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Research Report

**Polarity of processes with Golgi apparatus in a subpopulation
of type I astrocytes**

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Abstract

The Golgi apparatus-complex (GA), is a key organelle involved in several posttranslational modifications of polypeptides destined for lysosomes, plasma membranes and secretion. As reported from this laboratory, certain astrocytes in rat brain contain cisternae of the GA not only in perikarya, but also in processes. In order to further investigate which type of astrocytes contain GA in processes we conducted the present study using primary cultures of rat astrocytes and organelle specific antibodies against the GA and the rough endoplasmic reticulum (RER). While the perikarya of all cells contained elements of the GA, only a single process of a subset of type I astrocytes, negative to antibodies A2B5 and HNK-1, contained GA. In contrast, elements of the RER were found within perikarya and all processes. In order to confirm that the immunostained structures in processes indeed represent the GA, we exposed cultures to Brefeldin A (BFA), a secretion blocker which disperses the GA and redistributes it to the RER. We observed that BFA disrupted the GA of both perikarya and processes. However, astrocytes were resistant to prolonged incubations with BFA, while a similar treatment killed cultured fibroblasts and PC-12 cells. Furthermore, in astrocytes exposed to BFA for several days, the delicate network of glial fibrillary acidic protein (GFAP), was replaced by large perinuclear masses of the protein. These observations demonstrate that a subset of type I astrocytes have a single process with elements of the GA. We suggest that this specialization of the GA may be related to yet unrecognized secretory or protein processing functions of these cells. The resistance of astrocytes to BFA and the striking changes in their cytoskeleton induced by the drug, may contribute to studies on the mechanism(s) of action of BFA.

Key words: Golgi apparatus; Brefeldin A; Glial fibrillary acidic protein; Astrocyte type I; Astrocyte type II; MG-160; Electron microscopy; Immunohistochemistry; A2B5; HNK-1; Cell process

1. Introduction

The GA plays a pivotal role in secretion, plasma membrane biosynthesis and targeting of lysosomal enzymes [20]. The organelle is involved in numerous posttranslational modifications and in the sorting or targeting of processed polypeptides. In addition to the general properties of the organelle, cell specific features of the GA, such as selective production of certain enzymes (e.g. high PAPS cerebroside sulfontransferase in neuronal GA), have been identified [3,22,36,39,40,

49,62]. Astrocytes are actively involved in numerous secretory functions such as production of cytokines and growth factors [24,25,46]. It is therefore reasonable to speculate that the GA of astrocytes would express function-related properties of the GA.

Astrocytes, recognized by their specific cytoskeleton protein GFAP, are not a homogeneous population of cells [45,74]. In tissue sections, astrocytes can be divided by morphological criteria into 'fibrous' (mostly in the white matter) and 'protoplasmic' (mostly in the gray matter). Additional markers such as the monoclonal antibody A2B5 [15] and tetanus toxin are positive in cultures of astrocytes with numerous delicate branching processes (type II) and negative in flat astrocytes (type I) [58]. Additional evidence suggests a dual lineage of the two types of astrocytes in some culture

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systems [44,59]. Type I astrocytes and their processes envelop blood vessels, suggesting a function related to the blood brain barrier (BBB); also, type I astrocytes proliferate in response to injury, while type II astrocytes are associated with the nodes of Ranvier and die in response to axonal injury [52]. There is no clear parallel between the *in vivo* and *in vitro* classifications of astrocytes. Moreover, there is some question as to whether or not type II astrocytes have an *in vivo* analogue [53]. Astrocyte heterogeneity has been reported even within a morphologically distinct group, particularly for type I astrocytes cultures [74]. This includes heterogeneity in ion channel, expression of neurotransmitter receptor and glycoprotein, neuropeptide and cytoskeletal content, prostaglandin and peptide secretion, influence on neuronal differentiation, as well as morphology [4,29,45,51,74].

The use of organelle-specific markers is central to this study. Since the conceptualization of the secretory pathway [57], several groups have developed organelle-specific antibodies as a tool to study the GA and other organelles of the secretory-endocytic pathways [7–11,26,48,55,64,65,75]. In this laboratory an intrinsic membrane sialoglycoprotein (MG-160) of medial cisternae of the GA, was purified from rat brains [26]. The original monoclonal antibody, 10A8, used in the isolation of MG-160 from rat brains, reacts only with rat tissues. However, a polyclonal antiserum raised against immunoaffinity purified MG-160 reacts with several species and cell types, including human [11], suggesting that MG-160 or closely related molecules are conserved polypeptides of the GA.

In a previous ultrastructural, immunocytochemical study from this laboratory, using affinity-purified polyclonal sera specific for the GA, elements of the organelle were detected in peripheral segments of some astrocytic processes in sections of rat brains [68]. Other glial cells did not display extensions of the GA in processes. This localization of the GA in processes of astrocytes, and the investigation of other structural properties of the GA of astrocytes is the subject of the present study. In this study in order to further investigate the properties of elements of a putative GA in astrocytic processes, we used primary cultures of astrocytes and the organelle specific antisera against the GA and the RER [8,11,26]. The primary culture model offers the opportunity of experimental manipulations and better resolution of immunocytochemical staining, not easily obtainable in *in vivo* studies. To further ascertain whether immunostained elements indeed represented the GA, we exposed cultures to BFA, a secretion blocker, which disrupts the GA and induces its redistribution into the RER [12,47]. Results of this study indicate that processes of a subpopulation of astrocytes display elements of the GA in a polarized fashion.

2. Materials and methods

2.1. Cell cultures

Primary glial cell cultures were prepared from newborn Lewis rats (Charles River Laboratories) as previously described [41,42,70,71]. The method for the preparation of the glial cultures was adapted from the original method of McCarthy and de Vellis [50]. Careful removal of meninges resulted in minimal contamination of cultures by fibroblasts. Over 95% of the cultured cells stained with the monoclonal antibody against GFAP [43], an astrocyte specific intermediate filament protein. The cell lines of PC-12 cells (rat pheochromocytoma cells), and L-2 cells (rat fibroblasts) were originally obtained from American Type Culture Collection - ATCC (Rockville MD).

2.2. Organelle specific antibodies

The preparations of the anti-MG-160 monoclonal antibody (10A8), and the immunoaffinity purified anti-MG-160 polyclonal antibodies, were described in previous publications [10,11,26]. The preparation of the monoclonal antibody 2H1, a RER marker, has been previously described [8].

2.3. Immunohistochemistry

Cells, grown on poly-D-lysine treated coverslips, were fixed with 2% paraformaldehyde for 20 min at room temperature, washed 3 times in PBS, incubated for 30 min in 0.05% saponin/10% goat serum (GS) in PBS, washed 3 times in 10% GS in PBS, incubated with primary antibody (1:1000 dilution in PBS of immunoaffinity purified rabbit anti-MG-160 antibodies, or supernatant from the anti MG-160 hybridoma 10A8) overnight at room temperature. Cultures were then washed, incubated with a biotinylated goat anti-rabbit IgG antibody, incubated with the avidin-biotin complex (ABC), and stained with diaminobenzidine tetrahydrochloride (DAB) (5 mg DAB/10 ml Tris-saline containing 10 mM imidazole and 0.03% H₂O₂), according to standard methods [27,28].

2.4. Immunofluorescence

Cells, plated on poly-D-lysine treated coverslips, were rinsed with 20 mM phosphate buffer pH 7.2/0.45M NaCl (solution B), and fixed in 2% paraformaldehyde (20 min), incubated (30 min) with 20 mM phosphate buffer pH 7.2/5% saponin/0.45 NaCl/0.3% fish gelatin (solution A), rinsed with solution B, incubated with blocking solution made with 10% serum of the same species used for the secondary antibody in solution B (30 min), incubated with primary antibody in solution A, washed in solution B, and incubated with secondary antibody linked to FITC or RITC in solution A (30 min), washed with solution B (15 min), washed in 1:10 dilution of Solution B to remove non specific binding of antibodies in high salt, and mounted in Mowiol (Polysciences, Inc.). For the detection of antigens expressed on cell surfaces, cells were incubated with antibodies before fixation (supernatants from A2B5 hybridoma and HNK-1 hybridoma, obtained from American Type Culture Collection, Rockville, MD) [1,15]. Double staining was done by incubations with primary antibodies followed by secondary FITC-conjugated antibody, followed by a second primary antibody and then an RITC-conjugated secondary antibody. Cells were viewed with phase, rhodamine, and fluoresceine optics with a Zeiss microscope equipped with a filter system selective for RITC and FITC excitation and observation. In each experiment, negative controls of normal preimmune serum and supernatants from a non-productive myeloma cell line (SP2/0) were used.

2.5. Electron microscopy

Cells grown on Thermanox were fixed overnight at 4°C with 2.5% glutaraldehyde + 1% paraformaldehyde in 0.1% cacodylate buffer pH 7.4 + 0.002% CaCl₂. Subsequently cultures were postfixed in 1% osmium tetroxide + 1.5% potassium ferrocyanide, dehydrated in ethanol, and embedded in Araldite. Sections (8–10 nm thick) were stained with lead and uranyl salts and viewed in a transmission electron microscope (JEOL 100CX) at 80 kV [32,68].

2.6. Brefeldin A (BFA) treatment

BFA (Sigma) stock solution diluted in ethanol, was diluted in PBS and applied to cultures at a concentration of 5 µg/ml for periods of 30 min, or as specifically indicated in the text. Control experiments included incubation of cultures for the same time periods with the same dilutions of ethanol used to dissolve BFA.

3. Results

3.1. Detection of MG-160 in processes of astrocytes *in vivo*

To confirm and extend a previous ultrastructural immunocytochemical observation from this laboratory on the presence of GA in astrocytic processes in rat brain sections [68], we stained by the indirect immunoperoxidase method, using immuno-affinity purified rabbit polyclonal antibodies against MG-160, formalin-fixed, paraffin embedded, sections of rat brain and spinal cord. Immunostaining showed perinuclear GA staining in all cells. As expected, proximal neuronal dendritic processes contained short extensions of GA. In addition, areas of white matter (devoid of neuronal dendrites) also exhibited linear staining of occasional processes, consistent with those of astrocytes (Fig. 1).

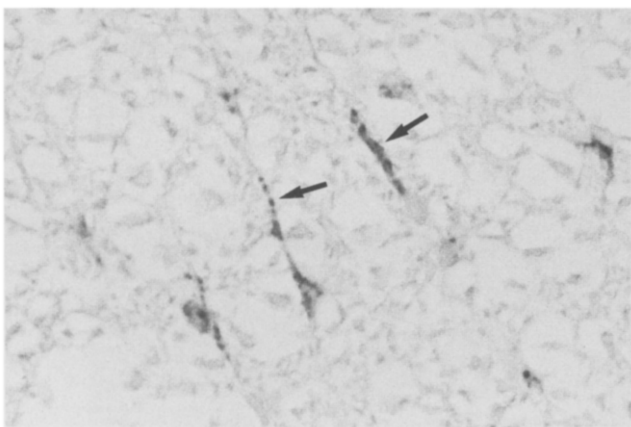


Fig. 1. Rat spinal cord sections stained by immunoperoxidase with polyclonal anti-MG-160, a marker for the GA. White matter (devoid of neuronal dendrites) exhibit linear staining of some glial cell processes (arrows). (DAB × 296).

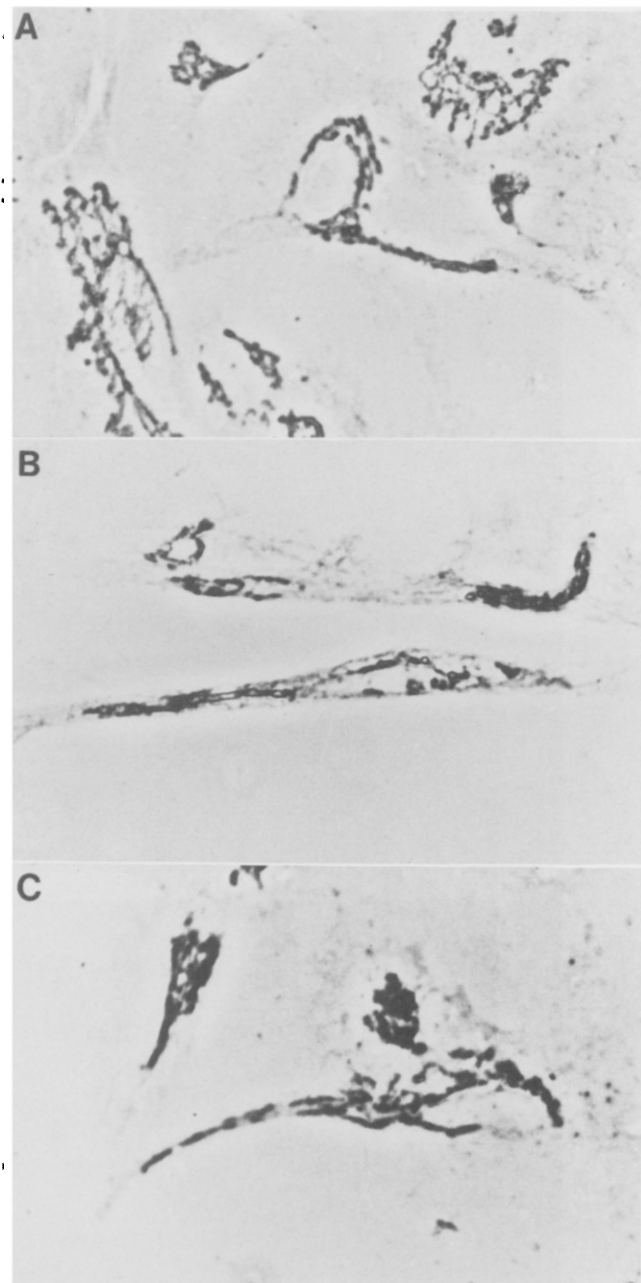
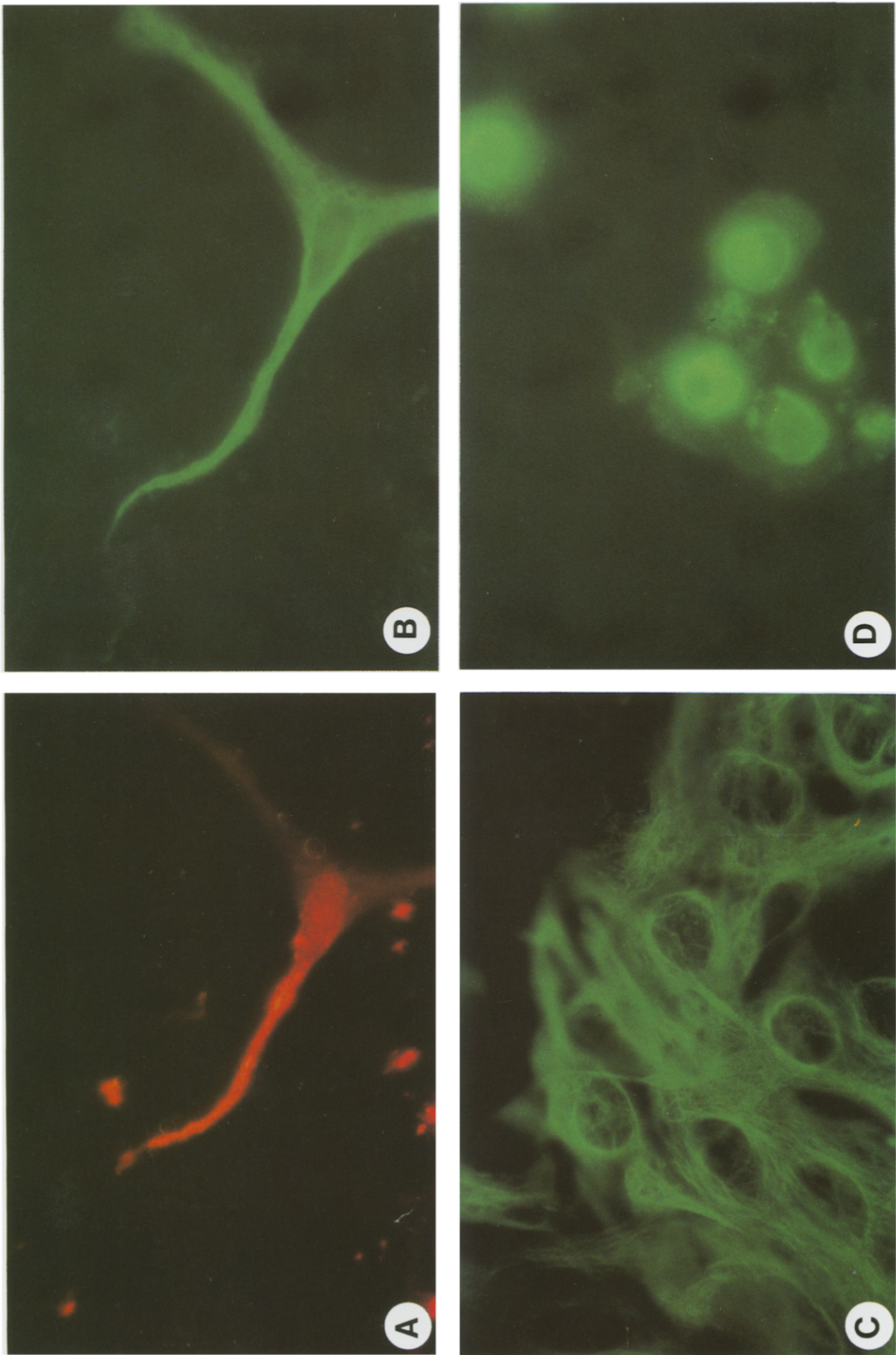


Fig. 2. A–C: examples of immunoperoxidase staining of primary rat astrocyte cultures with anti MG-160 antibodies exhibiting unique extension of the GA into processes (arrows). Note that only one process per cell contains GA staining (DAB without counterstaining × 304).

3.2. Detection of MG-160, a Golgi specific protein, in astrocytes in culture

The monoclonal (10A8) and polyclonal antibodies against MG-160 detected the GA in rat astrocytic cell cultures in virtually 100% of cells. The stain was cytoplasmic, mostly perinuclear and in a granular or reticular (network) form, consistent with the morphology and distribution of the GA (Fig. 2).



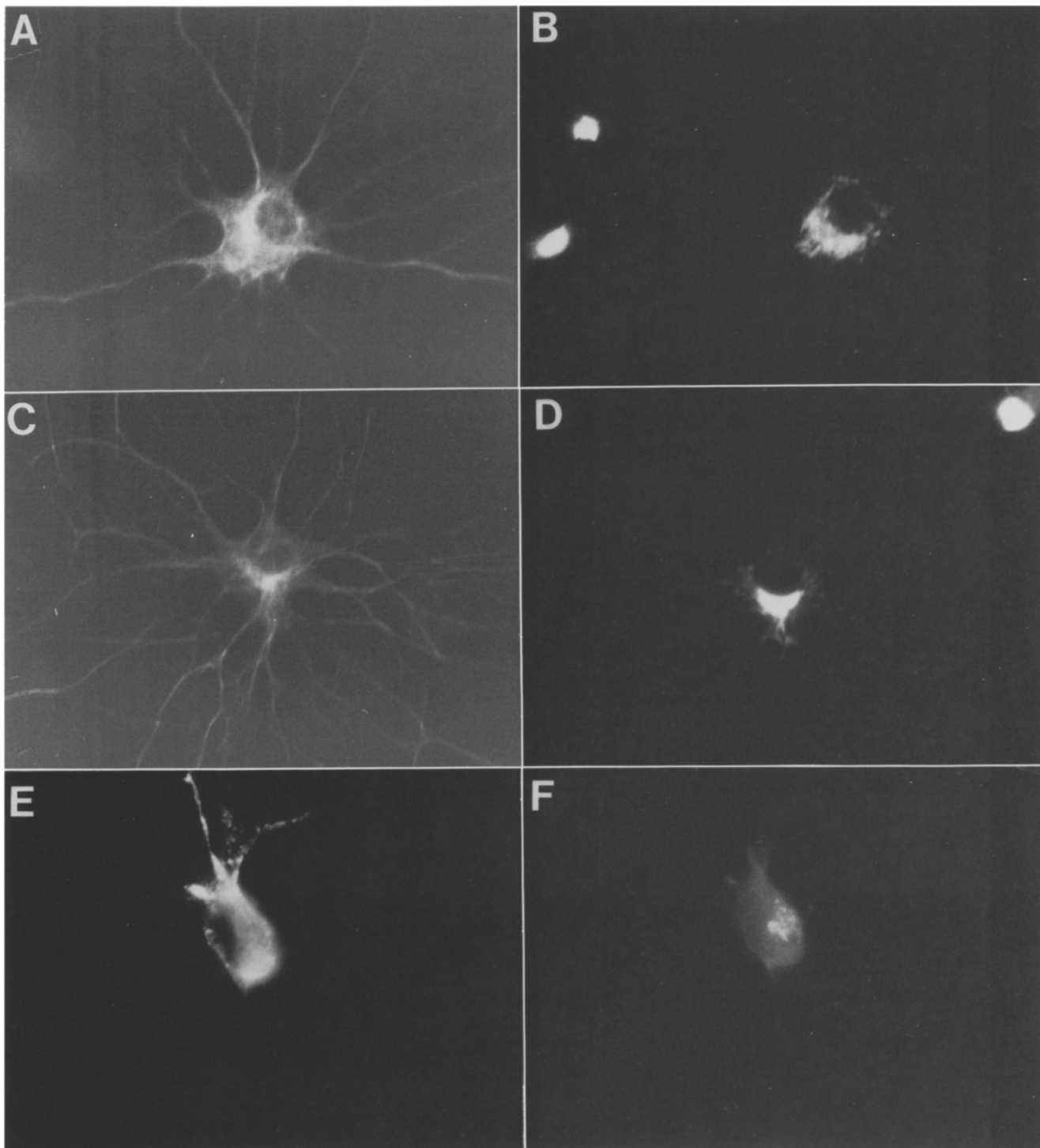


Fig. 4. Double immunofluorescence staining of type II astrocytes with delicate branching processes. GFAP staining (A- FITC) and anti MG-160 (B- RITC) are seen in the same cell. A2B5 staining (C- FITC) and anti MG-160 (D- RITC) are seen in a second cell. HNK-1 staining (E- RITC) and anti MG-160 (F- FITC) are seen in a third cell. Only perinuclear staining without extension into cell processes is seen in these type II astrocytes.

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Fig. 3. Double immunofluorescence staining of a subgroup of type I astrocyte with anti MG-160 (A- RITC) and GFAP (B- FITC). In this GFAP-positive cell with three processes, GA staining is seen only in one process ($\times 630$). The effect of BFA on the cytoskeleton of rat cultured astrocytes stained by immunofluorescence with anti GFAP antibodies before (C) and after (D) 48 h of BFA treatment (FITC $\times 630$).

Staining of MG-160 in processes in rat astrocyte enriched cultures was detected by immunohistochemistry and immunofluorescence with both polyclonal and monoclonal antibodies against MG-160. Staining of MG-160 in processes appeared in approximately 10–20% of the cells (in cultures ranging from 95% to 98% of GFAP-positive cells). Staining of GA in processes was also granular and in some cells in continuity with the perinuclear GA (Fig. 2). To confirm that cells which expressed MG-160 in processes were astrocytes, double-labeling immunofluorescence with anti-MG-160 and astrocytic-specific intermediate filament marker (GFAP) antibodies were performed. All cells with staining of the GA in processes were GFAP positive (Fig. 3A,B).

3.3. MG-160 expression in cultures enriched of astrocytic subtypes

The morphology of cells expressing MG-160 in their processes was consistently of type I astrocytes. In mixed glial cultures these cells were flat and constituted part of the adherent lower layer. These cells contained abundant cytoplasm and a small number of wide flat processes. The staining of the GA in perikarya of type I astrocytes was more abundant and in the form of a network while in type II astrocytes the staining of the GA was punctate (Figs. 2–4). MG-160 was detected only in wide, non-branching processes of type I astrocytes and not in delicate branching processes of type II astrocytes. To further characterize the astrocytic cell type expressing MG-160, the differential adhesion ('shake-off') technique was used, adapted from McCarthy and de Vellis [50]. Loosely attached cells were shaken off during incubation in an orbital shaker at 37°C/250 rpm for 18 h and then plated on coverslips.

In cultures of adherent cells, enriched in type I astrocytes, 90% of cells were positive for GFAP and negative for A2B5 antigens. On average, about 20% of type I astrocytes contained extensions of the GA in processes.

Following 'shake off' the loosely adherent layer of mixed glial cultures was transferred to coverslips and incubated with 10% fetal bovine serum to promote development of type II astrocytes instead of oligodendrocytes which require serum-free medium [58]. These cultures were enriched for type II astrocytes and had over 90% of A2B5-positive, GFAP-positive cells. Type II enriched astrocytic cultures had only a small perinuclear GA staining with anti-MG-160 antibodies. In contrast to type I astrocytes, in type II astrocytes there was no evidence for extensions of the GA into cell processes. Only rare cells in type II enriched cultures displayed GA staining in processes, however, these cells had the distinct morphology of contaminating type I flat astrocytes.

3.4. Subtyping by double labeling of cells containing peripheral GA

Since cultures were not 100% pure for any cell type we wished to further confirm that GA in cell processes was present only in type I astrocytes. Cells were double labeled with A2B5 and anti-MG-160 in both mixed glial and type-enriched cultures. In astrocytic cultures, A2B5 is considered to be a marker that identifies type II astrocytes and their precursors [54]. Only A2B5-negative cells (type I astrocytes) exhibited MG-160 staining in processes. These cells were GFAP positive when double stained with GFAP and anti-MG-160 (Fig. 3A,B). A2B5-positive cells which were GFAP positive had only a small perinuclear GA staining with anti-MG-160 antibodies (Fig. 4A–D). To further confirm that type II astrocytes did not contain GA in processes, another astrocyte type II marker, HNK-1, was used with essentially the same results (Fig. 4E,F). HNK-1 belongs to a group of markers (L-2/HNK-1/J1/Elec-39) against surface adhesion molecules such as myelin associated glycoprotein (MAG) which are present in a subtype of astrocytes (type II) associated with axons in perinodal locations [1,21,35,63].

3.5. Polarity of the GA in type I astrocytes

In tissue sections and culture, astrocytes have processes which do not display distinct patterns of polarization. Therefore, we asked whether an analysis of the orientation of the GA may reveal possible internal polarization. In type I astrocytes with either several or only two processes (bipolar, or spindle cells), MG-160 immunoreactivity was detected only in one process per cell (Figs. 2, 3 and 5A).

We then asked whether the polarity of the GA in one process of certain type I astrocytes was also displayed by other cell organelles. The most closely functionally and structurally related organelle to the GA is the RER. In order to examine the differential presence of GA vs RER in these cells we performed double labeling by immunofluorescence, using a polyclonal antiserum against MG-160 as a marker for the GA, and a monoclonal antibody against a RER antigen 2H1. Astrocytes expressing linear staining of MG-160 in one process exhibited RER staining in the entire cytoplasm and in all other processes including the one stained for MG-160 (Fig. 5). In unstained preparations the unique cell process containing the GA could not be distinguished from other cell processes not containing the organelle.

3.6. Short-term effect of Brefeldin A on the GA of astrocytes

In many mammalian cells, Brefeldin A, a secretion blocker, disperses the cisternae of the stack of the GA,

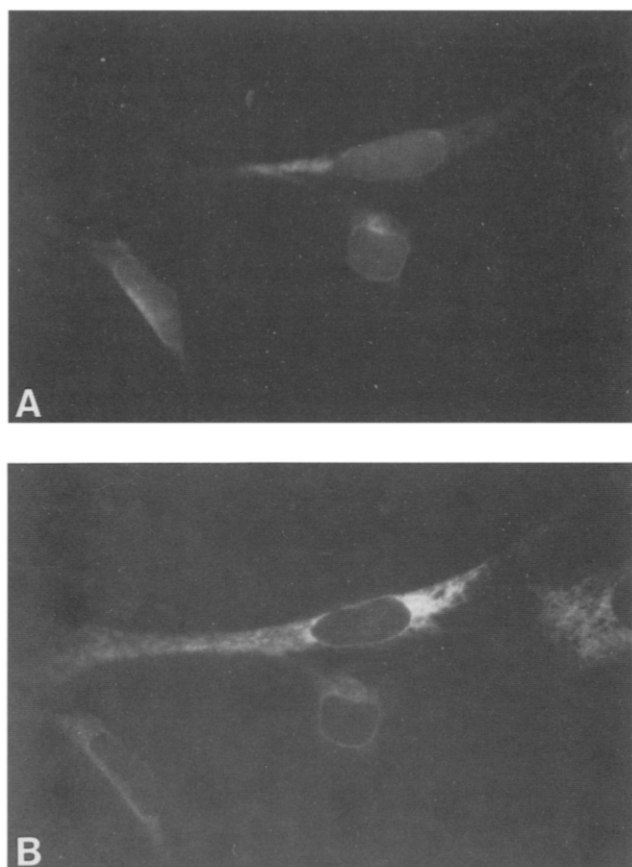


Fig. 5. Double labeling of the same cell by immunofluorescence, using RITC combined with anti-MG-160 as a marker for the GA (A), and FITC combined with 2H1 as a marker for the RER (B). A bipolar astrocyte is depicted, expressing linear staining of MG-160 in one process (A), and RER staining in the entire cytoplasm and both processes (B).

which subsequently fuse with membranes of the RER. In BFA-treated cells, the cisternae and vesicles of the Trans Golgi Network (TGN) aggregate in the form of tubules and vesicles around the centriole. Previous studies from other laboratories showed that enzymes of cisternae of the Golgi stack are redistributed to the RER [12,47]. In contrast to the Golgi stacks, which under the exposure of cells to BFA fuse with the RER, cisternae of the TGN under BFA treatment collapse around the microtubule organizing center [31,60].

In order to investigate whether the immunostained (MG-160) elements of astrocytic processes are indeed part of the GA, we treated cultures of astrocytes with BFA. Following treatment of astrocytic cultures with increasing amounts of BFA, a change in MG-160 distribution was observed (Table 1). Instead of the usual perinuclear staining of the GA which is in the form of coarse granules or network, BFA treatment caused a fine granular staining of MG-160, dispersed through the entire cytoplasm. A plateau of effect related to the

dose of BFA occurred with amounts greater than 1 $\mu\text{g}/\text{ml}$ (Table 1). A complete dispersion of the MG-160 immunostaining occurred with concentrations of 1 or 5 $\mu\text{g}/\text{ml}$ of BFA (Fig. 6). Since BFA was dissolved in ethanol we conducted control experiments in which cultures were incubated with the same concentrations of ethanol used to dissolve the BFA. In these experiments, MG-160 staining was not affected by the ethanol treatment.

The kinetics of GA dispersion were compared between astrocytes and other cells. Dispersion of the GA in astrocytes, PC-12 cells (rat pheochromocytoma cell line) and L2 cells (mouse transformed fibroblasts cell line) was complete 10–15 min after the beginning of treatment. Staining of the astrocytic cultures for the RER with monoclonal antibody 2H1 reacting with a 60–65 kDa polypeptide [8] revealed that the same concentrations of BFA which disrupted the GA did not affect the structure of the RER (Table 1). Both GA in astrocytic perikarya and the putative elements of the GA in processes dispersed with BFA treatment. This observation confirms that astrocytic processes indeed contain elements of the GA.

Since the effect of BFA is reversible, we examined the recovery of astrocytic and PC-12 cell cultures following BFA treatment. After a 30-min treatment with BFA, which caused complete dissociation of the GA as judged by the MG-160 stain, cells were washed and incubated with fresh medium. Reassembly of MG-160 was gradually observed and was complete within 60–120 min. The kinetics of BFA effect and recovery were similar in both astrocytes and PC-12 cells. The pattern of GA staining in astrocytes after recovery from a 30-min BFA treatment was identical to the GA staining before BFA treatment.

Since process formation in astrocytes can be a dynamic event modulated by various chemicals [19,66] we examined the effect of BFA on astrocytic processes after a longer exposure to BFA. Astrocyte cultures exposed to 5 $\mu\text{g}/\text{ml}$ of BFA once and examined 24 h later, or cultures exposed to the same amount of BFA

Table 1
Dose response of Brefeldin A (BFA) on the GA in astrocytes

Dose *	Effect
0.01 $\mu\text{g}/\text{ml}$	no effect on the GA
0.1 $\mu\text{g}/\text{ml}$	partial dissociation of the GA
1.0 $\mu\text{g}/\text{ml}$	complete dissociation of the GA
5.0 $\mu\text{g}/\text{ml}$	complete dissociation of the GA
5.0 $\mu\text{g}/\text{ml}$	no effect on the RER

* Primary newborn rat astrocytic cultures were incubated with each of the above doses of BFA for 30 min, then fixed in 5% GAA/95% ethanol and the effect of BFA was analyzed by indirect immunoperoxidase staining using rabbit-anti-MG-160 antibodies or 2H1, a monoclonal antibody against a RER protein.

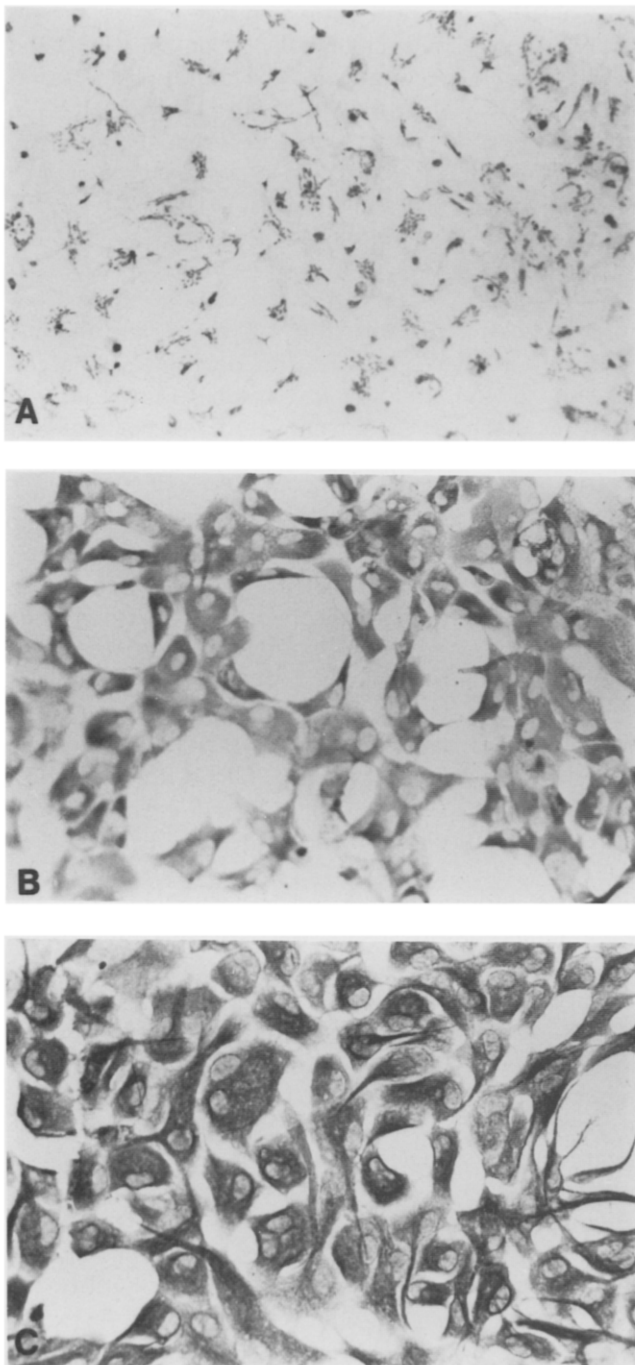


Fig. 6. The effect of Brefeldin A on primary rat astrocyte culture. Anti MG-160 staining without BFA treatment shows typical focal GA staining (A) while 24 h treatment of 1 $\mu\text{g}/\text{ml}$ BFA causes dispersion of the GA in the entire cytoplasm (C). Long term (72 h) exposure to 1 $\mu\text{g}/\text{ml}$ BFA shows the same diffuse pattern of GA distribution in surviving cells (B) while other cell types were completely destroyed by the same treatment after 48 h (DAB $\times 392$).

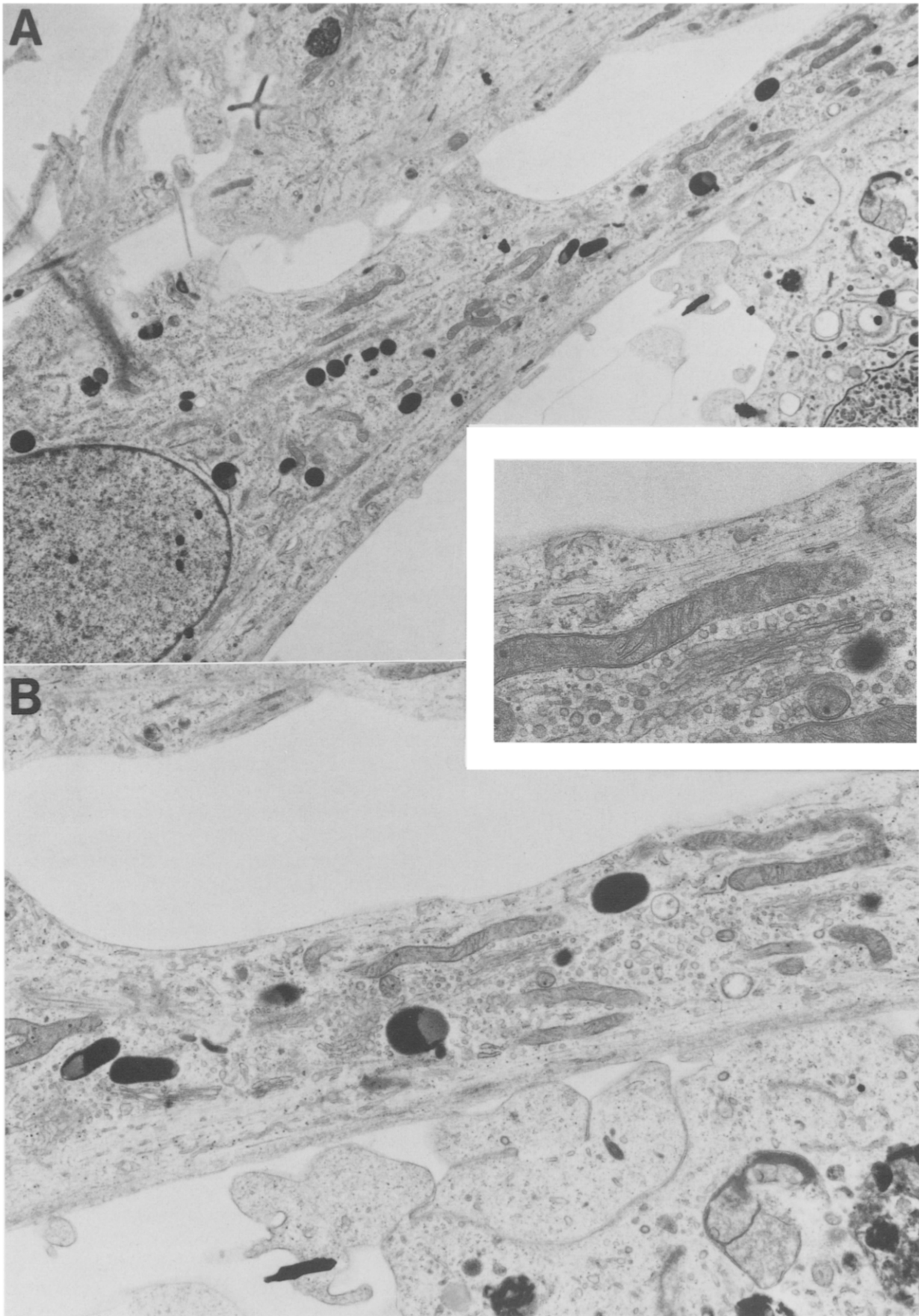
for four 2-h periods and examined at the end of the 8-h period, did not reveal any change in the number of processes, as judged by immunoperoxidase reaction against MG-160, an organelle specific marker of the GA.

3.7. Long-term effect of Brefeldin A on astrocytic MG-160

The effect of BFA on the redistribution of GA elements into the RER, although a reversible phenomenon when applied for short periods, has eventually detrimental consequences on the vitality and function of cells. When cultured L-2 cells or PC-12 cells were exposed to continuous 1 $\mu\text{g}/\text{ml}$ of BFA treatment for 24 h, the majority of cells exhibited degenerative changes consisting of shrinkage and hyperchromasia of nuclei, pyknosis of cytoplasm, and kariorrhesis, and cells completely disappeared by 48 h. In contrast, primary glial cell cultures survived the 1 $\mu\text{g}/\text{ml}$ of BFA treatment, and numerous cells were present after 72 h in BFA. Immunoperoxidase staining for MG-160 of cultures maintained in BFA for 72 h revealed that the effect of the drug on the dispersion of the GA was still present in all surviving cells similar to the effect after 24 h (Fig. 6). To analyze the nature of surviving cells under BFA, double-labeling by immunofluorescence was performed 48 h after the initial introduction of BFA (1 $\mu\text{g}/\text{ml}$) into the medium. All cells showed the BFA effect on the GA as expressed by a diffuse granular MG-160 staining. The majority of cells were GFAP positive, although occasional GFAP negative flat cells with abundant cytoplasm (presumably macrophage/microglia cells) were also present. Occasional HNK-1 positive cells were present. Thus both type I and type II astrocytes resisted the prolonged BFA treatment.

To test whether BFA is partially metabolized and 'detoxified' in the astrocytic culture, we compared primary glial cultures treated with 5 $\mu\text{g}/\text{ml}$ of BFA only once and kept for 96 h, to cultures that were washed every 24 h and replenished with the same dose of fresh BFA. Cultures with one BFA treatment survived during the 96-h observation period, while cultures which were supplied with new BFA died between 72–96 h. In both cultures the GA was dispersed as shown by staining for MG-160. Control experiments using similar dilutions of ethanol and PBS (which were used to dissolve and dilute BFA) did not affect the pattern of GA staining or the concentration of cells on the cover-

Fig. 7. Electron micrograph of astrocyte cultures demonstrating a type I astrocyte with a flat broad process extending from the cell body (A) ($\times 3300$). A higher magnification of a distal segment of the cell process (delineated by arrowheads) shows a linear GA within the astrocytic process in addition to bundles of glial filaments (B) ($\times 8300$). Inset shows higher magnification of the GA in astrocytic process.



slip. Thus in addition to its continuous effect on the GA, BFA or its metabolites may have other effects linked to the viability of cells in culture.

3.8. Long-term effect of Brefeldin A on the cytoskeleton of astrocytes

Cells exposed to BFA (one treatment of 5 $\mu\text{g}/\text{ml}$) for 2–4 days exhibited somewhat shorter processes. To test whether prolonged exposure to BFA affects the cytoskeleton of astrocytes, GFAP staining was performed at 24 and 48 h after an initial exposure of cultures to 5 $\mu\text{g}/\text{ml}$ of BFA. All cells showed the BFA effect on the GA as expressed by a diffuse granular staining for MG-160. After 24 h, double staining with GFAP and MG-160 showed a coarser network of GFAP staining in cells with dispersed GA, and the beginning of perinuclear condensation. After 48 h in BFA, the effect was more pronounced and occasional cells exhibited collapse of GFAP to a form of a perinuclear ‘ball’ while GFAP stain was absent in the rest of the cytoplasm and processes (Fig. 3C,D). Both the GA and GFAP network were reconstituted in cultures treated with BFA for 96 h, washed, placed in fresh media and observed 24 h later.

3.9. Electron microscopy

Astrocytic cultures were examined by transmission electron microscopy. Perinuclear GA with the characteristic membrane-bounded stack of flat cisternae was detected in all cells. In addition, flat cells with broad processes, exhibited characteristic stacks of cisternae of the GA which were parallel to the long axis of the process, and were approximately perpendicular to the orientation of the GA in the perikarya (Fig. 7). Only one process per cell showed evidence of GA elements. Thus, electron microscopy corroborated the immunohistochemical findings of polarity of the GA in type I astrocytic processes. Moreover, the ultrastructure of the GA in processes appeared identical to that in the perinuclear areas. Cells containing GA in processes were identified as astrocytes by the presence of bundles of intracytoplasmic intermediate filaments. Only broad processes of some cells (which belong to type I astrocytes) contained elements of the GA, while delicate branching processes (which belong to type II astrocytes) did not.

4. Discussion

Considerable differences exist among the GA of different cell types. For example, in contrast to liver Golgi and similar to renal Golgi, neuronal Golgi contains high PAPS cerebroside sulfontransferase [22,49]. Cell-specific distributions of enzymes (glycosyltrans-

ferases) of the GA have been shown in intestinal cells [62]. Also, the GA has polarized distributions in a variety of cell types. In certain epithelial cells the intracellular position of the organelle correlates with the direction of the vectorial transport and release of secretory products [3]. In pancreatic islet B cells, the GA is located in the juxtacapillary face of the cell [40]. In natural killer cells (NK) and in cytotoxic T cells there is adaptation of the polarization of the GA, in an orientation necessary for secretion of cytotoxic factors [36,39]. In Sertoli cells, polarization of the GA has been seen in cyclic fluctuations during the spermatogenic cycle [33]. Thus, the GA of several cells displays structure-function relationships which are specific to cell types.

The GA localization in polarized cells can be related to their function [36]. In morphologically non-polar cells, the location and orientation of the GA can serve as an indication of permanent or transient cell polarity [39]. It has been shown that polarity determined by the location of the GA is related to specific membrane recycling functions in the adjacent cytoplasmic membrane of the cell, and this has important role in cell motility [37].

The present study demonstrates several unique structural features of the GA of astrocytes. There is a unique polarization and distribution of the GA in processes of a subset of type I astrocytes. Thus, a subpopulation of type I astrocytes exhibit a polarized cell process similar to the axons and dendrites in neurons. These astrocytic processes contain elements of the GA as dendrites do. However, unlike the polarized nature of neuronal processes, which may be recognized with a Nissl stain and do not require organelle specific stains, the recognition of the polarized nature of astrocytic processes requires immunohistochemical staining for the GA. Additional support for the existence of polarity in astrocytic processes is derived from the report of specific distribution of sodium ion channels in astrocytic processes. Astrocyte processes forming the glial limitans and surrounding blood vessels display reduced immunoreactivity for a sodium channel specific protein, compared to longitudinally oriented or perinodal astrocytic processes, while focal regions of the glial limitans exhibit robust immunostaining [5]. The finding of increased density of potassium ion channels in the endfeet of astrocytic processes [56] provides further support for the possibility that astrocytic processes are actively involved in specialized functions. The subset of astrocytes with a polarized process may be involved in specialized secretory functions requiring rapid exchange of secretory products with the environment adjacent to this specialized cell process. It has been suggested that the GA plays a major role in cell motility [38], and that polarization of the GA in motile cells serves as a steering mechanism in the direction of

cell migration [37]. Therefore type I astrocytes with polarized GA may belong to a subpopulation of astrocytes that are motile and capable of migration in response to injury. Taken together the above evidence strongly suggests that the polarization of the GA in certain cells is not a random and insignificant phenomenon. However, the functional significance of the polarization of the GA in astrocytes remains to be investigated. The differential expression of GA in processes in the two distinct astrocytic types further emphasizes the hypothesis that functional, anatomical, and morphological differences separate these two cells which express the same intermediate filaments (GFAP). Furthermore it provides further evidence of the heterogeneity that exists among type I astrocytes.

The experiments comparing the BFA effect among various cells reveal that dissociation and reassembly of the GA in astrocytes, as revealed by the immunostaining against MG-160, is similar to that of other cells. These experiments suggest that the GA in processes has the same rate of dissociation as the perinuclear GA. The experiments of prolonged exposures of astrocytes to BFA show unique properties of these cells. Unlike other cells in culture tested in this study, and with the possible exception of macrophages, astrocytes do not succumb to GA disruption, and continue to survive in culture despite the dispersion of their GA. This observation is consistent with studies which showed that several Golgi enzymes redistributed into the ER during BFA treatment continue their functions [12]. Cell lines resistant to BFA have been described [30,34]; however, these cell lines do not exhibit dissociation of the Golgi back into the ER. Studies of astrocytic functions are now necessary to further explore the effect of BFA on astrocytes. The observation of the resistance of astrocytes to BFA may be also useful for future studies on the mechanism of the effect of BFA on these cells, and of its specific interaction with the GA.

The cytoskeleton which is intimately connected with the GA also shows changes under BFA effect. BFA treatment for 1–3 h did not destabilize the cytoskeleton (detyrosinated microtubule network) in GH3 cells derived from rat pituitary tumors [6]; however, this may be due to the fact that the effect of BFA on GFAP is evident after 48 h, at a time most other cells do not survive this treatment. BFA has also been shown to induce disassembly of actin microfilaments in BFA-sensitive cells, but not in BFA-resistant cells [69]. Our finding that BFA at 1 $\mu\text{g}/\text{ml}$ does not kill astrocytes but still affects the GA and the cytoskeleton may be useful in future studies of the interaction between GA and cytoskeleton. In that regard, it would be interesting to study whether astrocytes contain $\beta\text{-COP}$ and whether under BFA this protein dissociates from the GA within seconds as in other cell types [13,14].

Traditionally astrocytes have been considered as supporting cells. Astrocytes presumably fulfill functions such as regulation of cell migration and differentiation during development, support of neurons by regulating the extracellular environment for ion and neurotransmitter exchange, lipid metabolism, maintenance of the blood brain barrier and various interactions with endothelial cells and blood vessels [72].

Astrocytes are also known to be the major cells in the CNS that respond to various pathologic conditions including trauma, ischemia, inflammation, and degeneration. More recent studies define the changes that occur in reactive astrocytes: (1) proliferation and migration [17,61], (2) hypertrophy with increased cytoplasm and GFAP content [18,16], (3) changes in surface expression of MHC class I and II, and ICAM-1 molecules [73], [67], (4) production of cytokines and growth factors including IL-1, IL-3, IL-6, TNF, IFN, complement components, MIP-1, GM-CSF in response to various stimulations [25,46,24]. Cytokines are traditionally considered the product of cells which belong to the immune system. As evidenced by the nature of the reaction of astrocytes to injury, by their secretory function, by their possible ability to present antigen to lymphocytes [23], and by phagocytosis [2], astrocytes may now be considered as a potential component of the immune system intrinsic to the CNS. The entire spectrum of astrocytic functions in immune modulation and their role in immune-mediated CNS diseases is just beginning to emerge. The majority of functions described above probably occur in type I astrocytes since they are presumed to be involved in reactions to injury in the CNS [52]. In that regard, the demonstration of extensions of the GA in processes in a subpopulation of type I astrocytes may be relevant to their enhanced and specialized functions in regions adjacent to their processes.

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