**UNIVERSITY OF SZEGED • FACULTY OF MEDICINE •
LAKE ERIE COLLEGE OF OSTEOPATHIC MEDICINE (LECOM)**

SIGNIFICANCE OF NEUROTRANSMITTER INPUTS OF THE GONADOTROPIN-RELEASING HORMONE NEURONAL SYSTEM IN THE HUMAN DIENCEPHALON

Ph.D. Dissertation

by

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PAPERS ATTACHED TO THE DISSERTATION

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ABBREVIATIONS

AC	anterior commissure
AVPV	anterior ventral periventricular nucleus
CRH	corticotrophin-releasing hormone
EOP	endogenous opiate peptides
ER	estrogen receptor
GAL	galanin
GnRH	gonadotropin-releasing hormone
INF	infundibulum
IR	immunoreactive
LHRH	luteinizing hormone-releasing hormone
MB	mamillary body
NPY	neuropeptide Y
NT	neurotensin
Och	optic chiasm
PH	posterior hypothalamic area
POMC	pro-opiomelanocortin
PVN	paraventricular nucleus
S	septal area
SP	substance P
TIDA	tuberoinfundibular dopaminergic

CONTENTS

INTRODUCTION

Gonadotropin-releasing hormone (GnRH), also called luteinizing hormone-releasing hormone (LHRH) system is composed of loosely arranged neuronal elements distributed in the human diencephalon. Since the most significant role of GnRH is inducing the release of luteinizing hormone (LH) and folliculus stimulating hormone (FSH) via the hypophyseal portal circulation, GnRH system represents the final common pathway of a neuronal network that integrates multiple external and internal factors to control fertility. The release of GnRH into the hypophyseal portal circulation in the median eminence is pulsatile. Cyclic fluctuations in the amplitude and frequency of GnRH release, combined with changes in the secretory capacity of the pituitary gonadotrophs, are responsible for the generation of the LH secretion profile observed over the course of the ovarian cycle. The pulsatile pattern of GnRH release is critical for normal ovarian function; in females, the massive increase in GnRH release generates the LH surge necessary for ovulation.

Action of estrogen on the LH secretion in human

Among many factors that integrate the activity of the GnRH neuronal system, estrogens play the most important role. In the male, and for the greater part of the ovarian cycle in females, estrogen exhibit a negative feedback action on LH secretion. However, in the female, estrogen, in addition to the negative feedback, also exhibits a positive feedback influence upon the activity and output of GnRH neurons to generate the preovulatory LH surge and subsequent ovulation (1). Despite the critical effect of estrogen in ovulation, before 2000, the scientific community was exploring the mechanism of estrogen action based on the assumption that the GnRH neurons themselves do not express estrogen receptors (2). Therefore, it was postulated that the action of estrogen upon GnRH neurons was indirect involving several estrogen-sensitive neurotransmitter- and neuropeptide-synthesizing systems and glial cells that *trans*-synaptically regulate the activity of the GnRH neurons (1).

Hypothesis: role of interneurons inthe estrogen-modulated GnRH release

It has been recently reported that the GnRH neurons of the rat express estrogen receptor-beta (ERβ) mRNA, bind ^{125}I-estradiol (3), and possess ERβ but not ERα immunoreactivity

(4). Soon after these original reports, the presence of ERβ and the lack of ERα within GnRH neurons of rats was confirmed in other studies (5). Although both ERα and ERβ have been successfully localized in the human hypothalamus (6-8), no report indicates the presence of ERs in the GnRH neurons of humans. Whether this phenomenon is true or the lack of ERs in GnRH neurons in humans is due to technical difficulties remains to be determined. Therefore, until solid evidence is provided for the presence of ERs in GnRH neurons in humans, our current hypothesis is that the action of estrogen on GnRH neurons is mediated via interneurons and glial cells expressing ERs.

Potential neurotransmitter systems mediating the effect of ER on LH release in human

The identification of several neurochemically distinct nerve terminals synapsing on GnRH perikarya and dendrites (9) combined with the limited evidence for direct presynaptic inputs to GnRH nerve terminals in the median eminence (10) in other species than humans suggests that neurons and/or glial cells may act at both sites to regulate GnRH neuronal activity and GnRH release. An extensive body of literature is available with regard to the neurochemical regulation of LH and GnRH secretion in several species (11). Our focus is on morphological evidence indicating communication potential with GnRH neuronal systems in primates, particularly in humans. The afferents of GnRH neurons described below containing neuropeptides and catecholamines arise from multiple locations in the

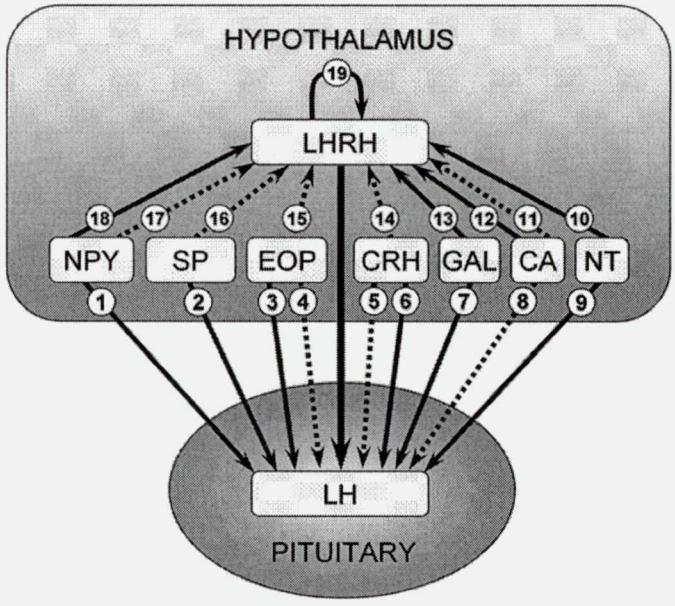

Fig. 1. Neurotransmitter systems influencing LH secretion directly (1-8) or via hypothalamic GnRH release (9-15). Some interactions are hypothetical. Full arrows represent augmentation, dotted arrows demonstrate attenuation of the neurotransmitter release. Abbreviations: CA, catecholamines; CRH, corticotrophin-releasing hormone; EOP, endogenous opiate peptides; GAL, galanin; NPY, neuropeptide Y; NT, neurotensin; SP, substance P.
References: 1,(137); 2, (138,139); 3,(94,140); 4,(140); 5,(94); 6,(95); 7,(141); 8,(116); 9, (161); 10, (160); 11,(142); 12,(137); 13,(29,30,115); 14,(97); 15,(98-100,143); 16,(52,61,63); 17,(53,54,144); 18,(31); 19, (145).

brain. Most of these areas are receptive for estrogen; however, no evidence is available indicating that the axon terminals contacting the GnRH neurons arise from cells expressing estrogen receptors. Moreover, most of the neuropeptides that contact GnRH neurons either at their perikarya, dendrites or at nerve terminals in the median eminence, are also released into the hypophysial portal circulatory system. Therefore, they may reach the anterior pituitary and directly regulate the secretion of LH. As shown in Figure 1, the neuropeptides and catecholamines in the hypothalamus may regulate LH secretion indirectly, by providing afferents to GnRH neurons or directly to the pituitary after being released into the hypophyseal portal circulation.

Specific Aims

- Comparative analysis of the pattern of GnRH-, CRH-, SP-, NPY-, galanin, β-endorphin-, leu-enkephalin-, and catecholaminergic [tyrosine hydroxylase-IR (TH-IR) structures in human hypothalamus

- Computer-aided modelling of the distribution of the perikarya of neurotransmitter systems on two- and three-dimensional stereoscopic images (Figs. 2-4).

- Analysis of the peptidergic and catecholaminergic afferents of GnRH neurons based on light microscopic double labelling immunocytochemistry (Fig. 5).

MATERIALS AND METHODS

Human samples

Human hypothalami were obtained from diagnostic autopsies on women and men with different ages in accordance with the regulation of the Ethics Board of Szent Györgyi Albert Medical University, Loyola University Chicago and Lake Erie College of Osteopathic Medicine (LECOM) (12). The clinical and pathological records of the individuals did not contain neurological or neuroendocrinological diseases.

Immunohistochemistry

Immunocytochemistry for single and double labelling was performed either with the PAP or the ABC method in combination with a modified silver intensification technology (13). For fluorescent immunohistochemistry, tetramethylrhodamine isothiocyanate (TRITC) was used to label GnRH-IR elements and fluorescein isothiocyanate (FITC) labelled secondary antibodies to stain the afferents of GnRH-IR neurons. The details of tissue procurement, preparation, the immunocytochemical detection and analysis have been described in detail previously (12).

Computer-aided three-dimensional modelling of the neurotransmitter systems

The hypothalamic sections were systematically scanned with the aid of a plain-scanner (Hewlett-Packard, Palo Alto, CA, USA). The outlines and the immunoreactive elements of the sections stained with single-label immunohistochemistry were traced by the CorelTrace software 4.0 (Corel Corporation, Ottawa, Ontario, Canada). The neurons and fibers were marked on these figures using a Zeiss Axiophot microscope with camera lucida, and Adobe Photoshop software, version 5.0. The 3D models were created by the computer-generated superimposition of the consecutive sections using VoxBlast NT/9x Version 3.0 Light (Vaytek, Image Analysis Facility, University of Iowa, Ames, IA, USA). For those unaccustomed to viewing stereoscopic figures by eye, the use of a stereoscopic image viewer is recommended.

Verification of the associations between the neurotransmitter systems

The words: "contact, juxtapose, abut" or "innervate" was used throughout the present study, although we are aware that the contacts observed at the light microscopic level may not always be indicative of classical synapses. Membranes of cells in the brain degrade during the relatively long post-mortem period of time (several hours to a day) to the extent that synaptic connections with electron microscopic immunocytochemistry cannot be identified. The detection of contacts or juxtapositions on 30 μm sections using high magnification (100X) with oil immersion were verified on semithin (0.5 μm) plastic-embedded sections. In addition, in many cases, these potential synapses were identified with confocal microscopy.

The terminology of the diencephalic structures was adopted from Braak and Braak (14), Saper (15) and Silverman (9).

RESULTS

Distribution of GnRH-IR neurons

(Fig. 2, 3)

The GnRH-IR neurons are not concentrated in one brain nucleus, but rather distributed widely in a loose network throughout the hypothalamus, as shown in Figs. 2 and 3. The number of labeled perikarya varies in the rostrocaudal and mediolateral directions. The labeled neurons are located in a 2-mm-wide periventricular zone of the hypothalamus, mainly in the preoptic region and in the anterior periventricular nucleus. The number of labeled cells gradually decreases in the mediolateral direction, and 16 mm laterally from the ventricular ependymal surface no GnRH-IR cell bodies are found. The cell bodies are located mainly in the anterior hypothalamus, along the diagonal band of Broca, in the periventricular regions and in the tuberal region. The organum vasculosum of the lamina terminalis (OVLT) contains relatively few GnRH-IR perikarya. Some GnRH-IR cell bodies are also present in the paraventricular and supraoptic nuclei, while only a few are seen in the posterior hypothalamus, where the labeled neurons are localized around the mamillary body. A similar distribution of GnRH neurons in the human hypothalamus has been reported by others (9-12,16-18).

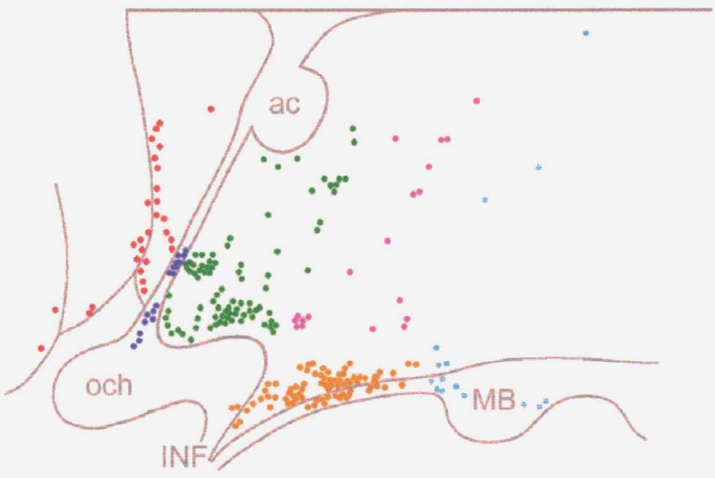

Fig. 2. Main subclasses of the gonadotropin-releasing hormone-(GnRH)-IR perikarya in the human diencephalon. Colored dots represent the cell bodies. The GnRH-IR cells appear to receive innervation from the NPY, SP, β-endorphin, leu-enkephalin, CRH, galanin, and TH (catecholaminergic) fiber varicosities, except the neurons in the septal area (red dots), that do not juxtapose with any of these neurotransmitter systems. The most heavily innervated GnRH subclasses are located in the infundibulum and median eminence (orange dots), followed by the one in the preoptic area (green dots). The GnRH-IR neurons in the posterior hypothalamus are only occasionally innervated. Color codes: red, septal area; blue, lamina terminalis; green, preoptic area; orange, infundibulum, median eminence; magenta, superior part of tuberal region; cyan, posterior hypothalamus. Abbreviations: ac, anterior commissure; INF, infundibulum; MB, mamillary body; och, optic chiasm.

Morphologically, the GnRH-IR neurons could be classified into two subtypes: fusiform and multipolar cells. The majority of the neurons are fusiform with thin cell bodies

and two processes emanating from opposite poles. The multipolar cells, with triangle-shaped or rounded cell bodies, are usually situated in the dorsal portion of the preoptic region.

Many fibers with infrequent branching points and varicosities are present in loose bundles within the diagonal band of Broca, seemingly directed towards the OVLT or they run above the dorsal surface of the optic chiasm through the medial preoptic area towards the tuberal region. The few GnRH-IR fibers of the posterior hypothalamus appear to be concentrated around the mamillary body.

Distribution of the NPY-IR neurons

(Fig. 3)

The NPY-IR neurons are scattered throughout the human hypothalamus (Fig.3). Numerous perikarya with intense NPY immunoreactivity are found in the septal region, rostrally to the lamina terminalis, the medial and lateral preoptic and periventricular areas, and the tuberal region. Few NPY-IR cell bodies are located in the posterior hypothalamus around the mamillary body. In the tuberal region and posterior hypothalamus, NPY-IR perikarya are often located periventricularly, whereas, in the medial preoptic area, cells were detected mainly in the medial hypothalamus. In general, the number of NPY-IR perikarya *en masse* decreases mediolaterally. The NPY-IR somata are identified as distinct fusiform and multipolar neurons. A similar distribution of the NPY neuronal system in the human hypothalamus has been reported by Escobar *et al.* (19).

NPY-IR fibers with many axonal swellings form dense networks along the diagonal band of Broca, in the periventricular area, and in the infundibular nucleus. The ventromedial and the paraventricular nuclei also contain NPY-IR fibers. Characteristically, the periventricular regions and the medial hypothalamus contain more fibers than the lateral hypothalamus. However, the lateral part of the preoptic area in the territory of the diagonal band is densely populated by NPY-IR fibers.

Juxtapositions between the GnRH-IR and NPY-IR neurons

Superimposition of the maps of GnRH-IR and NPY-IR structures reveal a partial overlap. The areas with the highest degree of overlap include the diagonal band of Broca, the periventricular areas (preoptic region, anterior periventricular nucleus), and the infundibular

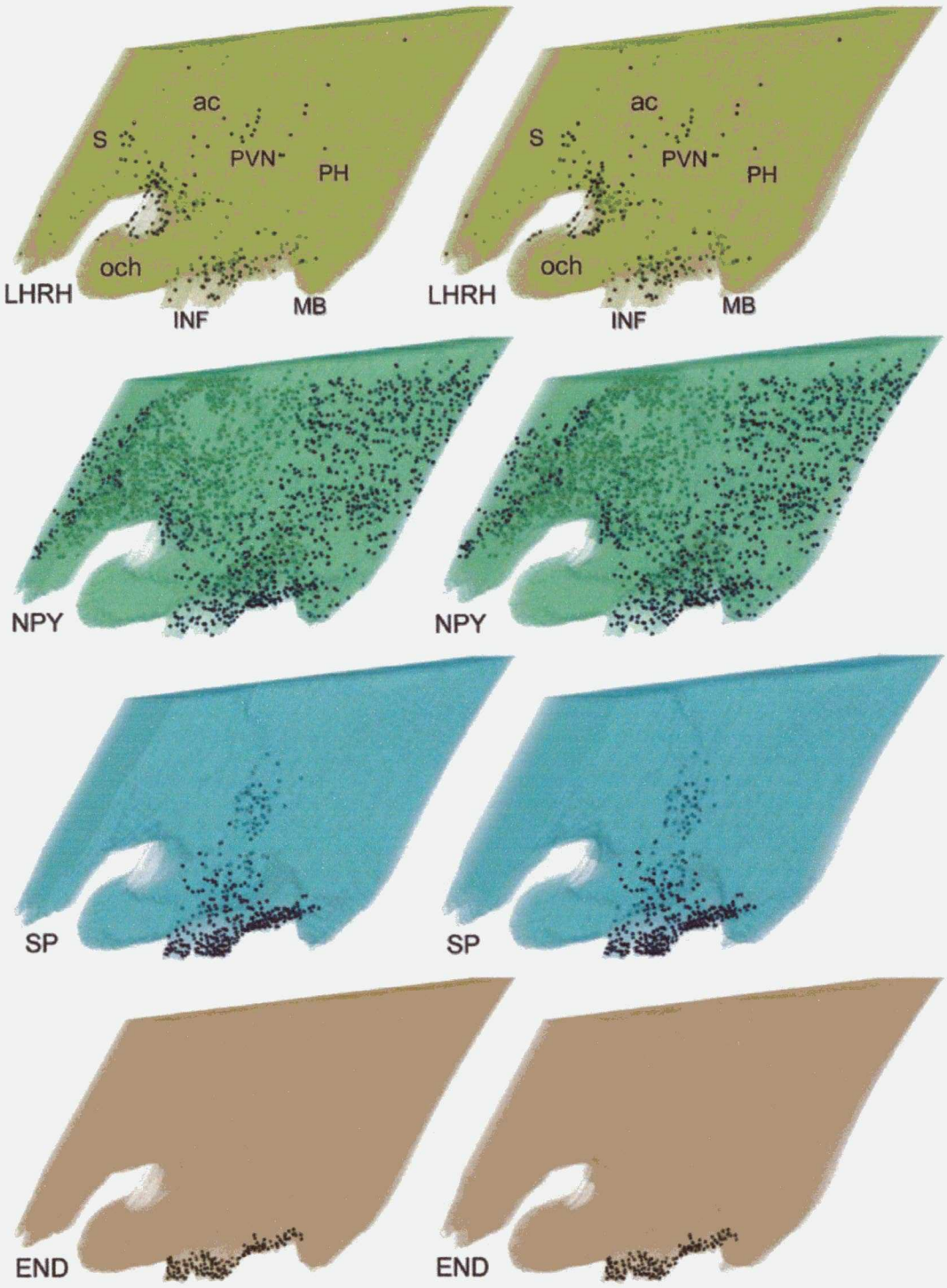

Fig. 3. Stereoscopic images of the human hypothalamus illustrating the distribution of the luteinizing hormone-releasing hormone (LHRH), neuropeptide Y (NPY), substance P (SP) and β-endorphin (END) perikarya (dots). Stereoscopic images can be seen using U or parallel vision. The eyes are relaxed to look into the distance until the pair of the images fuse, and then refocused by the brain. With this technique a 3D hypothalamus can be seen on the figure, floating in front of the paper, with the immunolabeled perikarya in it at different depth marked by the dots. The optimal viewing distance is approximately 20 inches from the paper surface (average reading distance). Stereoscopic magnifier is suggested to readers unfamiliar with U or parallel vision. Abbreviations: AC, anterior commissure; INF, infundibulum; MB, mamillary body; och, optic chiasm; PH, posterior hypothalamic area; PVN, paraventricular nucleus; S, septal area.

nucleus. A partial overlap can be observed in the paraventricular nucleus as well. At high magnification, NPY-IR varicose fibers are frequently observed to abut on GnRH-IR neurons in the preoptic region, periventricular and tuberal areas, but not in the diagonal band of Broca. NPY-IR varicosities are found in the immediate vicinity of the GnRH-IR stem dendrites and perikarya, which are either fusiform or multipolar in shape. The juxtapositions proved to be close axosomatic and axodendritic contacts (Fig. 5A and B). The individual GnRH-IR cells are often contacted at several sites by the axonal swellings of two or more NPY-IR fibers. In some cases, NPY-IR varicose fibers are observed to twine around GnRH-IR neurons (Fig. 5A). The juxtapositions between GnRH-IR perikarya and NPY-IR fibers are most numerous in the infundibular nucleus (64%) followed by the medial preoptic region (36%), and the anterior periventricular nucleus (25%).

Distribution of the substance P-IR neurons

(Fig. 3)

The majority (94%) of the SP-IR perikarya are located in the tuberal region (Fig.3). Only a few cell bodies are present in the periventricular zone of the preoptic area and in the basal part of the posterior hypothalamus. In the tuberal region, the neurons are arranged in several subclasses. Most of the perikarya are found in the infundibular region and the median eminence. The basal part of the periventricular area and the dorsomedial subdivision of the ventromedial nucleus contain an additional cell population. Finally, a well-defined cell group is distinguished in the basal perifornical area of the tuberal region. A similar distribution of the SP neuronal system in the hypothalamus has been reported by Chawla *et al.* (20).

Morphologically, the SP-IR neurons could be classified into two subtypes: fusiform or multipolar cells. The majority of the cells were fusiform, with oval-shaped cell bodies with a process emanating from each of the poles. The multipolar cells, with triangle-shaped or rounded cell bodies, are usually situated in the dorsomedial subdivision of the ventromedial nucleus.

SP-IR fibers form a loose network throughout the diencephalon. In the septal area, few fiber varicosities are present along the diagonal band of Broca and around the anterior comissure. In the preoptic area, SP-IR fibers are mainly periventricularly arranged, and a delicate fiber network appears around the fornix. In the lateral zone of the preoptic area, SP-

IR processes are located in the corpus striatum and in the basal part of the diencephalon running parallel with the surface of the brain. Most of the fiber varicosities are periventricularly arranged in the tuberal region of the diencephalon. A dense network of fibers is located around the portal vessels in the infundibulum/pituitary stalk. Few axons are present in the paraventricular nucleus. An additional SP-IR fiber population can be distinguished running to the infundibulum and the median eminence, passing over the optic tract and located laterally on the basal region of the diencephalon. Numerous SP-IR processes are located around the fornix in the tuberal region. In the posterior hypothalamus, the axon varicosities are found at the base of the 3rd ventricle and in the periventricular area. On the lateral part of the posterior hypothalamus, SP-IR fibers are observed at the region of the corpus striatum.

Juxtapositions between the GnRH-IR and SP-IR neurons

Superimposing the map of GnRH-IR and SP-IR neural elements reveals that the systems overlap in the medial preoptic area, infundibulum, and median eminence. Partial overlap occurs in the periventricular zone of the tuberal region and in the paraventricular nucleus. Dual-label immunohistochemistry and high magnification microscopic examination reveals that SP-IR fiber varicosities abut on GnRH-IR cell bodies in all of these regions (Fig. 5C and D). Quantitative analysis of the GnRH-SP juxtapositions shows that the majority of these structures (67%) are located in the infundibular area and in the median eminence. In these regions, 18% of the GnRH-IR perikarya are heavily innervated (more than three intimate contacts), 37% are lightly (one to three intimate contacts) innervated by SP-IR fibers, while 45% are not closely associated with SP-IR axon terminals. GnRH-SP juxtapositions are also present in the medial preoptic area (12%) and in the periventricular zone of the tuberal region (21%). Here, the GnRH-IR perikarya are contacted by one to three SP-IR axon varicosities.

The β–endorphin-IR neuronal system

(Fig. 3)

β–endorphin-IR perikarya form a single, well-circumscribed cell group located in the infundibulum/median eminence of the human diencephalon (Fig.3). The cell bodies are often found in close proximity to the portal vessels. The β–endorphin-IR neurons are usually

fusiform in shape with two processes emanating from the opposite poles of the cells. However, multipolar cells can be detected in the infundibular nucleus. A similar distribution of pro-opiomelanocortin (POMC) expression in the human hypothalamus has been reported by Sukhov *et al.* (21).

β–endorphin-IR fibers are not arranged into well-defined pathways; rather, they form a loose network in the diencephalon mainly populating the periventricular zone and the medial hypothalamus. In front of the lamina terminalis, β–endorphin-IR axon varicosities can be detected along the diagonal band of Broca and around the anterior commissure. A few fibers are found in the basal part of the lamina terminalis itself, where they often cross the midsagittal line and project contralaterally. In the suprachiasmatic region, most β-endorphin-IR processes are located periventricularly in the medial preoptic region and in the dorsomedial subdivision of the ventromedial nucleus. Only a few fibers are scattered in the lateral hypothalamus. The tuberal region contains delicate fiber network in the infundibulum/median eminence, where the β–endorphin-IR fibers are often in close association with the portal vessels. The lateral hypothalamic zone of the tuberal area contains few fibers. The most well-defined β–endorphin fiber system projects laterally from the infundibulum at the base of the diencephalon. In the posterior hypothalamus, β-endorphin-IR axons populate the area around the mamillary bodies and the tuberomamillary nucleus. Fibers are also detected in the periventricular region of the posterior hypothalamus.

Juxtaposition between the GnRH-IR and β–endorphin-IR neurons

The close associations between the β–endorphin-IR and GnRH-IR elements are mainly *en passant* type of contacts. The majority of these close associations are located in the infundibulum/median eminence (68%). Here, GnRH-IR neurons are usually lightly innervated (45%) receiving one to three contacts. Heavily innervated (more than three contacts) GnRH-IR cells represent a smaller percentage (26%), whereas 29% of the GnRH-IR perikarya do not receive any detectable β–endorphin-IR axon varicosities. A few juxtapositions occur between GnRH-IR and β–endorphin-IR fibers around the wall of the portal vessels. An additional subpopulation of β–endorphin-GnRH juxtapositions are observed in the medial preoptic area (21%), where the majority of GnRH cell bodies are lightly innervated by β–endorphin-IR axons. Finally, a few juxtapositions, with usually one to three contacts/GnRH perikarya, can

be found at the caudal part of the diagonal band of Broca in close proximity to the lamina terminalis, and at the basal part of the posterior hypothalamus, laterally to the mamillary bodies. Close examination of the β-endorphin-GnRH juxtapositions on semithin sections, utilizing oil immersion and high magnification, reveal no cleft between the contacting immunoreactive structures (Fig. 5E).

The distribution of Leu-enkephalin-IR neurons

(Fig. 4)

Leu-enkephalin-IR cell bodies are concentrated in three different regions: (i) the vast majority of leu-enkephalin-IR perikarya (82%) are observed in the periventricular area of the tuberal region; (ii) a few cells are found in the infundibulum in close proximity to the portal vessels; and (iii) periventricularly arranged in the medial preoptic area (Fig. 4). The leu-enkephalin-IR neurons are mainly fusiform in shape with processes emanating from the opposite poles of the cell body. The leu-enkephalin-IR cells are characteristically oriented with the axis of the fusiform cell bodies running parallel to the surface of the 3rd ventricle and the processes pointing dorsally and ventrally along the axis of the cells.

Leu-enkephalin-IR fibers are detected periventricularly, running along the medial surface of the hypothalamus. An additional fiber network projects from the infundibulum towards the lateral hypothalamic area. A dense leu-enkephalin-IR axonal network is revealed around the fornix and along the lateral part of the anterior commissure. Leu-enkephalin-IR fibers are also found in the dorsal part of the lateral hypothalamic area and around the portal vessels. Leu-enkephalin-IR fiber varicosities are observed to form fiber baskets in the periventricular area of the preoptic and tuberal region. Here, leu-enkephalin-IR axons abut on fusiform neurons, which do not show GnRH-immunoreactivity. The fibers are in intimate contact with the unidentified neurons and often cover the majority of their surface.

Juxtapositions between the GnRH-IR and leu-enkephalin-IR neurons

Leu-enkephalin-IR and GnRH-IR elements show overlap in the infundibulum/median eminence and the medial preoptic area after superimposing the maps of these two systems. Partial overlap is detected in the periventricular zone of the tuberal region and at the ventral

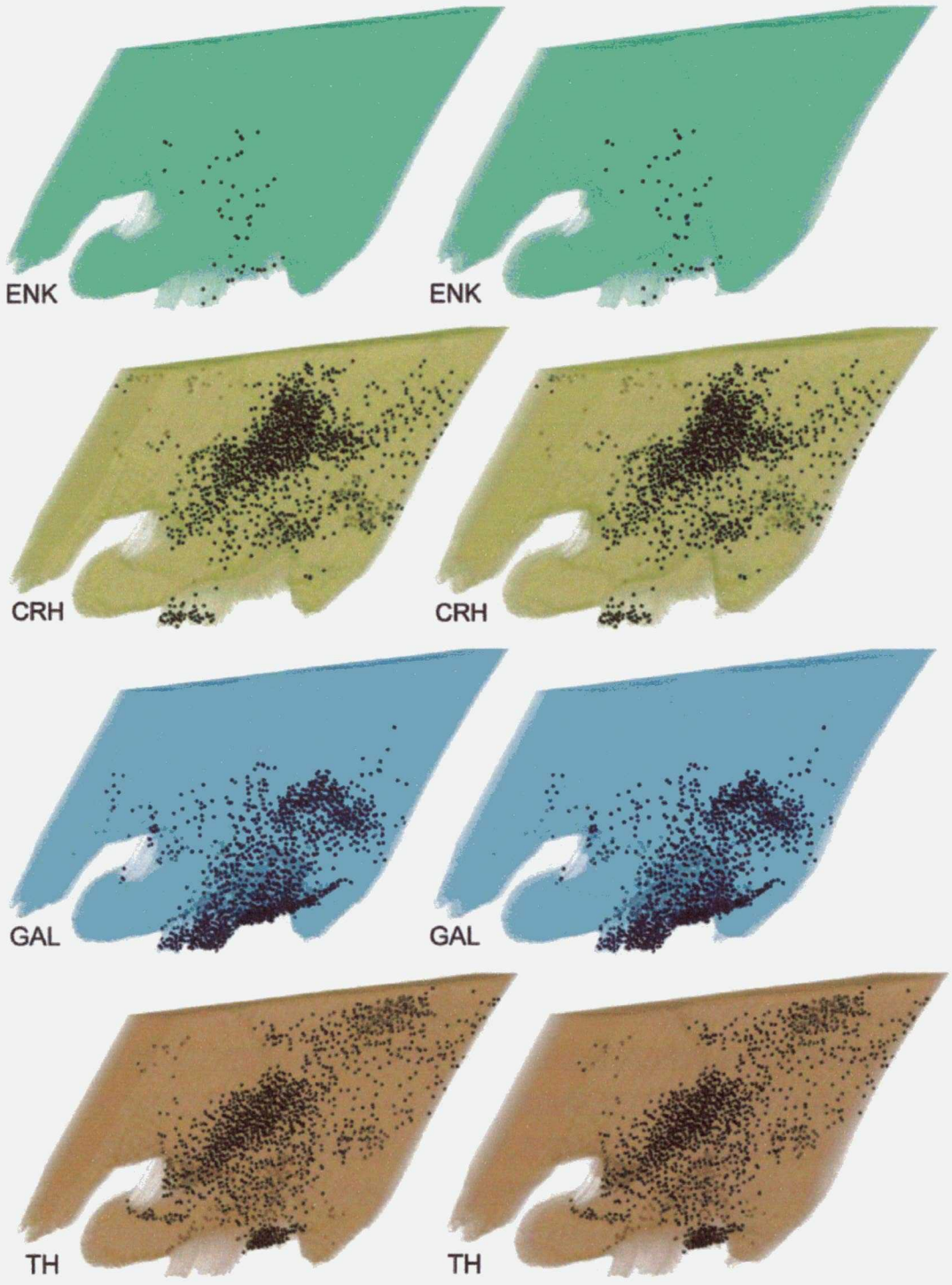

Fig. 4. Stereoscopic images illustrating the distribution of the leu-enkephalin (ENK), corticotropin-releasing hormone (CRH), galanin (GAL), and tyrosine hydroxylase (TH) (catecholaminergic) perikarya in the human hypothalamus (dots). Stereoscopic images can be seen using U or parallel vision. The eyes are relaxed to look into the distance until the pair of the images fuse, and then refocused by the brain. With this technique a 3D hypothalamus can be seen on the figure, floating in front of the paper, with the immunolabeled perikarya in it at different depth marked by the dots. The optimal viewing distance is approximately 20 inches from the paper surface (average reading distance).

zone of the posterior hypothalamus, around the mamillary body. Close examination of the overlapping areas reveal that leu-enkephalin-IR axons abut GnRH-IR cell bodies and axonal processes in these regions (Fig. 5F). The juxtapositions between leu-enkephalin-IR fibers and GnRH-IR neurons are detected mainly in the infundibulum and median eminence (78%), followed by the medial preoptic area and the periventricular area of the tuberal region (18%). A few juxtapositions between the leu-enkephalin and GnRH systems are revealed in other areas including the anteroventral periventricular nucleus and the tuberomamillary nucleus (4%). No juxtapositions are found rostrally to the lamina terminalis.

Distribution of CRH-IR neurons

(Fig. 4)

The vast majority of CRH-IR perikarya (97%) are located in the medial 4 mm zone of the human diencephalon (Fig. 4). In front of the lamina terminalis, the CRH-IR cells are concentrated in the superior part of the diencephalon around the fornix and laterally around the anterior commissure. In the preoptic area, the perikarya are periventricularly located spreading from the fornix to the optic chiasm. In the tuberal region, the CRH-IR cell bodies are concentrated in four areas: (i) the majority of the cells are located in the paraventricular nuclei; (ii) in the periventricular region, surrounding the 3rd ventricle from above the fornix to the infundibulum; (iii) in the dorsomedial subdivision of the ventromedial nucleus where a well-defined group of CRH-IR cells is found; and finally, (iv) a few immunoreactive cells are located in the infundibular region. In the posterior hypothalamus, CRH-IR perikarya are found in the periventricular region and around the mamillary body. The majority of the CRH-IR neurons are fusiform in shape with two to four processes emanating from the opposite poles. Numerous, star-shaped multipolar cells are found in the periventricular region.

CRH-IR fibers are organized in several well-defined pathways in the human diencephalon. Fibers originating from the infundibulum run laterally, close to the basal surface of the hypothalamus. This tract arches over the optic tract dorsally. The CRH-IR fibers arising from the paraventricular nucleus (PVN) follow three different directions: (i) a well-defined tract run horizontally from the PVN through the substantia innominata of the lateral hypothalamus; and (ii) fibers projecting to the base of the diencephalon surround the fornix laterally. This tract crosses the infundibular pathway in the tuberal region of the

hypothalamus, medially to the optic tract. (iii) Axons from the PVN surround the fornix medially and project towards the infundibulum/median eminence. An additional set of CRH-IR fibers is seen periventricularly. These fibers appear to arise from the periventricularly-arranged CRH-IR perikarya. Finally, a delicate fiber network is observed around the anterior commissure and the mamillary body. The CRH-IR fibers form a dense network around the portal vessels in the tuberal region and are often associated with small blood vessels in the PVN and in the periventricular region.

Juxtapositions between CRH-IR and GnRH-IR neurons

Superimposition of the CRH and GnRH maps reveals that these systems overlap mainly in the infundibular region, median eminence and medial preoptic area, while partial overlap is detected in the periventricular area of the tuberal region and in the basal part of the lateral preoptic area (Fig.3 and 4). Characteristically, the CRH-IR fiber varicosities abut fusiform GnRH-IR cells forming multiple axosomatic and axodendritic contacts as they pass along the neuron (Fig. 5G and H). The majority of the CRH-GnRH juxtapositions (82%) are found in the infundibulum and the median eminence followed by the preoptic region (11%), located periventricularly in the medial preoptic area and in the anteroventral periventricular nucleus. An additional population of juxtapositions is present in the basal part of the lateral hypothalamic region at the medial side of the optic tract. No contacts are present between the CRH-IR fibers and GnRH-IR cells in the rostral part of the diencephalon, anterior to the lamina terminalis. Few juxtapositions are observed between CRH-IR and GnRH-IR fibers. In some cases, CRH-IR and GnRH-IR axons target the same blood vessel in the periventricular area, forming multiple contacts around the vessel wall.

The distribution of galanin-IR neurons

(Fig. 4)

The majority of the galanin-IR perikarya (96%) are concentrated posteriorly to the lamina terminalis in different regions: (i) the infundibulum contains densely packed galanin-IR cell bodies that are often located around the portal vessels. (ii) Numerous immunoreactive perikarya are detected in a narrow zone at the basal surface of the tuberal region. This cell population extends from the infundibular nucleus to the optic tract. (iii) Several galanin-IR

perikarya are detected periventricularly in the chiasmatic and tuberal regions. The paraventricular nucleus also contains galanin-IR cell bodies. (iv) In the posterior hypothalamus, the galanin-IR neurons are concentrated in the posterior subdivision of the periventricular nucleus and in the tuberomamillary nucleus. Few perikarya can be detected in the periventricular region of the posterior hypothalamus. In front of the lamina terminalis, scattered galanin-IR cell bodies are observed along the diagonal band of Broca. Few cells are present in the lamina terminalis itself. A similar distribution of galanin-IR neurons in the human hypothalamus has been reported by Gentleman et al. (22).

The galanin-IR neurons are mainly fusiform in shape with processes emanating from the opposite poles of the perikarya. In the periventricular zone, galanin-IR cells are characteristically arranged with their longitudinal axis running parallel to the surface of the 3rd ventricle. Multipolar galanin-IR cells are present mainly in the infundibulum and in the posterior subdivision of the periventricular nucleus.

Galanin-IR fiber varicosities form a dense network in the infundibulum/median eminence. Numerous axons can be detected periventricularly in the chiasmatic and tuberal regions. A few galanin-IR fibers are present along the diagonal band of Broca, around the anterior comissure and fornix, and in the lamina terminalis. In the posterior hypothalamus, fibers can be seen in the posterior subdivision of the periventricular nucleus, in the tuberomamillary nucleus, and in the periventricular region. Sparse galanin-IR axon varicosities are observed in the lateral hypothalamus.

Juxtaposition between Galanin-IR and GnRH-IR neurons

Contacts between galanin-IR axon varicosities and GnRH-IR perikarya are present mainly in the infundibulum/median eminence of the human hypothalamus. Here, 77% of the GnRH-IR neurons are heavily contacted by galanin fibers receiving more than three contacts. The rest of the GnRH neurons in this region receive only a few (less then three) contacts per neuron. Numerous GnRH-IR cells receive both GnRH and galanin-IR axon varicosities in the medial basal hypothalamus. Galanin-GnRH juxtapositions are also present in the medial preoptic area and in the periventricular zone of the tuberal region where 24% of the GnRH-IR are contacted by galanin-IR nerve processes. A few GnRH-IR perikarya are juxtaposed by galanin-IR fibers (one to three contacts) in the posterior subdivision of the periventricular

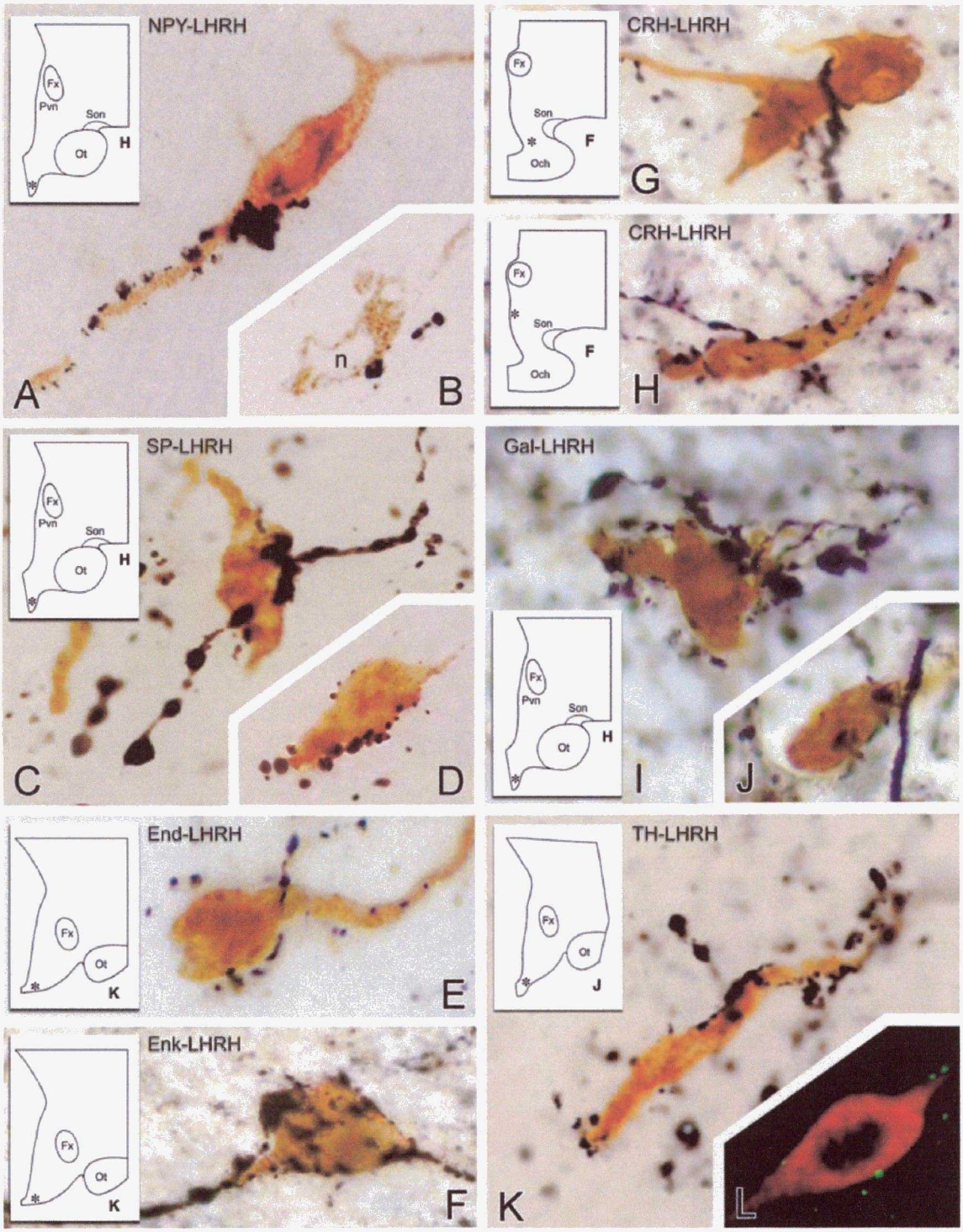

Fig. 5. Neurotransmitter systems providing input to the human gonadotropin-releasing hormone (GnRH)-immunoreactive (IR) neuronal elements. The position of the neurons is illustrated on schematic sections of the human hypothalamus. Double label immunohistochemistry (A-K) demonstrates black, silver impregnated axonal varicosities of the actual neurotransmitter systems abutting on brown, GnRH-IR perikarya visualized with DAB chromogen. Double label immunofluorescence combined with confocal microscopy at 1 µm virtual sectioning (L) provides further evidence of contacts between the intimately associated elements. The neurotransmitter-GnRH contacts have been further verified on 1.5 µm thick semithin sections (B). Abbreviations: CRH, corticotrophin-releasing hormone; end, ß-endorphin; enk, leu-enkephalin; gal, galanin; n, nucleus of the GnRH-IR neuron; NPY, neuropeptide-Y; SP, substance P; TH, tyrosine-hydroxylase (representing catecholaminergic neuronal elements).

nucleus, in the lamina terminalis, and along the diagonal band of Broca. Close examination of semithin sections of these intimate associations reveal no gap between the juxtaposing GnRH-IR and galanin-IR neural elements (Fig. 5I and J).

The distribution of catecholaminergic [tyrosine hydroxylase-IR (TH-IR)] neurons (Fig. 4)

In contrast to the GnRH-IR elements, the TH-IR perikarya often form clusters in the diencephalon (Fig.4). The majority of the cell bodies are located in the ventral part of the preoptic and tuberal areas, whereas few are present in the posterior hypothalamus. The septal area contains a small number of immunoreactive cells, concentrated on the medial-dorsal region. In the preoptic region, the TH-IR neurons are arranged periventricularly with a few cells located at the bottom of the optic recess sitting on the dorsal surface of the optic chiasm. The TH-IR cell bodies are densely packed in the paraventricular and supraoptic nuclei. In the tuberal region, a large number of cells are present in the median eminence and in the periventricular area, whereas the infundibular zone contains sparse TH-IR perikarya. Dorsal to the median eminence, a cluster of cell bodies is located between the fornix and the optic tract and in the dorsal part of the diencephalon, close to the midline. In the dorsal hypothalamus, the cells are arranged periventricularly, with some labelled neurons found around the mamillary body. Morphologically, the TH-IR neurons are mainly fusiform, with thin cell bodies and two processes emanating from their opposite poles. Smaller numbers of multipolar cells are in the paraventricular and supraoptic nuclei. The distribution of dopamine-I neurons in the human hypothalamus has been confirmed by Ciliax et al. (23) using antisera against the dopamine transporter and are consistent with the location of TH-IR cells identified in our studes.

TH-IR fibers are seen as a loose network in the periventricular and infundibular areas. Labelled axon varicosities are in the lateral hypothalamus and also in the septal area. Interestingly, the TH-IR processes form well-defined terminal fields in the paraventricular and supraoptic nuclei.

Juxtapositions between TH-IR and GnRH-IR neurons

Superimposition of the maps of GnRH-IR and TH-IR elements reveals regions of overlap

in the medial preoptic area and median eminence (Figs.3 and 4). Partial overlap occurs in the periventricular zone of the tuberal part of the hypothalamus and in the paraventricular and supraoptic nuclei. High magnification examination of the overlapping areas reveals TH-IR fibers on GnRH-IR neurons in the medial preoptic and infundibular areas and in the median eminence. Here the TH-IR varicose fibers target mainly fusiform GnRH-IR neurons and form intimate contact with the cell bodies and the stem dendrites.

The majority of juxtapositions between the GnRH-IR perikarya and TH-IR fibers (78%) are located in the infundibular region and median eminence. Here, 12% of the detected GnRH-IR cells are heavily (three to five contacts), and 54% are lightly (one to three contacts) innervated by TH-IR axon terminals, whereas 34% do not receive TH-IR fibers. The medial preoptic area contains fewer juxtapositions (22%) and in the majority of these cases, the contacting TH varicosities innervate the GnRH neurons with one to three contacts.

Examination of the juxtapositions with oil-immersion revealed no synaptic cleft between the associating TH-GnRH neural elements (Fig. 5K). Also, these interneuronal connections are mostly close axosomatic and axodendritic contacts when examined in fluorescent preparations with confocal microscopy (Fig. 5L).

DISCUSSION

The anatomical organization of the GnRH neuronal system in humans is quite different from that of rodents. The GnRH-IR perikarya in humans occupy two major regions: approximately half of the neurons are located in an anterior region that involves the preoptic-septal region and the other half in a posterior region involving the infundibulum (arcuate nucleus in rodents). In rodents, the vast majority (over 90%) of GnRH-IR perikarya are located rostrally in the diagonal band of Broca, medial septum, and medial preoptic area. Although substantial amounts of data indicate that the peptidergic neuronal systems described in the present studies innervate the GnRH neurons both in humans and rodents, the morphological differences are likely to translate into differences in the pattern of innervation. Thus, in the present work we focused on the pattern of juxtapositions between the GnRH system and various neurotransmitter sytems in the human hypothalamus. These juxtapositions

may be functional synapses and may represent the morphological substrate of GnRH release modulated by hypothalamic neurotransmitter systems.

Neurotransmitter systems influencing GnRH release in human - species differences

The peptidergic and catecholaminergic systems contacting the GnRH neurons convey a wide variety of inputs to GnRH neurons that are integrated and translated into an GnRH pulse and subsequent LH pulse. Estrogen, via its receptors (ERα and ERβ), also uses these neuronal systems to fine tune the activity of GnRH neurons. Moreover, estrogen seems to play a "gate-keeper" function, i.e. when it binds to its receptor present in peptidergic and catecholaminergic neurons, the activated ER permits stimulation of GnRH neurons by these signals (24). The rank order neuropeptides play in the regulation of GnRH neuronal activity is not known. However, in rodents glutamate, GABA, and neurotensin from estrogen-sensitive neurons in the anterior ventral periventricular nucleus (AVPV) and norepinephrine from estrogen-sensitive brainstem neurons all play an important role in mediating the positive feedback action of estrogen necessary for the GnRH and subsequent LH surges resulting in ovulation (1,25). The AVPV receives inputs from other brain regions that are critical for the LH surge (26), and therefore, integrates and transduces environmental and hormonal signals to GnRH neurons. Moreover, the AVPV is sexually dimorphic in rats and neurons in its medial division, which are present only in female rats, are coactivated with GnRH neurons at the time of the LH surge (27). However, we do not know whether the AVPV even exists in humans. In rats, β-endorphin in the arcuate nucleus also plays an important role in mediating the effect of estrogen to GnRH neurons whereas the regulatory function of NPY, SP, enkephalin, endorphin, CRH, galanin, and catecholamines on estrogen influenced GnRH activity appears to be secondary and, in most cases, dependent on the endocrine milieu of the animal; for review see Herbison (1). In contrast to rats, only minimal data are available on the functions of these neuronal systems in the regulation of GnRH neuronal activity in humans. Therefore we will focus our discussion on the peptidergic and catecholaminergic regulation of GnRH and LH secretion based on the morphological contacts that these neuronal systems make with the GnRH-IR neurons in humans as well as on the information available in the literature.

Regulatory role of hypothalamic neurotransmitter sytems on GnRH and LH release

NPY system

NPY may regulate LH secretion at the level of GnRH neurons or in the anterior pituitary gland. It facilitates both GnRH release and gonadotroph responsiveness to GnRH at the time of the LH surge in humans (28), primates (29,30) and rats (24,31); for recent review see Terasawa *et al.* (32). In the rat, NPY projections to GnRH neurons arise from two areas: one located in the arcuate nucleus and the other in the brainstem (33). Although NPY neurons in the arcuate nucleus of the rat express estrogen receptors (31), they do not appear to play a pivotal role in the generation of the preovulatory GnRH and subsequent LH surges (34,35) and several studies reported that the median eminence is the principal site where NPY stimulates GnRH release (34-36). The presence of NPY-IR nerve terminals on GnRH-IR perikarya and dendrites in the preoptic area of humans (12), but not in monkeys (37), suggests an action of NPY at proximal sites and not at nerve terminals of the human GnRH neuronal axis.

Substance P sytem

Substance P is an undecapeptide that modulates gonadal functions in several species including human (38-45). Although the exact mechanism of this modulation is not known, there is a general consensus that this phenomenon is based on the influence of SP on hypophyseal LH release. As for many neuropeptides, SP can alter gonadal function via a direct influence on pituitary LH secretion (46-51) and/or by regulation of the hypothalamic GnRH system. Subcutaneously administered SP inhibits GnRH-induced LH release via NK1 receptors (52, 53) and microinjection of the peptide into the medial preoptic area reduces LH and FSH levels in the plasma (54). A direct interaction between SP and GnRH systems, as first suggested by Tsuruo *et al* (55) based on electron microscopic observations in rats, may take place at the level of GnRH perikarya and dendrites in the medial preoptic area and infundibular stalk/median eminence regions in humans (46) and be the morphological substrate for the SP-controlled regulation of gonadal functions in humans.

β-endorphin system

Although previous studies described β-endorphin-IR elements in the hypothalamus of

numerous species including rat and human (47-49), only one study provided a high resolution mapping of the β-endorphin-IR system in the entire human hypothalamus (50). There is a general agreement that β-endorphin-IR perikarya are primarily found in the infundibulum/median eminence and β-endorphin-IR axon varicosities in the periventricular zone and medial hypothalamus. The relative small number of perikarya in contrast to the large number of β-endorphin-IR fibers suggests that either the sensitivity of detection in humans is not sufficient to visualize the entire β-endorphin-IR system or β-endorphin-IR processes may have extrahypothalamic origin. In rodents, the detection of β-endorphin-IR perikarya requires the use of colchicine, a drug that blocks axoplasmic transport and thereby accumulates the neuropeptide in the perikarya. As far as other locations of β-endorphin-IR perikarya are concerned, in rodents, neurons in the nucleus of the solitary tract/area postrema region also express β-endorphin (51).

Physical dependence to opiate drugs in humans is frequently associated with disruption of hypothalamo-pituitary-gonadal functions. Since β-endorphin, an endogenous opioid peptide, interacts with the same receptors (μ ipioid receptors) that are stimulated by opiate alkaloids, such as morphine, it is generally believed that β-endorphin is one of the major factors regulating gonadal homeostasis. Indeed, several studies revealed that β-endorphin is involved in the control of gonadal functions by influencing LH release of the pituitary gland in several species including humans (56).

Since neither basal nor GnRH-stimulated LH release in rat is blocked by endogenous opioid peptides when tested on hemipituitary tissue samples in vitro (57-59), and GnRH receptor antagonists block the naloxone-induced LH release (60), there is a general consensus that β-endorphin regulates LH secretion via GnRH release (59,61-64). β-endorphin nerve fibers communicate with GnRH neurons in rodents (65), monkey (66) and humans (50). β-endorphin has long been implicated in mediating the negative feedback action of estrogen on GnRH neurons since, in rats, a subpopulation of β-endorphin cells expresses estrogen receptors (67). However, to date, no μ, δ or κ opioid receptor mRNAs have been found within GnRH neurons. Thus, β-endorphin does not appear to play a primary role in mediating the negative feedback action of estrogen on GnRH secretion. Rather, the current concept proposed by Herbison (1) is that β-endorphin neurons form a component of the GnRH network that maintains a continuous inhibitory tone within the network and that this may be

involved in determining the dynamics of individual GnRH pulses (68). In contrast, β-endorphin is implicated in mediating stimulatory effects of estrogen on GnRH neurons (31,69).

There is disagreement at present over the participation of other neurotransmitter systems in the β-endorphin-controlled LH release. However, the close contacts between the β-endorphin-IR and GnRH-IR systems in humans (50), monkeys (66) and rodents (48) indicate that β-endorphin may directly regulate hypothalamic GnRH release.

In addition to contacts to GnRH-IR and other types of neurons in the hypothalamus, several β-endorphin-IR axon varicosities apposed portal vessels in the infundibulum and the median eminence (50). These observations suggest that β-endorphin in primates may be released into the portal blood stream and regulate the function of the anterior pituitary gland.

Leu-enkephalin system

Leu-enkephalin, another opioid peptide, may also have dual action on LH secretion acting at the level of the hypothalamus and on the anterior pituitary gland (Fig. 1). Although there are plenty of publications reporting the distribution of leu-enkephalin in the rodent brain (70-73), only one study has reported the distribution of leu-enkephalin in the human hypothalamus (74). In the human hypothalamus, the majority of leu-enkephalin-IR perikarya are present in the periventricular area of the hypothalamus and the infundibulum. In contrast to these findings, the leu-enkephalin-IR fibers form a delicate network throughout the entire hypothalamus without any well-distinguishable pathways. Although the periventricularly located leu-enkephalin fibers seem to originate from the periventricularly located leu-enkephalin-IR cell bodies, most leu-enkephalin-IR axon varicosities appear to be of extrahypothalamic origin.

As with β-endorphin, there is a general consensus that leu-enkephalin influences pituitary LH secretion via regulating hypothalamic GnRH release. However, the reported effects and sites of action of opiate agonists and antagonists on reproduction is controversial. Opioid agonists were reported to decrease the GnRH-controlled release of LH from the anterior pituitary gland in several species, including rats, monkeys (75) and humans (76,77). The release of GnRH from medial basal hypothalamic fragments can be inhibited by submicromolar concentrations of leu-enkephalin (78). Conversely, opiate antagonists

stimulate LH secretion in rats (57,79,80), sheep (81), and primates (82-84), including humans (76,77,85,86).

Although several studies reported other neurotransmitter systems may be involved in the leu-enkephalin-controlled GnRH secretion (87-90), direct innervation of GnRH neurons by the leu-enkephalin system has also been suggested (75). Our studies in humans revealed that the majority of the leu-enkephalin-GnRH juxtapositions (78%) were detected in the infundibulum, whereas in the medial preoptic area only a few GnRH cells were in close association with leu-enkephalin-IR axonal varicosities. These data indicate that modulation of GnRH by leu-enkephalin may be localized to the infundibular area and the median eminence. GnRH-IR nerve terminals in the median eminence receive presynaptic contacts from tuberoinfundibular dopaminergic (TIDA) neurons in the newt (91) and sheep (10). The TIDA neurons of hyperprolactinemic female rats (pregnant, lactating, aged or pharmacologically hyperprolactinemic) co-express leu-enkephalin (72,73). Therefore, it is possible that via these putative presynaptic connections between TIDA nerve terminals and GnRH-IR nerve terminals, enkephalin, colocalized with dopamine, may regulate GnRH release in the median eminence of the rat as well. The juxtapositions between leu-enkephalin and GnRH processes in the human infundibulum reported in the present studies and the presence of opioid receptors in the infundibulum and median eminence (92) suggest that a similar mechanism may also exist in the human. It may be speculated that, during lactation, leu-enkephalin, colocalized with dopamine, the physiological prolactin release-inhibiting factor, may either increase prolactin synthesis/secretion from the anterior pituitary gland and counterbalance the inhibitory action of dopamine and/or modulate the pulsatile pattern of GnRH release into the hypophysial portal circulation.

CRH system

Gonadal functions are suppressed by prolonged stress. Although it is generally believed that this phenomenon involves the inhibition of LH release by catecholamines, there is growing evidence that CRH plays a pivotal role in the stress-related suppression of reproductive functions (93). Although CRH directly influences LH release in rat and human pituitary cells *in vitro* (94,95), there is a general consensus that the effect of CRH on gonadal functions is based on the CRH-mediated inhibition of LH secretion via suppression of

hypothalamic GnRH release. Administration of CRH into the 3rd ventricle blocks the preovulatory GnRH-mediated LH surge in ewe (96). CRH infusion into the medial preoptic area or into the median eminence of the rat inhibits LH secretion through altering GnRH release (97-99). Moreover, CRH directly suppresses GnRH transcription in cell culture (100).

Although several studies indicated that endogenous opiate peptides mediate the regulation of gonadal functions by CRH (94,101,102), there is growing evidence that CRH may directly regulate GnRH release in the hypothalamus. Indeed, synaptic connections have been found between CRH- and GnRH-IR elements in the rat medial preoptic area (103). The infundibular region and the median eminence, two major locations of GnRH perikarya and fibers in humans, are targeted by CRH-IR fibers from the paraventricular nucleus and from periventricularly arranged CRH-IR perikarya. In these locations CRH-IR fibers are juxtaposed to GnRH-IR neurons (104) and may represent CRH-controlled GnRH release in the human hypothalamus.

Galanin system

Galanin, a 29 amino acid peptide (1,105) is widely distributed in the central and peripheral nervous systems. Centrally, galanin impairs cognitive performance, stimulates feeding behaviour, and inhibits spinal excitability (106-108). In the neuroendocrine system, galanin induces the release of prolactin (109-112) and growth hormone (113,114). Recent studies reveal that galanin also modulates gonadal functions via the hypothalamo-hypophyseal axis by regulating pituitary LH release (115).

Galanin may regulate LH secretion at the level of the pituitary (116), via regulation of GnRH release and/or in a paracrine fashion, since galanin is synthesized locally in the anterior pituitary (117,118). Galanin levels are higher in the hypophyseal portal blood than in the peripheral circulation, and galanin release into the hypophyseal portal circulation follows a pulsatile fashion (115). These observations suggest that galanin also functions as a hypothalamic, hypophysiotropic hormone. Tract-tracing studies in rats confirmed these functional observations by demonstrating retrogradely-labelled neurons in the arcuate and paraventricular nuclei from the median eminence (119)..

Galanin (120) and its receptors (121,122) are found in large concentrations in the preoptic area of the hypothalamus and galanin is co-expressed in a subpopulation of GnRH neurons of

the rat (123). The degree of co-expression is estrogen-dependent and sexually dimorphic (123,124). The stimulatory action of estrogen on galanin expression in rats is mediated via ERβ (125), also present in a subpopulation of GnRH-IR neurons (3,4). However, galanin has been shown to dramatically increase GnRH release from nerve terminals in the median eminence (115), suggesting that this is the site of action of galanin. However, the fact that the principal receptor expressed in GnRH neurons is the Gal-R1 (126) makes stimulation of GnRH release by galanin difficult to explain, since the Gal-R1 signaling pathways are inhibitory (127). As suggested by Steiner's group (156), it is possible that when GnRH neuronal activity is low, galanin, co-released with GnRH, blocks subsequent small amounts of GnRH released, however, when GnRH neuronal activity is elevated, galanin cannot suppress such large bulk release and creates a larger potential difference at the GnRH terminal. Via this mechanism, galanin may sharpen the pulsatile pattern of GnRH release into the hypophysial portal circulation necessary for induction of an LH surge and subsequent ovulation.

In addition to an autocrine action of galanin in GnRH neuronal activity, the observations that galanin-IR fiber varicosities abut GnRH-IR perikarya in the preoptic area of the human (105) and rat (123) brain suggest that galanin may control hypothalamic GnRH activity via synaptic communications. Although numerous data suggest that galanin is one of the major modulators of reproductive functions in the rat, it has not been shown to stimulate basal or GnRH-stimulated LH secretion in the pituitary of humans (128-130). However, the well-defined juxtapositions between galanin-IR and GnRH-IR neural elements in the human diencephalon (120) suggest galanin-controlled GnRH release. The recent discovery of galanin receptors and receptor subtype-specific agonists and antagonists should provide an impetus for further research aimed at dissecting out the function of galanin in central regulation of reproduction (127).

Catecholaminergic system

The connections between GnRH-IR and TH-IR neurons shown in this study could represent dopamine, epinephrine and/or norepinephrine, since TH is the first and rate-limiting enzyme for catecholamine synthesis. Although the morphology and distribution of GnRH (9,12,16-18,131) and catecholaminergic neuronal systems (132-135) have been mapped in humans, the structural relationship between these systems has not been studied. The role of all three of these neurotransmitters in the control of GnRH neuronal activity is controversial and

probably species-dependent (1, 165). It appears that stimulatory or inhibitory roles in LH secretion depend on the endocrine milieu and may change during sexual maturation. On the basis of recent molecular and neuroanatomical data, it is proposed that estrogen influences brainstem noradrenergic neurones specifically within the nucleus tractus solitarius (A2) to facilitate synaptic transmission within the GnRH network. In this manner, norepinephrine is likely to play a role in increasing GnRH mRNA expression and secretion necessary for ovulation. In addition to conveying the effects of estrogen, the A2 norepinephrinergic system integrates several visceral and peripheral inputs and, therefore, is well positioned to modulate the activity of GnRH and other neuropeptides/neurotransmitters interacting with GnRH neurons in response to homeostatic and environmental stimuli. Currently, norepinephrine is considered as a permissive factor promoting high output states of the GnRH neuronal network (136). This notion is based on the observations that adrenergic receptor antagonists block the actions of various neuropeptides/neurotransmitters on LH secretion (31). Although these findings originally were explained as a serial arrangement among the neuropeptides/neurotransmitters, norepinephrine and the GnRH neurones, it is now considered (1,136) that there is a parallel relationship between the actions of neuropeptides/neurotransmitters and norepinephrine. With removal of the permissive effect of norepinephrine within the GnRH network, the effect of neuropeptides/neurotransmitters contacting GnRH neurons becomes silent. Thus, the demonstration of TH-IR fibers contacting GnRH-IR neurons in the human hypothalamus suggests an evolutionarily conserved role for the norepinephrine system.

SUMMARY

- The present studies provided high resolution, three dimensional maps of NPY, SP, β-endorphin, leu-enkephalin, CRH, galanin and catecholaminergic neurotransmitter systems in the human hypothalamus.

- These neurotransmitter systems form intimate associations with the hypothalamic GnRH-IR neurons in humans mainly in the medial preoptic area and in the median eminence.

- Since close examination of these juxtapositions using semithin sections or confocal microscopy revealed no gaps between the contacting elements, these associations may be functional synapses, and may represent the modulation of GnRH secretion by various neurotransmitter sytems in humans.

- Although there are no data from the human brain as to whether these peptidergic and catecholaminergic neurons or the GnRH neurons express estrogen receptors, data from rodents and primates strongly suggest that the modulatory actions of estrogen are, indeed, mediated via these neurotransmitters/neuromodulators.

- In a series of studies we conducted in the human hypothalamus, we noticed that a subset of GnRH-IR neurons in front of the lamina terminalis did not have contacts from any of the neuropeptides (NPY, SP, leu-enkephalin, β-endorphin, CRH, galanin) or catecholaminergic systems suggesting that they form a subpopulation(s) that may have an entirely different regulatory mechanism and function than the remaining GnRH-IR perikarya.

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