Neuroscience Vol. 39, No. 1, pp. 209–224, 1990
Printed in Great Britain

0306-4522/90 $3.00 + 0.00
Pergamon Press plc
© 1990 IBRO

HISTOCHEMICAL AND ULTRASTRUCTURAL CHARACTERISTICS OF RAT BRAIN PERIVASCULAR MAST CELLS STIMULATED WITH COMPOUND 48/80 AND CARBACHOL

V. DIMITRIADOU, M. LAMBRACHT-HALL, J. REICHLER and T. C. THEOHARIDES*
Department of Pharmacology, Tufts University School of Medicine, 136 Harrison Avenue, Boston, MA 02111, U.S.A.

Abstract—Mast cells, known for their involvement in allergic reactions where they secrete numerous chemicals in response to immunoglobulin E and specific antigens, have recently been localized in the central nervous system. The function of these brain mast cells has remained speculative as they have not been the subject of any combined functional or detailed morphological studies. Here it is shown that these cells are primarily perivascular and stain metachromatically with Toluidine Blue, but red with Alcian Blue counterstained with Safranin, indicating that they contain proteoglycans quite similar to those of peritoneal, but not mucosal mast cells. Intracardiac administration of the classic mast cell secretagogue, compound 48/80, or the acetylcholine analog, carbachol, caused ultrastructural changes in brain mast cells consistent with secretion, but without exocytosis. However, it is known that the same concentration of carbachol has no effect on homogeneic peritoneal mast cells.

These results indicate that brain mast cells share histochemical characteristics with serosal mast cells, but differ in their reactivity to secretagogues, and apparently in the mechanism of secretion. Their ability to respond to neurotransmitters and their distinct mechanism of secretion, which may be selective, expands their possible role in brain pathophysiology.

Mast cells (MC) are secretory cells found primarily in connective tissues such as the lungs and skin and classically associated with the allergic response.[24,57] MC synthesize and secrete numerous powerful mediators,[74,83,84] such as endorphins, histamine, kinins, leukotrienes, prostaglandins, serotonin, vasoactive intestinal peptide, as well as proteolytic enzymes and phospholipases, which are well known to have significant pathophysiological effects on vascular and neuronal tissues.[4,30,56,66,72] Recent reports suggest a close relationship between the nervous and immune systems,[5,11,29,61,77,89,90] which could explain such pathological conditions as inflammatory processes,[5,18,48] asthma,[9] vascular headaches,[3,11,52] certain tumors,[1,53] and autoimmune diseases, such as multiple sclerosis.[2,58,63,68,82,86] All these cases appear to be associated with an increase in the numbers of MC, which can be stimulated to secrete by various neuropeptides[23,39,71,81] and maybe neurotransmitters such as acetylcholine,[20] even though this latter finding has been disputed.[5]

Although MC have been found in the brains of reptiles and amphibians[37,55] and in the mammalian peripheral nervous system, there has been considerable debate concerning their presence in the mammalian central nervous system, where they have been identified in the area postrema, hypophysis, pineal body and dura mater (for review see Ref. 62). In studies based mainly on light microscopy techniques, investigators such as Padawer,[64] Kiernan,[42] Kelsall and Lewis,[41] Cammermayer,[6] Krügger,[46,47] Dropp,[13] as well as Campbel and Kiernan,[7] described different types of brain cells which clearly resembled peripheral tissue MC. However, considerable confusion still exists concerning the identification and localization of these cells since they have also been characterized by others as: "perivascular microglia",[59,78] "vacuolated pericytes"[42] "granular pericyttes"[38,54] and "macrophages or lipofuscines".[43,44] Moreover, extensive histochemical and ultrastructural studies performed by Ibrahim[32,33] and Ibrahim *et al.*[34–36] in various mammalian species suggest that the mammalian brain contains two different types of MC, type I and type II. Such disagreement between investigators may be due in part to the improper utilization of fixatives or stains, or to the selection of unsuitable species or brain sites in which the observed MC seem to be rare or phenotypically different. In fact, it has been established that peripheral MC can vary phenotypically,[51] not only with respect to the species, but also to tissue localization, which allows peripheral MC to possess different functional potentialities. It is likely, then, that the phenotypic determination of MC depends on tissue microenvironmental factors which would drive MC differentiation according to specific tissue needs. The

*To whom correspondence should be addressed.
Abbreviations: CBF, cerebral blood flow; C48/80, compound 48/80; FAM, formaldehyde–acetic acid–methanol; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; MC, mast cell(s).

question of species–tissue relations for brain MC remains unanswered since brain parenchyma possesses little connective tissue and any phenotypic factors remain unknown.

The aim of this report, therefore, is to provide both morphological and functional criteria with which to identify rat brain MC and to determine their precise phenotypic and functional capacities. For this purpose, classical MC-specific histochemical, pharmacological, and standard electron microscopy techniques were used to identify MC and their secretory attributes in response to compound 48/80 (C48/80), the classic MC secretagogue and carbachol, the analog of the neurotransmitter acetylcholine.

EXPERIMENTAL PROCEDURES

Morphological characterization

Tissue preparation. Eighteen adult male Sprague–Dawley rats (250–350 g) were first anesthetized with ether and then fixed by direct cardiac perfusion through the left ventricle using a mixture of either 37% formaldehyde–glacial acetic acid–methanol (FAM; 1:1:8) for light microscopic preparations, or 2.5% glutaraldehyde with 0.5% tannic acid in 0.1 M phosphate buffer (pH 7.4) for electron microscopy. After decapitation, the brains were immediately removed, sectioned vertically anterior to the optic chiasm and posterior to the hypothalamus, and processed either for histochemistry (light microscopy) or ultrastructural observations (electron microscopy).

Histochemical procedures

Basophilia. After removal and sectioning, as described above, the brains ($n = 7$) were immersed for at least 48 h in FAM at 4°C and Vibratome thick (30–50 μm) sections were cut. Alternate sections were stained using either 0.25% Toluidine Blue–HCl (pH 2.5) for 15 min at 24°C, or 0.5% Alcian Blue–acetic acid (pH 2.5) for 30 min at 24°C, washed in acetic acid and counterstained for an additional 30–45 min with 0.1% Safranin–acetic acid to differentiate mucosal MC, which contain heparan sulfoconjugates (stained blue by Alcian Blue), from connective tissue (serosal) MC, which contain large amounts of heparin (a highly sulfated substance stained red by Safranin). Other sections were followed by dehydration in graded alcohols and the sections were mounted with Permount.

Lipid content. Two additional animals were fixed by perfusion using an aqueous mixture of 0.6% formaldehyde and 0.5% glacial acetic acid. The brains were treated as described above and the sections stained for 30 min in a saturated, freshly filtered Sudan Black–diacetine aqueous (1:1) solution to reveal lipids. Sections were then washed and mounted with an aqueous mounting medium.

Electron microscopy procedures

After perfusion and brain removal ($n = 9$), the cortex, thalamus and hypothalamus were dissected out, cut into 1-mm^3 pieces and immersed in 2.5% glutaraldehyde with 0.5% tannic acid in 0.1 M phosphate buffer for 24 h at 4°C. The samples were then washed, postfixed in 2% osmium tetroxide, dehydrated in graded alcohols and embedded in Epon. Semithin (0.5 μm) sections were stained using Toluidine Blue (pH 9.0) and ultrathin (75–85 nm) sections contrasted by uranyl acetate and lead citrate.

Pharmacological procedures

Ten adult male Sprague–Dawley rats were used for this portion of the study. Half of them were purfused, under either anesthesia for 30 min through the ascending aorta using C48/80 (1 μg/ml) or carbachol (10^{-6} M) in Locke's solution containing (in mM): NaCl 150, KCL 5, $CaCl_2$ and HEPES 5 1 g/l bovine serum albumin, 1 g/l dextrose, pH 7.4. The other half, used as controls, were perfused in the same way using Locke's solution. Thirty minutes later, the animals were fixed by intracardiac perfusion using either a mixture of FAM or a solution of 2.5% glutaraldehyde. After fixation, the brains were processed for light or electron microscopy, respectively, as described above.

All chemicals used were purchased from Sigma Chemicals Co. (St Louis, MO).

RESULTS

Histochemical characterization of brain mast cells

Granular cells showing characteristics of peripheral MC were observed in the parenchyma of rat brain. All histochemical staining procedures used in this study revealed the presence of numerous round or elongated granular cells (9–15 μm), exclusively localized around microvessels of the thalamic–hypothalamic areas, appearing to be most abundant within the dorsolateral thalamic nuclei. These cells displayed a centrally located oval or rounded nucleus which was often masked by the many isoform cytoplasmic granules present. When treated with acidified Toluidine Blue, cell granules stained in a specific metachromatic fashion characteristic of MC (Fig. 1A, B), while when stained with Alcian Blue and counterstained with Safranin, the granules appeared red (Fig. 1C, D). However, when treated with Sudan Black B, the cytoplasmic granules stained black (Fig. 1E, F), indicating that they contained considerable amounts of lipid, while homogeneic, peritoneal (serosal) MC did not.

Ultrastructural characterization of brain mast cells

At the electron microscopic level, these parenchymal granular cells were consistently localized around thalamic or hypothalamic microvessels (arterioles and venules) and were surrounded by a basement membrane which differed from that of the microvasculature (Figs 2, 4, 5). They contained a central or eccentric nucleus with peripherally condensed chromatin and a central chromatin mass (Figs 2, 4A, C, D). Nucleoli were rarely seen. These cells also contained a number of round or ovoid electron-dense

Fig. 1. Histochemical characterization of rat brain MC. Vibratome, 50-μm-thick rat brain sections. MC (arrows) appear round or elongated and contain a centrally located nucleus (never stained by these procedures), as well as many cytoplasmic granules. (A, B) Thalamic perivascular MC showing metachromasia after acidified Toluidine Blue staining: the granules are stained in violet-blue. (C, D) Thalamic perivascular MC, Alcian Blue-negative–Safranin-positive: the granules are stained in red. (E, F) Thalamic perivascular MC showing strong sudanophilia: the granules are stained in brown-black. Scale bars = 33 μm (A, C, E); 6.6 μm (B, D, F).

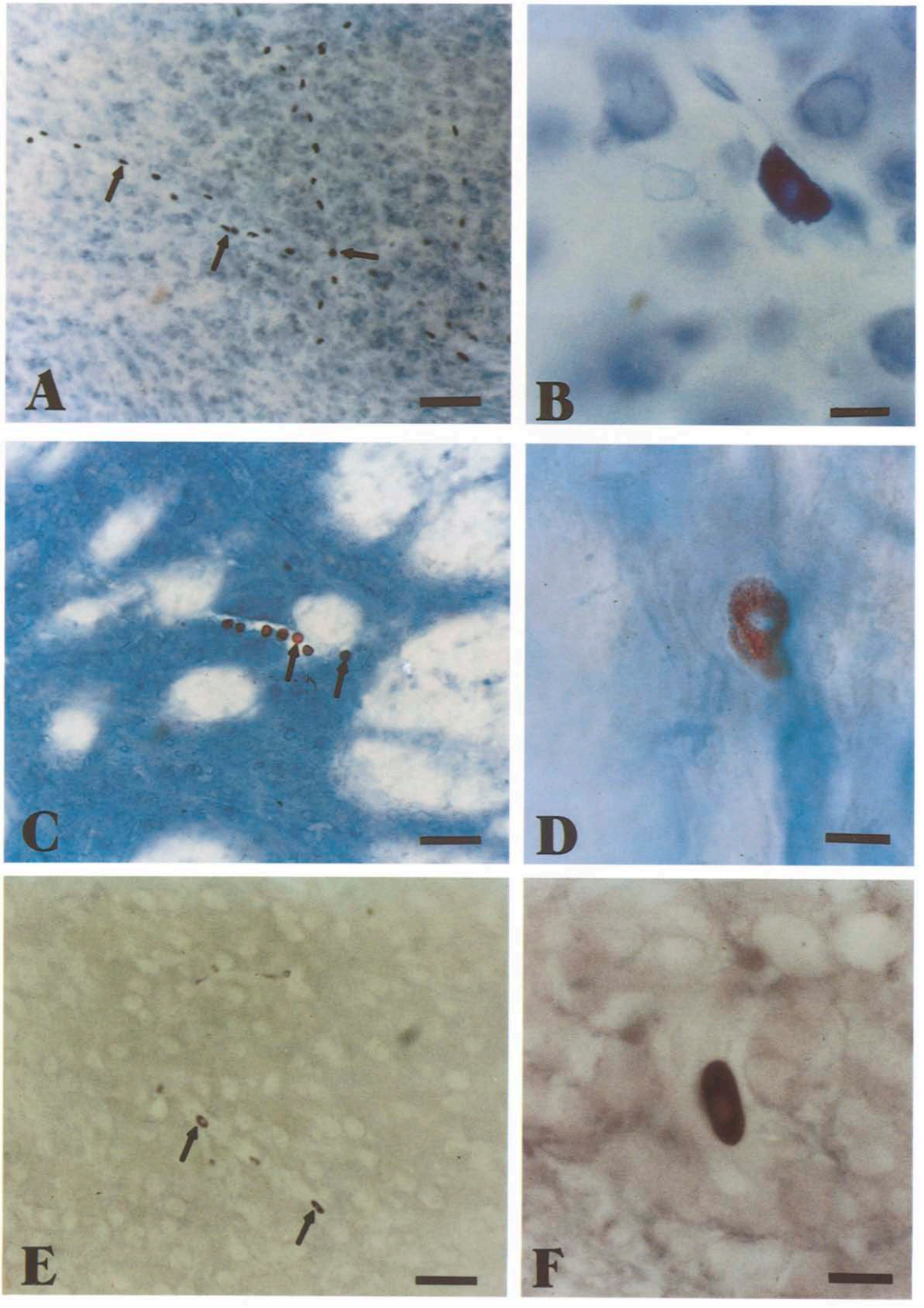

Fig. 1

cytoplasmic granules (Figs 2, 4, 5), the membrane of which often appeared associated with the rough endoplasmic reticulum (Fig. 2C, D). The electron-dense granules usually had a dark amorphous or lamellate texture, but sometimes immature granules exhibited a more electron-lucent composition. There were ovoid mitochondria with few cisternae (Fig. 2C), while the Golgi apparatus and smooth endoplasmic reticulum were poor (Fig. 2D). The rough endoplasmic reticulum was more developed and displayed irregular tubular profiles along with occasional circular ones. The smooth endoplasmic reticulum often contained some electron-dense material and small granules, an event that may represent the early stages of granule synthesis (Fig. 2C). Some clathrin-coated vesicles were seen infrequently, but lysosomes were never observed. In rare cases, the electron-dense granules appeared to contain empty globular vesicles, but this was determined to be an artifact of ineffective fixation.

Pharmacological characterization of brain mast cells: effect of compound 48/80 *and carbachol*

Brains of animals stimulated with C48/80 (Fig. 3B–D) or carbachol (Fig. 3H–F) and stained with acidified Toluidine Blue contained perivascular cells with metachromatic granules within the thalamus and hypothalamus (Fig. 3). However, in comparison with the control group (Fig. 3A, E), the granules appeared only lightly stained; some of them appeared "empty", were non-uniform in size and displayed "anisogranularity", a condition which may reflect fusion between the granules.

At the electron microscopic level, the group treated with C48/80, in comparison with the control group (Fig. 4A), showed gradual granule changes. In the early stages, a wider than normal range of granule sizes was seen and most of the observed electron-dense granules appeared fused (Fig. 4B). The cytoplasmic organelles showed no modifications, but some adjacent astrocytic feet appeared swollen. In more advanced stages, many granules appeared more electron-lucent, a condition that reflects the loss of electron-dense components (Fig. 4C). The granule matrix showed partial vacuolation with clusters of small peripheral vesicles (Fig. 4C), while other granules were entirely full of small vesicles. These latter stages were reminiscent of a "foamy" or "honeycomb-like" appearance (Fig. 4D, E). The granules never fused with the cytoplasmic membrane to form omega shapes indicative of exocytosis and no dispersed granular material was detected in the extracellular space.

The carbachol perfused animals, again in comparison with the control group (Fig. 5A), had similar ultrastructural modifications (Fig. 5B, C). The granules appeared electron-lucent or "empty", the smooth endoplasmic reticulum was enlarged and short cisternae filled with electron-dense material were occasionally observed in relation to the altered granules. However, these changes were not as pronounced as in the C48/80-treated group.

Ultrastructural characteristics of other brain granular or perivascular cells

In addition to the granular perivascular cells described above, macrophages and microglia contained granules, and these, as well as pericytes, were also observed perivascularly.

Microglia. Microglia (Fig. 6), a type of glial cell distinct from astrocytes and oligodendrocytes, were observed frequently, located immediately beneath the glial limitants (Fig. 6A) and also in the vicinity of blood vessels where they lay adjacent to the astrocytic feet surrounding a capillary (Fig. 6C). The microglia had an ovoid profile, displaying a number of cytoplasmic protrusions and processes, which gave them a somewhat irregular shape (Fig. 6A). They contained an oval nucleus with a central nucleolus and prominent masses of clumped chromatin distributed throughout the nucleoplasm, particularly adjacent to the nuclear envelope (Fig. 6A, C).

The density of the hyaloplasm, which formed a thin rim around the nucleus, was intermediate between astrocytes and oligodendrocytes. It contained a perinuclear, sometimes extensive Golgi zone, a few long and narrow rough endoplasmic reticulum cisternae, free ribosomes and oval mitochondria (Fig. 6B). There were no filaments, glycogen granules or microtubules.

The most characteristic feature of this type of cell was the population of dense inclusions, the smaller appearing to originate from the Golgi complex and lysosomes while the larger resembled lipofuscin granules (Fig. 6B, D).

Pericytes. Pericytes were non-granular ovoid cells within the basement membrane of capillaries and

Fig. 2. Ultrastructural characteristics of rat brain mast cells. (A, B) Ultrathin (80 nm) sections showing MC (M) localized around thalamic microvessels and separated from the endothelium (E) by astrocytic cytoplasmic projections (A). These cells contain a central nucleus (but not a nucleolus) and various amounts of cytoplasmic granules (g). Scale bars = 4 μm. (C, D) Ultrathin (80 nm) sections of the thalamus showing some of the cytoplasmic details. The granules have a lamellate (C) or amorphous (D) texture and their membrane appears in close association to the rough endoplasmic reticulum (curved arrow). Notice, in C, the presence of an immature granule surrounded by many Golgi-oriented granules (arrowhead). The cytoplasm contains many ribosomes (polysomes, arrows), some ovoid mitochondria (m), and some coated vesicles (D, small arrowheads). Notice also the presence of a basement membrane individually surrounding the mast cell. Scale bars = 1.16 μm. A, astrocyte; E, endothelium; n, nucleus; SM, smooth muscle cell.

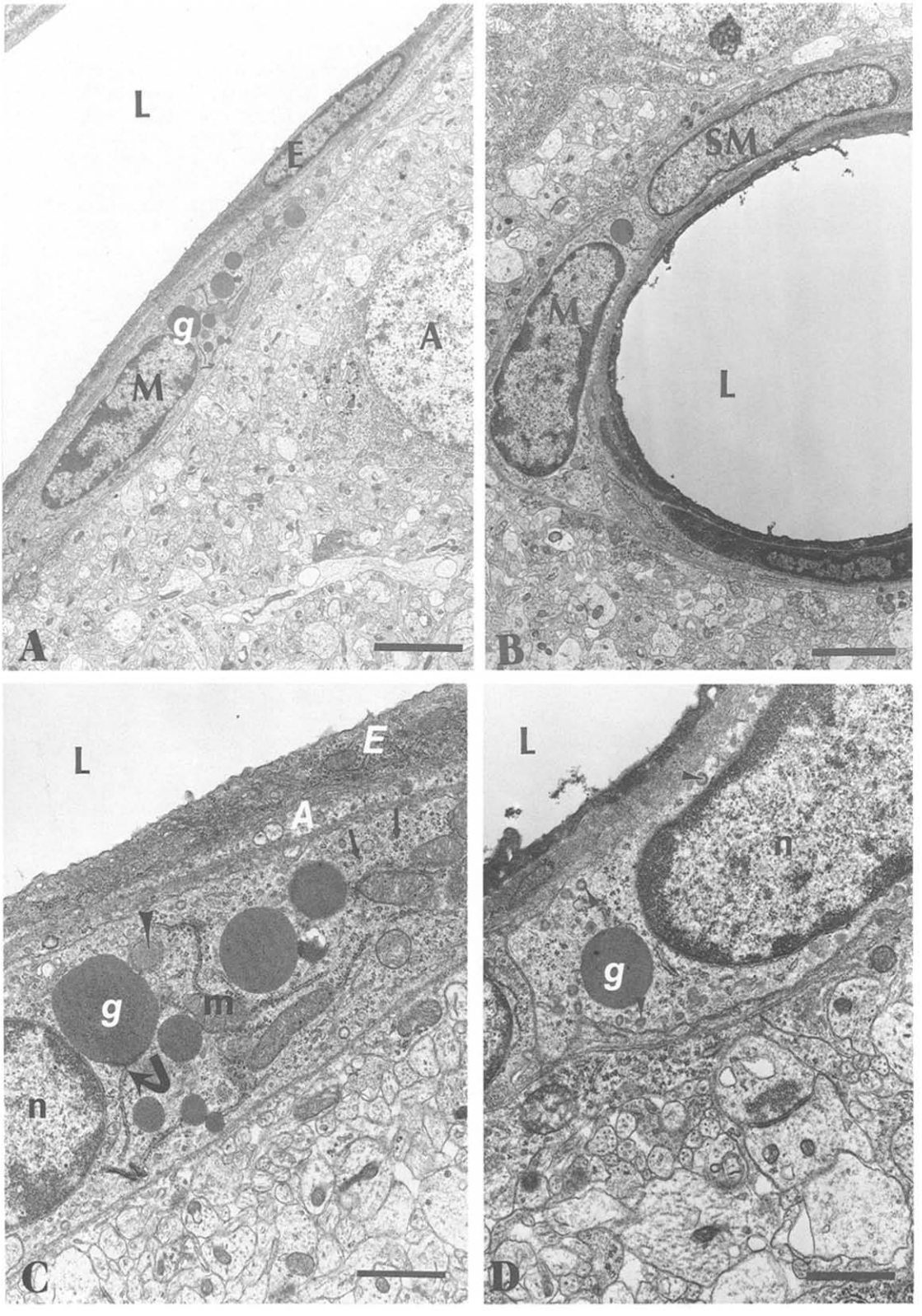

Fig. 2

displayed processes extending around the capillary wall (Fig. 7A). They contained an elongated nucleus that conformed to the shape of the underlying blood vessel with the chromatin clumped beneath the nuclear envelope. The hyaloplasm appeared less dense than that of the endothelial cells, there were few round or oval mitochondria, long and narrow cisterns of rough endoplasmic reticulum and numerous either free ribosomes or ribosomes forming clusters (polysomes). Pericytes did not contain any granules or lysosomes.

Macrophages. The macrophages observed (Fig. 7B) were elongated or spindle-like cells with thick pseudopodia present in the pia mater. They contained oval or irregularly shaped nuclei with a central nucleolus and abundant chromatin characteristically clumped around the nuclear membrane. The cytoplasm was moderately dense, containing a well-developed perinuclear Golgi apparatus with numerous small vesicles and dense granules of various shapes and sizes, large granules, characteristic of lysosomes, larger phagocytic vacuoles with a heterogeneous content of particulate, granular or crystalline material, myelin figures, as well as some pinocytic vesicles and lipid droplets. In addition, bundles of filaments, short cisternae of rough endoplasmic figures, free ribosomes, microtubules and a wealth of mitochondria were present throughout the cytoplasm. This type of granular or perivascular cell was not surrounded by a basement membrane, but appeared free in the pia mater between collagen fibers.

None of the cells described above showed any morphological modifications after treatment with C48/80 or carbachol (see Fig. 6C, D). In addition, these types of cells were not metachromatic or alcianophilic.

DISCUSSION

The histochemical, ultrastructural and pharmacological results described in this study allow the observed perivascular granular cells of the rat brain to be classified as MC, mainly because they display histochemical characteristics unique to these secretory cells; metachromasia when stained by acidified Toluidine Blue, which strongly suggests the presence within the granules of highly basophilic molecules such as heparan sulfoconjugates and heparin. This characteristic is classically described for peripheral MC, but never to our knowledge for other types of glial or nerve cells. These staining characteristics confirm the histological observations of many authors, including Dropp,[13] Krügger,[46] Ibrahim,[32] Edvinsson *et al.*,[17] Persinger[67] and Goldschmidt *et al.*[26,27] who described granular cells which stained with Toluidine Blue or Astra Blue, were highly localized in the thalamus and were similar to connective tissue MC.

In addition to the staining methods previously used to characterize MC in the brain, we identified these granular cells as MC with a histochemical technique currently used to distinguish between "serosal" and "mucosal" MC.[51] Alcian Blue, followed by Safrani counterstaining, stains red only granules of serosal but not mucosal MC. This technique stained the brain MC granules red, indicating that they are highly sulfated, whereas all other parenchymal cells stained lightly blue. Consequently, our histochemical results suggest that brain MC mimic phenotypically the connective tissue (or serosal) MC. Our ultrastructural observations of unstimulated brains agree with this classification since the brain MC exhibit all characteristics seen with typical connective tissue MC, such as those from tongue and skin. Such brain MC are described by Ibrahim[33] as "Type I" cerebral MC and are strictly localized around arterioles and venules, mainly within the thalamus and the hypothalamus. They should not be confused with pericytes since the latter are non-granular cells, surrounding capillaries and localized within the basement membrane of these microvessels.[21,70] The brain cells we identified as MC contain numerous, fairly homogeneous electron-dense cytoplasmic granules and are surrounded by their own basement membrane. Moreover, they are clearly situated in the thin external adventitial layer where they are separated from the endothelium by at least one layer of smooth muscle cells and by their own basement membrane.

According to Terry and Weiss,[80] Mato *et al.*,[54] and Cancilla *et al.*[8] there appears to be a sub-

Fig. 3. Pharmacological characterization of rat brain perivascular MC in light microscopy (Vibratome 5-μm-thick sections stained by acidified Toluidine Blue). (A) Control group: thalamic MC containing numerous metachromatic granules almost "masking" the nucleus. No dispersed or empty granules are present. Scale bar = 6.6 μm. (B) C48/80-treated group: thalamic perivascular MC showing weak metachromasia at low magnification. Scale bar = 16.5 μm. (C) C48/80-treated group: thalamic metachromatic MC containing some empty granules. Notice the presence of some larger (arrowheads) and of some dispersed metachromatic granules (arrows). Scale bar = 6.6 μm. (D) C48/80-treated group: thalamic mast cell containing only very few metachromatic granules. Some granules appear larger than the control ones (arrows). Scale bar = 6.6 μm. (E) Control group: thalamic MC containing numerous metachromatic granules. Notice the absence of any empty or dispersed granules. Scale bar = 6.6 μm. (F) Carbachol-tested group: thalamic perivascular MC showing weak metachromasia at low magnification. Scale bar = 16.5 μm. (G, H) Carbachol-tested group: thalamic metachromatic MC containing fewer granules in comparison to the control. Notice, in contrast, the presence of many empty granules (arrowheads). The nuclei are not masked by the granules. Scale bar = 6.6 μm.

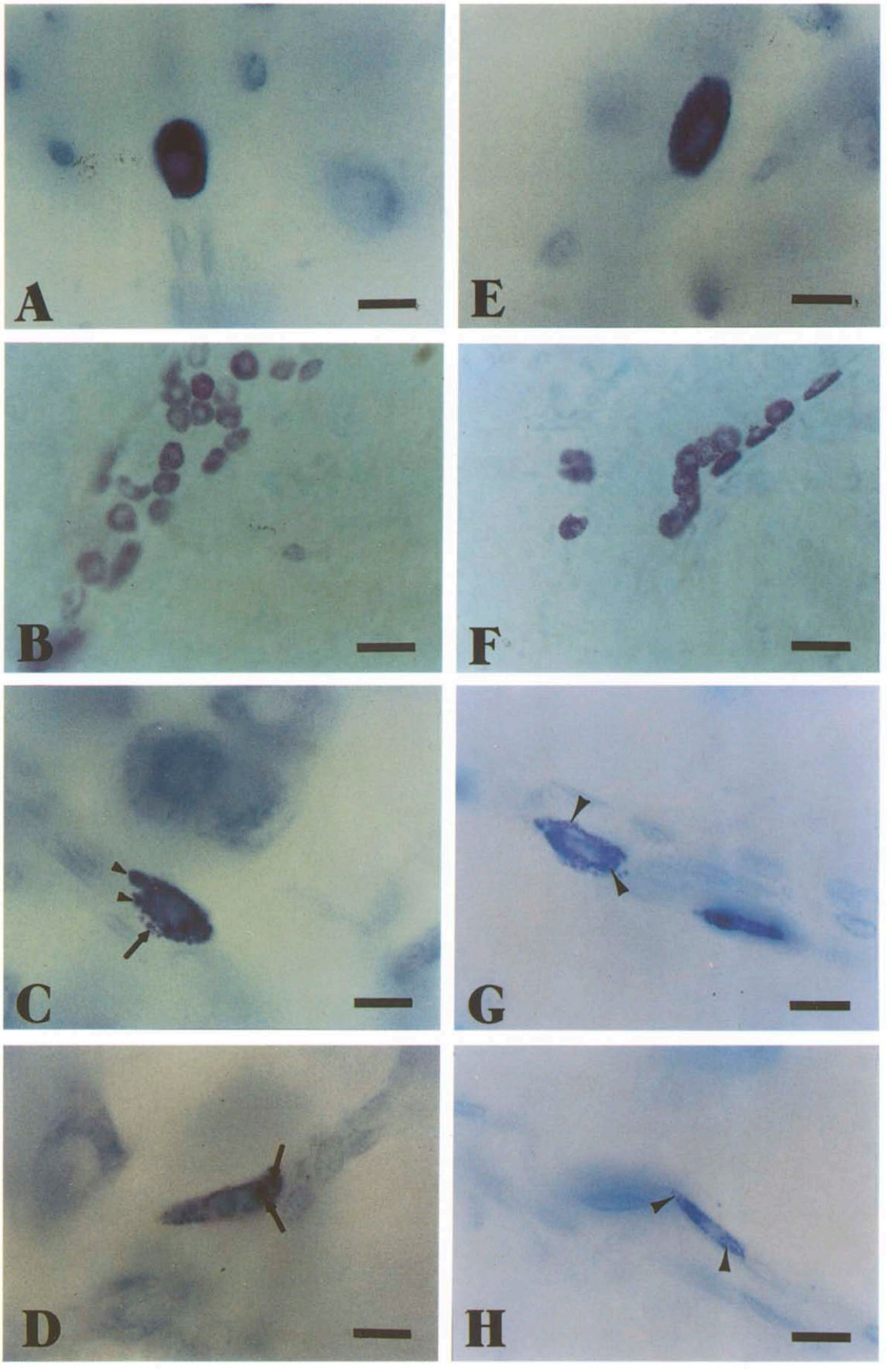

Fig. 3

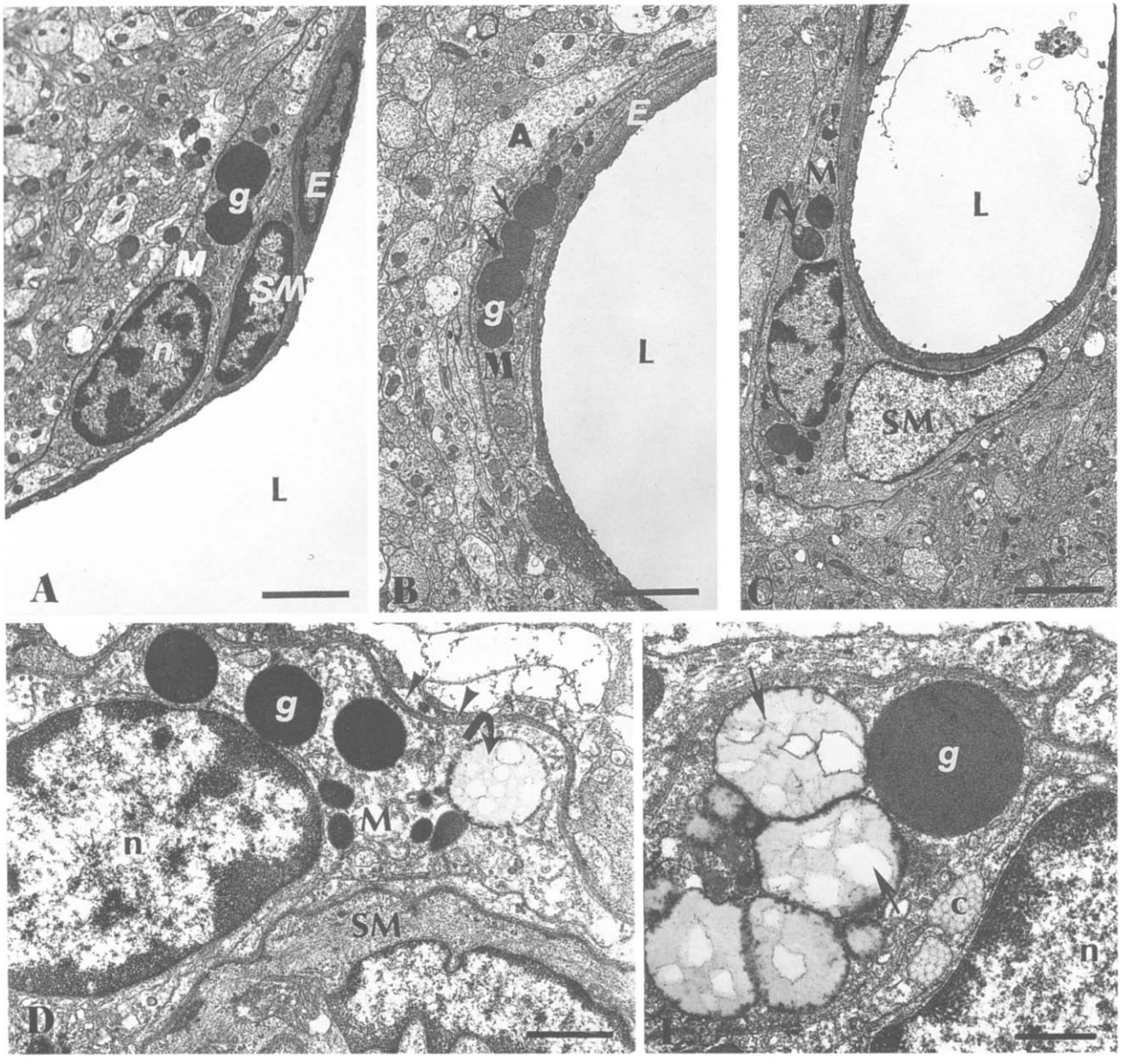

Fig. 4. Pharmacological characterization of rat brain perivascular MC at the electron microscope level: effects of C48/80 perfusion. (A) Control group: when the rat was perfused only with Locke's solution, the MC (M) granules (g) show no ultrastructural modifications. Notice the uniform and amorphous texture of the granule matrix. Scale bar = 2.8 μm. (B) C48/80-treated group: early ultrastructural changes. Notice the fusion of some granules and also the presence of some swollen astrocytic "feet" (A). Scale bar = 2.3 μm. (C) C48/80-treated group: the granules appear partly electron-lucent peripherally losing part of the components (curved arrows). Scale bar = 4 μm. (D, E) C48/80-treated group: notice the presence of completely vacuolated granules showing a "honeycomb-like" (D, curved arrows) or "foamy" (E, arrows) aspect. Scale bars = 1.16 and 0.58 μm, respectively. C, collagen fibers; E, endothelium; L, lumen; n, nucleus; P, pericyte; SM, smooth muscle cell; arrowheads, mast cell basement membrane.

population of pericytes, so-called "granular pericytes", believed to function as phagocytes as they have been initially observed after brain injury or intense irradiation. They were also observed in adult normal rabbits, but the histochemical criteria used[38] (autofluorescence, staining with Oil Red O and PAS positive) were very similar to those used to characterize the type II cerebral MC (or neurolipomastocytes).[33,34] Indeed, Ibrahim and Ibrahim *et al.*[34] found that the central nervous system, especially in the rabbit, contains different proportions and amounts of two types of cerebral MC: (a) "type I", which is phenotypically similar to the peripheral tissue MC; and (b) "type II" which is a

subpopulation sharing some, but not all, of the ultrastructural characteristics.

It has been reported, however, that type I cerebral MC can be differentiated from type II because the latter show weak metachromasia and their granules have a natural fluorescence and stain with Oil Red O and Sudan Black,[10,33] which is indicative of their presence of lipids. However, our results demonstrated that all the observed rat brain MC showed strong metachromasia with Toluidine Blue and stained with Sudan Black B, suggesting the presence of lipids within their secretory granules.

Such observations were unexpected since only type II cerebral MC had been shown to contain lipids and such cells had not been described in the rat brain. Since we have never observed any ultrastructural differences among rat cerebral MC to suggest the presence of two distinct cellular populations, one containing lipids, the other not, we believe that it is no longer appropriate to differentiate two types of rat cerebral MC only on their lipid content. There may, however, be reason to distinguish two types of cerebral MC, according to their amine content and their secretory characteristics. For instance, thalamic MC have been shown to contain 90% of the histamine in the rat thalamus and up to 50% of the total brain histamine.[16,26,48] In contrast, rabbit type II pial MC do not contain any detectable amount of histamine, while they are rich in serotonin.[69] Thus, two distinct types of cerebral MC could exist by biochemical criteria, and it would be of interest to determine if their features are species-specific or due to their articular tissue location.

Finally, a main reason for classifying the perivascular granular cells we describe as cerebral MC is their capacity to undergo granule changes, indicative of secretion, in response to the classic MC degranulation agent, C48/80. During such stimulation, rat cerebral MC gradually lose their intragranular electron-dense contents and finally appear empty, often with a honeycomb-like vesicular substructure. A similar appearance had been described by Ibrahim[32] and Ibrahim *et al.*[34,36] and is distinct from the type of degranulation known for peritoneal MC (fusion between the perigranular cytoplasmic membranes with exocytotic release of the granule content). However, secretion is not always accompanied by degranulation, as has been reported earlier by Dvorak *et al.*[14,15] Such a difference could be explained by local tissue factors, but this subject still needs further investigation. It is important to notice that none of the other cerebral granular cells, such as interparenchymal microglia and pial macrophages, were sensitive to C48/80. Consequently, cerebral MC can neither be identified as microglia,[59] nor as macrophages.[42,43] Moreover, these latter cells differ from the cerebral MC because of their location and their cytological aspects. For instance, the microglia not only lie within the cerebral parenchyma, often in distance, and without any obvious relationship with the blood vessels, but also their ultrastructural comparison with brain MC revealed important morphological differences. In fact, they are not surrounded by any basement membrane and their rough endoplasmic reticulum is not very developed in relation to the granules. On the contrary, these cells have an extensive Golgi apparatus, with many lysosomes and lipofuscine granules which have an ultrastructural aspect completely different from those described for cerebral or peritoneal MC.

Cerebral MC should not be confused with macrophages, which are described only in the subarachnoid space and morphologically they appear very different. In contrast to the cerebral MC, they have a very irregular spindle-like shape, they are not surrounded by any basement membrane, they contain more abundant chromatin, rich smooth and rough endoplasmic reticulum with cisternae that classically appear short, as well as a very well-developed perinuclear Golgi apparatus with many pleiomorphic vesicles, lysosomes and phagocytic granules containing crystal and myelin debris and lipid droplets. Finally, neither microglia nor macrophages can be metachromatically stained violet by low pH Toluidine Blue or red with Alcian Blue and Safranin counterstaining.

Rat brain MC should, therefore, be considered independent cellular entities, close to the peritoneal MC and, in any event, they should not be confused with any other perivascular or granular cerebral cellular types. However, their physiological significance within the cerebral parenchyma remains elusive. We believe that they could serve as a link between the nervous and the immune system,[84] thus explaining some specific pathophysiological conditions which could be better understood in the light of such an assocation. Knowledge of the perivascular localization and the vasoactive contents of these cells, as well as of their capacities for secretion suggests that they may have at least two functions: the control

Fig. 5. Pharmacological characterization of rat brain perivascular MC at the electron microscope level: effects of carbachol perfusion. (A) Control group: MC (M) localized around an arteriole and showing no noticeable ultrastructural modifications after perfusion by Locke's solution only. Notice the homogeneous and amorphous texture of the granule (g) matrix as well as the presence of some immature cytoplasmic granules (arrowhead). Scale bar = 2 μm. (B, C) Carbachol-treated group: the cytoplasmic granules (g) show gradual loss of their content. Some of them appear partly electron-lucent (B, arrows) while others appear empty (C, arrowheads). Notice also in C that the smooth endoplasmic reticulum appears enlarged (small arrow). Scale bars = 1.5 μm. L, lumen; SMC, smooth muscle cell.

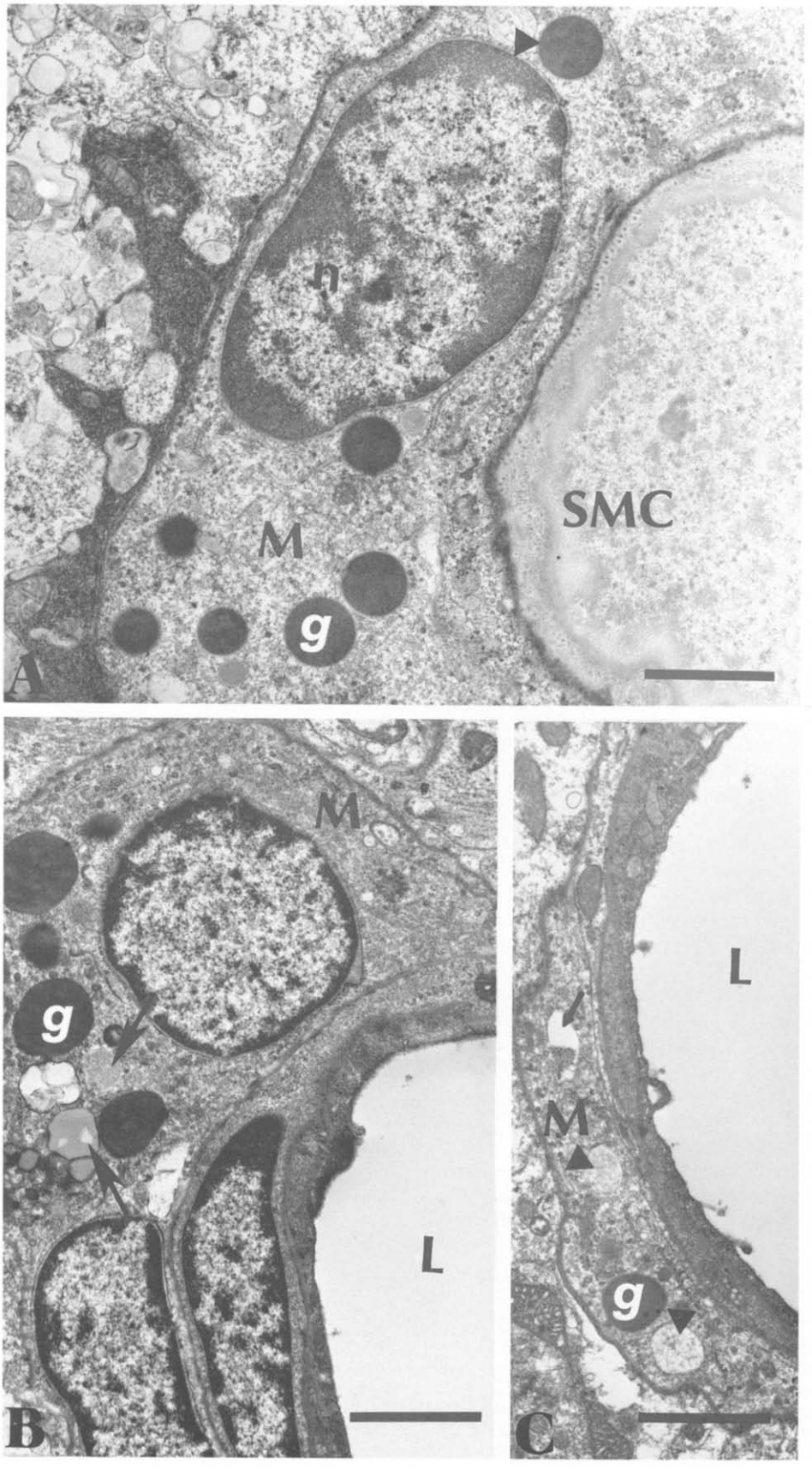

Fig. 5

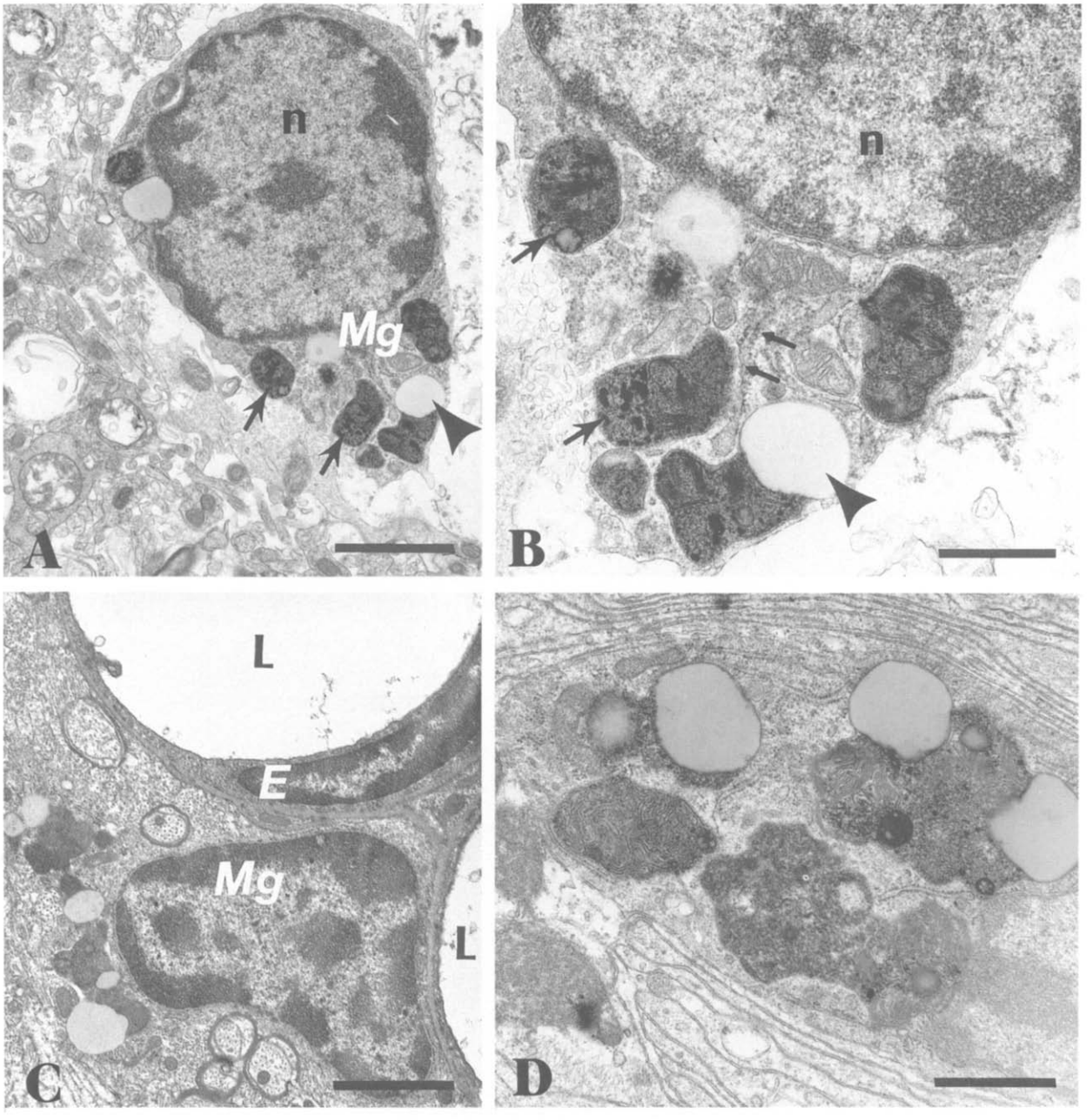

Fig. 6. Ultrastructural characterization of other perivascular and/or granular intraparenchymal cells: the microglia. (A) Ultrathin section of the thalamus showing an intraparenchymal microglia (Mg) cell non-associated to a blood vessel. The cell is characterized by its ovoid shape, a large oval nucleus (n) (that contains a prominent nucleolus), as well as by its numerous lysosomes (arrows) and lipofuscine granules (arrowheads). In contrast, it contains only a few mitochondria. Notice also the absence of any basement membrane. Scale bar = 2 μm. (B) Detail of the cytoplasmic organelles (of A). Notice the extreme heterogeneous texture of the granules that contain myeloid bodies, lipids and primary lyosomes. Notice also the presence of some short rough endoplasmic reticulum cisternae non-related to the granules (small arrows). Scale bar = 0.8 μm. (C) C48/80-treated group: ultrathin section of the thalamus showing a microglia cell (Mg) localized in close proximity to two capillaries. The cell has an irregular shape and shows no ultrastructural modifications in comparison to the cell described for the control group (A). Scale bar = 2 μm. (D) C48/80-treated group. Detail of the cytoplasmic granules. No ultrastructural changes are present in comparison to the control group. Scale bar = 0.8 μm. E, endothelium; L, lumen.

of the cerebral blood flow (CBF) and of the integrity of the blood–brain barrier.

Our evidence of ultrastructural changes indicative of secretion induced by carbachol, and the anatomical juxtaposition of cerebrovascular MC are consistent with a functional interaction. Such an association has been suggested for pial type II MC and adventitial nerve fibers.[10]

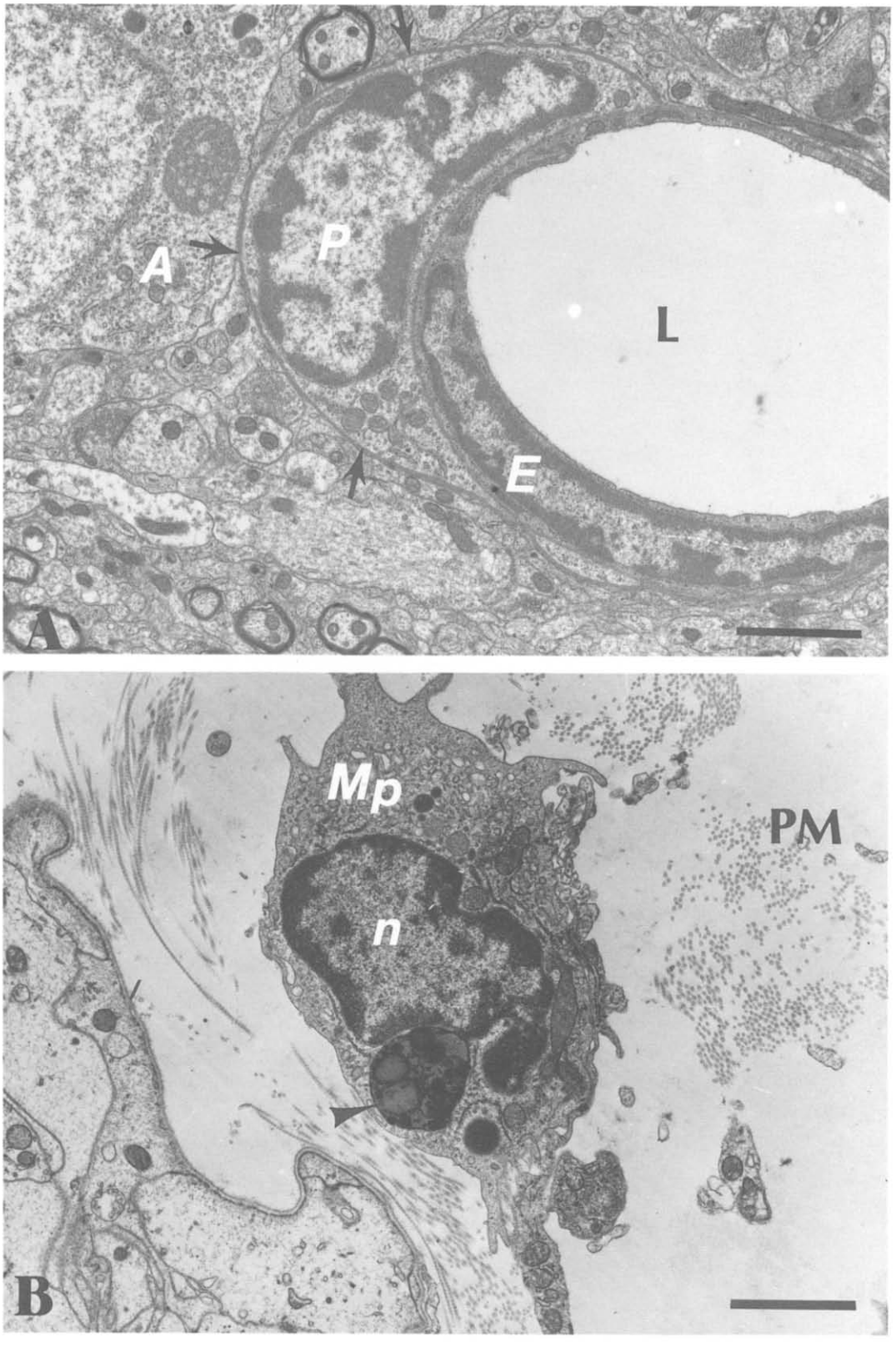

Fig. 7. Ultrastructural characterization of other perivascular or granular cells: pericytes and macrophages. (A) Ultrathin section showing a thalamic capillary surrounded by an ovoid non-granular cell enclosed in a common basement membrane (arrows). The cell, characterized as a pericyte (P), contains a large ovoid nucleus (with clumped chromatin beneath the nuclear envelope and an eccentric nucleolus), few round mitochondria but numerous ribosomes (free in "clusters"). Notice the absence of any cytoplasmic granule or lysosome. A, astrocyte; E, endothelium. Scale bar = 9 μm. (B) Ultrathin section of the pia mater (PM) showing a macrophage (Mp). The cell contains an irregular nucleus (n) and is characterized by its irregular spindle-like shape, numerous Golgi-orientated vesicles, primary and secondary lysosomes (arrowhead) and phagocytic vacuoles. It also contains short cisternae of rough endoplasmic reticulum and many free ribosomes. Notice the absence of basement membrane. Scale bar $\approx$ 2 μm.

Vasomotor local action

It is well known that serotonin[31] and histamine[75] have important effects on cerebral vasculature. It is also known that cerebral MC contain large amounts of these mediators which can be released in response to C48/80, carbachol and substance P.[49] As cerebral MC are preferentially located at the branching points of the cerebral vasculature, it appears possible that partial or total release of mediators could result in major vasomotor effects. Indeed, such an influence on the CBF has been suggested previously on different grounds.[19,72]

It is important to note that responsiveness of brain MC to carbachol may be another factor distinguishing them from peritoneal MC, for even though peritoneal MC have been reported to secrete in response to carbachol,[20] this result has been disputed repeatedly.[76]

The integrity of the blood–brain barrier

The close proximity between the cerebral MC and the cerebrovascular system, and their presence around the circumventricular organs suggests that these cells could have important functions in controlling the integrity of the blood–brain barrier. In fact, secretion of vasoactive molecules could make this barrier permeable to blood lymphocytes or pathogenetic agents.[12] Such a mechanism is suspected in a number of pathological cases, including for example, multiple sclerosis, the pathophysiology of which involves infiltration of brain white matter by lymphocytes and macrophages, leading to brain demyelination.[68] MC are known to contain phospholipases[84] which can degrade myelin,[40] and co-culture of MC and brain slices leads to demyelination.[4] The human demyelination disease, multiple sclerosis may be caused by the immune response to some latent viral infection,[87] and an experimental model of virus-induced encephalitis cannot develop in MC-deficient mice.[28] It has also been shown that MC can be degranulated both by different viruses,[65,79] as well as by myelin basic protein.[40,85] MC are also found around multiple sclerosis plaques,[63] which are infiltrated by substance P-immunoreactive astrocytes.[45] It is therefore of interest that substance P stimulates MC secretion,[22,49] and astrocytes are known to synthesize the MC growth factor interleukin-3.[23] Moreover, two drugs which are known to inhibit MC secretion have prevented experimental neuritis[73] and experimental allergic encephalomyelitis.[86] These data strongly suggest that cerebral MC could participate in the pathophysiology of MS.

Patients with multiple sclerosis also frequently experience allergies[2] and migraine headaches,[88] conditions which have both been implicated with MC. Moreover, an increased number of MC has been reported in cluster headache[3,52] and our recent ultrastructural studies on temporal arteries from cluster headache patients showed MC degranulation and a close relationship between adventitial nerve fibers and MC.[11] Peripheral MC have been shown to release histamine and/or serotonin in response to diverse neuromodulators such as acetylcholine,[20] noradrenaline,[30] as well as many neuropeptides,[39,77] including substance P,[27] somatostatin[81] and neurotensin;[71] consequently, the observation of MC secretion in cluster headache could be the result of a cascade of axonal reflexes, leading to painful inflammation.[11,52] MC may also be involved in migraine headaches, where both the trigeminal system and the thalamocortical axis are implicated.[25,60]

CONCLUSION

The present study clearly demonstrates the presence of MC within the cerebral parenchyma. These cells migrate through the pia mater[50] into the thalamus during development and are similar to serosal MC, but with a particular perivascular location and with secretory characteristics quite distinct from the latter. There is mounting evidence indicating that these extraordinary cells in the brain could serve as the anatomical and functional link between the immune and the nervous system and could also be involved the pathophysiology of many neuroinflammatory conditions.

Acknowledgements—This work was supported in part by grants from the National Multiple Sclerosis Society (U.S.A.) No. RG 1961-A-1 and Kos Pharmaceuticals, FL. Thanks are due to Ms Deirdre M. Ryan for her editorial comments and expertise in preparing this manuscript.

REFERENCES

1. Anderson A. C., Henningson S., Lundell L., Rosengren E. and Sundler F. (1976) Diamines and polyamines in DMBA-induced breast carcinoma containing mast cells resistant to compound 48/80. *Agents and Actions* **6/5,** 577–583.
2. Ansari K. A., Yokohama M. M. and Rand A. (1976) Circulating IgG, allergy and multiple sclerosis. Serum levels of IgG, other immunoglobulins and complement (C's) in patients with multiple sclerosis in-exacerbation and other neurologic diseases. *Acta neurol. scand.* **53,** 39–50.
3. Appenzeller O., Becker W. J. and Ragaz A. (1981) Cluster headache. Ultrastructural aspects and pathogenic mechanisms. *Archs Neurol.* **38,** 302–306.
4. Baloyannis S. J. and Theoharides T. C. (1982) Mast cells may induce demyelination of rat brain slices *in vitro*. *Clin. Res.* **30,** 407.
5. Bienenstock J., Tomioka M., Matsuda H., Stead R. H., Quinonez G., Simon G. T., Coughlin M. D. and Denburg J. A. (1987) The role of mast cells in inflammatory processes: evidenced for nerve/mast cell interactions. *Int. Archs Allergy appl. Immun.* **82,** 238–243.

6. Cammermeyer J. (1966) Reaction of mast cells identified in the rabbit brain. *J. Neuropath. exp. Neurol.* **25,** 130.
7. Campbel D. J. and Kiernan J. A. (1966) Mast cells in the central nervous system. *Nature* **210,** 756–757.
8. Cancilla P. A., Baker R. N., Pollock P. S. and Frommes S. P. (1972) The reaction of pericytes of the central nervous system to exogenous protein. *Lab. Invest.* **26,** 376–383.
9. Casale T. B. (1987) Neuromechanisms of asthma. *Ann. Allergy* **59,** 391–398.
10. Dimitriadou V., Aubineau P., Taxi J. and Seylaz J. (1987) Ultrastructural evidence for a functional unit between nerve fibres and type II cerebral mast cells in the cerebral vascular wall. *Neuroscience* **22,** 621–630.
11. Dimitriadou V., Henry P., Maalthiau P., Aubineau P. and Brochet B. (1989) Interrelations between mastocytes and adventitial nerves of the temporal artery in cluster headache. *Cephalgia* **9,** 37–38.
12. Domer F. F., Boertje S. B., Bing E. G. and Reddix I. (1983) Histamine and acetylcholine-induced changes in the permeability of the blood–brain barrier of normotensive and spontaneously hypertensive rats. *Neuropharmacology* **22,** 615–619.
13. Dropp J. J. (1972) Mast cells in the central nervous system of several rodents. *Anat. Rec.* **174,** 227–238.
14. Dvorak A. M., Dvorak H. F. and Galli S. J. (1983) Ultrastructural orientation for identification of mast cells and basophils in humans, guinea pigs and mice. *Am. Rev. resp. Dis.* **128,** 549–552.
15. Dvorak A. M., Galli S. J., Schulman E. S., Lichtenstein L. M. and Dvorak H. F. (1983) Basophil and mast cell degranulation: ultrastructural analysis of mechanisms of mediator release. *Fedn Proc. Fedn Am. Socs exp. Biol.* **42,** 2510–2515.
16. Edvinsson L., Owman C. and Sjoberg N. O. (1976) Autonomic nerves, mast cells and amine receptors in human brain vessels. A histochemical and pharmacological study. *Brain Res.* **115,** 377–393.
17. Edvinsson L., Cervos-Navarro J., Larsson L. I., Owman C. and Ronnberg A.-L. (1977) Regional distribution of mast cells containing histamine, dopamine, or 5-HT in the mammalian brain. *Neurology* **27,** 878–883.
18. Elson C. O., Kagnoff M. F., Fiocchi C., Befus A. D. and Targan S. (1986) Intestinal immunity and inflammation: recent progress. *Gastroenterology* **91,** 746–768.
19. Faleiro L. C., Machado C. R. S., Gripp A., Resende R. A. and Rodrigues P. A. (1981) Cerebral vasospasm: presence of mast cells in human cerebral arteries after aneurysm rupture. *J. Neurosurg.* **54,** 733–735.
20. Fantozzi R., Masini E., Blandina P., Mannaioni P. F. and BaniSacchi T. (1978) Release of histamine from rat mast cells by acetylcholine. *Nature* **273,** 473–474.
21. Fernando N. V. P. and Movat H. Z. (1964) The fine structure of the terminal vascular bed. III. The capillaries. *Expl molec. Path.* **3,** 87–97.
22. Fewtrell C. M. S., Foreman J. C., Jordan C. C., Oehme P., Renner H. and Stewart J. M. (1982) The effects of substance P on histamine and 5-hydroxytryptamine release in the rat. *J. Physiol., Lond.* **330,** 393–411.
23. Frie K., Bodmer S., Schwerdel C. and Fontana A. (1985) Astrocytes of the brain synthesize interleukin 3-like factors. *J. Immunol.* **135,** 4044–4097.
24. Galli S. H., Dvorak A. M. and Dvorak H. G. (1984) Basophils and mast cells: morphologic insights into their biology, secretory patterns and function. *Prog. Allergy* **34,** 1–141.
25. Goadsby P. J. and Lance J. W. (1988) Brain stem effects on intra- and extracerebral circulations. Relation to migraine and cluster headache. In *Basic Mechanisms of Headache* (eds Olsen J. and Edvinsson L.), pp. 413–427. Elsevier, Amsterdam.
26. Goldschmidt R. C., Hough L. B., Glick S. D. and Padawer J. (1984) Mast cells in rat thalamus: nuclear localization, sex difference and left–right asymmetry. *Brain Res.* **323,** 209–217.
27. Goldschmidt R. C., Hough L. B. and Glick S. D. (1985) Rat brain mast cells: contribution to brain histamine levels. *J. Neurochem.* **44,** 1943–1947.
28. Griffin D. E. and Mendoza Q. P. (1986) Identification of the inflammatory cells present in the central nervous system of normal and mast cell deficient mice during sinobis virus encephalitis. *Cell. Immunol.* **97,** 454–459.
29. Heine H. and Forster F. J. (1975) Relationships between mast cells and preterminal nerve fibers. *Z. mikrosk.-anat. Forsch.* **89,** 934–937.
30. Heitz D. C. and Brady M. J. (1975) Possible mechanism of histamine release during active vasodilatation. *Am. J. Physiol.* **228,** 1351–1357.
31. Hogestatt E. D., Anderson K. E. and Edvinsson L. (1983) Mechanical properties of rat cerebral arteries as studied by a sensitive device for recording of mechanical activity on mechanical activity in isolated small blood vessels. *Acta physiol. scand.* **117,** 49–61.
32. Ibrahim M. Z. M. (1974) The immediate and delayed effects of compound 48/80 on the mast cells and parenchyma of rabbit brain. *Brain Res.* **17,** 348–350.
33. Ibrahim M. Z. M. (1974) The mast cells of the mammalian central nervous system—I. Morphology, distribution and histochemistry. *J. Neurol. Sci.* **21,** 431–478.
34. Ibrahim M. Z. M., Tenekjian V. and Ulthman M. A. A. (1976) The effect of compound 48/40 on the perivascular granular cells (neurolipomastocytes of the rabbit brain). *Anat. Rec.* **184,** 434.
35. Ibrahim M. Z. M., Al-Wirr M. E. and Bahuth N. (1979) The mast cells of the mammalian central nervous system—III. Ultrastructural characteristics in the adult rat brain. *Acta anat.* **104,** 134–154.
36. Ibrahim M. Z. M., Uthman M. A. A., Tenekjian V. and Wiedman T. (1980) The mast cells of the mammalian central nervous system—V. The effect of compound 48/80 on the neurolipomastoid cells and related areas of the CNS: early changes. *Cell Tiss. Res.* **212,** 99–116.
37. Inoue Y., Akita M. and Shimai K. (1962) The mast cells in the brain of the *Triturus pyrrhogaster* (Boie). Part 1 (the histologic study). *Keio J. Med.* **17,** 235–249.
38. Jeynes B. (1985) Reactions of granular pericytes in a rabbit cerebrovascular ischemia model. *Stroke* **16,** 121–125.
39. Johnson A. R. and Erdös E. G. (1973) Release of histamine from mast cells by vasoactive peptides. *Proc. Soc. exp. biol. Med.* **142,** 1252–1256.
40. Johnson D., Seeldrayers P. A. and Weiner H. L. (1988) The role of mast cells in demyelination. 1. Myelin proteins are degraded by mast cell proteases and myelin basic protein and P_2 can stimulate mast cell degranulation. *Brain Res.* **444,** 195–198.
41. Kelsall M. A. and Lewis P. (1964) Mast cells in the brain. *Fedn Proc.* **23,** 1107–1108.
42. Kiernan J. A. (1972) Ultrastructure of mast cells in the diencephalon of the hedgehog. *J. Anat.* **211,** 347–348.

43. Kitamura T., Hattori H. and Fujita S. (1972) Autoradiographic studies on histogenesis of brain macrophages in the mouse. *J. Neuropath. exp. Neurol.* **31,** 502–518.
44. Koenig H. (1964) Studies of brain lysosomes. In *Response of the Nervous System to Ionizing Radiation* (eds Haley and Snider), pp. 403–412. Little, Brown, Boston.
45. Kostyk S. K., Kowall N. W. and Hauser S. L. (1988) Substance P immunoreactive astrocytes in multiple sclerosis plaques. *Neuroscience* **14,** 1057.
46. Krüger G. (1970) Mast cells in the brain of the hedgehog (*Erinaceus europaeushin*). Distribution and seasonal variations. *Acta Zool.* **51,** 85–93.
47. Krüger G. (1974) Demonstration of mast cells in the albino brain. *Experientia* **30,** 210–211.
48. Kowalski M. L. and Kaliner M. A. (1988) Neurogenic inflammation, vascular permeability and mast cells. *J. Immunol.* **140,** 3905–3911.
49. Lambracht-Hall M., Konstantinidou A. D. and Theoharides T. C. (1990) Serotonin release from rat brain mast cells *in vitro*. *Neuroscience* **39,** 199–207.
50. Lambracht-Hall M., Dimitriadou V. and Theoharides T. C. (1990) Migration of mast cells in the developing rat brain *Devl Brain Res.* (in press).
51. Levi-Schaffer F., Austen K. F., Gravallese P. M. and Stevens R. L. (1986) Coculture of interleukin 3-dependent mouse mast cells with fibroblasts results in a phenotypic change of the mast cells. *Proc. natn. Acad. Sci. U.S.A.* **83,** 6485–6488.
52. Liberski P. P. and Mirecka B. (1984) Mast cells in cluster headache. Ultrastructure, release pattern and possible pathogenic significance. *Cephalgia* **4,** 101–106.
53. Mahapatzo R. C. and Bowers H. M. Jr (1979) Distribution of mast cells in the axillary lymph nodes of breast cancer patients. *Cancer* **44,** 592–597.
54. Mato M. S., Ookawara E., Aikawa E. and Kawasaki K. (1981) Studies on fluorescent granular perithelium (F.G.P.) of rat cerebral cortex—especially referring to morphological changes in aging. *Anat. Anz.* **149,** 486–501.
55. Mazi V. (1954) Prime osservazioni sui mastociti nell'encephalo di alcuni bassi vertebrati. *Monit. Zool. Ital.* **62,** 56–66.
56. McCulloch J. and Edvinsson L. (1980) The effects of VIP upon pial arteriolar calibre, CBF, cerebral oxygen consumption and the electroencephalogram. *Am. J. Physiol.* **238,** 449–456.
57. Metcalfe D. D., Kaliner M. A. and Donlon M. A. (1981) The mast cell. *Crit. Rev. Immunol.* **3,** 23–74.
58. Mokhtarian F. and Griffin D. E. (1984) The role of mast cells in virus-induced inflammation in the murine CNS. *Cell. Immunol.* **86,** 491–500.
59. Mori S. and LeBlond C. P. (1969) Identification of microglia in light and electron microscopy. *J. comp. Neurol.* **135,** 57–80.
60. Moskowitz M. A., Buzzi G., Sakas D. and Linnik M. (1989) Pain mechanisms underlying vascular headaches. *Rev. Neurol., Paris* **145,** 181–193.
61. Newson B., Dahlström A., Enerbäck L. and Ahlman H. (1983) Suggestive evidence for a direct innervation of mucosal mast cells. *Neuroscience* **10,** 565–570.
62. Olsson Y. (1968) Mast cells in the nervous system. *Int. Rev. Cytol.* **24,** 27–70.
63. Olsson Y. (1971) Mast cells in plaques of multiple sclerosis. *Acta neurol. scand.* **50,** 611–618.
64. Padawer J. (1957) Studies on mammalian mast cells. *Trans. N.Y. Acad. Sci.* **19,** 690–713.
65. Padawer J. (1968) Uptake of virus by mast cells. *J. Reticuloend. Soc.* **5,** 578–579.
66. Parwaresch M. R., Horny H. P. and Lennert K. (1985) Tissue mast cells in health and disease. *Path. Res. Pract.* **179,** 439–461.
67. Persinger M. A. (1977) Mast cells in the brain: possibilities for physiological psychology. *Physiol. Psych.* **5,** 166–176.
68. Prineas B. S. and Wright R. G. (1978) Macrophages, lymphocytes and plasma cells in the perivascular compartment in chronic multiple sclerosis. *Lab. Invest.* **38,** 409–421.
69. Reynier-Rebuffel A. M., Aubineau P., Callebert J., Dimitriadou V. and Seylaz J. (1989) Cerebrovascular mast cells can release 5-HT during 48/80 or carbachol-induced degranulation. *J. cerebr. Blood Flow Metab.* **9,** *Suppl.* 1, 5505,
70. Rhodin J. A. G. (1968) Ultrastructure of mammalian venous capillaries, venules and small collecting veins. *J. ultrastruct. Res.* **25,** 452–500.
71. Rioux F., Kérouac R. and St-Pierre S. (1985) Release of mast cell mediators, vasoconstriction and edema in the isolated, perfused head of the rat following intracarotid infusion of neurotensin. *Neuropeptides* **6,** 1–12.
72. Rosenblum W. I. (1972) A possible role for rat mast cells in controlling the diameter of arterioles on the surface of the brain. *Brain Res.* **42,** 75–82.
73. Seeldrayers P., Johnson D. and Weinez H. L. (1988) Treatment of experimental allergic neuritis with a mast cell stabilizing drug. *Neurology* **38,** *Suppl.* 1, 238.
74. Serafin W. E. and Austen K. F. (1987) Mediators of immediate hypersensitivity reactions. *New Engl. J. Med.* **317,** 30–34.
75. Sercombe R., Verricchia C., Philipson V., Oudart N., Dimitriadou V., Bouchaud C. and Seylaz J. (1986) Histamine-induced constriction and dilation of rabbit middle cerebral arteries *in vitro*: role of the endothelium. *Blood Vessels* **23,** 137–153.
76. Shanahan F., Denburg J. A., Fox J., Bienenstock J. and Befus D. (1985) Mast cell heterogeneity: effects of neuroenteric peptides on histamine release. *J. Immunol.* **135,** 1331–1337.
77. Skofitsch G., Savitt J. M. and Jacobowitz D. M. (1985) Suggestive evidence for a functional unit between mast cells and substance P fibers in the rat diaphragm and mesentery. *Histochemistry* **82,** 5–8.
78. Stensaas L. H. (1975) Pericytes and perivascular microglial cells in the basal forebrain of the neonatal rabbit. *Cell Tiss. Res.* **158,** 517–541.
79. Sugiyama K. (1977) Histamine release from rat mast cells induced by Sendai virus. *Nature* **270,** 614–615.
80. Terry R. D. and Weiss M. (1963) Studies on Tay–Sachs disease. II. Ultrastructure of the cerebrum. *J. Neuropath. exp. Neurol.* **22,** 18–55.
81. Theoharides T. C. and Douglas W. W. (1978) Somatostatin induces secretion from rat peritoneal mast cells. *Endocrinology* **102,** 1143–1145.
82. Theoharides T. C. (1983) Mast cells and migraines. *Persp. Biol. Med.* **26,** 672–675.
83. Theoharides T. C., Bondy P. K., Tsakalos N. D. and Askenase P. W. (1982) Differential release of serotonin and histamine from mast cells. *Nature* **297,** 229–231.
84. Theoharides T. C. (1990) Mast cells: the immune gate to the brain. *Life Sci.* **46,** 607–617.

85. Theoharides T. C., Baloyannis S. J. and Manolidis L. S. (1990) Activated rat peritoneal mast cells can induce homogeneic *in vitro*. *Intl Archs All. appl. Immunol.* (in press).
86. Theoharides T. C. and Hauser S. L. (1988) Tricyclics inhibit brain mast cell secretion and neuronal demyelination. In *Proceedings of the International Workshop on Diagnostic Therapeutic Trials in Multiple Sclerosis: a New Look* (eds Weiner H. L. and Patty D. W.), pp. 44–46. National Multiple Sclerosis Society, New York.
87. Vandvik B. and Norrby E. (1989) Paramyxovirus SV5 and multiple sclerosis. *Nature* **338,** 769–771.
88. Watkins S. M. and Espir M. V. (1969) Migraine and multiple sclerosis. *J. Neurol. Neurosurg. Psychiat.* **32,** 35–37.
89. Wiesner-Menzel L., Schultz B., Vakilzadeh F. and Czarnetzki B. M. (1981) Electron microscopical evidence for a direct contact between nerve fibres and mast cells. *Acta derm.-vener., Stockh.* **61,** 465–469.
90. Zimmerman A. (1908) Über das vorkommen der mastzellen beim meezschwein-chen. *Arch. mikrosk. Anat.* **72,** 662.

(*Accepted* 29 *March* 1990)