Improved Visualization of Luteinizing Hormone Releasing Hormone Pathways by Immunocytochemical Staining of Thick Vibratome Sections

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Using 100-micron thick Vibratome sections and a modification of the peroxidase-antiperoxidase method of immunocytochemical staining we achieve a Golgi-like image of luteinizing hormone releasing hormone (LHRH) cells and fibers in mouse brain. Five LHRH pathways are described: 1) A dense projection of fibers from LHRH cells in the medial preoptic and septal areas to the wall of the third ventricle; 2) a projection of fibers from neurons in the bed nucleus of the stria terminalis and the nucleus of the anterior commissure to the subfornical organ; 3) projections of fibers from neurons in the medial septal nucleus and the diagonal band of Broca to the olfactory bulb; 4) fibers which travel within or just lateral to the wall of the third ventricle from the organum vasculosum laminae terminals to the median eminence; 5) cells and fibers located just dorsal to the optic tracts which project rostrally to the preoptic area and caudally to the level of the median eminence where they course medially to converge and enter the median eminence. KEY WORDS: Ventricles; Hypothalamic pathways; Organum vasculosum laminae terminals (OVLT).

Introduction

Immunocytochemical studies of luteinizing hormone releasing hormone (LHRH) cells and pathways report variable results depending on fixation, embedding, species, and antisera used (5). Dehydration through alcohols for paraffin embedding may wash out small peptides and in thin sections fibers appear as punctata of reaction product, making the description of pathways difficult.

In this report immunocytochemical staining of 100-micron thick Vibratome sections reveals LHRH cells and fibers which have not been seen in previous studies, simplifies the tracing of fibers, and provides some appreciation of the wide extent and relative densities of LHRH pathways in the brain.

Materials and Methods

Male Swiss mice were perfused with saline for 5 min followed by Zamboni's fixative for 25 min. Brains were removed and kept overnight in fixative. One hundred-micron thick Vibratome sections were stained in 5-mI polystyrene tubes according to Grzanna et al. (4). Sections were: 1) incubated in anti-LHRH (12) diluted 1:1000 in 0.05 M phosphate buffer, pH 7.6, containing 0.5 M NaCl and 0.4% Triton X-100 for 24 hr at 4°C; 2) washed for 3 hr in 10 changes of 0.05 M phosphate buffer, pH 7.6, containing 0.5 M NaCl and 0.02% Triton X-100 (PSX); 3) incubated for 1 hr in sheep anti-rabbit IgG diluted 1:10 in PSX; 4) washed for 1 hr in three changes of PSX; 5) incubated for 1 hr in rabbit peroxidase-antiperoxidase (PAP) diluted 1:50 in PSX; 6) washed for 1 hr in 3 changes of PSX; 7) rinsed in 0.05 M Tris-HCl devoid of Triton X-100; 8) incubated in 0.05% diaminobenzadine and 0.01% H2O2 in Tris-HCl, pH 7.6, for 14 min; 9) rinsed in 0.05 M Tris-HCl; 10) mounted on glass slides; 11) dried overnight; and 11) mounted in Permount.

In control animals, a 1:1000 dilution of anti-LHRH absorbed in solution by incubation with 100 µg/ml of LHRH (Bachem) was substituted for the primary antisem in alternate serial sections.

Results

In 100-micron thick sections, LHRH cells and fibers are darkly stained with very little background. In adjacent sections incubated with LHRH-absorbed anti-LHRH, no cells or fibers stained.

Ventricular Projections

Most LHRH cells in the medial preoptic area are bipolar. In sagittal sections through midline it is apparent that many of these cells project to the wall of the third ventricle. Small bipolar subependymal neurons send short processes toward the ventricular surface and longer processes toward the organum vasculosum laminae terminals (OVLT). Some bipolar neurons in the septal region send both processes toward the ventricular surface. There is an abundance of...
LHRH cells and fibers within the wall of the third ventricle and some fibers appear to travel for a distance along the surface of ependymal cells within the ventricular cavity.

**Subfornical Projection**

Neurons in the bed nucleus of the stria terminalis and the nucleus of the anterior commissure project fibers over the anterior commissure to the anterior wall of the third ventricle. These fibers travel dorsally to the subfornical organ where they form a dense plexus within the organ. Some fibers of this plexus appear to contact the ventricular surface. LHRH cells are present along this pathway.

**Olfactory Projections**

LHRH neurons in the medial preoptic and septal regions project fibers dorsally and then rostrally to the olfactory bulb. These fibers travel in dense bundles with many LHRH neurons interspersed along the pathway. Cells of the diagonal band of Broca project fibers medially and rostrally to the olfactory bulb. Most LHRH neurons of these olfactory pathways are bipolar, however, occasional multipolar cells are seen.

**Medial Hypothalamic Fibers**

From the combined study of frontal and sagittal sections it is apparent that many fibers from the OVLT travel caudally within or just lateral to the wall of the third ventricle. Retrochiasmatically these fibers fan out dorsally within the wall of the ventricle and can be traced for long distances within sagittal sections. These fibers converge caudally and pass ventrally within the wall of the infundibular recess to the median eminence.

**Lateral Hypothalamic Fibers**

The combined study of frontal and sagittal sections also reveals a lateral pathway from the preoptic area to the median eminence. Spindle-shaped cells which lie just dorsal to the optic tracts project fibers parallel to the tracts, rostrally to the preoptic area, and caudally to the level of the median eminence where dense bands of fibers travel medially to converge and enter the median eminence.

**Discussion**

**Ventricular Fibers**

The demonstration of an abundance of LHRH cells and fibers within the wall of the third ventricle and in close association with the ventricular surface suggests that the cerebral spinal fluid–ventricular system may participate in neuroendocrine function. Subependymal bipolar neurons which send short processes towards the ventricular surface may be specialized sensory units which monitor levels of bioactive molecules within the cerebral spinal fluid. The morphology of these LHRH bipolar neurons in the septal region that send both processes to the ventricular surface suggests a short-loop single-cell control mechanism. In 100-micron thick sections many LHRH cells and fibers appear to contact the ventricular surface. Other neuronal systems are closely associated with the ventricles. Fluorescent and autoradiographic studies demonstrate serotonergic fibers in the third and lateral ventricles (1, 3, 10) and immunocytochemical studies demonstrate sparse somatostatin fibers between ependymal cells (17).

**Subfornical Projections**

The system of LHRH neurons that projects over the anterior commissure to form a dense plexus of fibers in the subfornical organ suggests a pathway for the delivery and release of LHRH into the organ and possibly into the third ventricle. This pathway has also been described in monkey (2).

**Medial Hypothalamic Projections**

LHRH fibers which travel within the wall of the third ventricle from the OVLT to the median eminence may or may not functionally interact with the CSF. The numbers of neurons we see in the retrochiasmatic area and along this pathway is not sufficient to account for the number of fibers we see. We suggest that many cell bodies located in the medial preoptic and septal regions send fibers within the ventricular wall directly to the median eminence or alternatively to the OVLT and from there via this medial pathway to the median eminence.

Figure 1. In a 100-micron thick frontal section through the third ventricle at the level of the median eminence in rat, LHRH fibers extend between ciliated ependymal cells (arrowheads) to the ventricular surface (arrow). ×610.
Lateral Hypothalamic Pathway

The dense bands of LHRH fibers entering the median eminence from the lateral hypothalamic pathway indicate that it is a major source of LHRH fibers in the median eminence. Marshall et al. (11) describe LHRH cells and fibers dorsal to the optic tract in baboon, but do not follow the pathway to the median eminence. Krisch, however, describes a similar ventrolateral somatostatin pathway from the preoptic area to the median eminence in rat (9).

Literature Cited

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