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Rich ependymal investment of luliberin (LHRH) fibers revealed immunocytochemically in an image like that from Golgi stain

(luteinizing hormone releasing hormone/unlabeled antibody method/cerebrospinal fluid/neuroendocrine control mechanisms/third ventricle)

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ABSTRACT **Immunospecific staining in 100-μm-thick Vibratome section by the unlabeled antibody method resembles Golgi staining and reveals an abundance of luliberin- (luteinizing hormone releasing hormone, LHRH) positive cells and fibers in close contact with the surface of the third ventricle. The polarity of LHRH cells can be seen and processes can be traced for several millimeters. In the medial preoptic and suprachiasmatic areas bipolar LHRH neurons send short stout processes to the ventricular surface and long processes toward the organum vasculosum laminae terminalis. These cells resemble the receptor cells contacting the cerebrospinal fluid that have been described by Vigh and Vigh-Teichmann [Vigh, B. & Vigh-Teichmann, I. (1973) *Int. Rev. Cytol.* 35, 189–251]. In the septal region some bipolar neurons send both of their processes towards the ventricular surface. LHRH neurons in the nucleus of the anterior commissure and the bed nucleus of the stria terminalis project over the anterior commissure to form a dense plexus of fibers in the subfornical organ. The proximity of LHRH perikarya and fibers to the ventricular surface supports the hypothesis [Knigge, K. M., Joseph, S. A., Scott, D. E. & Jacobs, J. J. (1971) in *The Neuroendocrinology of Human Reproduction*, eds. Mack, H. C. & Sherman, A. J., (Thomas, Springfield, IL), pp.6–22.] that the cerebrospinal fluid functions in neuroendocrine control mechanisms.**

Cerebrospinal fluid (CSF) contains several neuropeptides and neurotransmitters (1, 2). Immunocytochemical studies have revealed luliberin- (luteinizing hormone releasing hormone, LHRH) reactive cells near the third ventricle, and puncta of reaction product suggest a ventricular projection (3). These studies have been limited to the examination of relatively thin sections with only small segments of cells and fibers present in each section. A recent modification of the peroxidase-antiperoxidase method allows for penetration of its reagents through 100-μm-thick Vibratome sections and reveals immunospecific neuronal profiles like the profiles obtained with the Golgi method (4). In such sections the polarity of neurons is seen and fibers can be traced for several millimeters. The present report examines the relationship of LHRH-immunoreactive cells and fibers to the third ventricle by using this method.

MATERIALS AND METHODS

Mature male Swiss mice were anesthetized with pentobarbital and perfused through the ascending aorta with saline for 5 min followed by Zamboni's fixative at room temperature for 25 min. Brains were removed and kept overnight in fixative. Sagittal and frontal Vibratome sections, 100 μm thick, were stained in 5-ml polystyrene tubes according to Grzanna *et al.* (4). The sections were incubated for 24 hr at 4°C in anti-LHRH (5) diluted 1:1000 in 0.05 M sodium phosphate buffer, pH 7.6, containing 0.5 M NaCl and 0.4% Triton X-100. Sections were washed for 3 hr in 10 changes of 0.05 M sodium phosphate buffer, pH 7.5, containing 0.5 M NaCl and 0.02% Triton X-100 (PSX), incubated for 1 hr in sheep anti-rabbit IgG diluted 1:10 in PSX, and washed for 1 hr in 3 changes of PSX. Sections were then incubated with peroxidase-antiperoxidase complex (6) diluted 1:50 to contain 0.020 mg of peroxidase and 0.055 mg of rabbit antiperoxidase per ml and again washed for 1 hr in PSX. Sections were rinsed in 0.05 M pH 7.6 Tris·HCl devoid of Triton X-100, incubated with 0.05% diaminobenzidine and 0.01% H_2O_2 in Tris·HCl for 14 min, rinsed in Tris·HCl, mounted on glass slides, dried overnight, and mounted in Permount.

To control for nonspecific staining, a 1:1000 dilution of anti-LHRH serum absorbed with LHRH (Bachem, Fine Chemicals, Torrance, CA) at 100 μg/ml was substituted for the primary antiserum in the staining procedure.

RESULTS

In 100-μm-thick sections a dense network of LHRH-specific cells and fibers is strongly stained with little background. No cells or fibers are stained in adjacent sections in which LHRH-absorbed anti-LHRH was used. Many LHRH neurons in the preoptic area are oriented in the sagittal plane and, in parasagittal sections, processes can be traced for several millimeters within the same section.

An abundance of LHRH cells and processes in the medial preoptic and septal regions are in direct contact with the third ventricle (Fig. 1). Small bipolar neurons within the ventricular wall send short processes to the ventricular surface and longer processes to the organum vasculosum laminae terminalis (Fig. 2). Other LHRH-immunoreactive perikarya appear to be in direct contact with the ventricular cavity (Fig. 3). Several bipolar cells in the septal region that send both processes toward the ventricular surface have been seen (Fig. 4). In general, ependymal cells do not stain. However, light staining of occasional tanycytes has been observed.

Some neurons in the bed nucleus of the stria terminalis and in the nucleus of the anterior commissure send fibers over the anterior commissure to the third ventricle (Fig. 5). These fibers travel ventrally along and within the wall of the third ventricle to the thalamic recess, where they form a dense plexus in the subfornical organ (Fig. 6). The beaded fibers of this plexus appear to terminate at the ventricular surface and perhaps within the ventricle.

The walls of the third ventricle are rich with LHRH-immunoreactive fibers. Some fibers perpendicular to the wall of the ventricle appear to extend between ependymal cells to the ventricular surface. Other fibers extend for some distance

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Abbreviations: CSF, cerebrospinal fluid; LHRH, luliberin (luteinizing hormone releasing hormone); PSX, 0.05 M sodium phosphate buffer, pH 7.5/0.5 M NaCl/0.02% Triton X-100.

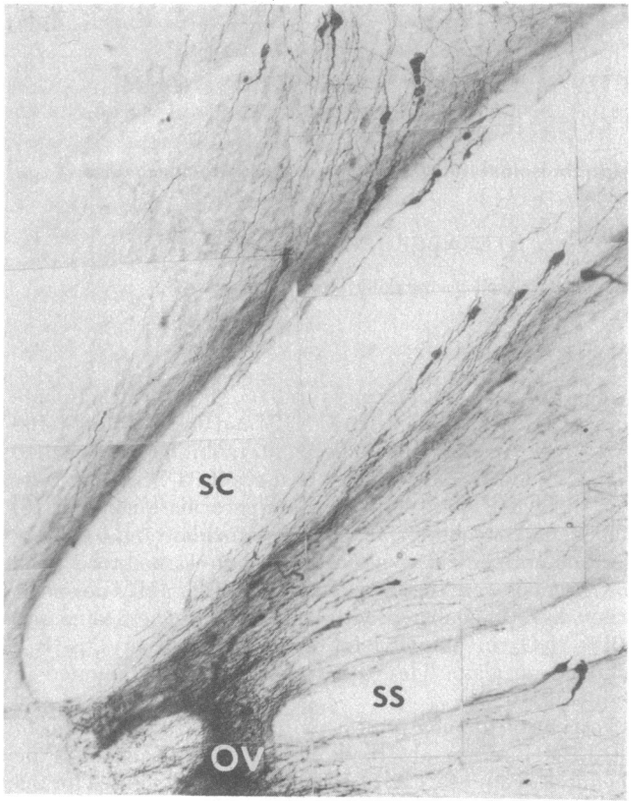

FIG. 1. In a sagittal section near midline, a photomontage of the suprachiasmatic and medial preoptic areas shows an abundance of LHRH-immunopositive cells and fibers within the ventricular wall and in direct contact with the ventricular surface. ov, Organum vasculosum laminae terminalis; sc, suprachiasmatic recess of third ventricle; ss, suprasellar recess of third ventricle. (×130.)

within the walls of the ventricle. In sections through the midline in which the wall of the ventricle is cut in a tangential plane, beaded fibers appear to extend from the organum vasculosum laminae terminalis to the median eminence within the wall of the ventricle. In addition, some fibers appear to extend along the surface of the ependymal cells within the ventricular cavity.

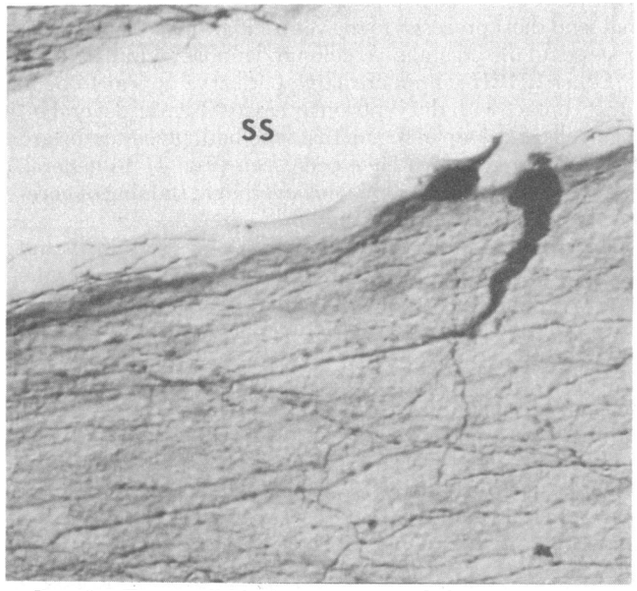

FIG. 2. Two small bipolar neurons send short stout processes toward the ventricular surface of the suprasellar recess (ss) of the third ventricle. (×630.)

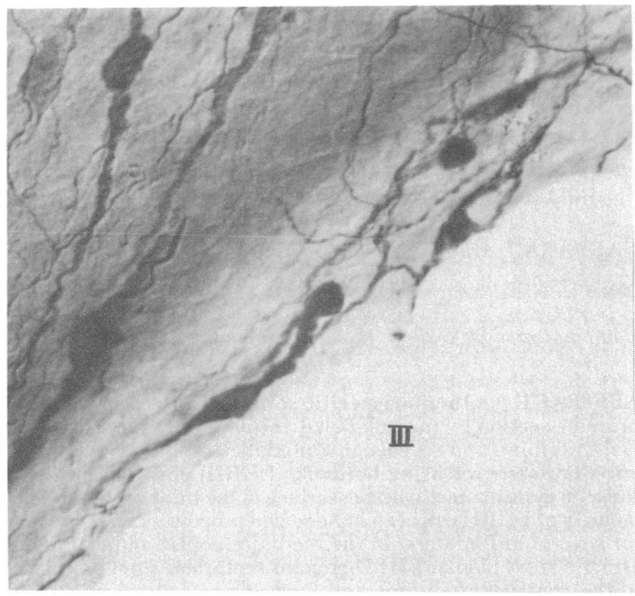

FIG. 3. LHRH-immunoreactive cells and fibers appear to be in direct contact with the ventricular cavity. III, Third ventricle. (×630.)

DISCUSSION

Nerve fibers in contact with and within the ventricular cavity have been seen by light and electron microscopy (7, 8). Fluorescence and autoradiographic studies demonstrated supraependymal serotonergic fibers in the third and lateral ventricles (9–11), and immunocytochemical studies revealed a relationship of LHRH (12–14), neurophysin (14), and angiotensin II (15) fibers with tanycytes and a relationship of somatostatin fibers with ependymal cells (16, 17).

The resolution obtained with thick Vibratome sections in the present paper does not permit the conclusion that LHRH fibers come in direct contact with the CSF. The possibility of separation of the fibers from the lumen of the third ventricle by flattened glial or ependymal cell processes must also be considered. In any event, the apposition of LHRH cells and fibers to the ventricular surface reinforces the notion that the

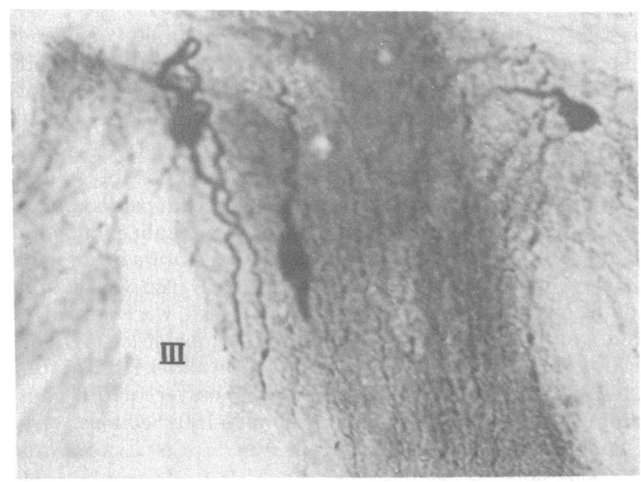

FIG. 4. In frontal section a bipolar neuron in the septal region sends both processes toward the triangular recess of the third ventricle (III). (×630.)

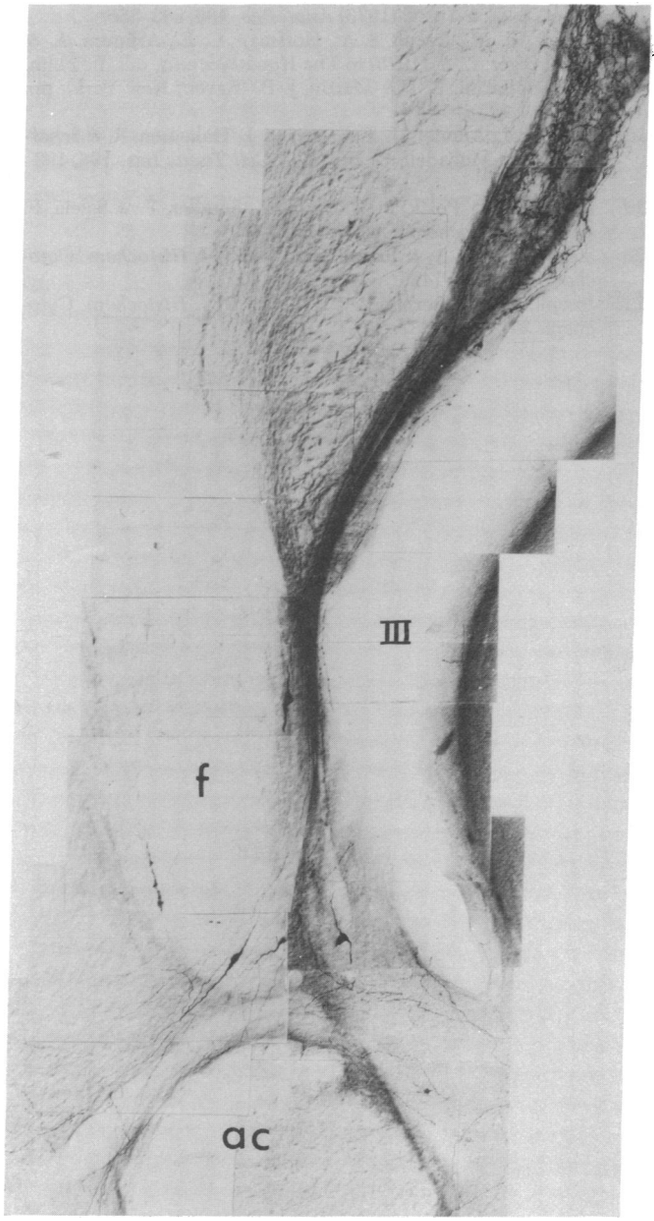

FIG. 5. Neurons in the nucleus of the anterior commissure and the bed nucleus of the stria terminalis send processes to the subfornical organ. ac, Anterior commissure; f, fornix; III, third ventricle. (×100.)

CSF–ventricular system influences neuroendocrine or perhaps other functions of these neuropeptide-producing cells. The morphology of LHRH neurons that contact the third ventricle permits several modes of interaction. Small bipolar neurons that send short processes to the ventricular surface may be specialized sensory units that monitor levels of bioactive molecules within the CSF. Vigh and Vigh-Teichmann (7) describe the ultrastructure of small, bipolar, CSF-contacting neurons that are cytologically similar to special sensory elements of both vertebrates and invertebrates. The morphology of LHRH bipolar neurons in the septal region that send both of their processes to the ventricular surface suggests a short-loop single cell control mechanism. In addition, the LHRH system of neurons that projects over the anterior commissure to form a dense plexus of fibers in the subfornical organ of the third ventricle may represent one pathway for the delivery and release of

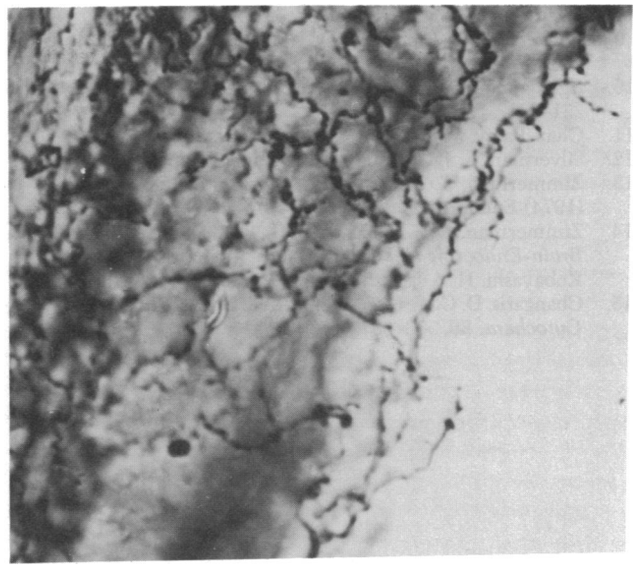

FIG. 6. LHRH fibers form a dense plexus in the subfornical organ. Some fibers appear to contact the ventricular surface. (×630.)

LHRH into capillaries of the organ and possibly into the third ventricle.

Because of the short half-life of neuropeptides in blood, feedback of neurohormones may perhaps be exerted via nonvascular paths. The presently described terminals in close association with the CSF could conceivably provide receptors that partake in such extravascular feedback regulation.

Finally, the possibility could be conceived that neurons delineated with antiserum to LHRH may not represent LHRH but a peptide related to it. Immunocytochemistry cannot prove identity of antigens, although it can, by the combined use of several antisera, demonstrate diversity (6, 18, 19). Such studies have recently revealed diversity of corticotropin- and β-lipotropin-related peptides in different regions of the hypothalamus (20, 21) and suggested that neurons may express individual idiotypic specificities of proteins produced and peptides cleaved therefrom. One may have to consider, therefore, that the LHRH projections to periventricular organs or to the ventricular system may not necessarily be true LHRH projections and may not necessarily partake in reproductive mechanisms. Perhaps the ventricular termination of fibers as unrelated in their origin as the LHRH fibers described here and the 5-hydroxytryptamine fibers described by Lorez and Richards (9) and Chan-Palay (11) allude to a yet undiscovered function of the ventricles.

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