Lipocortin 1 (Annexin 1): A Candidate Paracrine Agent Localized in Pituitary Folliculo-Stellate Cells*

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ABSTRACT

It is now well established that lipocortin 1 (LC1) plays an important role as a mediator of early delayed glucocorticoid feedback action in the hypothalamo-hypophysial system. In both the hypothalamus and anterior pituitary gland, LC1 mimics some of the actions of glucocorticoids; moreover, glucocorticoids stimulate the synthesis of LC1 and cause the translocation of intracellular LC1 to the outer cell surface. The mechanism by which LC1 acts in these tissues is only partially understood, but may involve paracrine and/or autocrine actions. To address these possibilities we have investigated the localization of LC1 in the rat anterior pituitary gland, using double labeling immunohistochemistry to identify the pituicytes and thereby access the paracrine cell types that express LC1. At the light microscopic level LC1 was not detected in the endocrine cells in the pituitary gland, but LC1 was found in abundance in the surrounding folliculo-stellate (FS) cells. In the anterior and intermediate pituitary lobes, there was a near total colocalization of LC1 and S100, a specific marker of FS cells. By contrast, in the posterior pituitary gland, LC1 immunoreactivity was not colocalized with S100, which labeled most pituicytes, or with OX-42 monoclonal antibody, a marker of the microglial cells. Immunogold electron microscopy confirmed that LC1 is present in the nongranulated FS cells. LC1 immunoreactivity was also present in a mouse pituitary FS-like cell line (Tt/TgF), particularly in the periphery of the cytoplasm. The localization of LC1 in the FS cells of the anterior pituitary gland defines LC1 as a new marker of the FS cell population. These results support our hypothesis that LC1 acts as one of the paracrine agents liberated by FS cells that modulate the release of pituitary hormones. (Endocrinology 140: 4311–4319, 1999)

G LUCOCORTICOID hormones and their synthetic analogs have many powerful actions and are widely used for the treatment of inflammation, allergy, and autoimmune diseases. They also exert many effects on central endocrine mechanisms, including powerful inhibitory effects on the hypothalmo-pituitary-adrenocortical (HPA) axis (1, 2). Regulation of the HPA axis by steroids is not fully understood, but there is substantial evidence that they act in a paracrine manner, modulating many actions of hypothalamus and pituitary gland (3). There is increasing evidence that paracrine controls of hormone secretion, although poorly understood, are of considerable importance in the anterior pituitary gland (20). We therefore propose that LC1 exported by target cells in response to a glucocorticoid challenge depresses peptide release by binding to cell surface LC1 receptors on endocrine cells and thereby serves as an autocrine and/or paracrine agent. There is increasing evidence that paracrine controls of hormone secretion, although poorly understood, are of considerable importance in the anterior pituitary gland (20). LC1 immunoreactivity has been detected in the hypothalamus and pituitary gland (21, 22), and Western blot analysis shows that rat anterior pituitary tissue is rich in LC1 (25). However, previous studies on the cellular localization of LC1 in the brain have produced conflicting results, which almost certainly reflect the diversity of the antibodies and fixation procedures employed (27). We have, therefore, undertaken a light and electron microscopic immunohistochemical analysis of the pituitary gland to determine the cell types that express LC1.

Materials and Methods

Preparation of tissues for light microscopy

Adult Sprague Dawley rats (200–250 g) were terminally anesthetized by ip injection of 30 mg sodium pentobarbital (Sagatal, Rhone Merieux, France) and perfused through the heart with heparinized saline (0.9% NaCl and 10 U/ml heparin) followed by periodate-lysine-paraformal-
dehydrated via a graded series of ethanol concentrations, cleared in 70% methanol for 48 h, and embedded at 20°C with a Vibratome (Camden Instruments, Sileby, UK). Slabs were then freeze-cryoprotected in liquid nitrogen-cooled isopentane. Cryostat sections, 6–8 μm thick, were mounted on gelatin-coated glass slides, air-dried, and stored at −20°C.

Immunostaining for light microscopy

For visualization of LC1 immunoreactivity by fluorescence, the sections were rinsed in PBS, blocked with 10% normal bovine serum (BS) in PBS at room temperature for 1 h, and incubated overnight at 4°C with a 1:6000 dilution of a well characterized sheep polyclonal antiserum raised against the full-length human recombinant LC1 molecule (29). The mouse monoclonal coded 1B against human recombinant LC1 (30) was also used. All sera were diluted in PBS containing 10% BS. Immunoreacted sections were washed with PBS, then incubated for 1 h at room temperature with a fluorescein-conjugated donkey anti-rabbit IgG (Sigma Chemical Co., Poole, UK). Preincubation of the anti-LC1 serum or monoclonal antibody with an excess of human recombinant LC1 (gift from Dr. J. Browning) overnight at 4°C abolished the immunostaining.

For colocalization studies, sections in which LC1 immunoreactivity had been demonstrated were incubated with one of the following antisera, all raised in rabbits: against ACTH (dilution, 1:4000), against GH (dilution, 1:8000), against PRL (dilution, 1:8000), against LH (dilution, 1:4000; all from the National Hormone and Pituitary Program, Rockville, MD), against S100 protein (dilution, 1:4000; DAKO Corp., Cambridge, UK), or monoclonal mouse antibody against glial fibrillary acidic protein (GFAP; dilution, 1:50; Roche Molecular Biochemicals, Lewes, UK). The sections were then washed and incubated for 1 h at room temperature with a rhodamine-conjugated goat antibody directed against rabbit IgG or mouse IgG Fc as appropriate. Nonspecific immunostaining and background were assessed by substitution of nonimmune rabbit serum for primary antisera. A double immunoenzyme reaction was used to study colocalization of LC1 and the macrophage marker OX42. Sections were treated for 5 min with 1% sodium borohydride solution, washed, incubated with 0.3% hydrogen peroxide in 40% methanol-PBS for 15 min to eliminate endogenous peroxidase activity, then blocked with 10% BS-PBS at room temperature for 1 h. LC1 immunoreactivity was localized by use of the sheep polyclonal antiserum described above. Immunoreacted sections were washed with PBS then incubated for 1 h at room temperature with an alkaline phosphatase-conjugated donkey anti-rabbit IgG (Sigma Chemical Co.) for 1 h at room temperature. Phosphatase activity was revealed with 5-bromo-4-chloro-3-indolyl phosphate/nitro-blue tetrazolium (Roche Molecular Biochemicals) containing 1 mM levamisole to inhibit endogenous phosphatase activity. Sections were then washed and incubated for 2 h at room temperature with the microleral marker OX42 (1:300; Serotec, Kidlington, UK), then with biotinylated horse antimouse IgG Ig (Vector Laboratories, Inc., Burlingame, CA), washed in PBS, and incubated with avidin-biotin-peroxidase complex (Vector Laboratories, Inc.) for 45 min. Peroxidase activity was revealed by use of 3,3′-diaminobenzidine tetrahydrochloride solution (0.5 mg/ml) in the presence of 0.02% H2O2 in PBS. The sections were then washed in distilled water, dehydrated via a graded series of ethanol concentrations, cleared in xylene, and mounted in DePeX (BDH, Poole, UK). The other monoclonal antibodies used to detect microglia were OX1 (against rat leukocyte-common antigen) and OX30 (mixed 1:1), both provided by Dr. V. H. Perry (Department of Pharmacology, University of Oxford, Oxford, UK).

Cell culture and immunostaining

The folliculo-stellate (FS)-like cell line Tt/GF, established from a murine pituitary thyrotrophic tumor (31), was provided by Dr. C. A. McArdle (Department of Medicine, University of Bristol, Bristol, UK). Tt/GF cells were cultured in DMEM-Ham’s F-12 medium containing 10% FCS, 2.5 mM l-glutamine, 100 IU/ml-penicillin, and 100 μg/ml streptomycin. For immunohistochemistry, cells were plated at 100 cells/mm² on collagen-coated coverslips. After 5 days of culture, the cells were fixed and permeabilized by methanol at −20°C for 5 min, blocked with 3% BSA, and incubated with the anti-LC1 serum diluted 1:6000, followed by fluorescent-conjugated donkey antirabbit secondary antibody. For immunoelectron microscopy, cell suspensions were fixed with 4% paraformaldehyde and 0.2% picric acid, cryoprotected with sucrose, impact-frozen, and then spread on 200-mesh nickel grids, incubated for 30 min with 1% sodium borohydride solution, washed, incubated for 1 h at room temperature with a rhodamine-conjugated goat antibody directed against rabbit IgG or mouse IgG Fc as appropriate. Nonspecific immunostaining and background were assessed by substitution of nonimmune rabbit serum for primary antisera.

Results

LC1 immunoreactivity (LC1-ir; single labeling) in rat pituitary

LC1-ir of varying intensity was detected in all three parts of the pituitary gland (Fig. 1). Fluorescence microscopy revealed the strongest immunoreactivity in the anterior pituitary lobe, in a network of irregularly shaped cells with elongated processes, distributed evenly across the tissue at relative high density. LC1-ir was also detected in cells lining both sides of the hypophyseal cleft. In the intermediate lobe, strong LC1-ir was found in tissue surrounding the lobules of endocrine cells and in a very few parenchymal cells. In the neural lobe, weaker LC1 immunoreactivity was present in widely dispersed cells with very fine radiating cytoplasmic processes. The polyclonal sheep antiserum and the monoclonal 1B antirecombinant human LC1 gave identical results.

| TABLE 1. Density of lipocortin 1-immunogold particles over cellular compartments of different anterior pituitary cell types |
|---------------------------------|----------------|----------------|
| **Gold particles/μm² over:**   | **Cytoplasm** | **Nucleus**    |
| Fulliculo-stellate cells        | 39 ± 6        | 27 ± 5         |
| Somatotrophs                    | 5 ± 2         | 7              |
| Gonadotrophs                    | 1             | 42 ± 8         |
| Lactotrophs                     | 1             | 26 ± 6         |

Data were derived from seven representative micrographs.
cytoplasmic processes. (IP) in tissue surrounding the lobules and in a few cells in the parenchyma, and in the posterior pituitary (PP) in radiating cells with long

in many irregularly shaped cells with fine elongated processes, in cells lining both sides of the hypophyseal cleft (HC), in the pars intermedia (IP) in tissue surrounding the lobules and in a few cells in the parenchyma, and in the posterior pituitary (PP) in radiating cells with long cytoplasmic processes. Scale bar, 50 μm.

Double immunolabeling studies

To identify the cell types that express LC1 in the anterior pituitary gland, we compared LC1-ir with that obtained for its various hormones. Anterior pituitary endocrine cells (somatotrophs, corticotrophs, lactotrophs, gonadotrophs, and thyrotrophs) were identified from morphology and hormone content of their secretory granules. Double immunofluorescence labeling revealed no overlap between the distribution of LC1 immunoreactivity and the somatotroph, gonadotroph, corticotroph or lactotroph cells (data not shown). FS cells were distinguished from endocrine cells by their morphology (a network of stellate cell bodies with elongated processes that form follicles with microvilli and junctional complexes; absence of secretory granules) and their content of S100 protein, a nonhormonal marker (32, 33). However, with the exception of a few cells (Fig. 2, A and D, arrowheads), there was nearly total colocalization of LC1-ir and S100-ir in the anterior pituitary and in cells lining the hypophyseal cleft (Fig. 2, A, B, D, and E, and Fig. 3). In the intermediate lobe, although, in general, S100-ir was stronger than LC1-ir, occasional cell bodies and processes immunopositive for LC1 coexpressed S100 protein both in the parenchyma of the lobules and in tissue surrounding the lobules. However, in the tissue surrounding the lobules, the outer layer contained only LC1 (Fig. 2, B and E).

In the neural lobe LC1-ir and S100-ir were differently distributed. The anti-S100 serum strongly stained many pituicytes in which no LC1-ir could be detected. However, some isolated cells and very thin processes forming a fine network were positively marked by the anti-LC1 serum (Fig. 2, C and F). The strong colocalization of LC1 and S100 immunoreactivity in the pars distalis and intermedia indicates their colocalization in FS cells. However, double exposure micrographs in particular showed no such colocalization in neural lobe pituicytes (Fig. 3).

No immunolabeling for GFAP (another marker for FS cells and the filament-rich fibrous type of neurohypophyseal pituicyte) (34) was observed in the rat adenohypophysis. In the neural lobe, the anti-GFAP serum marked a subpopulation of the pituicytes recognized by anti-S100, but not the cells and processes identified by the anti-LC1 serum (data not shown).

To determine whether cells of the macrophage lineage express LC1 in the pituitary we used the OX42 antibody, which recognizes the complement type 3 receptor CR3 (35). No overlap was observed between CR3-ir and LC1-ir in either the neuro- or adenohypophysis (Fig. 4).

Ultrastructural analysis

Ultrathin sections of freeze-substituted anterior pituitary tissue immunostained with the S100 antiserum confirmed the ultrastructural identification of FS cells as stellate-shaped cells with elongated cytoplasmic expansions, but with no secretory granules and few other cytoplasmic organelles. Very strong S100 immunogold labeling was observed in both the cytoplasm and nuclei of FS cells and could be traced into their long, thin processes between the various granule-containing endocrine cells (Fig. 5). No S100 immunoreactivity could be detected over endocrine cells. Immunogold localization of LC1 with the sheep polyclonal antibody revealed diffuse LC1-ir over both the cytoplasm and nuclei of FS cells (Fig. 6); the monoclonal antibody IB did not produce detectable labeling. The LC1 immunostaining was always less intense than the S100 immunostaining (suggesting that LC1 antigenicity is more sensitive than that of S100 to the preparative procedures). The secretory granules of some endocrine cells, particularly somatotrophs, were also marked by the LC1 immunogold procedure. This reaction was more intense in terms of gold particle density than that over FS cell cytoplasm. However, the gold particles were restricted to the dense cores of the secretory granules and were not present over the cytosol of the endocrine cells.

Quantitative analysis of the immunogold-LC1 labeling is summarized in Table 1. The cytoplasm of the FS cells was about 8-fold more strongly labeled for LC1 than the cytoplasm of somatotrophs; similarly, LC1 immunoreactivity was 4-fold stronger in nuclei of FS cells than in the nuclei of somatotrophs. Preincubation of the LC1 antiserum with human recombinant LC1 diminished, but did not abolish, the gold labeling over the somatotroph granules, whereas it abolished the labeling over FS cells. Preincubation of the LC1 antiserum with either GH or PRL had no consistent effect on
FIG. 2. Double immunofluorescence labeling for LC1 by the LC1pAb (A–C) and S100 (D–F). A and D, In the anterior pituitary, with the exception of few cells (arrowhead), LC1 is colocalized with S100. B and E, In the intermediate lobe, LC1-ir and S100-ir are nearly totally colocalized in tissue surrounding the intermediate lobules and in some cells inside the parenchyma lobules. C and F, In the posterior pituitary, LC1-ir is not colocalized with S100-ir. Scale bar, 40 μm.
In freeze-substituted posterior pituitary tissue, very weak LC1 immunogold marking was apparent over some protoplasmic pituicytes, but no cells with a strong immunoreactivity could be detected.

**LC1 in the TtT/GF cell line**

Cells of the mouse pituitary FS TtT/GF cell line showed a very strong immunofluorescent staining for LC1, which extended throughout the cytoplasm of all TtT/GF cultured cells (Fig. 7A). As described previously (31), TtT/GF cells were also immunopositive for S100 (weak staining in all cells) and GFAP (some cells strongly stained; data not shown). Immunogold detection of LC1 showed gold particles scattered over the cytoplasm and nucleus of TtT/GF cells, with a marked concentration near the plasma membrane (Fig. 7B).

**Discussion**

This study presents immunohistochemical evidence that LC1 is localized in the FS cells of the rat anterior pituitary. The finding of strong LC1 immunoreactivity in the TtT/GF FS cell line strengthens this. An identical immunostaining pattern was obtained for LC1 and S100 protein in the anterior pituitary gland and in the epithelial cells lining the Rathke’s cyst, which, like FS cells, have few organelles and no secretory granules. S100 is the most suitable specific marker for detecting FS cells in the anterior pituitary. GFAP, an astrocytic marker protein, is another reported component characteristic of FS cells, but is less suitable because it is present in only a subpopulation of the cells (34, 36–38). Like Redecker and Morgenroth (38), but in contrast to the human data (36, 37), we could not detect GFAP in anterior pituitary FS cells. Similarly, the cytoskeletal proteins vimentin and cytokeratin 7, 8, 18, and 19 (39, 40), which are also present in FS cells, are less suitable markers, because vimentin is also present in endothelial cells and infiltrated macrophages (37) and cytokeratins 8 and 18 in endocrine cells (39).

In the neural lobe, pituicytes, which are the major nonneuronal cellular elements, are known to express S100 protein (32, 41). In accord with these data, we observed S-100-ir...
pituicytes in the neurohypophysis, but, in contrast to our observations on the adenohypophysis, these S-100-ir cells appeared not to show LC1-ir. We also confirmed that a subpopulation of pituicytes contain GFAP-ir (42), but LC1- and GFAP-ir did not overlap. In addition, no overlap was found between LC1-ir and OX42-ir, which labels all types of microglia.

Preliminary investigations of LC1-ir have been made in human (21) and rat pituitary tissues (23, 24). Our results accord with the finding of LC1-ir in the epithelial cells lining the hypophyseal cleft (in human and rat), but not in the neurohypophysial pituicytes. Variable immunoreactivity was reported in stellate cells distributed throughout the human pars distalis (21). However, no systematic comparison was made of LC1 and S100 immunoreactivities in the pars distalis, although some overlap was reported in the pars intermedia. Other contradictory results appear in the literature concerning S100 staining in goat pituitary (43).

These differing results might reflect species variation, but could also be due to the problem of antigen preservation. McKanna and Zhang (27) carried out a very elegant series of experiments in which they analyzed in detail the sensitivity of LC1 in the brain to different fixatives, freezing steps, pH, and acid-alcohol treatment. Brain LC1-ir was highly sensitive to the fixative used and appeared to be restricted to cells characteristic of microglia in the normal brain. We have tested different fixatives, but, using the sheep polyclonal antiserum as the probe, LC1-ir in FS cells did not seem to be affected by the pH of the fixative. Apart from the pH, the fixative that we used (a neutral aldehyde fixative) was very similar to that recommended by McKanna. Moreover, the immunostaining in a parallel study in which we used the 1B monoclonal antibody (17) was indistinguishable from that obtained with the sheep antiserum, although the monoclonal antibody gave staining at the light microscopic level only.

Our electron microscopic analysis confirmed that LC1 is a very labile protein. S100, but not LC1, immunoreactivity could be detected after standard fixation, dehydration, and embedding at −20 °C, and we had to use freeze-substitution to detect LC1-ir. This repeats our finding for the preservation of LC1 immunoreactivity in a lung adenocarcinoma model cell system (A549 cells) (44).

Whereas our light microscopic study suggests that in the pars distalis, LC1 is restricted to the FS cells, our immunogold study and the similar findings of Woods et al. (24) raise the possibility that LC1 is also present in endocrine secretory granules (especially in somatotrophs). Moreover, in a study in which we measured intracellular LC1 by fluorescence-activated cell sorting analysis, we detected LC1 in approximately 80% of the secretory cells in the adenohypophysis,
where its expression was glucocorticoid regulated (45). However, whereas preincubation of the sheep anti-LC1 serum with an excess of human recombinant LC1 abolished the light and electron microscopy-specific immunostaining of FS cells, it only diminished the gold labeling over endocrine (especially somatotroph) granules. In contrast, preincubation of the anti-LC1 serum with a 100-fold excess of GH or PRL had no consistent effect on the gold labeling over somatotroph granules. Immunocytochemistry, therefore, does not allow us to determine whether the somatotroph granule labeling reflects the presence of LC1 in the granules or is due to a nonspecific attachment of the antibody to the high concentration of protein in the granules. We are not aware of any similarity of sequence between LC1 and other proteins in somatotroph granules. It is highly unlikely that LC1 is synthesized and packaged in endocrine cell granules, because it lacks the signal for segregation into the secretory pathway, and drugs that block various steps in the exocytotic pathway do not alter the cellular disposition of LC1 (46). It is possible that a related peptide with a secretory signal sequence is present in somatotroph granules, but why this could not be detected by immunofluorescence is, then, equally inexplicable, given that the fixation procedures were very similar. It is also possible that the LC1 immunoreactivity endocrine cell secretory granules represents LC1 externalized by FS cells and endocytosed by the endocrine cells, which have binding sites for LC1 (16); such internalization has been shown for GnRH and CRH. In situ hybridization studies are in progress to clarify this question.

The localization of LC1-ir cells in the neural lobe raises another problem. The LC1 is not colocalized with S100 or GFAP, which mark the pituicytes that constitute around 80% of the nonendothelial cells. The remaining 20% of cells are microglia, but LC1-ir was also not colocalized with microglial OX4, OX1, or OX30-ir, which showed that LC1-positive cells did not belong to this microglial population. Neurohypophysial pituicytes are a heterogeneous cell type (47), and the possibility that LC1-ir cells are a subpopulation of pituicytes not recognized by the S100 antiserum would be consistent with the weak immunogold reactivity of some protoplasmic pituicytes. It is less likely that the cells are a subpopulation of microglia not recognized by OX42, as morphologically identified microglia were not marked by immunogold.

Several functions have been attributed to FS cells, including supportive and trophic effects, stem cell function, roles in ion transport, and phagocytic and catabolic activities. However, there is now increasing evidence that FS cells modulate the release of pituitary hormones by exerting a paracrine influence on the surrounding endocrine cells (48, 49).
The nature of that influence is, however, unclear. Conditioned medium from a clonal strain of S-100-expressing pituitary cells can stimulate the release of PRL from clonal (IG4) cells (50). Conversely, using in vitro pituitary cell reaggregate cultures, a FS cell-enriched fraction inhibited the secretory responses of GH, PRL, and LH cells to hypothalamic releasing factors and local releasing factors (51).

There are several candidates for the paracrine agents produced by FS cells. FS cells in rat and mouse pars distalis in vitro synthesize and secrete interleukin-6 (IL-6) (52), which stimulates the release of PRL, GH, and LH from rat anterior pituitary cells in primary culture (53) and ACTH secretion in vitro (54). Furthermore, IL-1 can stimulate IL-6 release from rat anterior pituitary cells in vitro (55). IL-1 may, therefore, play a role in the regulation of endocrine functions via the immune-hypothalamic-pituitary-adrenal axis (56, 57). However, the time scale of the inhibitory actions of glucocorticoids on the HPA responses to cytokines in vivo and in vitro that are mimicked by LC1 suggests that these actions of IL-1 occur predominantly in the hypothalamus (11, 58). Nitric oxide is another potential paracrine agent produced by FS cells because neuronal nitric oxide synthase immunoreactivity is also present in these cells (59).

The present report adds LC1 to the list of putative paracrine agents produced by the FS cells. It appears to play a role primarily in the inhibitory actions of glucocorticoids on the HPA axis and suggests, for the first time, that some regulatory actions of steroids on pituitary hormone release may not be exerted directly on the endocrine cells, but indirectly via the FS cells. This possibility is strengthened by the recent demonstration that FS cells possess intracellular glucocorticoid receptors (60). Paracrine interactions among the anterior pituitary cells are undoubtedly complex. The TtT/GF cell line (31), which expresses LC1, should prove a useful model system in which to study the externalization of LC1 from FS cells, the influence of glucocorticoids on FS LC1, and the paracrine role played by LC1 in the regulation of secretion from the endocrine cells.

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References

3. Dayanithi G, Antoni FA 1989 Rapid as well as delayed inhibitory effects of glucocorticoid hormones on pituitary adrenocorticotropin hormone release are mediated by type II glucocorticoid receptors and require newly synthesized messenger ribonucleic acid as well as protein. J Endocrinol 125:308–313
15. John C, Cover PO, Morris JF, Flower RJ, Buckingham JC 1998 Inhibitory effects of hormones derived from the N-terminus of lipocortin 1 on anterior
29. Smith T, Flower RJ, Buckingham JC, McLean IW, Nakane PK
39. Philip JG, Flower RJ, Buckingham JC 1998 Blockade of the classical pathway of protein secretion does not affect the cellular exportation of lipocortin I. Regul Pept 73:133–139
46. Spangelo BL, Judd AM, Isackson PC, MacLeod RM 1989 Interleukin-6 stimulates the secretion of adrenocorticotropic hormone in vivo. J Endocrinol 125:575–577