Histochemistry (1987) 88:1–10

Histochemistry
© Springer-Verlag 1987

Hypophysiotrophic thyrotropin releasing hormone (TRH) synthesizing neurons*

Ultrastructure, adrenergic innervation and putative transmitter action

Zs. Liposits[1], W.K. Paull[1], P. Wu[2], I.M.D. Jackson[2], and R.M. Lechan[3]**

[1] Department of Anatomy, University of Missouri-Columbia, M314, Columbia, MO 65212, USA
[2] Division of Endocrinology, Brown University, Rhode Island Hospital, Providence, RI 02902, USA
[3] Department of Medicine, Division of Endocrinology, New England Medical Center and Hospitals, Boston, MA 02111, USA

Accepted July 29, 1987

Summary. The neuropeptide thyrotropin releasing hormone (TRH) is capable of influencing both neuronal mechanisms in the brain and the activity of the pituitary-thyroid endocrine axis. By the use of immunocytochemical techniques, first the ultrastructural features of TRH-immunoreactive (IR) perikarya and neuronal processes were studied, and then the relationship between TRH-IR neuronal elements and dopamine-β-hydroxylase (DBH) or phenylethanolamine-*N*-methyltransferase (PNMT)-IR catecholaminergic axons was analyzed in the parvocellular subnuclei of the hypothalamic paraventricular nucleus (PVN). In control animals, only TRH-IR axons were detected and some of them seemed to follow the contour of immunonegative neurons. Colchicine treatment resulted in the appearance of TRH-IR material in parvocellular neurons of the PVN. At the ultrastructural level, immunolabel was associated with rough endoplasmic reticulum, free ribosomes and neurosecretory granules. Non-labelled axons formed synaptic specializations with both dendrites and perikarya of the TRH-synthesizing neurons. TRH-IR axons located in the parvocellular units of the PVN exhibited numerous intensely labelled dense-core and fewer small electron lucent vesicles. These axons were frequently observed to terminate on parvocellular neurons, forming both bouton- and en passant-type connections. The simultaneous light microscopic localization of DBH or PNMT-IR axons and TRH-synthesizing neurons demonstrated that catecholaminergic fibers established contacts with the dendrites and cell bodies of TRH-IR neurons. Ultrastructural analysis revealed the formation of asymmetric axo-somatic and axo-dendritic synaptic specializations between PNMT-immunopositive, adrenergic axons and TRH-IR neurons in the periventricular and medial parvocellular subnuclei of the PVN.

These morphological data indicate that the hypophysiotrophic, thyrotropin releasing hormone synthesizing neurons of the PVN are directly influenced by the central epinephrine system and that TRH may act as a neurotransmitter or neuromodulator upon other paraventricular neurons.

* Supported by NIH research grants NS19266 and DK34540
** Reprint requests should be sent to: Dr. Zsolt Liposits, Department of Anatomy, University Medical School, H-7643 Pécs, Szigeti u. 12, Hungary

Introduction

The tripeptide, thyrotropin-releasing hormone (TRH) (Burgus et al. 1969; Schally et al. 1969) is widely distributed within the brain and spinal cord as determined by radioimmunoassay (Jeffcoate et al. 1973; Jackson and Reichlin 1974; Winokur and Utiger 1974; Brownstein et al. 1974) and immunocytochemical techniques (Hökfelt et al. 1975a, b; Johansson et al. 1980; Lechan and Jackson 1982; Lechan et al. 1983, 1984; Ishikawa et al. 1984). From among TRH-synthesizing neurons, however, only those establish connections with capillaries of the pituitary-portal system that reside in the parvocellular subdivisions of the hypothalamic paraventricular nucleus (PVN) (Greer 1957; Martin and Reichlin 1972; Jackson and Reichlin 1979; Aizawa and Greer 1981; Lechan and Jackson 1982; Lechan et al. 1983).

Despite the importance of TRH-synthesizing neurons of the PVN, little information is known about their intranuclear organization (Johansson et al. 1980; Hisano et al. 1986) and regulation by afferent systems (for reviews see: Krulich 1982; Reichlin 1986; Jackson and Lechan 1987). Although there is evidence for a direct alpha-adrenergic regulation of hypothalamic TRH secretion (Montoya et al. 1979) the effect of catecholamines upon TRH release remains contradictory (Grimm and Reichlin 1973; Schaeffer et al. 1977; Hirooka et al. 1978; Joseph-Bravo et al. 1979; Maeda and Frohman 1980; Chen and Ramirez 1981). There is morphologic evidence for catecholaminergic innervation of TRH-containing neurons of the PVN (Shioda et al. 1986), however, it is uncertain whether it is derived from noradrenergic or adrenergic sources.

To further characterize TRH-IR neurons of the PVN and clarify the nature of their catecholaminergic innervation, we have performed single and double immunocytochemical labellings on hypothalamic sections using antisera against TRH and catecholamine-synthesizing enzymes, dopamine-β-hydroxylase (DBH) and phenylethanolamine-*N*-methyltransferase (PNMT). A preliminary report of these findings has appeared in abstract forms (Liposits et al. 1987a, b).

Materials and methods

Animals

This study was carried out on intact (No.: 4) and colchicine pretreated (No.: 25) male Wistar rats. In the latter group, the animals

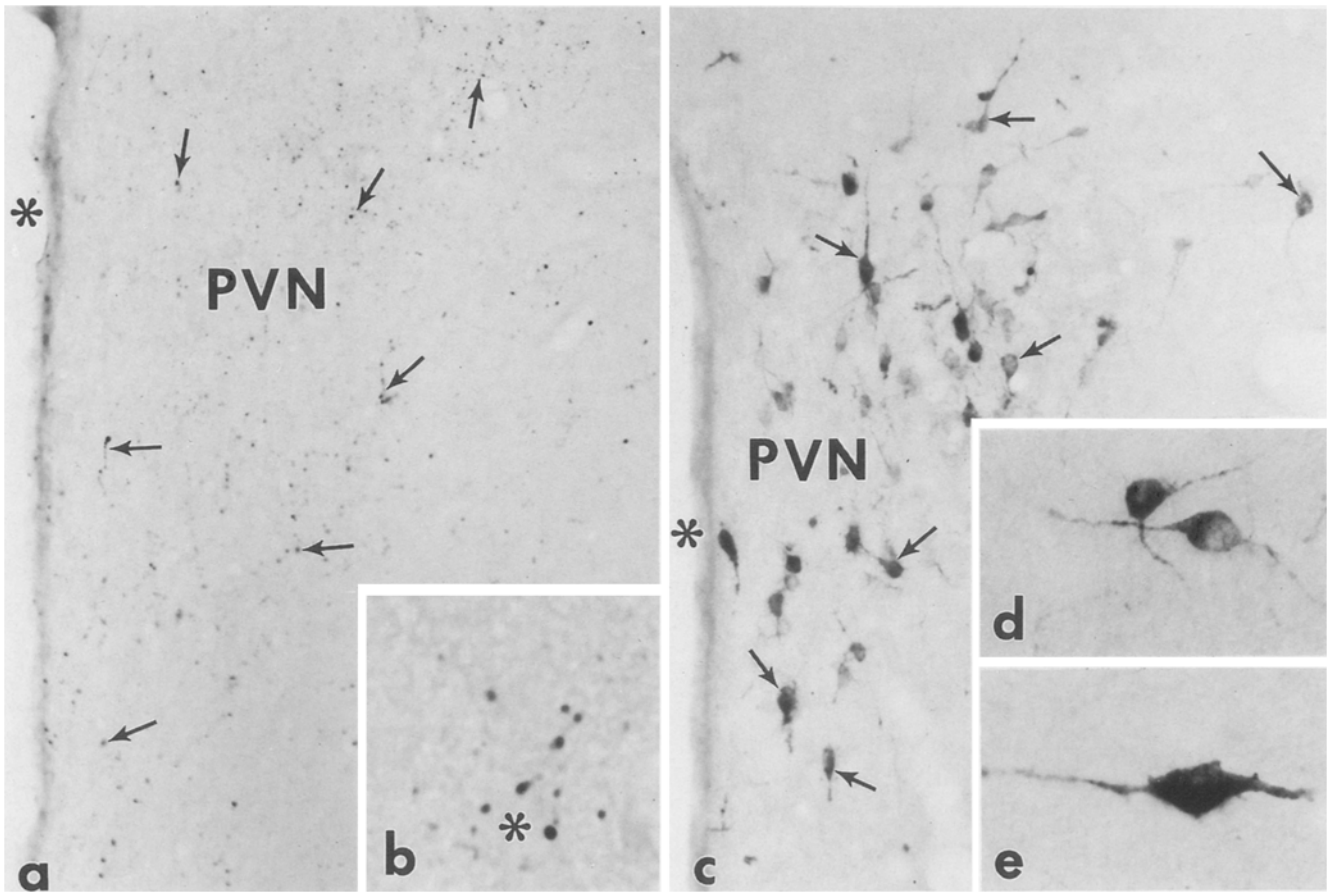


Fig. 1a–e. TRH-immunoreactive neuronal structures located in the hypothalamic paraventricular nucleus (*PVN*). *: third ventricle. **a and b** intact animal. **c–e** colchicine treated rat. **a** Varicose TRH-IR axons (*arrows*) distributed in the paraventricular nucleus. ×140. **b** TRH-IR axon varicosities outlining the border of a non-labelled parvocellular neuron (*). ×630. **c** Parvocellular neurons, expressing TRH-immunoreactivity (*arrows*) in the medial part of the PVN. ×140. **d** Two TRH-immunoreactive neurons exhibiting smooth surface contour. ×280. **e** Rough contoured TRH-synthesizing neuron with somatic and dendritic appendages. ×420

received a single dose of colchicine (80 μg/100 g b.w. dissolved in 5 μl of saline), injected into the central part of the lateral cerebral ventricle, under Nembutal anesthesia (40 mg/kg; i.p.). The colchicine treated animals were allowed to survive for 30–36 h.

Fixation and section preparation

Re-anesthesized (Nembutal) animals were perfused through the ascending aorta first with 80–100 ml of 0.1 *M* phosphate buffered saline (PBS: pH 7.4), and then with 350–500 ml of fixative solution. Two types of fixative were used with similar success. One of these was prepared according to the original formula of Zamboni and DeMartino (1967) and 0.02% glutaraldehyde was added to the final solution. The other fixative was a 4% paraformaldehyde solution, used at variable pH (6.5 and 8.6) according to the concept of Berod et al. (1981). The satisfactory visualization of TRH antigen required the addition of glutaraldehyde at a concentration of 0.02% to the pH 8.6 component of the fixative. Using either of these fixatives, as a final step, the vasculature of the brains was perfused with the high pH fixative solution but in the absence of glutaraldehyde. The same glutaraldehyde-free solutions were used for overnight post-fixation of the brains. Thereafter, the brains were removed from the skull and coronal, 40 μm thick sections were cut on a Lancer vibratome.

Immunocytochemical labelling

Detection of single tissue antigens. Preembedding immunocytochemical techniques were used for the visualization of thyrotropin releasing hormone (TRH), dopamine-β-hydroxylase (DBH) and phenylethanolamine-N-methyltransferase (PNMT) antigens in the paraventricular nucleus of the hypothalamus. Both the traditional peroxidase-antiperoxidase complex (PAP)-3,3′-diaminobenzidine (DAB) technique of Sternberger et al. (1970) and a modification of this procedure (Liposits et al. 1984), which amplifies the final DAB-reaction product by silver-gold intensification (Gallyas 1982) were employed. All primary antibodies used in this study were generated in rabbits. The generation and characterization of anti-TRH sera have been previously published (Jackson and Reichlin 1974; Lechan and Jackson 1982). The anti-TRH serum #31 was used at a 1:2000 working dilution. The marker sera for the central noradrenergic and adrenergic systems were generated against DBH and PNMT, purified from bovine adrenal medulla (Eugene Tech Int. Inc., Allendale, NJ) and units of these commercial antibodies were diluted in 10 ml of PBS, containing 1% normal goat serum and 0.1% sodium azide. The sections were kept in the primary antibodies for 36–48 h and for 1–1$^1/_2$ h in both the bridging antibody (goat, anti-rabbit IgG, 1:100, Antibodies Incorporated, Davis, CA) and PAP-complex (1:100, Sternberger-Meyer Immunocytochemicals, Jarrettsville, MD). The antigen-antibody sequences were visualized according to the method of Streit and Reubi (1977). The laboratory protocol of the silver-gold intensification has previously been published (Liposits et al. 1984).

Sequential detection of two tissue antigens. In order to elucidate the relationship of DBH and PNMT-IR axons *with* TRH-synthesizing neurons, DBH or PNMT antigens were simultaneously localized with TRH-immunoreactive sites in the PVN, using a recently

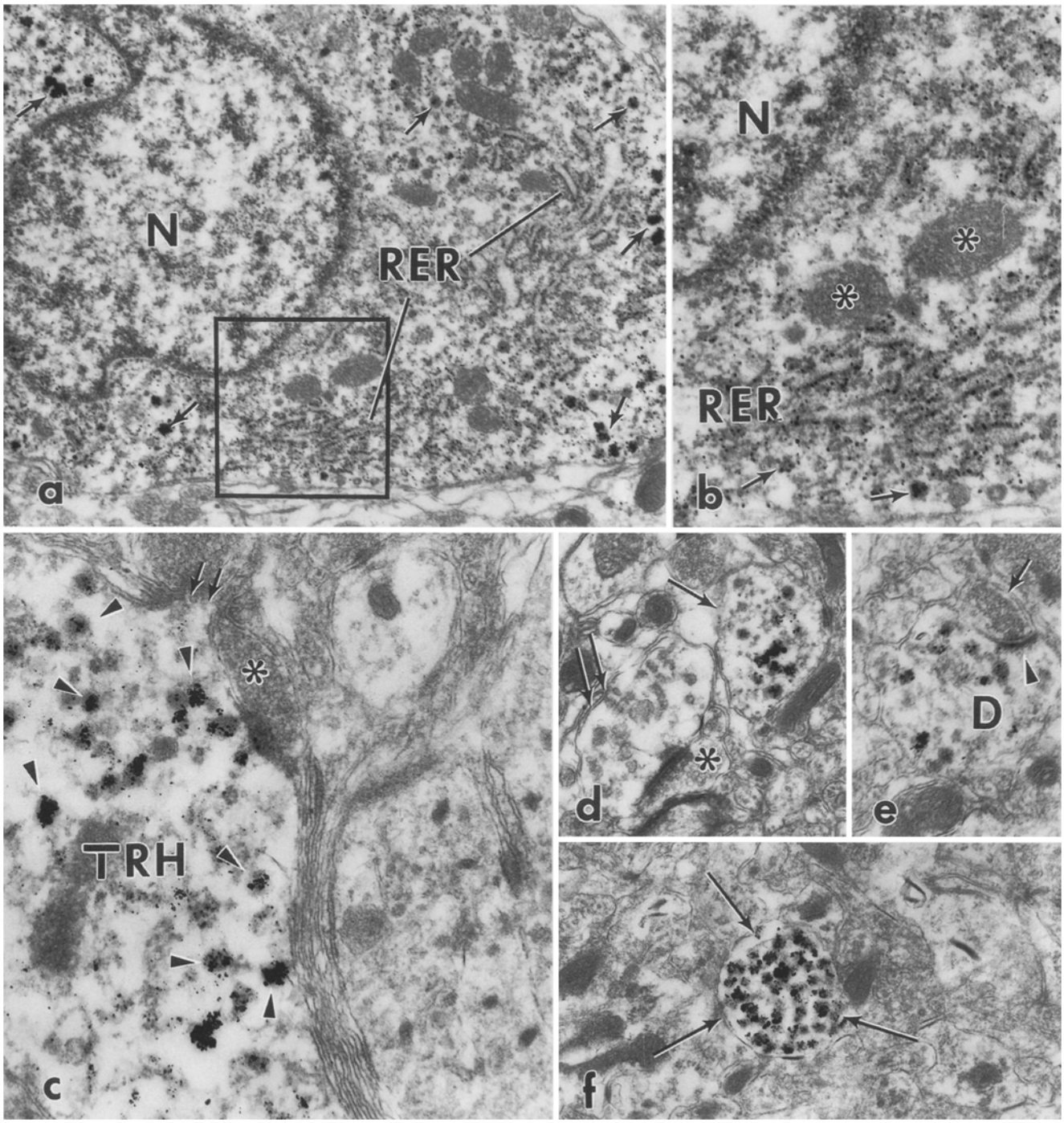


Fig. 2a–f. Ultrastructural detection of TRH-immunoreactivity in perikarya (**a–c**) and processes (**d–f**) of parvocellular neurons obtained from the periventricular and medial parvocellular subnuclei of the PVN. Colchicine treated animal, silver-gold postintensified DAB-chromogen. **a** TRH-synthesizing perikaryon characterized by indented nucleus (*N*), immunolabelled neurosecretory granules (*arrows*), free and membrane-bound (*RER*) ribosomes. ×15500. **b** High power micrograph of the enframed area shown in a. In contrast to the mitonchrondria (*) and nucleus (*N*), the rough endoplasmic reticulum (*RER*) and the neurosecretory granules (*arrows*) are intensely labelled by metal particles. ×39000. **c** Marginal cytoplasmic zone of a TRH-IR neuron (*TRH*) containing many labelled secretory granules (*arrowheads*). A non-labelled axon terminal (*) establishes a synaptic specialization with the cell. Double arrows point to a somatic appendage. ×38500. **d** TRH-immunoreactive dendrite (*arrow*) in the vicinity of an immunonegative dendritic process (*double arrows*), which receives a synapsing axon terminal (*). ×25500. **e** An axon terminal (*arrow*) forming synaptic connection (*arrowhead*) with a TRH-containing dendrite (*D*). Note the labelled neurosecretory granules in the dendrite. ×40000. **f** Cross sectioned axon (*arrows*) filled with numerous TRH-immunopositive neurosecretory granules. ×25000

developed double labelling technique (Liposits et al. 1983a, 1986b). First, DBH or PNMT-IR axons were detected by the black silver-gold intensified (SGI)-DAB-endproduct. Thereafter, TRH-containing neuronal elements were identified by the natural brown DAB chromogen. The high contrast of color and electron density between the chromogens allows the distinctive separation of neuronal structures that express different immunoreactivities at both the light and electron microscopic levels.

Some of the immunostained sections were mounted on glass slides for light microscopic evaluation while the majority of them were processed for flat embedding in epon resin. The ultrathin sections were placed on Formvar-coated single-slot grids contrasted with uranyl acetate and Reynolds' lead citrate and examined with a Zeiss EM-10 CR transmission electron microscope.

Results

Thyrotropin releasing hormone (TRH)-immunoreactive elements of the hypothalamic paraventricular nucleus

Light microscopy. In control animals, TRH-immunoreactivity was found exclusively in varicose axons and their terminals. Labelled fibers were located in the periventricular and medial parvocellular subnuclei of the PVN (Fig. 1a). TRH-containing axon varicosities were noted to be associated with non-labelled parvocellular neurons (Fig. 1b), in addition, to TRH-IR fibers leaving or entering the paraventricular nucleus. As a result of colchicine treatment, the TRH-immunoreactive material became detectable in dendrites and parvocellular perikarya of the PVN (Fig. 1c). Both fusiform and multipolar neurons demonstrated TRH immunoreactivity. The TRH-synthesizing neurons exhibited either a smooth (Fig. 1d) or a rough surface contour (Fig. 1e) due to the presence of dendritic and somatic appendages. The cytoplasm of the immunolabelled cells was densely packed with neurosecretory granules.

Electron microscopy. Silver-gold intensified diaminobenzidine chromogen was used to demonstrate the TRH-immunoreactivity at the ultrastructural level. TRH-synthesizing parvocellular neurons showed a very intense immunolabelling. The metallic grains were deposited in neurosecretory granules (Fig. 2a–c) and were also seen in association with free ribosomes and the rough endoplasmic reticulum system (Fig. 2a and b). The cell nuclei, mitochondria and cisternae of the Golgi complex were immunonegative. Dendrites of TRH-IR neurons contained microtubules and intensely stained neurosecretory granules (Fig. 2d and e). Both perikarya (Fig. 2c) and dendrites (Fig. 2e) of the TRH-synthesizing neurons received immunonegative synapsing axon terminals.

Numerous immunolabelled neurosecretory granules were located in TRH-IR axons (Figs. 2f and 3) and, as suggested by the light microscopic observations, they formed bouton terminale (Fig. 3b and c) and en passant-type (Fig. 3e) synapses with paraventricular neurons. Axo-somatic (Fig. 3b) and axo-dendritic (Fig. 3c, d, e) communications of the asymmetric type were observed. Within the terminating TRH-IR axons, electron dense neurosecretory granules (75–90 nm in diameter) and small electron lucent vesicles (30–40 nm in diameter) demonstrating a fine immunolabelling were seen (Fig. 3b, c, d, f).

In some instances, the structural preservation of the immunolabelled tissue allowed a high resolution analysis of the TRH-IR synapse. Occasionally TRH-immunopositive neurosecretory granules accumulated around the presynaptic membrane specialization (Fig. 3e and f) and the presynaptic dense material contained TRH-immunoreactive synaptic vesicles (Fig. 3f and g). In one instance, (Fig. 3g) the immunolabelled vesicle (30 nm in diameter) was attached to the inner face of the presynaptic membrane. The synaptic cleft was 20 nm wide and contained a dense intercellular material. The postsynaptic membrane also demonstrated a prominent density.

Light microscopic indications for catecholaminergic innervation of TRH-synthesizing neurons

The periventricular and medial parvocellular subnuclei of the PVN, received a very intense noradrenergic and adrenergic innervation, as visualized using antisea against dopamine-β-hydroxylase (DBH) (Fig. 4a) and phenylethanolamine-*N*-methyltransferase (PNMT) (Fig. 4b). The simultaneous light microscopic localization of these catecholamine-synthesizing enzymes and TRH revealed a congruency of the immunoreactive neuronal elements. In vibratome sections, brown-colored TRH-synthesizing cells were located among black, DBH (Fig. 4c) and PNMT (Fig. 4d) containing axons. At high magnification, both DBH-IR fibers (Fig. 5a) and PNMT-containing axons (Fig. 5b) appeared to form multiple contacts with TRH-producing neurons. Since dopamine-β-hydroxylase occurs in both noradrenergic and adrenergic axons, and PNMT is predominantly present in adrenergic fibers, we used the anti-PNMT serum to visualize the adrenergic system and to address the possibility of an interaction of the central epinephrine system with the TRH-IR cell populations of the PVN.

Semithin sections (1 μm) demonstrated more convincingly (Fig. 5c) that PNMT-IR axons and TRH-synthesizing neurons had a similar distribution pattern within the medial part of the PVN. Adrenergic fibers contacted the cell bodies (Fig. 5d) and dendrites (Fig. 5e) of TRH-IR neurons.

Synaptic interaction between adrenergic, PNMT-IR axons and TRH-IR neurons

At the ultrastructural level, PNMT-IR fibers were recognized by the presence of silver-gold grains in their axoplasm. The natural, non-intensified DAB-endproduct ensured the identification of TRH-containing neurons. Because of the different properties of the chromogens used, the PNMT- and TRH-containing profiles were easily distinguishable (Fig. 6b and c). Adrenergic fibers formed asymmetric synapses (Fig. 6a and b) with non-labelled paraventricular neurons and were also found in juxtaposition to TRH-IR cells (Fig. 6d). The detection of these chemically identified, apposed neuronal elements in series of ultrathin sections revealed that they are interconnected via synapses. Adrenergic, PNMT-IR axons were noted to form asymmetric synapses on both the cell bodies (Fig. 6d, e, f) and dendritic processes (Fig. 6g) of TRH-synthesizing neurons.

Discussion

In this study, the location of TRH-synthesizing neurons in the PVN, one of the "hypophysiotrophic" regions of the hypothalamus (Greer 1957; Aizawa and Greer 1981) is in agreement with data from previous immunocytochemical studies (Lechan and Jackson 1982; Lechan et al. 1983; Nishiyama et al. 1985). Morphologically, two types of neurons were observed, including cells with smooth surface contour and those exhibiting somatic and dendritic appendages. The latter is also characteristic of other hypothalamic neurons synthesizing luteinizing hormone-releasing hormone (LHRH) (Krisch 1980; Liposits et al. 1984) and corticotropin releasing factor (CRF) (Liposits et al. 1983c). At

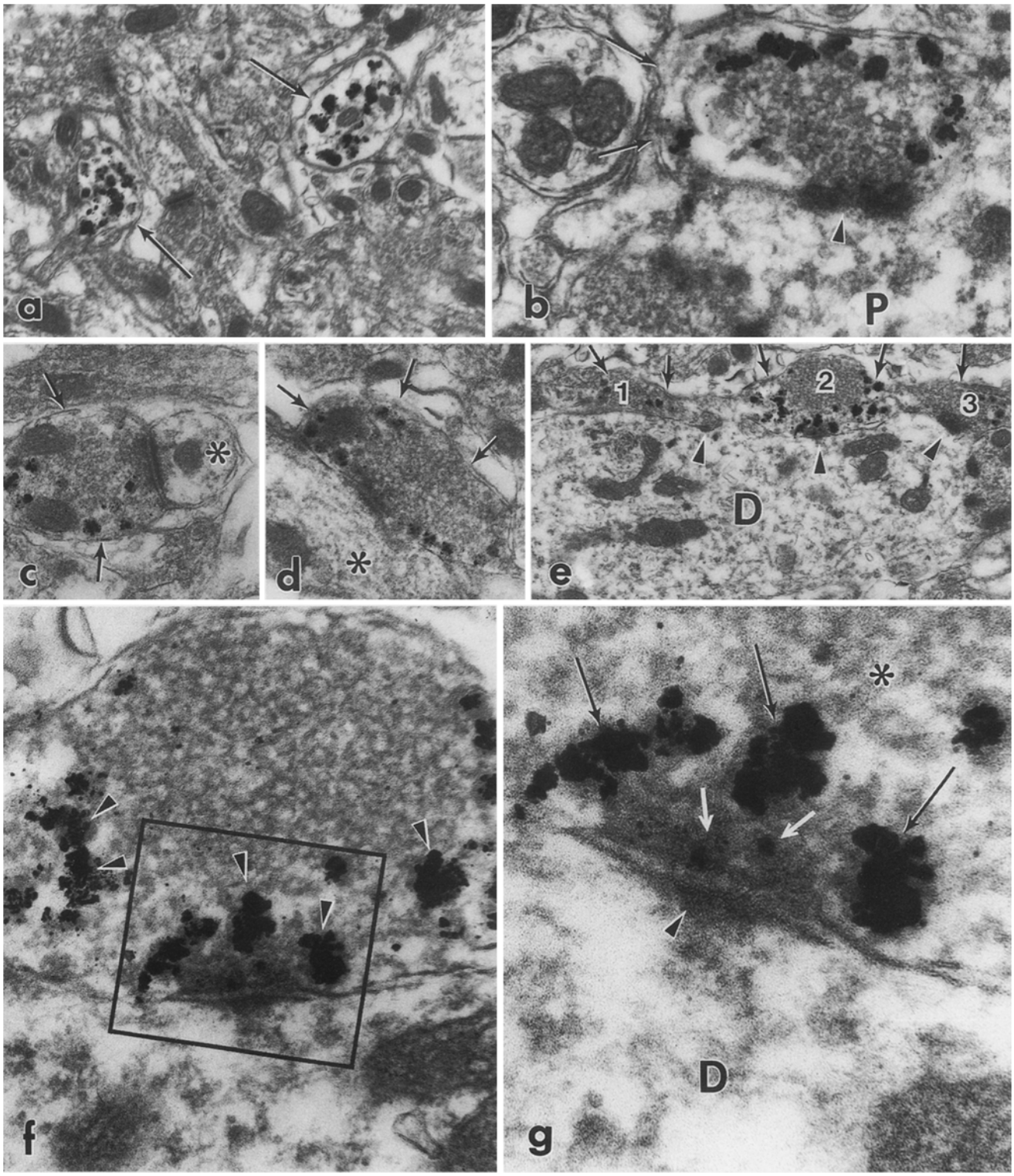
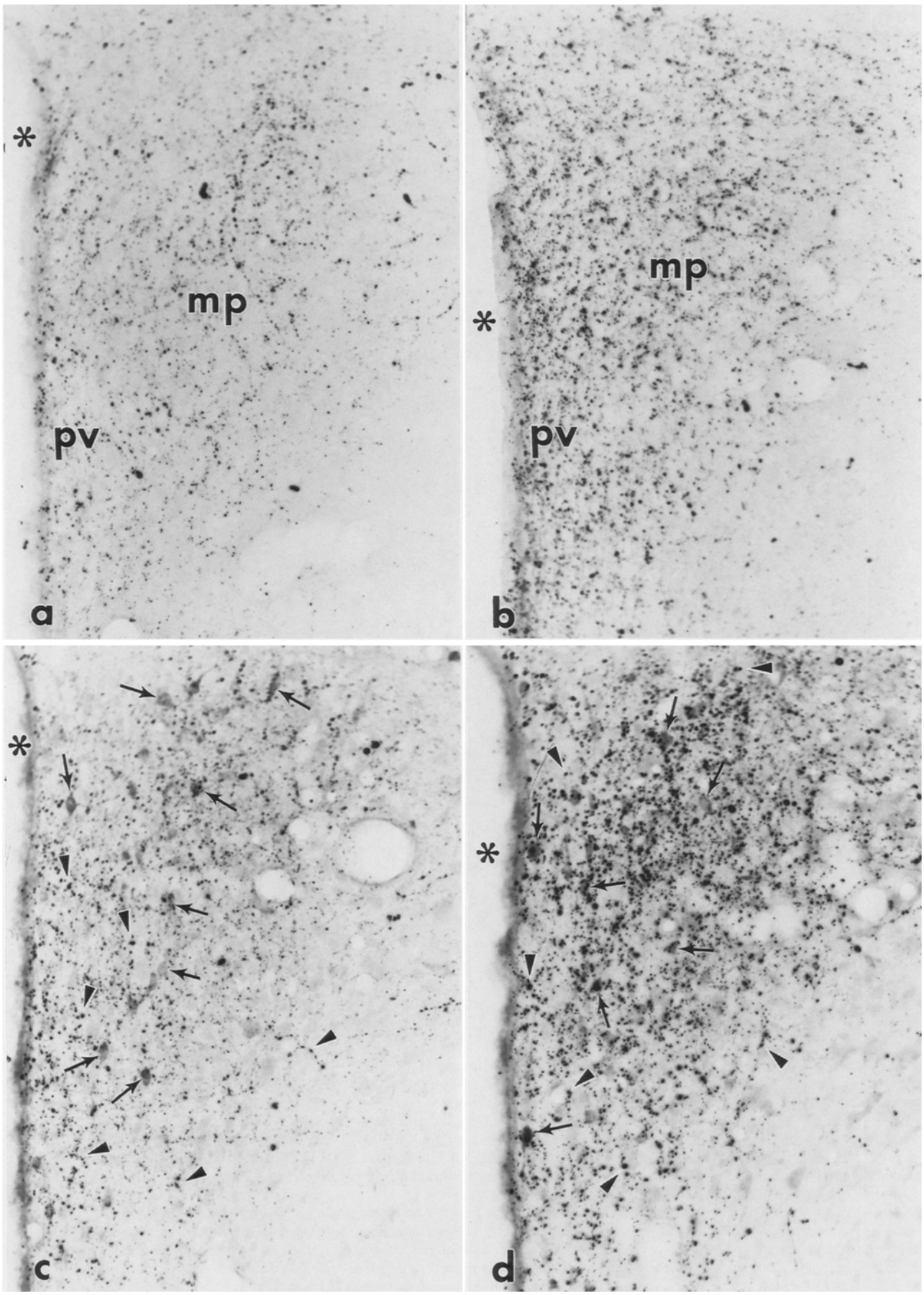


Fig. 3a–g. Ultrastructural features indicative of a neurotransmitter action of TRH upon neurons of the PVN. Silver-gold postintensification. **a** Cross-sectioned TRH-IR neuronal process (*arrows*) located within the medial parvocellular subnucleus. There are no signs of synaptic communication. × 25000. **b** TRH-immunoreactive axon (*arrows*) establishes synaptic specialization (*arrowhead*) with a parvocellular perikaryon (*P*). The neurosecretory granules are heavily labelled. Fine metallic grains are also present over the small electron lucent vesicles. × 54000. **c and d** TRH-containing axons (*arrows*) synapse on non-labelled dendrites (*). $\times_c$39000; $\times_d$39000. **e** Longitudinally sectioned, TRH-IR axon (*arrows*) is juxtaposed to a non-labelled dendrite (*D*). The labelled axon establishes three en passant-type synapses (*arrowheads*). Note also that the axon demonstrates three varicosities (*1,2,3*). × 19000. **f** High power micrograph of the axodendritic synapse established by varicosity No. 2 of the TRH axon, shown in e. The majority of the immunolabel is in neurosecretory granules (*arrowheads*). A delicate labelling is also associated with some of the electron lucent vesicles. × 100000. **g** Micrograph showing the enframed area in f at higher magnification. The asymmetric synaptic specialization (*arrowhead*) found between the TRH-IR axon (*) and the dendritic process (*D*) is in the center of the micrograph. Note that both the pre- and postsynaptic membranes are well preserved. The presynaptic active zone is surrounded by three neurosecretory granules (*large arrows*). Two immunolabelled vesicles (*small arrows*) of 30 nm diameter can be observed in the pre-synaptic dense material. The lower vesicle on the left side is attached to the inner face of the presynaptic membrane. × 200000


*
mp
pv
a
mp
*
pv
b
*
c
*
d

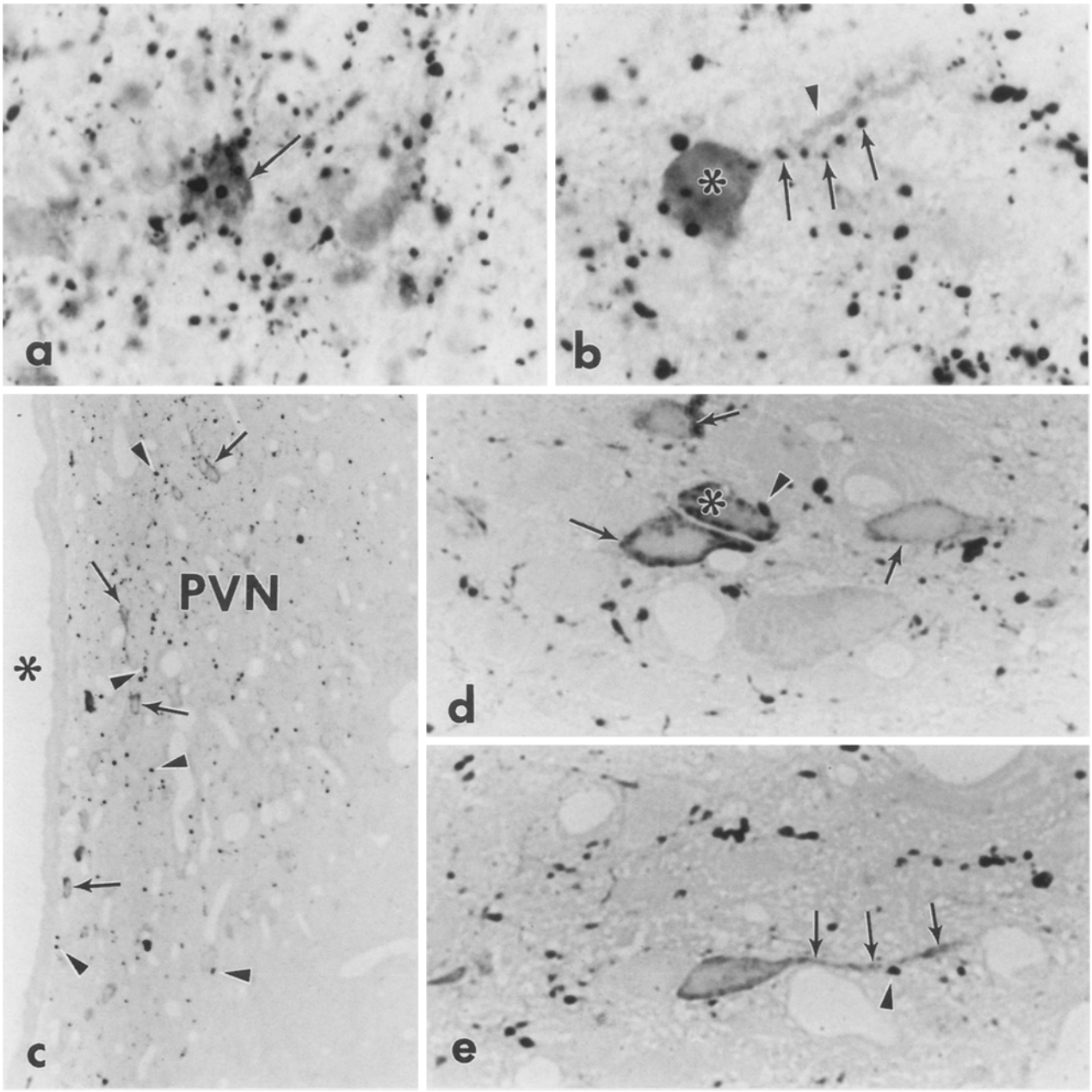


Fig. 5a–e. High power light microscopic demonstration of contacts between catecholaminergic axons and TRH-IR neurons in 40 μm thick vibratome (**a and b**) and semithin (**c–e**) sections of the paraventricular nucleus. Dual antigen detection. **a** TRH-synthesizing neuron (*arrow*) heavily contacted by DBH-IR axon varicosities. × 700. **b** PNMT-IR axons apposed to the cell body (*) of a TRH-producing neuron. A PNMT-IR axon (*arrows*) runs parallel to the dendritic process (*arrowhead*) of the immunolabelled neuron. × 700. **c** Simultaneous localization of PNMT-IR axons (*arrowheads*) and TRH-synthesizing neurons (*arrows*) in a 1 μm thick plastic section. *: third ventricle. × 140. **d** One (*) of the TRH-synthesizing perikarya (*arrows*) is contacted by a PNMT-IR axon (*arrowhead*). × 700. **e** PNMT-IR axon varicosity (*arrowhead*) is juxtaposed to the dendrite (*arrows*) of a longitudinally sectioned TRH-synthesizing neuron. × 700

◀ **Fig. 4a–c.** Distribution of catecholaminergic fibers within the paraventricular nucleus (**a and b**) and their relationship to TRH-synthesizing neurons (**c and d**). *: third ventricle. **a and b** Dopamine-β-hydroxylase (DBH) immunoreactive (**a**) and phenylethanolamine-N-methyltransferase (PNMT)-containing (**b**) axons innervating the periventricular (*pv*) and medial parvocellular (*mp*) subnuclei of the PVN. $\times_a 140$; $\times_b 140$. **c** Simultaneous detection of DBH-IR axons (*arrowheads*) and TRH-synthesizing neurons (*arrows*) in the hypophysiotrophic areas of the PVN. Note the congruency of the systems. × 140. **d** TRH-IR neurons (*arrows*) located in the "bed" of PNMT-containing, adrenergic axon terminals (*arrowheads*) within the PVN. Even at this low magnification, it is possible to observe that most of the TRH cells are surrounded by PNMT-IR axon varicosities. × 140

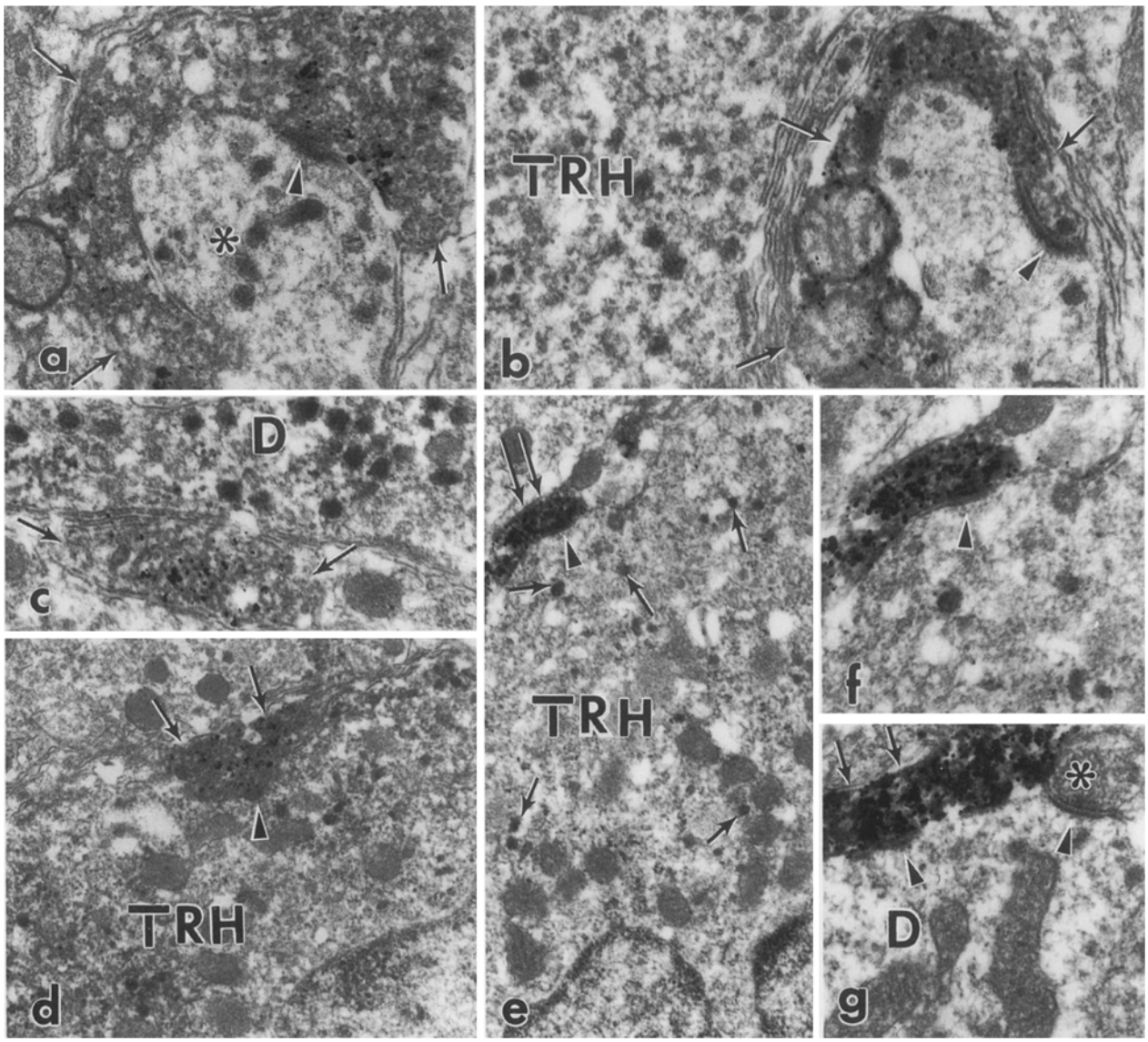


Fig. 6a–g. Simultaneous localization of PNMT-IR and TRH-containing structures in the PVN at the ultrastructural level and demonstration of synaptic specializations between the structures. **a** PNMT-IR axon (*arrows*) synapses (*arrowhead*) on a non-labelled profile (*). ×46000. **b** A terminating (*arrowhead*) PNMT-IR axon (*arrows*) in the vicinity of a TRH-IR perikaryon (*TRH*). ×47000. **c** PNMT-IR axon (*arrows*), labelled by metallic grains, in the vicinity of a TRH-IR dendrite (*D*) that is identified by the presence of diaminobenzidine chromogen. ×37500. **d** PNMT-IR axon (*arrows*) forming a synapse (*arrowhead*) with a TRH-synthesizing perikaryon (*TRH*). ×24000. **e** Axo-somatic synapse (*arrowhead*) established between an adrenergic, PNMT-IR axon (*double arrow*) and a TRH-producing parvocellular neuron (*TRH*). Note the presence of many neurosecretory granules (*arrows*) in the cytoplasm of the labelled cell. ×24000. **f** High power micrograph of the synapsing profiles shown in e. Note the asymmetric character of the synapse (*arrowhead*) and the different appearance of the pre- and postsynaptic immunolabels. ×47000. **g** TRH-IR dendrite (*D*) receives both adrenergic, PNMT-immunopositive (*arrows*) and non-labelled (*) axon terminals. *Arrowheads* indicate the synaptic specializations. ×40000

the ultrastructural level the TRH synthesizing neurons demonstrated the general morphological features of peptidergic hypophysiotrophic hormone producing cells of the diencephalon (Krisch 1980; King and Anthony 1983; Liposits et al. 1983b; Beauvillain et al. 1987). As a special characteristic, however, TRH-IR neurons contain numerous intensely labelled neurosecretory granules. This contrasts with LH-RH, CRF and growth hormone-releasing factor (GRF) producing neurons, in which immunolabelled granules predominate in axons and their terminals following colchicine administration.

The intense immunolabelling of neurosecretory granules supports the assumption that processing of pro-TRH occurs primarily in the cell bodies of the neurons and the mature TRH is then transported to axons and terminals (Jackson et al. 1985; Lechan et al. 1986a, b), since the TRH antiserum used in this study recognizes only the fully processed tripeptide-amide (Jackson and Reichlin 1974) and not any portion of its precursor molecule (Wu et al. 1987). In addition to large, granular vesicles, however, reaction product was also seen in association with ribosomal elements of TRH-IR cells. The explanation for this labelling will require further studies but could indicate an alternate mechanism for pro-TRH processing in the endoplasmic reticulum.

TRH-IR neurons of the PVN were observed to establish

axo-dendritic and axo-somatic synapses with non-labelled axons, a finding in agreement with a recent report of Hisano et al. (1986). The synaptic specializations observed in our material, however, were mainly asymmetric. Conversely, TRH-IR granules and moderately-staining small vesicles were also observed in axon terminals presynaptic to other, non-labelled paraventricular neurons. The presence of some immunopositive vesicles embedded in the presynaptic dense material and attached to the inner face of the presynaptic membrane indicates that TRH may be secreted into the synaptic cleft and function as a neurotransmitter in the PVN. The origin of these terminals is unknown, although they may arise from the PVN, itself through axon collaterals (van den Pol 1982). This raises the possibility that some TRH-IR neurons may serve a dual function by modulating neuronal activity of certain paraventricular neurons in addition to regulating TSH secretion from the anterior-pituitary gland.

A number of studies have indicated a role for catecholamines in the regulation of the hypothalamo-hypophysial-thyroid axis, with actions both at the hypothalamic and pituitary levels (for reviews see Montoya et al. 1979; Krulich 1982; Jackson and Lechan 1987). Interruptions of noradrenergic neurotransmission causes a decrease of serum TSH levels and blocks cold-induced stimulation of TSH secretion (Krulich 1982). These data support a stimulatory role for norepinephrine on the hypothalamic TRH-synthesizing system and is consistent with ultrastructural studies showing synaptic specialization of 5-OHDA and ^{3}H-NA-labelled axons on TRH-IR neurons (Shioda et al. 1986). Catecholaminergic neurons which synthesize epinephrine also contain DBH and can accumulate ^{3}H-NA and incorporate 5-OHDA. Therefore, we studied the synaptic association of axon terminals containing PNMT, a marker specific for epinephrine, to TRH-IR neurons of the PVN. These studies showed PNMT-containing axons synapse on both dendrites and somata of TRH-synthesizing neurons, supporting a role for epinephrine in the neurotransmitter regulation of TRH-IR neurons. This is in keeping with observations by Terry (1986), suggesting a stimulatory role for epinephrine in the regulation of thyrotropin secretion. It is likely, however, that epinephrine may have several sites of action for the regulation of anterior pituitary TSH secretion, including the PVN, median eminence and anterior pituitary. This is based on preliminary evidence of the close proximity of TRH-IR fibers to PNMT-containing axons in the median eminence (personal observation) and previous reports that epinephrine causes a significant increase in TSH from anterior-pituitary cell cultures (Klibanski et al. 1983) and has a synergistic action with TRH on inducing TSH secretion (Dieguez et al. 1984).

The adrenergic innervation of TRH-producing neurons is not a unique phenomenon in the hypothalamus. We have recently reported an intense PNMT-immunoreactive innervation of corticotropin releasing factor synthesizing neurons of the PVN (Liposits et al. 1986a). Our latest findings indicate that the adrenergic innervation of hypophysiotropic neurons seems to be a general phenomenon, observed for LH-RH, somatostatin and GFR-synthesizing neurons of the diencephalon (Liposits et al. 1987a).

Acknowledgements. The authors wish to express their appreciation to D. Sherman and J. Lipscomb for technical assistance and to C. Swanson for typing the manuscript.

References

Aizawa T, Greer MA (1981) Delineation of the hypothalamic area controlling thyrotropin secretion in the rat. Endocrinology 109:1731–1738

Beauvillain JC, Tramu G, Mazzuca M (1987) Fine structural studies of growth-hormone-releasing factor (GRF)-immunoreactive neurons and their synaptic connections in the guinea pig arcuate nucleus. J Comp Neurol 255:110–123

Berod A, Hartman BK, Pujol JF (1981) Importance of fixation in immunohistochemistry: use of formaldehyde solutions at variable pH for the localization of tyrosine hydroxylase. J Histochem Cytochem 29:884–850

Brownstein MJ, Palkovits M, Saavedra JM, Bassiri RM, Utiger RD (1974) Thyrotropin-releasing hormone in specific nuclei of rat brain. Science 185:267–269

Burgus R, Dunn TF, Desiderio D, Guillemin R (1969) Structure moleculaire du facteur hypothalamique hypophysiotrope TRF d'origine ovine: mise evidence par spectrometrie de masse de la sequence. CR Acad Sci, Ser D 269:1870–1893

Chen YF, Ramirez VD (1981) Serotonin stimulates thyrotropin-releasing hormone release from superfused rat hypothalami. Endocrinology 108:2359–2366

Dieguez C, Foord SM, Peters JR, Hall R, Scanlon MF (1984) Interactions among epinephrine, thyrotropin (TSH)-releasing hormone, dopamine, and somatostatin in the control of TSH secretion in vitro. Endocrinology 114:957–961

Gallyas F (1982) Suppression of argyrophil III reaction by mercapto-compounds. A prerequisite for intensification of histochemical reactions by physical developers. Acta Histochem 70:99–105

Greer MA (1957) Studies on the influence of the central nervous system on anterior pituitary function. In: Pincus G (ed) Recent progress in hormone research. Academic Press, New York, pp 67–104

Grimm Y, Reichlin S (1973) Thyrotropin-releasing hormone (TRH): neurotransmitter regulation of secretion by mouse hypothalamic tissue in vitro. Endocrinology 93:626–631

Hirooka Y, Hollander CS, Suzuki S, Ferdinand P, Juan S-I (1978) Somatostatin inhibits release of thyrotropin releasing factor from organ cultures of rat hypothalamus. Proc Natl Acad Sci USA 75:4509–4513

Hisano S, Ishizuka H, Nishiyama T, Tsuruo Y, Katoh S, Daikoku S (1986) Immunoelectron microscopic observations of hypothalamic TRH-containing neurons in rats. Exp Brain Res 63:495–504

Hökfelt T, Fuxe K, Johansson O, Jeffcoate S, White N (1975a) Distribution of thyrotropin-releasing hormone (TRH) in the central nervous system as revealed with immunohistochemistry. Eur J Pharmacol 34:389–392

Hökfelt T, Fuxe K, Johansson O, Jeffcoate S, White N (1975b) Thyrotropin releasing hormone (TRH)-containing nerve terminals in certain brain stem nuclei and in the spinal cord. Neurosci Lett 1:133–139

Ishikawa K, Inoue K, Tosaka H, Shimada O, Suzuki M (1984) Immunohistochemical characterization of thyrotropin-releasing hormone-containing neurons in rat septum. Neuroendocrinology 39:448–452

Jackson IMD, Reichlin S (1974) Thyrotropin-releasing hormone (TRH): Distribution in hypothalamic and extrahypothalamic brain tissues of mammalian and submammalian chordates. Endocrinology 95:854–862

Jackson IMD, Reichlin S (1979) Distribution and biosynthesis of TRH in the nervous system. In: Collu R, Barbeau A, Ducharme JR, Rochefort JG (eds) Central nervous system effects of hypothalamic hormones and other peptides. Raven Press, New York, pp 3–54

Jackson IMD, Lechan RM (1987) Thyrotropin releasing hormone. In: Martin J, Brownstein M, Krieger D (eds) Brain peptides update, vol 1. John Wiley & Sons, New York, pp 101–125

Jackson IMD, Wu P, Lechan RM (1985) Immunohistochemical

localization in the rat brain of the precursor for thyrotropin-releasing hormone. Science 229:1097–1099
Jeffcoate SL, Fraser HM, Gunn A, White N (1973) Radioimmunoassay of thyrotrophin releasing hormone. J Endocrinol 59:191–192
Johansson O, Hökfelt T, Jeffcoate SL, White N, Sternberger LA (1980) Ultrastructural localization of TRH-like immunoreactivity. Exp Brain Res 38:1–10
Joseph-Bravo P, Charli JL, Palacios JM, Kordon C (1979) Effect of neurotransmitters on the in vitro release of immunoreactive thyrotropin-releasing hormone from rat mediobasal hypothalamus. Endocrinology 104:801–806
King JC, Anthony ELP (1983) Biosynthesis of LHRH: inferences from immunocytochemical studies. Peptides 4:963–970
Klibanski A, Milbury PE, Chin WW, Ridgway EC (1983) Direct adrenergic stimulation of the release of thyrotropin and its subunits from the thyrotrope in vitro. Endocrinology 113:1244–1249
Krisch B (1980) Two types of luliberin-immunoreactive perikarya in the preoptic area of the rat. Cell Tissue Res 212:443–455
Krulich L (1982) Neurotransmitter control of thyrotropin secretion. Neuroendocrinology 35:139–147
Lechan RM, Jackson IMD (1982) Immunohistochemical localization of thyrotropin-releasing hormone in the rat hypothalamus and pituitary. Endocrinology 111:55–65
Lechan RM, Molitch ME, Jackson IMD (1983) Distribution of immunoreactive human growth hormone-like material and thyrotropin-releasing hormone in the rat central nervous system: Evidence for their coexistence in the same neurons. Endocrinology 112:877–884
Lechan RM, Snapper SB, Jacobson S, Jackson IMD (1984) The distribution of thyrotropin-releasing hormone (TRH) in the Rhesus monkey spinal cord. Peptides 5 (Suppl): 185–194
Lechan RM, Wu P, Jackson IMD, Wolf H, Cooperman S, Mandel G, Goodman RH (1986a) Thyrotropin-releasing hormone precursor: characterization in rat brain. Science 231:159–161
Lechan RM, Wu P, Jackson IMD (1986b) Immunolocalization of the thyrotropin-releasing hormone prohormone in the rat central nervous system. Endocrinology 119:1210–1216
Liposits Zs, Görcs T, Török A, Domány S, Sétáló G (1983a) Simultaneous localiation of two different tissue antigens based on the silver intensified PAP-DAB and on the traditional PAP-DAB method. Acta Morphol Hung 31:365–369
Liposits Zs, Görcs T, Sétáló G, Lengvári I, Flerkó B, Vigh S, Schally AV (1983b) Ultrastructural characteristics of immunolabelled, corticotropin releasing factor (CRF)-synthesizing neurons in the rat brain. Cell Tissue Res 229:191–196
Liposits Zs, Lengvári I, Vigh S, Schally AV, Flerkó B (1983c) Immunohistological detection of degenerating CRF-immunoreactive nerve fibers in the median eminence after lesion of paraventricular nucleus of the rat. A light and electron microscopic study. Peptides 4:941–953
Liposits Zs, Sétáló G, Flerkó B (1984) Application of the silver-gold intensified 3,3′-diaminobenzidine chromogen to the light and electron microscopic detection of the LH-RH system of the rat brain. Neuroscience 13:513–524
Liposits Zs, Phelix C, Paull WK (1986a) Adrenergic innervation of corticotropin releasing factor (CRF)-synthesizing neurons in the hypothalamic paraventricular nucleus of the rat. Histochemistry 84:201–205
Liposits Zs, Sherman D, Phelix C, Paull WK (1986b) A combined light and electron microscopic immunocytochemical method for the simultaneous localization of multiple tissue antigens. Histochemistry 85:95–106
Liposits Zs, Sievers L, Paull WK, Lechan RM, Coen CW, Culler MD, Merchenthaler I, Jackson IMD (1987a) Monoamine and peptidergic innervation of chemically characterized hypophysiotrophic neurons. Abst of sec world congr of neurosci, Budapest, 1987. Neuroscience 22 (Suppl):810
Liposits Zs, Lechan RM, Paull WK, Wu P, Jackson IMD (1987b) Adrenergic innervation of thyrotropin releasing hormone (TRH) synthesizing neurons of the rat hypothalamus. Soc Neurosci Abst 17 (in press)
Maeda K, Frohman LA (1980) Release of somatostatin and thyrotropin-releasing hormone from rat hypothalamic fragments in vitro. Endocrinology 106:1837–1842
Martin JB, Reichlin S (1972) Plasma thyrotropin (TRH) response to hypothalamic electrical stimulation and to injection of synthetic thyrotropin releasing hormone (TRH). Endocrinology 90:1079–1085
Montoya E, Wilber JF, Lorincz M (1979) Catecholaminergic control of thyrotropin secretion. J Lab Clin Med 93:887–94
Nishiyama T, Kawano H, Tsuruo Y, Maegawa M, Hisano S, Adachi T, Daikoku S, Suzuki M (1985) Hypothalamic thyrotropin-releasing hormone (TRH)-containing neurons involved in the hypothalamic-hypophysial-thyroid axis. Light microscopic immunohistochemistry. Brain Res 345:205–218
Reichlin S (1986) Neuroendocrine control of thyrotropin secretion. In: Ingbar SH, Braverman LE (eds) Werner's the thyroid. JB Lippincott, Philadelphia, pp 241–266
Schaeffer JM, Axelrod J, Brownstein MJ (1977) Regional differences in dopamine-mediated release of TRH-like material from synaptosomes. Brain Res 138:571–574
Schally AV, Redding TW, Bowers CY, Barrett JF (1969) Isolation and properties of porcine thyrotropin-releasing hormone. J Biol Chem 244:4077–4088
Shioda S, Nakai Y, Sato A, Sunayama S, Shimoda Y (1986) Electron-microscopic cytochemistry of the catecholaminergic innervation of TRH neurons in the rat hypothalamus. Cell Tissue Res 245:247–252
Sternberger LA, Hardy PH Jr, Cuculis JJ, Meyer HG (1970) The unlabelled antibody enzyme method of immunochemistry. Preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-antiperoxidase) and its use in identification of spirochetes. J Histochem Cytochem 18:315–333
Streit P, Reubi JC (1977) A new and sensitive staining method for axonally transported peroxidase (HRP) in the pigeon visual system. Brain Res 126:530–537
Terry LC (1986) Regulation of thyrotropin secretion by the central epinephrine system. Neuroendocrinology 42:102–108
van den Pol AN (1982) The magnocellular and parvocellular paraventricular nucleus of rat: Intrinsic organization. J Comp Neurol 206:317–345
Winokur A, Utiger RD (1974) Thyrotropin-releasing hormone: Regional distribution in rat brain. Science 185:265–267
Wu P, Lechan RM, Jackson IMD (1987) Identification and characterization of thyrotropin-releasing hormone precursor peptides in rat brain. Endocrinology 121:108–115
Zamboni L, DeMartino L (1967) Buffered picric acid-formaldehyde: a new rapid fixative for electron microscopy. J Cell Biol 35:148A