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Mast cells with gonadotropin-releasing hormone-like immunoreactivity in the brain of doves

(sexual behavior/habenula/immune–neuroendocrine interactions)

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ABSTRACT **Using an antiserum (LR-1) raised against mammalian gonadotropin-releasing hormone (GnRH), we previously identified a nonneuronal cell that was more numerous in the medial habenula (MH) of courting ring doves than in individuals housed in visual isolation. The current studies suggest that they are mast cells. Both acidic toluidine blue and toluidine blue dissolved in water/butanediol revealed metachromatic cells with a distribution and morphology similar to that obtained by immunostaining with the GnRH antiserum in the MH. Some cells had granules reactive to safranin in the presence of alcian blue, indicative of a highly sulfated proteoglycan of the heparin family. Immunocytochemical studies demonstrated that all MH cells containing GnRH-like immunoreactivity contained histamine, another mast cell marker. The GnRH-immunoreactive cells had a unilobular, ovoid nucleus. Secretory granules within the cells were electron dense and displayed a variety of internal structures. Fine filamentous processes appeared evenly distributed on the cell surface whether cells were located on the pial surface or within the brain parenchyma. All of these features are characteristic of mast cells. To test whether the epitope recognized by the GnRH antiserum was produced by the mast cells or endocytosed from the cerebrospinal fluid, an iodinated GnRH analog was injected intracerebroventricularly at the initiation of courtship. Radioautography revealed no radioactive cells in the brain, indicating that the GnRH antibody recognized a molecule synthesized by the nonneuronal cells rather than internalized by a receptor-mediated mechanism. These observations suggest an interaction between a component of the immune network and specific regions of the central nervous system.**

A population of cells, recognized by gonadotropin-releasing hormone (GnRH)-like immunoreactivity, is present in large numbers in the medial habenula (MH) of the ring dove after a brief period of courtship (1). The cells were classified as nonneuronal because they lacked axonal or dendritic processes and had heterochromatic rather than euchromatic nuclei. There were fewer such cells in birds housed in isolation, and cell numbers were further reduced or not detectable in long-term castrates (2). The object of the present studies was to identify these nonneuronal elements. We previously suggested that they might be of either monocyte/microglia or mast cell lineage. Both of these cell types are derived from the bone marrow. Mast cells generally circulate in precursor form and enter tissues where they complete their differentiation; basophils, which share some characteristics with mast cells, are granulated leukocytes that circulate in fully differentiated form and rarely enter tissues (3). Mast cells enter the brain during the neonatal period (4) and are usually associated in the adult with the blood vessels; they lie just inside the blood–brain barrier (5–13). After insult to the brain or damage of brain tissue by disease (5), mast cells enter deeper into the neuropil of the central nervous system (CNS). The present report characterizes the nonneuronal cells within the MH and indicates that they are mast cells.

METHODS

Animals. Subjects were adult male ring doves (*Streptopelia roseogrisea*), at least 1 year old, with previous breeding experience. They were housed for ≥3 weeks in visual, but not auditory, isolation from other birds and were kept on a 14-hr light/10-hr dark cycle. At the beginning of the experiment, each male was placed with an adult female in a cage provided with straw and a nest bowl, and the pair was permitted to court uninterruptedly. Animals given radioactively tagged GnRH were permitted to court for 2 hr to 5 days. In all other experiments, animals were paired for only 2 hr and immediately sacrificed under deep chloral hydrate/pentobarbital anesthesia prior to intracardiac perfusion with fixative.

Mast Cell Markers. *Toluidine blue.* Tissue sections prepared for immunocytochemistry, radioautography, or EM (see below) and blood samples obtained from the wing vein were stained with acidic toluidine blue (14). Blood smears were dried at room temperature, fixed briefly in Carnoy's fixative (14), and then exposed to the stain. In a second staining procedure, the dye (0.05%) was dissolved in distilled water/butanediol (50:50, vol/vol) (15).

Alcian blue/safranin. Brains were fixed with 4% (wt/vol) paraformaldehyde/0.1% glutaraldehyde. Vibratome sections (50 μm) were stained in 1% (wt/vol) alcian blue and counterstained using 0.5% safranin (16).

Immunocytochemical Procedures. *Reagents.* Immunocytochemical staining for histamine- (17), serotonin- (18), and GnRH-like immunoreactivity was performed. The latter used an antibody (LR-1) directed against GnRH (1, 19).

Tissue preparation. For the light microscopic studies involving serotonin and GnRH-like immunoreactivity, animals were perfused with 4% paraformaldehyde. Brains, liver, lung, and the gastric gland of the intestinal tract were cut on a vibratome at 40–50 μm. Detection of histamine required fixation in 4% (wt/vol) 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (Sigma) (17). These brains were cut on a cryostat (30 μm).

Immunocytochemical processing. Localization of GnRH- or serotonin-like immunoreactivity was accomplished separately in sections treated with Triton X-100 (0.1%) at dilutions of 1:20,000 and 1:2000, respectively. We used appropriate biotinylated secondary antibodies (Vector Laboratories) and an avidin-biotin-horseradish peroxidase conjugate (Vector Laboratories) with 3,3′-diaminobenzidine as the chromogen

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Abbreviations: GnRH, gonadotropin-releasing hormone; MH, medial habenula; CNS, central nervous system; CSF, cerebrospinal fluid.

(Sigma) (1). For the visualization of histamine alone, antibody incubations (dilution of 1:4000) were carried out on cryostat sections. All other steps were identical to those listed above. Simultaneous localization of histamine- and GnRH-like immunoreactivity entailed sequential incubation in (*i*) the antiserum to histamine, (*ii*) biotinylated goat anti-rabbit IgG, (*iii*) avidin-aminomethylcoumarin acetate (Jackson ImmunoResearch), (*iv*) avidin-biotin blocking reagents (Vector Laboratories), (*v*) the LR-1 antiserum, (*vi*) biotinylated goat-anti rabbit IgG, and (*vii*) avidin-Texas Red (Vector Laboratories). In some experiments the order of the primary antibodies was reversed or the second primary antibody was replaced with normal rabbit serum.

EM. Birds were perfused with 2% paraformaldehyde and 2.5% glutaraldehyde. The MH was postfixed for 4 hr. Vibratome sections (40 μm) were cut, and the MH was dissected. The MH was treated with 2% (wt/vol) OsO_4 in 0.9% NaCl containing 1.5% $K_3Fe(CN)_6$ and embedded in Epon 812. Cells (n = 10) were identified by their purple granules in toluidine blue-stained 1-μm sections. Each cell was examined at three levels 700 nm apart. Ultrathin sections were mounted on slot hole grids and viewed without counterstaining with a JEOL 1200EX.

Injection of Radioactive GnRH Analogue. *Injection protocol.* To determine if the GnRH-like immunoreactivity entered by endocytosis from the cerebrospinal fluid (CSF), an ^{125}I-labeled GnRH agonist [D-Ala^6,MeLeu^7,Pro^9]GnRH ethylamide was prepared, purified, iodinated (21), and administered in 7 μl of avian saline over 7 min at the time of pairing via an indwelling cannula in the lateral ventricle (22). Animals (n = 9) received between 250,000 cpm and 2 × 10^6 cpm and were killed after 2 hr to 5 days of pairing.

Radioautography. For radioautographic localization of the iodinated GnRH analog, 30-μm vibratome sections were mounted onto slides, air-dried overnight, then dipped at 41°C in Kodak NTB-2 emulsion diluted 1:1 with distilled water, and exposed for 2–4 weeks. Sections were developed in D19 (Kodak; 15°C, 4 min), fixed with Rapidfix (Kodak; 2 min), and counterstained with acidic toluidine blue (14).

RESULTS

Numerous small, round nonneuronal cells of 8–10 μm in diameter that were immunoreactive to the LR-1 antiserum were observed in the MH of all courted birds (1, 2). Immunoreactive neurons were distributed in the dove brain as described (19). The nonneuronal cells in the MH were easily distinguishable from the fusiform immunoreactive neurons of the preoptic area.

Mast Cell Markers. *Acidic toluidine blue.* Sections from courted birds used in the radioautographic studies (see below), sections from the tissue processed for light microscopic immunocytochemistry and conventional EM, and blood smears were stained with toluidine blue at pH 2.5. In all preparations small, round granulated cells displaying metachromatic properties (i.e., alteration of the color of the dye) were observed. The color and the degree of vacuolation of the cytoplasm varied with fixation and/or tissue preparation. In tissue (MH, lung, and liver) fixed with 4% paraformaldehyde, the cells had a pink cytoplasm and deep blue nuclei (data not shown). In the brain, this staining pattern was easily distinguished from that of either the surrounding neurons with their pale blue cytoplasm and clear nuclei containing deep blue nucleoli or the nuclear staining produced in glial cells. When toluidine blue staining was carried out on the 1-μm plastic-embedded sections of tissue prepared for conventional EM (see *Methods*; Fig. 1*a*), these same cells contained deep purple/violet granules. Such granules were absent from surrounding neurons whose cytoplasm was a uniform pale blue and whose nuclei contained a prominent, deep blue nucleolus (Fig. 1*a*). To determine if the GnRH$^+$ cells accounted for all of the nonneural elements, we prepared 20 1-μm sections from tissue processed for immunocytochemistry, counted all of the LR-1$^+$ cells, and then counterstained the sections with acidic toluidine blue. No additional metachromatic cells were evident within the tissue sections.

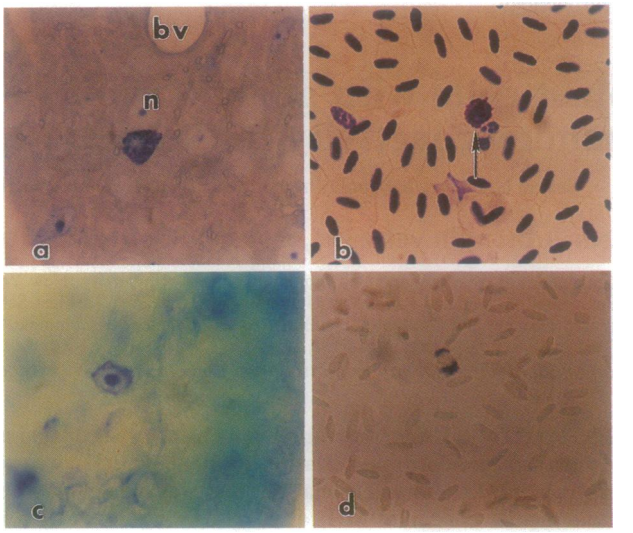

FIG. 1. Toluidine blue-stained sections of the brain (*a* and *c*) and blood (*b* and *d*). (*a*) Acidic toluidine blue revealed a population of metachromatic cells with deep purple granules and heterochromatic nuclei. These are distinct from the surrounding neurons (n) with very pale cytoplasm and a prominent nucleolus. Note that the metachromatic cell is not near blood vessels (bv). Tissue was fixed with a mixture of aldehydes that best preserves the granules. (*b*) When the same dye is applied to blood smears, a population of slightly smaller cells, presumably basophils, with pink to purple granules and a heterochromatic nucleus are present (arrow). Also stained are numerous nuclei of the red blood cells. (*c*) When toluidine blue is dissolved in water/butanediol, nonneuronal cells in the MH have a deeply stained nucleus and pale pink granules. This fixative did not preserve granular structure as well as the aldehyde mixture illustrated in *a*. (*d*) A rare granulated blood cell is revealed by staining with the toluidine blue in water/butanediol. Not only was this cell less frequently encountered than the cell in *b* but also the cell shape appeared different. Both blood smears were fixed briefly in Carnoy's solution. (×550.)

In blood smears (Fig. 1*b*), the nuclei of the red blood cells were stained deep blue. Neutrophil nuclei (not shown) were a pale gray and heavily lobulated. Additional granulated leukocytes (5–10 per slide), presumably basophils with a deep blue, heterochromatic nucleus and deep pink/purple granules, were also visible (Fig. 1*b*).

Toluidine blue in butanediol. When the toluidine blue dye was dissolved in water/butanediol, the cells in the MH had a distribution similar to that seen with the acidic dye or with the LR-1 or histamine antiserum (see below). Both the nucleolus and cytoplasmic granules were a blue/purple (Fig. 1*c*). In the blood neither red blood cells nor neutrophil nuclei were stained (Fig. 1*d*). An additional rare cell type (a total of five observed on as many slides) was found, which had very small blue/purple granules (Fig. 1*d*).

Alcian blue/safranin. With this mixture of reagents, small, round cells with a similar morphology and distribution to that observed with acidic toluidine blue were found. The cells contained either all red, all blue, or a combination of red and blue granules (not shown).

Immunocytochemistry. Single-label studies indicated that the histamine and LR-1 antisera recognized cells with a similar rounded morphology in the brain, as well as in the lung and liver. The gastric gland of the intestinal tract was not tested for the presence of histamine because no LR-1-positive cells were

found. The MH contained histaminergic- and LR-1-positive axons but no positive neurons. The serotonin antiserum stained enteroendocrine cells in the gastric gland and nerve fibers in the brain, including the MH (not shown). No other elements were immunoreactive for serotonin in the tissues studied.

Double-label studies carried out on the MH (Fig. 2 *a–c*), the adjacent pia, lung, and liver (not shown) indicated that all nonneuronal cells (lacking dendritic and axonal processes) that reacted with the LR-1 antiserum also contained histamine-like immunoreactivity. The immunofluorescence was punctate, and most puncta appeared to fluoresce at both emission spectra when appropriately excited (Fig. 2*c*). Control sections in which the second primary antibody was replaced with normal rabbit serum contained only single-labeled cells, indicating that the double-labeling was not due to binding of the second fluorophore to the initial layer of reagents.

EM. The apparent degranulation of the LR-1$^+$ cells noted earlier (1) led us to examine tissue in which ultrastructural preservation was maximized. This degranulation was artifactual since in tissue fixed with a mixture of aldehydes and processed without detergents, mast cells were not vacuolated. Their granules had a wide range of sizes (0.5–1.5 μm), electron densities, and substructure, indicative of different degrees of activation and secretion (Fig. 3). Granular patterns included dense homogeneous, scroll, and particle types. Fine filamentous cell surface extensions were clearly evident and uniformly distributed on cells resident within the CSF (between the thin pial covering and the habenula proper) (Fig. 3*a*). Interestingly, cells within the parenchyma also had these clearly defined processes though they tended to be more convoluted (Fig. 3 *b* and *c*) as if compressed by the surrounding tissue. In some cases, extracellular space between the neuronal elements and these nonneuronal cells was evident. Of the 50 nonneuronal cells examined ultrastructurally, only 2 were observed adjacent to the endothelial cells of brain capillaries. Sections taken at different depths indicated that they did not give rise to any dendritic or axonal processes and received no synaptic input.

Occasional lipid droplets were identified within the cytoplasm. Glycogen was absent. There was little rough endoplasmic reticulum and a small Golgi apparatus (Fig. 3*c*).

Endocytosis of GnRH Analog. Radioactive neuropeptide, administered into the CSF and detected in tissue sections by radioautography, did not accumulate in any cells within the brain, although abundant silver grains were found in the ventricular space.

DISCUSSION

Mast Cell Markers. The aim of the present study was to characterize the nonneuronal elements of the ring dove MH.

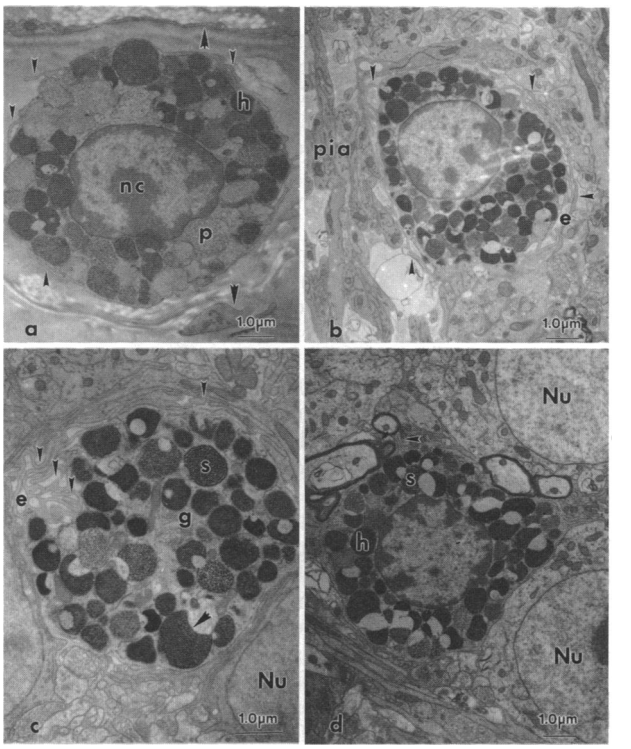

FIG. 3. Electron micrographs of mast cells near (*a*) or in (*b–d*) the MH. Tissue was fixed with 2% paraformaldehyde/2.5% glutaraldehyde and processed solely for electron microscopy. (*a*) This cell is free in the CSF and associated with the thin pia (large arrowheads). The ovoid, unilobed nucleus has heterochromatin condensed along the inner surface of the nuclear envelope. Granule substructures range from homogeneously (h) electron-dense (sometimes with a semicircular concavity), particle (p), or mixed scroll and particle. Fine filamentous processes (small arrowheads) are seen evenly distributed over the cell surface. nc, Nucleolus. (*b*) This cell lies just within the CNS proper, below the pia. Note the abundant processes on the parenchymal side of the cell (arrowheads) and the extracellular space (e) between the cell and the neuronal elements. (*c*) In this cell, the scroll pattern (s) of granules and the presence of semicircular concavities (large arrowhead) are obvious. A portion of the Golgi apparatus (g) is present in this plane of section. Even though the cell is embedded in the CNS, the cytoplasmic extensions are still prominent (small arrowheads) and enclose extracellular space (e). A neuronal nucleus (Nu) is seen in the lower right. (*d*) This cell is deeper in the parenchyma, and there is no extracellular space separating the mast cell from the brain tissue. However, the cell still has some filamentous processes (arrowhead). Note the difference between the nucleus of the mast cell and the nuclei of two adjacent neurons (Nu). s, Scroll granule; h, homogeneous granule.

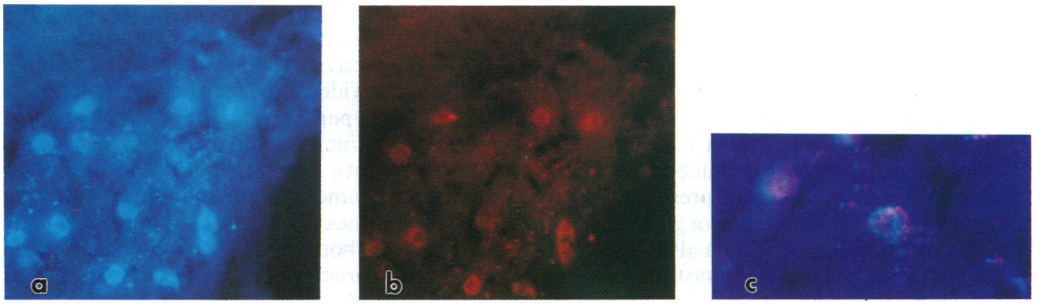

FIG. 2. Double-label immunocytochemical staining for histamine- and GnRH-like immunoreactivity using aminomethylcoumarin acetate and Texas Red fluorophores, respectively, in the MH of an animal that had courted for 2 hr. In *a* and *b*, exposures for the two fluorophores were taken on separate transparencies. All cells appear to contain both the peptide and the amine. In *c* a double exposure was made with excitation/emission cubes slightly misaligned. This permits one to see more clearly that granules within the cell fluoresce at both wavelengths. (×550.)

The use of mast cell markers and immunocytochemical and ultrastructural methods indicate that the cells are mast cells. Mast cells are known to contain sulfated proteoglycans including heparin (3). When cells containing polyanionic polymers such as heparin are stained with basic aniline dyes, these molecules alter the staining characteristics of the dye by forming aggregates that absorb at a lower wavelength (23). For example, toluidine blue stains mast cells violet or purple while neurons are stained pale blue. This dye revealed a population of mast cells in the MH with different staining properties than surrounding neurons, best seen in 1-μm sections in tissue fixed for conventional EM. Metachromatic cells were distributed in a manner similar to those observed following immunocytochemical detection of the GnRH-like peptide or histamine. In 1-μm sections, immunocytochemically processed with the LR-1 antiserum and subsequently counterstained with toluidine blue, there were no additional metachromatic cells. Hence, the LR-1 staining accounts for all mast cells in this brain region.

Acidic toluidine blue also stains the granules of basophils (24). These granulocytes also contain sulfated proteoglycans though usually of a lower sulfation level than heparin (3), designated heparans. Blood smears had metachromatic cells under these staining conditions. These occurred at a higher frequency than in mammals, as found in other birds (15, 25).

Toluidine blue dissolved in water/butanediol distinguishes tissue mast cells (positive) from circulating basophils (negative) in birds (15, 26). The MH contained a single population of metachromatic cells with a distribution and morphology similar to that obtained with the acidic dye. In contrast, the blood smears contained a very rare cell type, with a morphology different from that observed in smears stained with acidic toluidine blue and also differing from that obtained in brain with the butanediol procedure. These cells were elongated, rather than ovoid (compare *b* to *d* in Fig. 1). We suggest that in blood acidic toluidine blue stains basophils and the butanediol mixture stains another blood cell that differs from the blood basophils and the tissue mast cell.

We employed a second histochemical marker that reacts with the highly sulfated form of glycosaminoglycans found in mature mast cells. Alcian blue reacts with the more lightly sulfated heparans; safranin competes with the alcian blue only when the mature, highly sulfated heparins (27) are present. Basophils derived from human fetal liver stem cells are alcian blue-positive/safranin-negative (28). In contrast, the nonneuronal cells in courted birds contained safranin-positive granules. Interestingly, cells with similar morphology show the same developmental pattern in doves as found in mammals (29) and chickens (30, 31)—with a developmental switch from being only alcian blue-positive to gradual acquisition of safranin staining (32).

Immunocytochemistry. An antibody directed against histamine further delineated the granule contents of the LR-1$^+$ mast cells. In the MH (as well as lung and liver, see below), these two antigens were colocalized, most likely in the same granules (Fig. 2*c*). These cells lacked serotonin, a constituent of mast cells in some but not all species.

Ultrastructure. Mast cells and basophils can also be distinguished ultrastructurally (26, 33, 34). Human mast cells are characterized by a monolobed rather than multilobed nuclei, by evenly distributed fine surface projections, the presence of lipid droplets, variegated granules, and the absence of glycogen (see table 3 in ref. 35). This constellation of criteria also marks the nonneuronal cells of the dove brain. Avian mast cell granule morphology includes the presence of semicircular concavities (30, 31, 34, 36) and is similar to that presented here. Furthermore, circulating basophils in domestic fowl have a bilobed nucleus (26) distinct from the tissue cells. Although more functional assays on isolated presumptive mast cells from the ring dove will be essential in making a final diagnosis, the data suggest that the cells described in this and our previous reports (1, 2) are mast cells. This is further supported by the presence of LR-1$^+$ cells in organs such as lung and liver, which are known to contain mast cells in avian species (36). In humans and mice, basophils do not enter tissue space in nondisease states, but whether they can do so in birds is not known.

Location in Brain. In the normal adult mammalian brain, mast cells have been reported in close association with endothelial cells of capillaries (5–11). In the MH of courted birds, this association of mast cells and endothelial cells is not obligatory. Ultrastructural observations carried out on successive sections indicate that mast cells located just below the ependymal wall were primarily in contact with glial processes. Those at least one cell diameter deeper into the brain were surrounded by nonvascular elements of the neuropil. Only occasional mast cells (2 out of 50 studied) were located adjacent to capillaries. The present report is, to the best of our knowledge, the first demonstration that mast cells in the normal adult brain are not necessarily associated with blood vessels.

Origin and Migration. In mammalian species, mast cells are derived from the bone marrow and circulate as committed precursors in the blood (3). They complete their specific differentiation program upon entering the tissues. In the adult dove, prior to courtship, a few LR-1$^+$ mast cells are observed in the choroid plexus (37) and in the pia between the tectum and the habenula. Whether cells in the MH observed following courtship (1) originate from those resident in the choroid or pia or are derived from less mature precursors present in the blood is not known. The morphology of mast cells found just below the pial or ependymal surface suggests migration. These cells still maintain numerous filamentous processes, and there is considerable extracellular space between them and the neuropil. These processes appear collapsed on cells lying deeper in the parenchyma, and the extracellular space is obliterated. Definitive proof of transmigration must await experiments in which cells can be prelabeled prior to courtship.

Degranulation. Although the cells observed following immunocytochemical detection of LR-1 appeared extensively degranulated (1), this is likely an artifact of tissue preparation. In sections prepared for conventional EM, the granules had a very different appearance (vide supra). Many were homogeneously electron dense; other granules showed internal patterns described for cells that had undergone prior stimulation and granule release (35). However, the morphological appearance of the granules (especially since the unstimulated state is unknown in doves) can only suggest that prior release of material had occurred. If secretion does take place, it would presumably result in release of histamine and a GnRH-like peptide, making these biologically active substances more available as courtship proceeds. Since axons immunoreactive for each of these substances are found within this area (19), it is reasonable to suppose that specific histamine and GnRH receptors are also present.

Other Tissues. Mast cells containing histamine and LR-1 immunoreactivity were also found in the lung and liver. Mast cells are divided into many subcategories and can vary in their amine, peptide, and proteoglycan contents (reviewed in ref. 3). Distinctions have been made in mammals between mucosal (gut), serosal (lung), and brain types (6), and the differences among types is thought to be due to local environmental cues (3). It has been suggested that brain mast cells differ from both serosal and mucosal mast cells based on staining characteristics (including the presence of safranin staining in serosal cells) and response to secretagogues (3, 5, 6). In ring doves, the cells in the MH and those in the lung appeared very similar in size, morphology (at the light microscopic level), and codistribution of amine and peptide. Hence, in this animal, brain and serosal mast cells seem to share some biochemical properties.

Radioautography. To determine if the GnRH-like immunoreactivity in the MH mast cells was endogenous to these cells or arose from uptake of the peptide epitope from the CSF, a ^{125}I-labeled GnRH analogue was administered at the time of courtship. The chicken pituitary GnRH receptor binds both chicken I GnRH and mammalian GnRH with the same affinity (21). The analogue used in these studies has increased activity in the chicken (R.P.M. and J.A.K., unpublished results) and is resistant to degradation by the endopeptidase 24.15 cleaving at Gly—Leu and to the post-proline cleavage enzyme (see ref. 38). Since little is known about the specificity of GnRH receptors in the avian CNS, this analogue seemed to be a reasonable candidate for uptake studies. The absence of accumulated radioactive material suggests that mast cells synthesize a GnRH-like peptide. The presence of the peptide epitope in both lung and liver (where there are no other GnRH$^+$ elements and where circulating GnRH is likely to be very low) also strongly supports the hypothesis of prior synthesis of the peptide within the mast cells. It is, however, possible that GnRH itself cannot enter these cells but that they endocytose another peptide or protein recognized by the LR-1 antiserum. It is known that in mammals mast cells are capable of endocytosing peroxidase derived from eosinophils (3).

In many regions, including the CNS, mast cells are innervated or in close proximity to nerve terminals (9) and can be stimulated to release their granular contents by neuropeptides (39, 40). Mast cells can contain at least 20 mediators (41) including interleukins, granulocyte/macrophage colony-stimulating factor (42), and a variety of proteoglycans, proteases, and neuropeptides (3, 39). Of particular interest for this and related studies (1, 2) is the clinical observation that histamine secretion from mast cells and cutaneous anaphylaxis can be induced with GnRH (43) and GnRH agonists and antagonists (43–45). Mast cells respond not only to GnRH and its analogues but also to gonadal steroids. Amine secretion can be triggered directly by progesterone in the rodent (46), and induced release can be augmented by estradiol (47, 48). Estrogen receptors have been demonstrated in mast cells of rats (48).

Conclusions. The current results support the hypothesis that the nonneural cells that are immunoreactive to the LR-1 antiserum and identified in the MH of ring doves should be classified as mast cells. This classification is based on their size, metachromatic properties, staining with safranin in the presence of alcian blue, the presence of histamine-like immunoreactivity, their ultrastructural appearance including the morphology of their nucleus, the distribution and shape of their cytoplasmic extensions, and the character of their granules. This identification is further supported by the occurrence of similar cells in connective tissue of the lung and liver. Their origin and the nature of their recruitment into the gray matter of brain remains to be determined. It is possible that mast cell secretion into the brain represents an additional delivery system for biologically active substances.

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