanatomy of TRH cells in the human hypothalamus. Okon and Koch (1976) demonstrated TRH in the human hypothalamus and in the upper part of the pituitary stalk by

Abbreviations	
AC	anterior commissure
BST	bed nucleus of the stria terminalis
CM	corpus mamillare
CRH	corticotropin-releasing hormone
CSF	cerebrospinal fluid
DB	diagonal band of Broca
DM	dorsomedial nucleus
FO	fornix
IF	infundibular nucleus
III	third ventricle
INAH	interstitial nuclei of the anterior hypothalamus
MPN	medial preoptic nucleus
NTL	lateral tuberal nucleus
OC	optic chiasm
OT	optic tract
OXT	oxytocin
PAP	peroxidase-anti-peroxidase
PVN	paraventricular nucleus
RIA	radioimmunoassay
SCN	suprachiasmatic nucleus
SDN	sexually dimorphic nucleus
SDN-POA	sexually dimorphic nucleus of the preoptic area
SON	supraoptic nucleus
TBS	Tris-buffered saline
TMN	tuberomammillary nucleus
TRH	thyrotropin-releasing hormone
TSH	thyroid-stimulating hormone
VM	ventromedial nucleus
VP	Vasopressin

Fig. 1. A–I: Schematic illustration of the distribution of immunoreactive thyrotropin releasing hormone (TRH) cell bodies and fibers in frontal sections of the human hypothalamus at intervals of 800 μ m. Each dot corresponds to one cell body. Parvicellular cell: spherical dot; bipolar cell: oval dot; magnocellular cell: large dot; 1, low fiber density; 2, intermediate fiber density; 3, high fiber density.

Figure 1

Figure 2

Subsequently, free-floating sections were washed overnight in TBS at 4°C.

Immunocytochemistry

Immunocytochemistry was performed with the unlabeled peroxidase-anti-peroxidase (PAP) technique (Sternberger et al., 1970) and consisted of the following steps:

1. pretreatment with 10% methanol and 3% H₂O₂ in TBS (30 minutes, room temperature)
2. rinsing in TBS (3 × 10 minutes, room temperature)
3. rinsing in TBS, containing 0.5% Triton X-100 (TBS-Triton) (30 minutes, room temperature)
4. incubation with the first antiserum (1 hour at room temperature, and overnight at 4°C)
 - a) rabbit TRH antiserum (#4319), 1:1,000 in TBS-Triton, or
 - b) rabbit TRH antiserum (#8880), 1:4,000 in TBS-Triton, or
 - c) rabbit vasopressin (VP) antiserum (Truus, 10-04-86), 1:500 in TBS-Triton, or
 - d) rabbit TRH antiserum (#8880), 1:4,000 in TBS-Triton, following preadsorption with TRH (vide infra)
5. rinsing in TBS-Triton (4 × 10 minutes, room temperature)
6. incubation with goat anti-rabbit serum (Betsy, 1:100 in TBS-Triton (1 hour, room temperature)
7. rinsing in TBS-Triton (4 × 10 minutes, room temperature)
8. incubation with PAP, 1:1,000 in TBS-Triton (1 hour, room temperature)
9. rinsing in TBS-Triton (2 × 10 minutes, room temperature)
10. rinsing in TBS (10 minutes, room temperature)
11. 0.05% 3,3'-diaminobenzidine tetrahydrochloride (DAB), activated with 0.001% H₂O₂ (10 minutes, room temperature)
12. rinsing in TBS (2 × 10 minutes)

The sections were mounted on chrome alum-coated glass slides, air-dried, dehydrated in graded ethanol series and mounted in Entellan (Merck).

Specificity

For details on the specificity of TRH antiserum #4319 as tested by means of RIA, the reader is referred to Visser and Klootwijk (1981). Specificity tests of #4319 in immunocytochemical studies have been described by other investigators (e.g., Arvidsson et al., 1992). Although antiserum #4319 recognizes alterations in both C- and N-terminal amino acids of TRH (pGlu-His-Pro-NH₂), it is much less sensitive to changes in the middle position and shows quantitative cross-reactivity as measured by RIA with the analogs pGlu-Phe-Pro-NH₂ and pGlu-Glu-Pro-NH₂. Antiserum #8880 is much more specific, showing only 5% and < 0.1% cross-reactivity with these peptides, respectively. In the present study, the specificity of TRH-antiserum #8880 was tested in sections following preadsorption of the diluted antiserum (1:4,000) with synthetic TRH (Hoechst AG) at a concentration of 2 ng/ml for 30 minutes at room temperature. A second adsorption test was performed by binding synthetic TRH (Hoechst AG) to a gelatin-coated nitrocellulose membrane with the fixative used in the present study, followed by preadsorption with TRH antiserum (#8880) 1:4,000 (for details see Buys et al., 1989). The two preadsorbed TRH antisera were used as first antiserum in

In the present report, we describe the distribution of TRH-containing cells and fibers in the human hypothalamus, using a fixative containing paraformaldehyde, glutaraldehyde and picric acid (cf. Arvidsson et al., 1992). The results are compared with earlier data obtained in the rat, and possible functional implications are discussed.

MATERIALS AND METHODS

For the present study, the brains of 14 subjects were obtained from the Netherlands Brain Bank (coordinator Dr. R. Ravid; Table I). Separate permission for brain autopsy was obtained either from the patients themselves or from partners or relatives. The series included seven patients with dementia, five of whom suffered from Alzheimer's disease. Patients with Alzheimer's disease fulfilled the NINCDS-ADRDA criteria of probable Alzheimer's disease according to McKhann et al. (1984), a diagnosis that was made definite by neuropathological confirmation. Neuropathology was performed on all subjects in a systematic way by Dr. W. Kamphorst (Free University, Amsterdam) or Prof. F.C. Stam (Netherlands Brain Bank, Netherlands Institute for Brain Research, Amsterdam).

Tissue treatment

Autopsy was performed according to the protocols of the Netherlands Brain Bank, which includes measurement of the cerebrospinal fluid (CSF) pH (see Table I) in order to estimate agonal state (Hardy et al., 1985; Ravid et al., 1992). The hypothalamus was dissected at autopsy and cut mid-sagittally. One hemihypothalamus was immersed in a fixative containing 4% paraformaldehyde, 0.2% picric acid, and 0.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.2 (Arvidsson et al., 1992). Within 12 hours the hypothalamus was immersed in fresh fixative at 4°C for seven days. The tissue was rinsed overnight in Tris-buffered saline (TBS, 0.05 M Tris and 0.13 M NaCl, pH 7.6). Fifty µm sections were cut in the frontal plane on a vibratome.

Fig. 2. The PVN of subject 93.047. A: Vasopressin (VP) staining positive cells are present. Asterisk: blood vessel. B: TRH staining positive cells are present. Note large number of darkly stained, parvocellular TRH-positive neuron. Note large, unstained nucleus and small, darkly stained perikaryon. D: Detail from the same section as B. Magnocellular TRH-positive neuron. Note large cell body and prominent nucleolus. Bars = 100 µm for A and B, 10 µm for C and D.

Figure 3

Fig. 4. Detail from the SDN of subject 93.005 (male, 72 years). TRH staining (#8880, 1:4,000), counterstained with hematoxylin-eosin. Note darkly stained TRH-positive cell (arrowhead) and moderate TRH fiber density. Bar = 50 μ m.

sections of subjects 93.003 and 93.048, respectively. For details on the specificity of the VP antiserum Truus, the reader is referred to Van der Sluis et al. (1988).

Neuroanatomy

We analyzed the hypothalamus in 50 μ m serial sections from the lamina terminalis to the level of the corpus mamillare (CM). Thionine staining was performed in each tenth section for orientation. In these sections, the sexually dimorphic nucleus (SDN; Swaab and Fliers, 1985), which is also called sexually dimorphic intermediate nucleus (Braak and Braak, 1992), was clearly visible. In addition, VP staining was used to identify the PVN, SCN, and SON as anatomical landmarks. The anatomical nomenclature was adopted from Braak and Braak (1992). The DM, which is poorly differentiated in the human brain (Braak and Braak, 1992), could not clearly be distinguished in our sections from the surrounding hypothalamic gray. In many of the hypothalami studied, the median eminence was damaged due to both midsagittal cutting at autopsy and to vibratome sectioning. When present, this part of the section was often not optimal. The SDN was localized in TRH-stained sections by counterstaining with hematoxylin-eosin. We adopted the anatomical schemes of Braak and Braak (1992) and added an extra figure at the most rostral level (see Fig. 1).

RESULTS

Procedure

In a first series of pilot experiments, brain tissue was used that had been fixed in 4% buffered formalin for four

weeks. Immunocytochemistry was performed in 6 μ m paraffin sections. No staining was obtained with TRH antiserum #4319 or #8880 (1:1,000–1:4,000), while excellent staining of VP cells was observed after staining with VP antiserum (Truus, 1:500).

In a second pilot experiment, one hypothalamus was cut midsagittally at autopsy. One hemihypothalamus was fixed in 4% buffered formalin for four weeks, while the other half was fixed in the paraformaldehyde, glutaraldehyde and picric acid fixative as described in Materials and Methods. Staining was performed on vibratome sections of both halves. In formalin-fixed vibratome sections, no TRH staining was obtained, but in the contralateral part the TRH staining was excellent. VP staining was intense on both sides. We therefore used the paraformaldehyde, glutaraldehyde and picric acid fixative for this study.

No clear effects of CSF pH, clock time of death, season of death, or postmortem delay on the staining intensity or distribution of TRH cells and fibers were observed, although the small number of subjects studied did not allow for a systematic study of these variables. There was a complete absence of immunostaining at all hypothalamic levels following preadsorption of the two antisera using either of the two preadsorption methods. The distribution of TRH cells and fibers as revealed by TRH antisera #4319 and #8880 was similar. Antiserum #8880 was subsequently used for the present study.

General distribution

Throughout the hypothalamus many TRH-containing cell bodies and fibers were found. A schematic representation of the neuroanatomy of TRH cells and fibers is given in Figure 1. Small numbers of TRH cells were present throughout the hypothalamic gray. The general distribution of TRH cells and fibers was similar in all subjects studied. There were, however, strong interindividual variations in staining intensity and cell number. In two Alzheimer patients studied (93.003 and 93.004), staining intensity of

Fig. 3. The SCN of subject 92.071. **A:** VP staining (Truus, 1:500), overview. A large number of VP-positive cells is visible. **B:** TRH staining (#4319, 1:1,000). Note occasional TRH-positive cells (arrowheads) in the lateral and medial portion of the SCN. **C:** Detail from B. TRH-positive cell body from the lateral portion of the SCN. The cell nucleus is clearly visible (arrowhead). Bars = 100 μ m for A and B; 10 μ m for C.

Fig. 5. The SON of subject 92.071. **A:** VP staining (Truus, 1:500), overview. Note many magnocellular VP-positive neurons. **B:** TRH staining (#4319, 1:1,000), overview. No TRH-positive cells and fibers

are present in the SON. Some corpora amylacea are visible in the ventrolateral portion of the SON. Arrowhead points to TRH-positive cell just medial to the SON. Bars = 100 μ m.

both TRH cells and fibers was much lower than in any other subject in all areas studied.

The TRH fibers formed a loose network throughout the hypothalamus, whereas a high fiber density was observed in the median eminence (Fig. 6), the VM (Fig. 7B), the IF and the perifornical area (Fig. 7A). In several nuclei, unstained cell bodies were directly surrounded by beaded fibers, suggesting TRH-positive nerve endings in contact with TRH-negative perikarya (Fig. 7B).

Paraventricular nucleus (PVN; Fig. 1A–G). The PVN contained many TRH-immunoreactive cells, especially in the dorsocaudal portion. The majority of TRH cells was parvocellular, although some magnocellular neurons were occasionally found (see Fig. 2). Both bipolar cells with an oval and spindle-shaped form, and spheric multipolar cells were seen in this nucleus. In addition, a dense network of both beaded and unbeaded TRH-immunoreactive fibers was observed in the PVN.

Suprachiasmatic nucleus (SCN; Fig. 1A). A few bipolar TRH cells were found in the lateral and medial portion of the SCN, but not in its central part (Fig. 3). These cell bodies were small and had intermediate staining intensity. No immunoreactive TRH fibers were present in the SCN.

Sexually dimorphic nucleus (SDN; Fig. 1A). A number of parvocellular TRH cells were present in each section through the SDN (Fig. 4). The SDN contained only few TRH fibers as compared with its direct surroundings.

Supraoptic nucleus (SON; Fig. 1A–G). The SON did not contain any TRH immunoreactivity (Fig. 5). However, some small bipolar TRH cells were found just dorsomedially of the rostral part of the SON (Fig. 1a and b).

Bed nucleus of the stria terminalis (BST). The BST did not contain any TRH cells or fibers.

Median eminence. The median eminence contained many TRH fibers (Fig. 6). A number of these fibers outlined the contours of capillary vessels.

Fig. 6. The median eminence of subject 92.071. TRH staining (#8880, 1:4,000). Note the high density of TRH-positive fibers surrounding capillary vessels. Asterisk: blood vessels. Bar = 50 μ m.

Tuberomammillary nucleus (TMN; Fig. 1G–I). The TMN showed dense accumulations of TRH fibers, which were heavily stained (Fig. 7C). Clusters of TRH fibers were seen outlining cell contours, suggesting synaptic endings in the TMN.

Lateral tuberal nucleus (NTL; Fig. 1F, G). The NTL did not contain any TRH cells or fibers.

DISCUSSION

In the present study, the distribution of TRH cells and fibers in the human hypothalamus is described for the first time. The lack of earlier data on the neuroanatomy of TRH cells in the human brain has been attributed to insufficient postmortem stability of the highly soluble peptide (e.g., Kopp et al., 1992). However, remarkable postmortem stability of TRH in human hypothalami was reported between 2.5 and 21 hours postmortem as measured by RIA, while the properties of TRH as tested by high performance liquid chromatography in extracts of human brain tissue appeared to be similar to synthetic TRH (Parker and Porter, 1982). In the present study, we found remarkable differences in staining intensity of TRH-containing cells using different fixatives. In addition, no effect of postmortem delays between 4 and 9 hours was observed (see Table I). Therefore, fixation rather than postmortem stability seems to be the critical factor in antigen preservation of TRH for immunocytochemistry. In this respect, TRH appears to be no different from other neuropeptides in human autopsy material: excellent immunocytochemical staining of VP

cells in the human hypothalamus has been described with postmortem delays of more than 40 hours (Fliers et al., 1985; Swaab et al., 1985).

Another important aspect with regard to immunohistochemical studies of the human brain is agonal state, which has been shown to influence a large number of postmortem parameters in autopsy material. Since the pH of the CSF has been found to reflect the agonal state and not the postmortem delay (Hardy et al., 1985; Ravid et al., 1992), measurement of the CSF pH has been adopted as a routine procedure by the Netherlands Brain Bank. In the present material, there was no difference in staining intensity of TRH cells and fibers according to a high or low pH of the CSF (pH 8.3 and pH 6.1, respectively; see Table I). Therefore, an important effect of agonal state on TRH immunoreactivity seems unlikely.

For the present study, two different rabbit TRH antisera were used, i.e., #4319 and #8880. The first antiserum was used by other groups in a number of immunohistochemical studies in the rat and was shown to result in complete absence of staining after preadsorption with the appropriate antigen (e.g., Arvidsson et al., 1992). In RIA studies, however, antiserum #8880 has been shown to be more specific for TRH than #4319 (see Materials and Methods). Since staining with #8880 was as good as with #4319, it was also used for the present study. Two different preadsorption tests were performed with #8880, both leading to a complete absence of immunostaining in the two subjects studied (93.003 and 93.048). No differences were observed between #4319 and #8880 with respect to the distribution

Figure 7

of TRH-positive cells and fibers, giving support to the specificity of the immunohistochemical localization of TRH.

The anatomical distribution of TRH cells and fibers as found in the present study is in general agreement with the early RIA data on human tissue of Okon and Koch (1976), who showed the highest concentration of TRH to be present in a hypothalamic strip of the posterior portion of the wall of the third ventricle. This strip can be assumed to contain the posterior part of the PVN. Kubek et al. (1979), measuring TRH concentrations in micropunched human hypothalamic areas of sudden death cases, also found high concentrations of this neuropeptide in the PVN. The median eminence contained most of the TRH measured, which can be explained by the presence of a large number of TRH fibers of PVN neurons terminating on the capillaries of the portal system as demonstrated in the present study. Kubek et al. (1979) found hardly any TRH in the SON as measured by RIA, which is also confirmed by our immunocytochemical study. Interestingly, TRH-positive neurons were present dorsomedially of the SON. Borson-Chazot et al. (1986) found intermediate concentrations of TRH as measured by RIA in the PVN and high concentrations in the VM and DM. In the present study, a high density of TRH fibers was observed in the VM, explaining the high concentration of TRH as measured by RIA in this nucleus. The 50 μ m section thickness, however, does not allow for quantitative morphometrical analysis of cell numbers or fiber density because of the probability of incomplete penetration of the antisera.

The neuroanatomical distribution of TRH and fibers in the human hypothalamus was generally in agreement with earlier studies using immunocytochemistry following colchicine pretreatment in the rat brain. Lechan and Jackson (1982) demonstrated medium-sized TRH-positive cells in the SCN and a large number of TRH-positive cells in the parvicellular portion of the PVN in rats after colchicine pretreatment. In the present study, TRH cells were observed in the same nuclei in the human hypothalamus without any pretreatment, although the number of TRH-positive neurons in the SCN was relatively small. A similar species difference in stainability has been described for other neuropeptides. The neuroanatomy of corticotropin-releasing hormone (CRH)-positive cells in the human hypothalamus was recently described using immunocytochemistry (Raadsheer et al., 1993), while in rats colchicine administration or adrenalectomy is required for visualization of CRH-containing cells.

In contrast to the rat brain (Tsuruo et al. 1987, 1988) the human SON contains no TRH-positive perikarya, supporting the absence of cross-reactivity of the antiserum with neurohypophyseal hormones or their precursors. The occasional magnocellular TRH-positive perikarya in the human PVN are therefore no artifact, and may rather suggest colocalization with other neuropeptides such as VP or OXT. In the rat PVN, Meister et al. (1990) demonstrated a small number of both VP- and OXT-containing magnocellular

neurons colocalized with TRH. That this colocalization may have functional implications can be inferred from studies showing effects of intracerebroventricular administration of TRH on neurohypophyseal and hypothalamic VP and OXT content (Ciosek and Guzek, 1992). In addition, VP has been demonstrated to cause a dose-related release of TSH from rat pituitary cells in vitro (Lumpin et al., 1987). In the clinical setting, the combination of hyponatremia and severe hypothyroidism is not uncommon and has been ascribed to inappropriately increased VP secretion (Skowsky and Kikuchi, 1978), also pointing towards functional interaction between the hypothalamo-neurohypophyseal system and thyroid regulation. Therefore, the possible colocalization of TRH and VP in the human PVN will be a subject of further study in our group.

The TRH-containing neurons as described in the present study represent the first identification of a population of neuropeptidergic cells in the human SDN. Striking neuroanatomical sex differences have been reported in the human anterior hypothalamic area (Swaab and Fliers, 1985; Allen et al., 1989; Braak and Braak, 1992), but different nomenclatures are used. The SDN was first reported to be sexually dimorphic by Swaab and Fliers (1985) and was suggested to be homologous with the SDN of the preoptic area (SDN-POA), as originally described by Gorski et al. (1978), on the basis of its localization. The SDN-POA is part of the rat medial preoptic nucleus (MPN), the medial subdivision of which contains TRH-immunoreactive cells (Simerly et al., 1986). Subsequently, Allen et al. (1989) described four nuclei in the human anterior hypothalamus and proposed the term interstitial nuclei of the anterior hypothalamus (INAH 1-4). INAH 1 is the same nucleus as the SDN described by Swaab and Fliers (1985) or the intermediate nucleus (Braak and Braak, 1992). No sex difference in INAH 1 volume was found by Allen et al. (1989), raising doubt on the homology of this nucleus in the human hypothalamus with the SDN-POA in the rat hypothalamus. The conflicting data on the sexual dimorphism of the SDN may, however, be due to the age dependency of the sex differences in this nucleus (for discussion see Swaab et al., 1992). The presence of TRH cells in both the rat MPN and the human SDN supports homology between these nuclei.

In rats, changes occur in hypothalamic-pituitary regulation of TSH during aging. The amplitude of the circadian periodicity of serum TSH decreases (Klug and Adelman, 1979). This might be the result of altered SCN function in senescence, as in both the human (Swaab et al., 1985) and rat SCN (Roozendaal et al., 1987) decreased cell numbers have been found in aged subjects. Although the levels of thyroid hormone decrease in senescence, average TSH concentration remains unchanged, which also suggests changes in hypothalamic TSH regulation (Klug and Adelman, 1979). In man, decreased serum concentrations of triiodothyronine (T₃) have been reported in senescence (Petersen et al., 1991). This might, however, be the reflection of diseases associated with old age, rather than being a manifestation of the aging process itself (cf. Loosen, 1992). In the present study, no young subjects were included. In the small age range studied (67–92 years), no clear age differences in TRH cell number or staining intensity were observed.

The diurnal pattern of serum TSH levels in the rat has been demonstrated to depend on season (Wong et al., 1983). Marked seasonal variations in volume and VP cell number in the human SCN have recently been found. Such seasonal

Fig. 7. TRH-positive fibers in the human hypothalamus. **A:** Dorsal part of the perifornical area of subject 93.047 (#8880, 1:4,000). Note very high TRH fiber density. Arrowheads point to varicosities around blood vessel. Asterisk: blood vessel. **B:** Detail from the VM of subject 93.047 (#8880, 1:4,000). Arrowhead points to TRH-negative cell body directly surrounded by TRH-positive beaded fibers, suggesting nerve endings. **C:** Detail from the TMN of subject 93.005 (#8880, 1:4,000). Typical cluster of heavily stained TRH-positive fibers. Bars = 100 μ m for A; 50 μ m for B; 250 μ m for C.

variation was not present in the human PVN (Hofman and Swaab, 1992). In the present study, no effect of season of death could be detected. However, TRH cell numbers and TRH fiber density were not quantified, leaving open the possibility of subtle seasonal variation.

In two subjects (93.003 and 93.004), staining intensity of TRH cells throughout the hypothalamus was lower as compared with the other subjects studied. In both cases, the diagnosis was Alzheimer's disease. Thyroid disease has been reported to be a possible risk factor for Alzheimer's disease (Heyman et al., 1984; Breteler et al., 1991; Landin et al., 1993), although other studies have not confirmed this association (Broe et al., 1990). The present study suggests that Alzheimer's disease itself might lead to alterations in TRH cell function, resulting in altered thyroid regulation. It should be noted, however, that altered hypothalamic regulation of TSH secretion might also be secondary to diseases or conditions associated with Alzheimer's disease, such as cachexia, rather than being a consequence of Alzheimer's disease per se. In this respect it is of interest that Romijn et al. (1990) demonstrated decreased T3 concentrations and a decreased nocturnal TSH surge during fasting. Rondeel et al. (1992) studied the effects of starvation in rats and found decreased hypothalamic TRH release, probably leading to decreased thyroid function.

Future studies will be aimed at the assessment of TRH cell activity under various clinical conditions. In particular the relationship between altered TRH cell function and Alzheimer's disease will be the subject of further investigation.

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