Physiology of Microglia

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I. The Discovery and Definition of Microglia 462
II. Microglia: Origin and Development 466
III. Evolutionary Origins of Microglia 466
IV. Microglia: From the Warden (Surveillance) to Cleaner (Macrophage): The Process of Activation 468
V. Morphology and Identification of Microglia 471
A. Morphology 471
B. Identification 472
VI. General Physiology of Microglia 474
A. Membrane properties of microglial cells in normal tissue 474
B. Membrane properties of microglial cells in pathological tissue 474
C. Membrane properties of microglial cells in culture 475
VII. Calcium Signaling in Microglia 477
A. Intracellular Ca\(^{2+}\) stores 477
B. Store-operated Ca\(^{2+}\) entry 478
C. Calcium extrusion 480
D. Ca\(^{2+}\) homeostasis and microglial cell death 480
VIII. Ion Channels in Microglia 480
A. Sodium channels 480
B. Calcium-permeable channels 483
C. Potassium channels 484
D. Anion channels 487
E. Proton channels 488
F. Aquaporins 488
G. Connexons 489
IX. Microglial Receptors: Concept of “On” and “Off” Receptor-Mediated Signaling 489
X. Neurotransmitter Receptors 493
A. Purinoceptors 493
B. Glutamate receptors 501
C. GABA receptors 502
D. Cholinergic receptors 503
E. Adrenergic receptors 504
F. Dopamine receptors 504
XI. Receptors for Neurohormones And Neuromodulators 505
A. PAF receptors 505
B. Bradykinin receptors 505
C. Histamine receptors 505
D. Endothelin receptors 505
E. Cannabinoid receptors 506
F. Angiotensin II receptors 507
G. Somatostatin receptors 507
H. Glucocorticoid and mineralocorticoid receptors 507
I. Opioid receptors 507
J. Neurokinin (substance P) receptors 508
K. Vasoactive intestinal polypeptide receptors 508
L. Neurotrophin receptors 508
XII. Cytokine and Chemokine Receptors 508
A. Chemokine receptors 508
B. TNF-α receptors 510
I. THE DISCOVERY AND DEFINITION OF MICROGLIA

Pio del Rio-Hortega introduced the concept of microglia (Fig. 1) as a defined cellular element of the central nervous system in a book chapter called “Microglia” (208) written for the landmark publication Cytology and Cellular Pathology of the Nervous System, edited by Wilder Penfield in 1932. In this visionary article, which we strongly recommend to read in original, del Rio-Hortega postulated the following: 1) microglia enter the brain during early development. 2) These invading cells have amoeboid morphology and are of mesodermal origin. 3) They use vessels and white matter tracts as guiding structures for migration and enter all brain regions. 4) They transform into a branched, ramified morphological phenotype in the more mature brain (known today as the resting microglia). 5) In the mature brain, they are found almost evenly dispersed throughout the central nervous system and display little variation. 6) Each cell seems to occupy a defined territory. 7) After a pathological event, these cells undergo a transformation. 8) Transformed cells acquire amoeboid morphology similar to
the one observed early in development. 9) These cells have the capacity to migrate, proliferate and phagocytose.

All these statements are perfectly valid today and could appear in a modern textbook of neuroscience without the smallest change. Del Rio-Hortega had based his postulates on studies published in a series of articles between 1919 and 1927 (202–207, 209, 210) in which he used a modified silver carbonate impregnation to label the microglial cells. The technique was tedious, varied in efficiency between species, but when working yielded excellent images of these cells.

Del Rio-Hortega called the newly discovered cell class microglia and the individual cell “microgliocyte.” In some of the contemporary publications, these cells were even referred to as “Hortega cells” (583). In the first two glial books published after the second world war, these cells were already termed microglia cells (324, 992), the name which (being slightly modified to microglial cells) has remained in the present day.

The concept of neuroglia was introduced by Rudolf Virchow (963, 964; for historical overview, see also Refs. 443, 956), and his first drawings of glia do not resemble the modern image of an astrocyte or an oligodendrocyte, but look rather similar to an activated microglial cell. It is therefore unclear what Virchow has depicted. At that time and for the next 50 years or so, different types of glial cells were not well defined despite the introduction of the term astrocyte in 1891 (516, 517). Nonetheless, pathologists had noted the appearance of morphologically dis-

Distinct cells in pathological tissue, cells which differed very much from those in the normal brain. The origin of these “pathological” cells was heavily debated. In 1878, Carl Frommann (300) identified cellular changes in defined areas of the brain and spinal cord when examining tissue from a deceased 22-yr-old multiple sclerosis patient (Fig. 2). Frommann had the most modern vision at the time in postulating that the glial cells change their morphology. He described that their soma became larger and the cellular processes shorter and less numerous. Frommann also noted a higher density of glial cells in the “epicentres of damage” (Heerdsubstanz), and moreover, he found that some of the glial cells in these “epicenters” had a granulated soma containing inclusions. It seemed obvious to him that the glial cells transformed in the multiple sclerosis pathology.

With later studies, the field became more unclear. In the late 19th to early 20th century, several scientists found and described microglial cells in the pathological brain (Figs. 2 and 3) without clearly understanding their origin. Nissl, Alzheimer, and Merzbacher (14, 15, 582, 647) described cells in patients with neurodegenerative diseases such as syphilitic paralysis, which they termed rod cells (Stäbchenzellen), grid cells (Gitterzellen), or clearance cells (Abräumzellen).

The origin of these cells was debated. Conceptually, glial cells were believed to be of ectodermal origin, although Nissl concluded that pia cells contribute to the formation of rod cells and considered them as mesodermal (647). Robertson, a Scottish pathologist, described a new form of glia termed mesogial cells (762), which he also considered to be of mesodermal origin. He also noted that unlike the macroglial cells (most likely he referred to astrocytes), the mesogial cells do not contact blood vessels. He also mentioned in his Textbook of Pathology (763) that mesogial cells withdraw their processes and transform into granule cells in response to injury. He was close to unraveling the mystery of the microglia, and therefore, Glees (324) suggested to name microglia the “Robertson-Hortega” cells.

Also with his developmental concept, del Rio-Hortega had predecessors. Campobianco and Fragnito (117) and Campobianco (116) reported that at the early

![Fig. 2. Neuroglial cells in pathological context. A and B: different types of glial cells found in multiple sclerosis plaques of human cortex. C: glial cell close to a 14-day-old hemorrhage in human white matter. Axons pass through the network of the cell. [A and B from Frommann (300); C from Alzheimer (14).]
embryonic stages a number of mesoblastic cells migrate into the nervous system and are transformed into neuroglia. Hatai (362) described two types of glial cells in the early postnatal brain of mouse and rat. One form he termed the type "a" cell, which he considered to be the ectodermal derived glial cells. The second type, the type "b" cell, was morphologically distinct. Based on his morphologic studies, Hatai concluded that these "b"-type cells separated from the vessel wall, became amoeboid, and migrated away from the capillary. He concluded that the brain contains two types of glial cells, one of ectodermal and the other of mesodermal origin. All in all, the time was ripe for the discovery of microglia. What should, however, be emphasized is the fact that it was the introduction of a novel staining technique which enabled del Rio-Hortega to finally convince his peers.
In the years to follow, del Rio-Hortega’s observations were confirmed and further elaborated. Kershman (441) studied the infiltration of microglia in embryonic human development and described hot spots of immigrations such as at the plexus chooroideus and coined the term microglia fountains for these sites. For many years, however, the field did not advance beyond the statements of del Rio-Hortega formulated in 1932. A detailed historical account on the concept of mesodermal cells in the CNS can be found in References 38 and 758.

The modern era of microglial research started in the late 1960s when Georg Kreutzberg introduced the facial nerve lesion model (71). This preparation allowed studying microglial responses to injury in tissue with an intact blood-brain barrier. It also provided the possibility to distinguish between responses of intrinsic microglia and invading monocytes and helped to establish the concept that microglial cells are important for both de- and regeneration of the brain. Despite the fact that in vitro cultures of microglial cells were described as early as 1930 by Costero (172), their introduction as a wide-spread tool to study microglial properties and functions happened almost half a century later after the development of a method of obtaining large numbers of microglia from the postnatal brain (319). This has led to an explosion of studies on cultured microglia. Unfortunately, microglial cells recognize the cell culture environment as alien with the consequence that all these in vitro studies may not faithfully reflect properties of microglia in the normal, non-pathological brain. The combination of advanced imaging techniques with the use of genetically based cell-specific markers has recently allowed investigations of microglia in the undisturbed tissue (190, 645).

II. MICROGLIA: ORIGIN AND DEVELOPMENT

The origin of microglia has been debated for a long time. There were arguments that these cells stem from the neuroectoderm (271). Today there is general consensus that microglial cells are derived from progenitors that have migrated from the periphery and are from mesodermal/mesenchymal origin (for review, see Ref. 138). In rodents, these cells immigrate from the blood system as monocytes. Early reports indicated that monocytes invaded the brain also in adulthood (376, 837). Recent studies, however, demonstrated that the exchange of microglial cells in the normal undisturbed brain is almost negligible (10, 586). An essential step in the latter study was the protection of the brain from irradiation. After blood-brain barrier damage, a subpopulation of monocytes enters the brain and transforms into microglia (586). The obvious conclusion is that in a healthy, intact brain, the microglial cells exist as a stable population. As reviewed by Chan (138), there is also an embryonic invasion of cells that gives rise to a microglial population. This occurs from the middle of the first trimester and throughout the early part of the second trimester in human and between embryonic days 10 and 19 in rodents. There is evidence for at least two separate populations of microglial cells. One population is derived from progenitors that are of myeloid/mesenchymal origin (not necessarily derived from monocytes). The second population represents a developmental and transitory form of fetal macrophage, and this cell is related to amoeboid microglial population as described in the postnatal brain of rodents (757).

After invading the brain parenchyma, microglial cells transform into the ramified phenotype. We know much more what controls the activation of microglia compared with what converts them to the “resting,” ramified phenotype. Some cell culture studies provide certain hints: astrocyte conditioned medium increases ramification of blood monocytes in culture (835). ATP or adenosine mimicked the astrocyte conditioned medium, but could not be confined to a distinct purinoceptor subtype. Combining astrocyte conditioned medium with ATP or adenosine yielded a phenotype with more extensive ramification, indicating that purines are not the only ramification-inducing factors of microglia (997). Further candidates are cytokines released from astrocytes, namely, transforming growth factor-β (TGF-β), macrophage colony-stimulating factor (M-CSF), and granulocyte/macrophage colony-stimulating factor (GM-CSF). The ramification in response to astrocyte medium was prevented by neutralizing antibodies against TGF-β, M-CSF, or GM-CSF (804). The activity of Cl⁻ channels seems to be a requirement for the cells to make the morphological transformation. Pharmacological blockade of Cl⁻ channels prevented the ramification process (242). It should, however, be noted that the Cl⁻ channel pharmacology is not very specific.

III. EVOLUTIONARY ORIGINS OF MICROGLIA

The evolutionary origins of microglia remain largely unexplored; however, it is conceivable to assume that
they appeared simultaneously with an emergence of compact neuronal masses and were represented by myeloid cells entering the neuronal ganglia. The evidence for microglial cells is available for leech (Annelids), bivalves and snails (Molluscs), and insects (Arthropods).

Microglial cells of invertebrates were best studied in the leech. Following injury, the leech nervous system has the capacity to regenerate as evidenced by axonal sprouting. In the injured nervous system, small amoeboid cells that have the capacity to phagocyte and migrate had been observed. These cells can be labeled by weak silver carbonate, a classical stain for vertebrate microglia and, thus, these leech cells were also termed microglia. Within 24 h after the injury, these cells accumulate at the lesion site, indicating that they respond to nerve injury (612). Live imaging in leech nerve cords revealed that only a fraction of microglia, typically <50%, moved at any time with a speed of up to 7 μm/min. Cells were moving within 15 min of crushing directly toward the lesion along axons or axon tracts (565). The leech microglial cells were also implicated in production of antimicrobial peptides in response to infectious attack (800).

Cell culture experiments revealed that leech microglial cells maintained on the plant lectin concanavalin A (Con A) had a rounded shape, remained stationary, and were avoided by growth cones (50). In contrast, microglia cultured on extracts of leech extracellular matrix were mobile, spindle-shaped with long processes, and did not influence neurite growth (557). Both types of microglia, the round and the spindle-shaped, had similar membrane properties in culture. They were characterized by a high input resistance similar to microglial cells from acutely isolated slices of rodents and distinct from cultured rodent microglia. A small cationic conductance, which has not been described for rodents, was also present (865).

In response to tissue damage, leech microglia, similarly to vertebrates, migrate to the site of injury, change their morphology (248, 556), and acquire phagocytic properties (706). When the leech nerve cord is injured, microglia migrate towards the lesion and accumulate at the damage site (145, 482, 556, 970). In response to injury, leech connective glia and some microglia express endothelial nitric oxide synthase (eNOS) as revealed by immunolabeling. Minutes after the injury, eNOS activity could be detected as indicated by a histochemical labeling for NADPH diaphorase (822). The NO could mediate the accumulation of microglia at the lesion site in the leech: a NOS inhibitor significantly reduced microglial accumulation (640). Thus, in the leech nervous system, microglial cells are important elements to control regeneration (631).

Similar mechanisms are also present in molluscs. Microglial cells can be observed to egress from excised ganglia of the mussel Mytilus edulis, a marine bivalve, and this migratory activity was naloxone-sensitive. The authors conclude that morphine can stimulate NO release, which counteracts the egress of microglia from the ganglia (531). The morphine-induced NO release mediated by activation of the opiate alkaloid-selective, opioid peptide-insensitive micro3 receptor, which is similar in human monocytes and invertebrate immunocytes, indicating that functional coupling of morphine to NO production has been conserved during 500 million years of evolution (545). Invertebrates and vertebrates share even more signaling pathways: anandamide, an endogenous cannabinoid receptor ligand, is found in invertebrate immunocytes and mammalian monocytes. Anandamide triggered the release of NO both from Mytilus edulis microglia and human macrophages. The release was blocked by a NOS inhibitor and a cannabinoid antagonist (855). Moreover, recombinant human interleukin-10 (IL-10) inhibits the migration of microglia from excised ganglia of Mytilus edulis. A substance immunoactivity similar to human IL-10 was detected in ganglia homogenates. This IL-10 is another example of a signaling pathway in microglia, which has evolved early in evolution. A third common signaling pathway found in invertebrates and vertebrates is represented by 17β-estradiol. This estrogen inhibits the migration of Mytilus edulis microglia from the ganglia and is also coupled to the release of NO. Indeed, the authors could identify a fragment of the estrogen receptor-beta gene with 100% sequence identity to the human counterpart (856). The egress from the excised ganglia as an assay of microglial activity was also described for the snail Planorbarius corneus and the insect Leucophaea maderae (847). Traumatic injury triggers proliferative response in microglial cells from Planorbarius corneus (712); activation of microglia was associated with expression of inducible NOS (iNOS) (711). Evidence also exists that opiate signaling regulates microglial activities in this mollusc (848).
Similar to the vertebrate system, apoptosis occurs in the nervous system during *Drosophila* development. Apoptotic neurons are engulfed by a variety of glia including midline glia, interface (or longitudinal tract) glia, and nerve root glia. However, the majority of apoptotic cells in the CNS are engulfed by subperineurial glia in a fashion similar to the microglia of the vertebrate CNS. In a *Drosophila* mutant, which lacks macrophages, subperineurial glia contain an abundance of apoptotic cells, indicating that there is a cross-talk between macrophages and subperineurial glia resulting in the removal of apoptotic cells (849). Inhibition of a receptor required for macrophage-mediated engulfment of dead cells causes CNS defects, indicating that macrophage-mediated clearance of cell corpses is required for proper morphogenesis of the *Drosophila* CNS (817).

IV. MICROGLIA: FROM THE WARDEN (SURVEILLANCE) TO CLEANER (MACROPHAGE): THE PROCESS OF ACTIVATION

Microglia in the healthy mature CNS, including the brain, spinal cord, as well as the eye and optic nerve, have a ramified morphology, a small soma with fine cellular processes. This typical appearance, which is quite different from a classical macrophage, has been associated with microglial “resting” state. Infection, trauma, ischemia, neurodegenerative diseases, or altered neuronal activity, that is any disturbance or loss of brain homeostasis indicating real or potential danger to the CNS can evoke rapid and profound changes in the microglial cell shape, gene expression and the functional behavior which sum- marily is defined as “microglial activation” (72, 169, 192, 334, 351, 479, 869, 949). Phenotypically, the complexity of the cellular processes is reduced, and microglia revert to an amoeboid appearance (Fig. 4). Microglia can become motile and actively move to a lesion or herd of infectious invaders following chemokinetic gradients. Local densities of microglia can also increase by proliferation, to provide more cells for the defense against invading germs and to organize for the protection and restoration of tissue homeostasis. Induction and rearrangement of surface molecules for cell-cell and cell-matrix interactions, changes in intracellular enzymes as well as release of multiple factors and compounds with proinflammatory and immuno-regulatory effects are additional elements of the activation process. Microglia can unfold their phagocytotic activities to clear tissue debris, damaged cells, or microbes. Release of chemoattractive factors recruits and guides immune cell populations to the CNS, and presentation of antigens to T cells can subsequently aid the adaptive immunity in fight against viral or bacterial invasion. The range of microglial activities also covers production of neurotrophic factors and the physical association with endangered neurons.

The stages of microglial activation were defined based on morphological, molecular, and functional characteristics, with fully activated microglia presenting themselves like other macrophages (169, 192, 351). Yet these cells are embedded in a specialized tissue (brain parenchyma) which also has to protect itself from the potentially damaging consequences of an immune reaction. This special condition has been described as the “immune privilege” of the CNS (306). The microglial activation is thus a highly regulated process. The CNS is a complex organ with regional variations in glial and neuronal cell populations as well as the biochemical milieu conditioned by and for them. Microglia may thus not necessarily represent a homogeneous population. This has been impressively shown in a study on the role of the chemokine/receptor system CXCL10/CXCR3 for neuronal cell death and glial activation in the mouse hippocampus (951). Based on organotypic slice cultures and N-methyl-D-aspartic acid (NMDA)-induced excitotoxicity, the authors found that the different vulnerability of CA1 versus CA3 neurons depends on microglia. Constitutive heterogeneity as a kind of diversity “to start with” may thus predestine microglial performance and overlay with the inducible diversity upon stimulations (351).

Until recently, microglia of the healthy adult CNS were considered quiescent, i.e., functionally dormant, due to the low or absent expression of activation-associated molecules and their “immobile” ramified morphology (479). In reality, however, these “resting” cells actively scan their environment. With motile processes, and by constant integration and interpretation of environmental cues, microglia monitor their extracellular space and cellular neighborhood, always ready to transform to executive states of activity. The in vivo imaging studies employing mice with EGFP-expressing microglia demonstrated that their fine cellular processes are in constant motion (190, 645). Moving the fine processes the territorially faithful microglia can scan the environment without disturbing the fragile neuronal circuitry. The “resting” microglia thus actively survey the tissue, being ready to rapidly transform to “activated” states upon appearance of signs indicating a threat to the CNS. “Activated” microglia will then primarily serve for a support and protection of the structural and functional integrity of the CNS.

Nevertheless, while assuming that the surveillance function of microglia is important for the maintenance of CNS homeostasis, the housekeeping activity of microglia may remain largely unnoticed (351). A proper “physiological” performance of microglia could be simply overlooked. Microglia would, in case of a small vascular defect or an isolated neuronal impairment, act immediately to offer protection and trophic support, even reduce activating synaptic input by the phenomenon of synaptic strip-
Such locally restricted and transient activities would, however, not be recognized. In other words, little is known about the impact of the daily function of microglia. On the contrary, failure to offer protection, generation of maladapted responses, or excessive and chronic activation is detected when surfacing with clinical symptoms or, in the worst case, by histopathological evidence. The neuropathological records hence bias for failure and harmful contributions as they have no balance for the beneficial outcome of microglial engagement. It would be important to focus in the future on the role of microglia in health to better unravel the deviations in cellular function that, occasionally, cascade into disease.

The transition from the surveillance mode of a “resting” cell to the executive states thereby represents more
of a shift in activities rather than “activation” per se. This view acknowledges that microglia have a constant functional importance. There are no periods of inactivity. Moreover, the term activation does not contain any information about the functional orientation, which decides on the CNS consequences (919). Instead, there is increasing evidence that microglial activation is not an “all-or-none” process, neither is it a linear path with a fixed uniform outcome, to the contrary, activated microglia can acquire distinct functional states (128, 166, 351, 705, 816).

There is ample evidence that microglia undergo changes in shape and expression patterns during the course of an activation in vivo. Findings derive from responses to injury (152, 488, 743), ischemia (778), or autoimmune challenges (722). Microglial activation may start with an early emergency response, such as defense-oriented functions, to fight off an infection or to limit further damage after an injury. The activated population with an initially chosen program may eventually convert to a repair-oriented support for tissue restoration. The scheme in Figure 4 highlights the major components of such a dynamic response.

While still a lot has to be learned about the events affecting microglia throughout the activation process, even less is known about the period after. A terminated microglial response, in the best case successful and not even much noticed, may still leave traces (351). Are there subtle alterations within a previously activated microglia? The postactivated microglia may remain undistinguishable by morphology or isolated criteria of detection (via marker staining) from the “resting” cells in nearby populations, while still bearing long-lasting adjustments. Receptors with regulatory influences on the transcriptional control in macrophages have been reported, and these regulations could be selective for sets of genes (197, 289, 962). Epigenetic mechanisms organizing longer-lasting adjustments are already known. The experienced cell could then behave differently when being challenged again. As a local population, such a microglia could influence the likelihood of a tissue region to develop age-related dysfunction or to succumb to neurodegenerative processes (72, 170). Conceivably, this principle could also have some protective effect, when the former activation prepares the affected population to respond faster or more efficient upon a second encounter.

When discussing the process of activation, the functional heterogeneity of microglia has to be also considered. Regional heterogeneity and populational segregation of microglia in tissues and preparations were demonstrated by morphology, selective detection of constitutive or inducible mRNAs and proteins [e.g., for TNF-α, IL-6, IGF-I, integrins, CD4, CD11c, CD34, CD40, CD45, CD86, major histocompatibility complex (MHC) class II, FcγRII, iNOS, and members of the neurotrophin family], or differences in the proliferative potential (6, 111, 128, 192, 247, 287, 327, 351, 438, 475, 485, 487, 488, 552, 755, 809, 810, 825, 854, 872, 993). Heterogeneity was also observed in microglia from the aging brain where surprising differences between individuals were found (579, 834).

An influence on the expression profiles and functional properties could result from the major tissue architecture. White and gray matter microglia were recently reported to differ in the expression of the immunoregulatory receptor Tim-3 (16). Rapid ex vivo methods now allow for “snapshot” analyses of multiple protein expressions by microglia of different CNS regions (193). Here, too, first dissociations in populational profiles seem to follow a sorting by white matter index, indicating that the myelin environment requires and/or dictates a special microglial setting that is different from that in gray matter. These differences could impact on development, normal functionality, as well as vulnerability to inflammation (390). Interestingly, the heterogeneity of microglia is mirrored by heterogeneity of oligodendroglial subpopulations in subregions of gray and white matter (450).

The exposure to neurotransmitters, the proximity to blood vessels, and properties of the blood-brain barrier controlling the microenvironment (3) may associate with “provincial” adaptations of microglia (192). Whether these specificities concern mainly housekeeping activities, such as the scanning of neuronal structures or whether they become important only upon further activation is not known. The more information will become available on the constitutive heterogeneity of “the” microglia by anatomical divisions, the more studies on inducible diversity have to respect that microglial behavior can vary by location, i.e., superimposing constitutive and inducible diversity.

Being consequent, the demonstration of a white-versus-gray-matter distinction in receptor expression (16) could lead down the road to the hypothesis that specialized cells reside even among a regional population. Not only barely visualized by the conventional detection toolkit, individual microglial cells could be predisposed to certain functions. Indeed, population heterogeneity was reported already in one of the first papers dealing with microglial protein expression by anatomical subdivisions (247). Neurotrophins were seen in microglia with a region-specific pattern, but also within a region, only subpopulations contributed to their production. Strong support for such individuality came more recently from studies on TREM-2 (triggering receptor expressed on myeloid cells-2) (809, 810). Found as being expressed by inactivated microglia, and downregulated upon lipopolysaccharide (LPS) or interferon (IFN)-γ encounter, TREM-2 varied in its expression by microglia not only from region to region, but also within each. The authors interpreted these observations as a sign for the role of the microenvironment in instructing a microglial phenotype. Cell-to-cell variability,
indeed, is gaining attraction (842), and the novel technical approaches will certainly contribute to evaluate the extent as applying to microglia.

V. MORPHOLOGY AND IDENTIFICATION OF MICROGLIA

A. Morphology

It was through the study of morphological changes that a microglial role in the diseased CNS was recognized (351, 479). At first glance, the microglial appearance in the healthy matured tissues of the brain, spinal cord, or the retina does not suggest an immediate association with a macrophage nature (Fig. 5). The cellular processes branching off from the small soma with further distal arborization are typical for the “ramified” microglia. This term has been almost synonymously used with “resting” microglia, implying an intimate link between morphology and function (872). Even though time-lapse analyses on microglial morphology had been conducted already (861), it was the elegant application of in vivo two-photon imaging to the monitoring of resting microglia in the mouse neocortex which revealed, surprisingly, the dynamics of the processes (190, 645). Upon challenges, the process of microglial transformation from resting to activated states is accompanied by marked morphological changes. Microglia reduce the complexity of their shape by shortening (retracting) the branches of their processes so that they are resorbed into the cell body. Several steps and intermediate stages can be identified, including characteristics of process withdrawal, transition or hyper-ramification, and subsequent formation of new protrusions along with motility and locomotion, i.e., microglial movement in the tissue (539, 861, 872). Interestingly, in ageing, microglia gain morphological markers characteristic for senescence and functional deterioration (587, 868, 872).

Microglial cells in vitro usually do not have the ramified structure typically seen in the normal CNS. They show heterogeneous shapes, ranging from spindle and rod-shaped or amoeboid versions with short thick processes expanding as lamellipodia to even round cells, but morphological reorganization can still be imposed by treatment with typical activating agents, such as bacterial LPS (6). Morphological responses differ depending on the type of insult (872, 873). Cells can acquire bushy or bi- and tripolar, spindle- or rod-shaped morphologies (635, 759). The heterogeneous forms may interchange, while transition from the ramified microglia to the amoeboid macrophage-like appearance is thought to occur as a stereotypic sequence (861).

FIG. 5. Morphology of resting and activated microglia. Mouse microglia in brain tissue and cultures. A: staining for Iba1 in mouse cortex reveals microglia with their processes. B: a microglial cell visualized in a brain tissue slice by filling with Lucifer Yellow through a micropipette. C: upon stimulation with bacterial cell wall components, such as LPS, microglia in a primary culture present with the typical morphology of activation. Cells were stained with FTC-conjugated ILB4. D: microglia in culture stained with FTC-ILB4 and showing phagocytosed rhodamine-labeled myelin (made by coupling of rhodamine to a mouse myelin preparation). [Images are from Denise van Rossum, University of Göttingen (A, C, and D) and Clemens Boucsein, MDC Berlin (B).]
The remarkably fast and drastic transformations in cell shape indicate that they might be required for functional adjustments. For example, filopodia protrusion and dynamic rebuilding are needed for motility and directed migration. Similarly, appearance of "foamy" cells with lipid- or myelin-loaded organelles in brain tissue sections points to phagocytic activity, for example, in a myelin lesion. Yet morphology is not always a reliable reflection of functional orientation. Certain gene inductions or activities can occur in the absence of obvious morphological transitions (254), whereas a ramified shape can be experimentally induced even under conditions that do not support ramification (400, 886). As a consequence, loss of ramified and gain of more amoeboid cell formats do not tell much about the actual reactive phenotype (551).

B. Identification

The identification of microglial cells beyond cytomorphological criteria was facilitated by the development of staining procedures that take advantage of the exclusive expression of certain molecules in specific cell types (Table 1). The suitability of the various markers and methods is determined by the ability 1) to discriminate microglia from other CNS-resident cells, like neurons, astrocytes, oligodendrocytes, or endothelial cells; 2) to distinguish parenchymal microglia from other resident or infiltrating monocytes/macrophages; and 3) to either reveal the entire population of microglia or to display a bias for certain activity states. The mere issue of technical aspects that affect the success and quality of staining, like tissue collection, fixation, and embedding as well as choice of staining tools and procedures, is out of the scope of this review. Below we may rather focus on selected molecules that have been valuable to identify microglia and to reveal their morphology.

Microglia can be visualized in human and animal brain tissue sections and in cultures by a variety of cell surface-associated or intracellular/cytosolic molecules. In some cases, the exact identity and function of the respective protein or carbohydrate structure is not known, such as for surface-expressed glycan moieties identified by *Griffonia simplicifolia* isoolectin B₄ (ILB4) or tomato lectin (84, 870). Nonetheless, these are useful markers in lectin-histochemical or antibody-based procedures. Although blood vessels can be stained by ILB4 with supreme intensity as well, a differentiation is possible by morphological criteria and even by automated image analysis. In other cases, the molecules targeted by staining have an established function as receptors, adhesion molecules, or enzymes in macrophages, for example, the α3β2 integrin (CD11b/CD18, complement receptor 3, CR3, MAC1), which binds complement C3b, plasma, and extracellular matrix proteins and serves in the phagocytotic clearance of opsonised and nonopsonized material (542). Antibodies against Iba1, a protein with a suggested role in calcium homeostasis (406, 407), have proven most helpful in visualizing microglia with details of their processes.

In the neuropathological routine and experimental research, immunoglobulin receptors (CD16/32/64, FcyRII/III/II, I), CD45 (leukocyte common antigen, LCA), CD68 (macrophasin, CD163 (scavenger receptor M130, ED2), CD169 (sialoadhesin, siglec-1), CD204 (MSR), F4/80 antigen, β-glucan receptor dectin-1, or mannose receptor (CD206) are useful targets, with more or less prominent and overlapping recognition of other tissue macrophages/macroglial-like cells. The expression level of many of these molecules, such as for example CD11b or Iba1, mostly increases with microglial activation (420, 421). MHC class II structures are only expressed by activated microglia including the expression of the accessory molecules for antigen presentation, namely, B7.1 or B7.2 (CD80/86).

While microglial cells can be easily distinguished from other brain cells, this is not always possible with respect to non-CNS monocyes. Costaining of other CNS macrophage populations, like perivascular cells or the macrophages of the meninges (912), comes with a (micro)anatomical separation. The situation is, however, different when injured and diseased tissues are examined. Virtually all common macrophage markers, e.g., CD11b, Iba1, or F4/80, will simultaneously report all macrophages present in the CNS tissue (128). Despite considerable efforts, hitherto a clear distinction of (activated) microglia from infiltrating monocytes is hampered by the overlap in the expression of macrophage-associated factors typically serving microglial staining. The CNS-invading cells from the periphery share lineage origin and features with the resident microglia, and both major populations intermingle in a CNS lesion by both morphological and biochemical properties. To some extent, the expression level of CD45 has been exploited to discriminate between them. CD45⁺ cells in the CNS reveal a low (CD45lo, parenchymal microglia) or intermediate (CD45int, other CNS-associated macrophages), but rarely a high (CD45hi) expression level (1029). Infiltrating cells, on the contrary, seem to be either of the CD45hi phenotype. Separation of the CD11b⁺CD45lo and CD11b⁺CD45hi populations, for example, in FACS analyses, can thus be used to differentiate parenchymal microglia from the infiltrating macrophages. However, with lesion progression, the clear demarcation may fade, rendering an unequivocal identification more difficult. Other distinctions have been made by superoxide dismutase expression (250), whereas expression of the calcium binding macrophage related proteins MRPs8/S100A8 and MRp-14/S100A9 is thought to identify circulating and more recently invaded monocytes/macrophages (375, 490), while delayed expression in microglia can occur as well (249).
More recently, new antigens for immunolabeling have been introduced which promise the selectivity for microglia among cells of the mononuclear phagocyte system (790). The glucose transporter 5 (GLUT5), at least in human tissue, is suggested as a useful marker for resting and activated microglia (387), as this isoform seems to be restricted to microglia (546, 952). Antibodies against GLUT5 can apparently stain human microglia to even allow for a distinction from other related cell types (peripheral macrophages). Yet the suitability of the marker in reliably identifying only microglia could be limited to human material. Discrete expression patterns of the various GLUT isoforms seem to vary in other species. Rodent macrophages may not be excluded from GLUT5 staining. On the other hand, antibodies against the keratin sulfate epitope 5D4 can serve such discrimination in the rat (54).

More recently, generation of genetically modified animals expressing fluorescent proteins under the control of
macrophage/microglia-expressed factors, such as the fractalkine receptor (CX3CR1), have been employed as microglia-indicator mice. In this mouse line, EGFP was inserted into the fractalkine receptor locus, and heterozygous mice express both EGFP and fractalkine (430). Whether the missing gene copy may influence microglial function could be a concern. A transgenic mouse line expresses the enhanced green fluorescent protein under control of the Iba1 promoter (379). The fluorescence of this line is, however, not as bright as the one of the fractalkine mouse.

VI. GENERAL PHYSIOLOGY OF MICROGLIA

A. Membrane Properties of Microglial Cells in Normal Tissue

The majority of studies on microglial physiology were performed on cultured microglial cells. These cells exhibit amoeboid morphology and therefore cannot be considered as a model for the resting microglial cells. The cells in culture can be stimulated and thus triggered to acquire a proinflammatory phenotype. A classical tool to activate microglial cells is LPS; within 24 h, microglial cells are considered as activated and release a number of proinflammatory substances such as TNF-α, IL-6, or nitric oxide. Microglial activation is, however, not an all-or-none process, but can vary depending on the stimulation context (for review, see Ref. 351). Therefore, much of the physiological recordings in vitro are from moderately to fully activated microglial cells.

There are also an ample number of investigations performed on various types of microglial cells lines. These cell lines, however, cannot be considered as a proper model of microglial cells due to substantial and inconsistent modifications in physiological properties; therefore, in the present review we deliberately omitted results obtained from cell line preparations.

So far, we have no physiological recordings from microglial cells in vivo, neither of their membrane potential nor of currents nor of intracellular Ca2+ concentration. The data that come closest to the in vivo situation are recordings from acutely isolated brain slices (Fig. 6). Microglial cells were studied in slices from cortex, striatum, and facial nucleus from young-adult (8–12 wk old) rats. Cells were identified by specific labeling with tomato-lectin coupled to Texas Red. The labeled cells retained their ramified morphology within the first hours after slice preparation. These cells were characterized by high input resistance and little, if any, voltage-gated membrane currents and a very low membrane potential (~20 mV; range ~2 to ~40 mV) (81). Clamping the membrane potential of microglial cells in slices at ~70 mV led to a rapid (10 s to a couple of minutes) cell damage and loss of a giga-seal.

Similar results were obtained in forebrain slices from adult (6–8 wk old) mice: cells commonly lacked voltage-gated currents, while the membrane potential recordings distinguished two subpopulation of cells with resting potential around ~52 and ~29 mV, respectively (82). In ramified microglial cells in acute slices from juvenile mice, the resting potential was around ~38 mV (802).

Microglial cells during early postnatal development were studied in the corpus callosum of 6- to 9-day-old mice. This area is one of the “microglial fountains” (i.e., the sites of microglia progenitors invasion) as described by Kershman (441). These cells had amoeboid morphology; they rapidly migrated within the slice and commonly accumulated at the slice surface within one to several hours. The amoeboid cells were characterized by only minute if any membrane currents with depolarizing voltage steps, while the hyperpolarizing voltage steps induced large inward currents (Fig. 7). These currents exhibited properties of the inwardly rectifying K+ current in that the reversal potential depended on the transmembrane K+ gradient, inactivation time constants decreased with hyperpolarization, and the current was blocked by tetraethylammonium (86). The membrane potential varied considerably between ~10 and ~70 mV (with the mean value of ~42 mV).

B. Membrane Properties of Microglial Cells in Pathological Tissue

The changes in microglial membrane currents in response to a pathological event have been studied in acutely isolated slices containing the facial nucleus. Using the classical paradigm developed by the Kreutzberg group (71), the facial nerve was cut, thus triggering microglial activation in the facial nucleus without the disturbance of the blood-brain barrier; microglial cells were studied 12 h, 1 day, 3 days, and 7 days after the lesion. At the 12-h time point, the cells expressed predominantly inward rectifying currents. One day after the lesion, the membrane conductance was dominated by delayed rectifying outward currents and inward rectifying currents. After 3 days, these voltage-gated currents became smaller in amplitude, and after 7 days, currents were only slightly larger than in the control cells (81).

A similar observation was obtained in microglial cells studied after an ischemic insult. To distinguish peripheral leukocytes from microglia, the blood cells were prelabeled in vivo with a fluorescent dye rhodamine. Six hours after ischemia, the microglial cells were still lacking voltage-gated channels or exhibited only an inward rectifier current. After 48 h, the intrinsic microglial population expressed inward and outward currents similar to those observed 1 day after facial nerve axotomy (540). This pattern was further confirmed by a study using a mouse model of status epilepticus.
induced by intraperitoneal kainate injections. In 24–48 h after the injection, microglial cells in the hippocampal slices were characterized by the appearance of voltage-activated inward and outward potassium currents (27). A study on human microglia obtained from epilepsy patients who underwent surgery indicated a nonactivated, normal pattern. These human microglia had a high input resistance, a low resting membrane potential of \(-110\) mV, and lacked Na\(^+\) currents as well as inwardly rectifying and delayed rectifying K\(^+\) currents similarly to a nonactivated microglia from mouse and rat (77).

The cell membrane capacity (which is proportional to cell membrane surface area) of ramified microglia in acutely prepared slices was \(-12\) pF; the activation of microglia by LPS resulted in significant increase in cell capacitance (to \(-43\) pF in 48 h) after triggering the activation process (42). In the acutely prepared hippocampal slices from juvenile (P5–P9) mice, the membrane capacity of microglial cells (which generally were in resting ramified state) was \(-12.4\) pF, which was significantly smaller than the capacitance of activated microglial cells from the same slices organotypically cultured for 3–5 days (\(-16\) pF) (802). The difference in the input resistance was even greater: resting microglia in acutely isolated slices had input resistance of \(-2.4\) GΩ, whereas in activated microglia in cultured slices, the input resistance increased to 4.0 GΩ (802).

C. Membrane Properties of Microglial Cells in Culture

Most studies on cultured microglia follow the procedure introduced by Giulian and Baker (319, 320), in which postnatal CNS tissue is used to isolate microglial cells from rats and mice. Initial physiological experiments on these cultures indicated that inward rectify-
K+ currents dominate the membrane conductance of cultured microglial cells (442). The resting membrane potential of microglia cultured from rodents or humans was about −50 mV (442, 658). Thus the cultured cells have a membrane current pattern different from microglial cells in slices from normal brain tissue. This current pattern matches that of the amoeboid, invading microglial cells in situ or that of microglia 12 h after a pathological insult. Stimulation of cultured microglial cells with bacterial LPS or IFN-γ induced the expression of an additional outward current (656). The resting membrane potential of LPS-activated cultured rat microglial cells showed two levels in distinct cell subpopulations at around −48 and −70 mV; the resting membrane potential of −48 mV is mostly determined by the delayed rectifier K+ channels which have a “window” current region around −45 mV (159), whereas in cells with the more negative membrane potential the dominant K+ permeability was represented by the inward rectifier (160). This pattern is similar to microglial cells 24 or 48 h after a pathological insult. Incidentally, the acidification of an extracellular solution led to depolarization of resting potential of cultured rat microglia (from −45 at pH 7.4 to −29 at pH 6.4) probably due to the shift of delayed rectifier potassium current (IKDR) activation curve (162).

FIG. 7. Method of isolation of single amoeboid microglial cells from the surface of corpus callosum slice and inward rectifier K+ currents recorded from these cells. A–D: combinations of images taken by means of infrared video microscopy (left panels) and schematic drawings showing the method of cell isolation. Video images were captured with an intensified CCD camera (Hamamatsu, Japan) and digitized by a frame grabber connected to the PC. A: initial position of amoeboid microglial cells situated on the surface of a corpus callosum slice. B: a single microglial cell was approached with a micropipette, and a whole cell patch-clamp configuration was established. C: after 2–3 min, the cell partially spread over the pipette, intensifying the cell-to-pipette contact. D: finally, the cell was lifted for 200 μm over the slice surface. Scale bar in D = 10 μm. E and F: voltage-activated whole cell currents recorded from a single cell at stages indicated in B and D, respectively. Currents were activated by depolarization and hyperpolarization voltage steps (duration 200 ms, increment 10 mV) from a holding potential of −70 mV. The lifting of the cell did not affect the ionic current pattern. During the experiments, slices were held in a recording chamber mounted on the stage of an upright microscope (Axioscope, Zeiss, Oberkochen) and continuously superfused with HEPES-buffered salt solution, containing (in mM) 150 NaCl, 5.4 KCl, 2 CaCl2, 1 MgCl2, 5 HEPES, and 10 glucose, pH 7.4. Membrane currents of amoeboid microglial cells were recorded using a standard patch-clamp technique in whole cell configuration. Current signals were amplified with conventional electronics (EPC-7 amplifier; HEKA, Germany), filtered at 3 kHz, and sampled at 3–5 kHz by an interface connected to an AT-compatible computer system, which also served as a stimulus generator. Experiments were controlled by Wintida software (HEKA, Germany). Recording pipettes were fabricated from borosilicate capillaries (Hilgenberg, Germany), with resistances of 5–10 MΩ. The pipette solution contained (in mM) 130 KCl, 0.5 CaCl2, 5 EGTA, 4 MgCl2, 10 HEPES, 2 Na2ATP; pH 7.3. [From Haas et al. (342), with permission from Elsevier.]
The majority of subsequent studies on microglia confirmed this current pattern (for review, see Ref. 264). Differences were described for human brain macrophages (microglia) isolated from explants of neurosurgical adult human tissue in that some of these cells expressed Na\(^{+}\) currents (658).

VII. CALCIUM SIGNALING IN MICROGLIA

Calcium signaling/homeostatic system, which is operative in most living cells, is controlled by several evolutionary conserved molecular cascades responsible for Ca\(^{2+}\) transport across cellular membranes and intracellular Ca\(^{2+}\) buffering (130, 713). Conceptually, intracellular Ca\(^{2+}\) signals are shaped by electrochemically driven Ca\(^{2+}\) diffusion through membrane channels and Ca\(^{2+}\) transport against the concentration gradient, which is accomplished by several families of Ca\(^{2+}\) pumps and exchangers (120, 714). These channels and transporters are differentially distributed within the cell, thus enabling different intracellular compartments to handle Ca\(^{2+}\) in a distinct way. Generally, neuroglial cells use intracellular Ca\(^{2+}\) signals as the substrate for their excitability, whereas propagating Ca\(^{2+}\) waves enable long-range signaling in astroglial syncytia (954, 955, 958, 960). For electrically nonexcitable microglial cells, the primary route for Ca\(^{2+}\) signal generation is associated with Ca\(^{2+}\) release from the intracellular stores and with Ca\(^{2+}\) entry through the plasmalemma via ligand-gated (see section X for detail) and store-operated Ca\(^{2+}\)-permeable channels (263, 600; and Fig. 8).

A. Intracellular Ca\(^{2+}\) Stores

Microglia contain at least two types of dynamic Ca\(^{2+}\) stores: the endoplasmic reticulum (ER) and mitochondria. The ER is one of the major cellular organelles, formed by the endomembrane, which extends from the nuclear envelope down to the finest cellular processes. The ER lumen is internally continuous, thus providing the intracellular communication system through which various molecules are transported to their destinations (715). The functions of the ER are diverse, from protein synthesis and protein posttranslational folding to a generation of...
various intracellular signaling molecules. The intra-ER free Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) varies between 0.2 nM and 0.8 mM thus providing electro-driving force for Ca\(^{2+}\) release (598, 844, 846); the high [Ca\(^{2+}\)]\(_i\) is maintained by sarco(endo)plasmic reticulum Ca\(^{2+}\)-ATPases (SERCA), the activities of which are precisely controlled by free Ca\(^{2+}\) in both cytosol and ER lumen (99). The release of Ca\(^{2+}\) is accomplished by several families of endomembrane-resident Ca\(^{2+}\) channels (see Refs. 61, 473, 957, 958), from which the most characterized are Ca\(^{2+}\)-gated Ca\(^{2+}\) release channels, generally referred to as ryanodine receptors (RyRs) and inositol 1,4,5-trisphosphate (InsP\(_3\))-gated Ca\(^{2+}\) release channels commonly known as InsP\(_3\) receptors (InsP\(_3\)Rs). Importantly, both RyRs and InsP\(_3\)Rs are capable to undergo regenerative activation (52), which together with rapid intra-ER Ca\(^{2+}\) equilibration (“Ca\(^{2+}\) tunneling”; Refs. 715, 845) create a substrate for intracellular propagating Ca\(^{2+}\) waves.

Microglial cells contain both types of intracellular Ca\(^{2+}\) release channels. Microglial cultures obtained from the fetal human brains expressed mRNA for all three RyRs subunits, RyR1–3, whereas adult human microglia had only RyR1/2 mRNAs (402). Functionally, Ca\(^{2+}\) signaling in these cultured microglial cells can be induced by very high concentrations of 4-chloro-m-creosol (4-Cmc; 1–5 mM), this effect being antagonized by 1,1’-diheptyl-4,4’-bipyridinium dibromide (DHBP, 10 \(\mu\)M). Both DHBP (10 \(\mu\)M) and 4-Cmc (100 \(\mu\)M) when administered together with microglia-activating agents (LPS and IPN-\(\gamma\) or IPN-\(\gamma\) and \(\alpha\)-synuclein) reduced neurotoxicity of conditioning media obtained from microglial cultures and applied to cultured neuroblastoma (462). The Ca\(^{2+}\) release following activation of the RyRs by natural agonist cyclic ADP ribose (cADPR) was also demonstrated in human cultured microglia (826). In primary microglial cultures, the cADPR signaling cascade was involved in LPS-induced activation process. The cADPR was produced by ectoenzyme CD38, and the cADPR-dependent events were inhibited by ryanodine and cADPR antagonist 8-bromo-cADPR, thus indicating possible involvement of RyRs (293, 562). Nonetheless, the functional characterization of microglial RyRs is yet to be done, and so far it seems that RyRs are not particularly important for Ca\(^{2+}\) signaling in these cells.

The main route for the generation of microglial Ca\(^{2+}\) signals is associated with InsP\(_3\)Rs. The InsP\(_3\)Rs are part of a widely present intracellular signaling cascade, which starts from G protein-coupled metabotropic receptors linked to phospholipase C (PLC); activation of the latter produces two intracellular second messengers the diacetyl-glycerol (DAG) and the InsP\(_3\) (see Refs. 51, 53, 714, 957 for review). The InsP\(_3\) in turn, activates InsP\(_3\)Rs of the ER, thus producing Ca\(^{2+}\) release and [Ca\(^{2+}\)]\(_i\) elevation, which then effects on numerous cellular functions. Microglial cells express a multitude of metabotropic receptors, which are discussed in detail in sections X–XIV of this review.

Apart from metabotropic receptors, which are the primary triggers for the activation of InsP\(_3\)-induced Ca\(^{2+}\) release, the InsP\(_3\) signaling cascade can be affected by several other factors. For example, the exposure of primary cultured rat microglia to pure albumin or albumin associated with immunoglobulins and fatty acids (albumin fraction V) triggered [Ca\(^{2+}\)]\(_i\) elevation, which was mediated through Src tyrosine kinase, PLC, and InsP\(_3\) and was coupled to microglial proliferation (386). Interestingly, albumin did not trigger Ca\(^{2+}\) signals in peritoneal macrophages (386). Similarly, the application of ammonium to primary cultured mouse microglia triggered ER Ca\(^{2+}\) release through direct activation of PLC/InsP\(_3\) signaling cascade (589).

Some chronic pathological conditions may also affect the status of the ER Ca\(^{2+}\) store. In particular, the InsP\(_3\)-mediated Ca\(^{2+}\) release [following the stimulation of purinoceptors or platelet-activating factor (PAF) receptors] was reduced by more than 50% in microglial cells obtained from the brains of Alzheimer’s disease patients, possibly indicating chronic depletion of the ER store (569).

The inhibition of SERCA pumps by thapsigargin induced morphological transformation of amoeboid microglia in culture to a ramified phenotype (1014).

B. Store-operated Ca\(^{2+}\) Entry

Store-operated Ca\(^{2+}\) entry (SOCE) was initially contemplated by Jim Putney as a “capacitative Ca\(^{2+}\) influx” mechanism (732, 733), which coupled depletion of ER stores with the activation of the plasmalemmal Ca\(^{2+}\) entry pathway. This initial hypothesis gained a universal acknowledgment since. The SOCE pathway was identified in the majority of nonexcitable and many excitable cells (520, 521, 692, 731). The SOCE pathway is mediated through plasmalemmal channels, most probably of several subtypes. The most characterized are the calcium release-activated Ca\(^{2+}\) (CRAC) channels (389, 691), although other channels (e.g., TRPC) can have store-operated gating (734). Properties of CRAC channels in microglia are discussed in detail in section VIII. Conceptually, the SOCE channels are open upon the depletion of intracellular Ca\(^{2+}\) stores and aid in replenishing the Ca\(^{2+}\) stores. In different cell types, depletion of ER can activate SOCE either in a graded or in a threshold manner; in the latter case, only a substantial depletion of the store (or a depletion of specific portion of the store) triggers plasmalemmal Ca\(^{2+}\) influx (231, 358, 690). Essentially, the activation of SOCE often substantially outlasts the period of initial stimulation and Ca\(^{2+}\) release, therefore providing a long-lasting Ca\(^{2+}\) influx. The mechanisms of ER to
SOCE coupling are not yet completely understood, although recently two molecules were discovered to be major players in both the signaling to and permeation mechanisms of the store-operated channels. These molecules are Stim1 and the Orai proteins, with Stim1 acting as an ER Ca^{2+} sensor and Orai as the pore-forming subunit of CRAC channel (280, 723, 734, 772).

The first evidence for functional SOCE in microglial cells was obtained by [Ca^{2+}] imaging experiments investigating the response of microglia to complement fragments or endothelin (603, 605). In both sets of experiments, the plateau phase of the agonist-induced cytoplasmic Ca^{2+} signal was completely dependent on the presence of extracellular Ca^{2+}. Subsequently, it became evident that activation of microglial metabotropic receptors linked to Ca^{2+} release from internal stores activates SOCE, which can be recorded as a plateau phase of the ligand-induced Ca^{2+} response. Examples are many; the SOCE is activated following stimulation of metabotropic purinoceptors, PAF receptors, lysophosphatidic acid receptors, etc. (See sects. X, XI, and XVI for detailed examples). The SOCE appear to play an important role in human microglia, where several reports indicated the strong dependency of metabotropic agonist-induced [Ca^{2+}]_i transients on external Ca^{2+} (326, 573, 982, 985, 1031). However, the SOCE in human microglia is not only coupled to P2Y purinoceptor-dependent depletion of internal stores, but is also modulated by membrane depolarization either following the activation of Cl^- channels (570) or ionotropic P2X purinoceptors (568). Extracellular acidification (from pH 7.4 to 6.2) resulted in almost instant and pronounced (by 87%) inhibition of SOCE activated following PAF-induced ER depletion (447), which may be explained by rapid cell depolarization triggered by pH lowering.

The maximal depletion of ER Ca^{2+} store in cultured microglia by overstimulation of P2Y_{2,4} metabotropic purinoceptors resulted in the chronic activation of SOCE (914). In these experiments, the Ca^{2+} release was induced when bathing cells in Ca^{2+}-free external solution. After the period of stimulation, the external Ca^{2+} was reintroduced, which resulted in strong rebound [Ca^{2+}]_i increase; the Ca^{2+} remained at an increased level for tens of minutes and reflected the persistent activation of SOCE, as changes in extracellular Ca^{2+} were mirrored in rapid changes of [Ca^{2+}]_i (914). During this period, the ER stores remained depressed as neither the stimulation of purinoceptors nor administration of SERCA blocker thapsigargin induced any [Ca^{2+}]_i responses. The long-lasting basal [Ca^{2+}]_i elevation can be also induced by treatment with phorbol ester (600), and with BDNF (597) that may result from chronic activation of SOCE. Whether such a chronic activation ever occurs in vivo remains, however, unknown.

Incidentally, activation of microglial cells in vitro by LPS leads to an elevation of basal [Ca^{2+}]_i, which is associated with suppression of evoked calcium signaling, as indicated by reduced [Ca^{2+}]_i transients in response to UTP or complement factor 5a (382). Preventing the LPS-induced increase in basal [Ca^{2+}]_i, by BAPTA strongly attenuated the (LPS-induced) release of NO and certain cytokines and chemokines. Ionomycin, an ionophore elevating basal [Ca^{2+}]_i, failed to induce release activity. Elevated [Ca^{2+}]_i, is obviously required, but it is not sufficient to trigger the release of NO and certain cytokines and chemokines. Elevation of basal [Ca^{2+}]_i, was suggested to be a central element in the regulation of executive functions in activated microglia (382). It is not yet resolved whether the persistent elevation of basal [Ca^{2+}]_i, in activated microglial cells is associated with long-term activation of SOCE. In human embryonic cultured microglia, activation of metabotropic purinoceptors with adenosine 5’-O-(2-thiodiphosphate) (ADPβS) triggered a biphasic [Ca^{2+}]_i elevation with the second plateau phase lasting several minutes. The plateau phase was sensitive to the SOC inhibitor SKF96365. This Ca^{2+} elevation was due to store-operated Ca^{2+} entry and was instrumental in increasing production of COX-2 (153).

The IFN-γ, another substance to activate microglia, led to slowly developing steady-state [Ca^{2+}]_i, increase in cultured human microglia (rate of increase 0.8 nM/s and maximal amplitude 102 nM; Ref. 292). Likewise, elevated basal [Ca^{2+}]_i, was found in activated microglia isolated from the human post mortem brains from Alzheimer’s disease patients (569) or in cultured microglia exposed to Aβ_{35–35} (472).

There is a link between mitochondria and the SOCE in microglial cells. The SOCE induced following stimulation of PAF receptors or the depletion of ER after exposure to cyclopiazonic acid (CPA) can be effectively (IC_{50} ~9 μM and 20 μM, respectively) blocked by PK11195, a ligand of mitochondrial peripheral benzodiazepine receptor. This inhibition was also accompanied with a reduced expression of COX-2. This peculiar pharmacology led to the suggestion that there is a direct link between mitochondria and SOC entry pathways (385), which has been considered previously as a general mechanism of CRAC channels regulation (316, 689).

The LPS was reported to trigger direct, transient Ca^{2+} increases in 10–12% of microglial cells from primary cultures of the rat area postrema and from rat brain. The LPS-induced Ca^{2+} signaling in cultured microglia was abolished by ruthenium red and preincubation with caffeine, thus suggesting the involvement of RyRs and caffeine-sensitive ER Ca^{2+} store (29, 1007). This response was not confirmed by others including our own observations.

Significant abnormalities are present in the Ca^{2+}-mediated signal transduction in microglia isolated from...
Alzheimer’s disease (AD) patients; these microglial cells were characterized by significantly higher (20%) basal (resting) Ca$^{2+}$ relative to the cells from individuals not suffering from dementia. The peak amplitude of ATP and initial phase of PAF responses, which reflect rapid depletion of Ca$^{2+}$ from the ER, were reduced by ~60% in AD cells relative to amplitudes recorded from normal microglia. Additionally, AD microglia showed diminished amplitudes (by ~60%) of SOCE following PAF stimulation and prolonged time courses (increase by 60%) of ATP responses compared with healthy cells (569).

C. Calcium Extrusion

Calcium extrusion following [Ca$^{2+}$]$_i$ elevation is accomplished by plasmalemmal Ca$^{2+}$ pumps of the PMCA (plasmalemmal Ca$^{2+}$-ATPase) family and the sodium/calcium exchanger (NCX). The Na$^+$/Ca$^{2+}$ exchangers, represented in mammals by three subtypes NCX1, NCX2, and NCX3, are members of Ca$^{2+}$/cation antiporter superfamily (541). These microglial PMCAs have not been yet characterized in detail. Operational Na$^+$/Ca$^{2+}$-dependent uptake (as assayed by $^{45}$Ca$^{2+}$ technique) was detected in cultured microglial cells in 2001 (559). Subsequent experiments revealed an expression of all three NCX isoforms, NCX1, NCX2, and NCX3, at both translational and protein levels in cultured rat microglia (624). At the mRNA level, expression of NCX1 was dominating and was larger than in neurons from the same animals; expression of NCX2 and NCX3 was weak, being much less that in nerve cells (624). Treatment with IFN-γ caused an increase in Na$^+$/Ca$^{2+}$-dependent $^{45}$Ca uptake (under conditions favoring the reverse mode of operation) and stimulated NCX protein synthesis. This stimulation was governed by two distinct intracellular signaling cascades: the early increase in NCX activity was sensitive to inhibitors of PKC (staurosporine and GF109203X) and tyrosine kinase (herbimycin A), whereas delayed effects of NCX synthesis were in addition sensitive to the protein synthesis inhibitors cycloheximide and actinomycin D, indicating de novo synthesis (624), which affected all three NCX isoforms. The activity of microglial NCX was also positively regulated by NO, the latter even causing depletion of the ER and subsequent ER stress, which are somehow connected to the NCX function (560) because inhibition of NCX prevented NO-induced ER stress and microglial death (626). The NCX is also involved in NADPH-mediated respiratory burst induced by phagocytotic activity: inhibition of the reversed mode of Na$^+$/Ca$^{2+}$ exchanger with KB-R7943 decreased the respiratory burst in dose-dependent manner (639); similarly, the reverse mode of NCX action was instrumental for bradykinin-induced microglial migration (397). Ischemic insult (middle cerebral artery occlusion, MCAO) significantly increased the expression of NCX1 in microglial cells penetrating into the infarct core 3–7 days after the surgery (79). A similar increase in NCX1 expression was observed in microglial cells cultured from the infarction core (79).

D. Ca$^{2+}$ Homeostasis and Microglial Cell Death

Calcium ions are intimately involved not only in physiological signaling but also in triggering various types of cell death (644). Disruption of microglial Ca$^{2+}$ homeostasis triggers activation of death programs, which are regulated by the microglia activation status. Treatment of primary cultured microglial cells by thapsigargin or ionomycin induced apoptosis, whereas the same agents applied to LPS-activated microglia resulted in necrotic cell death (625). Both apoptotic and necrotic pathways were regulated by [Ca$^{2+}$]$_i$ because the treatment of cultures with BAPTA-AM reduced cell death (625).

VIII. ION CHANNELS IN MICROGLIA

A. Sodium Channels

The presence of sodium channels in microglial cells is controversial. In microglial cells in situ and in the majority of studies in cultured microglia, rapid inward currents typical for voltage-gated sodium channels were not observed. Sodium currents ($I_{Na}$) were described in cultured microglial cells prepared from rat (471, 812) and human (658) brains (see Table 2). Sodium currents in cultured microglial cells were similar to a typical neuronal Na$^+$ channels; they were highly sensitive to TTX (complete inhibition at 2–5 μM), had a very rapid kinetics, and had characteristic voltage dependence (threshold approximately ~40 mV and peak ~0 mV). With peak amplitude of ~50 pA, they were much smaller than in neurons. Voltage-gated Na$^+$ currents were expressed only in 20% of cultured rat microglial cells; the cells with ramified morphology had a higher incidence of $I_{Na}$ detection (471). In contrast, in human microglial cells, cultured from explants obtained during surgery on patients suffering from brain tumors, the majority of cells (30 of 32 recorded) demonstrated TTX-sensitive $I_{Na}$ (658). Incidentally, coculturing microglial cells with astrocytes, which promotes ramified morphology of the former, significantly increased the proportion of cells expressing $I_{Na}$ (812). Very recently, three types of sodium channels, namely, TTX-sensitive Na$_{a,1,1}$ and Na$_{a,1,6}$ and TTX-resistant (TTX-R) sodium channel Na$_{a,1,5}$ were identified, by using specific antibodies, in purified rat microglial cultures (70). The inhibition of Na$^+$ channels in LPS-activated microglia with use-dependent blocker phenytoin (1 μM) decreased
**TABLE 2. Ion channels in microglia**

<table>
<thead>
<tr>
<th>Channel Type/Subunit</th>
<th>Experimental Preparation/Technique</th>
<th>Pharmacology</th>
<th>Biophysical Properties and functional relevance</th>
<th>Reference Nos.</th>
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<tr>
<td><strong>Sodium channels</strong></td>
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<tr>
<td>TTX-sensitive $I_{\text{Na}}$</td>
<td>Rat, human/cultured primary microglia/whole cell voltage clamp</td>
<td>TTX</td>
<td>In rat cultures, $I_{\text{Na}}$ was detected 471, 658, 812 in ~20% of cells; in human; in ~95% human cultures however were prepared from patients with brain tumors. Coculturing rat cells with astrocytes increased proportion of cells with $I_{\text{Na}}$.</td>
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<tr>
<td>Na$<em>{\text{v},1.1}$, Na$</em>{\text{v},1.5}$ (TTX-sensitive), Na$_{\text{v},1.6}$ (TTX-resistant)</td>
<td>Rat/cultured primary microglia/whole cell voltage clamp/RT-PCR, specific antibodies</td>
<td>TTX, phenytoin</td>
<td>Treatment of LPS-activated microglia with TTX or phenytoin reduced IL-$\alpha$, IL-$\beta$, and TNF-$\alpha$ secretion and decreased motility responses to ATP 70</td>
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<tr>
<td>Na$_{\text{v},1.6}$</td>
<td>EAE mice/postmortem MS human spinal cord/immunocytochemistry/in situ hybridization</td>
<td>Phenytoin</td>
<td>The protein and mRNA for Na$_{\text{v},1.6}$ were detected in EAE mice spinal cord and optic nerve, as well as in MS-affected human spinal cord. Phenytoin reduced microglial activation in EAE model. 180</td>
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<tr>
<td><strong>Calcium channels</strong></td>
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<tr>
<td>ORAI/CRAC</td>
<td>Rat/cultured primary microglia/whole cell voltage clamp/RT-PCR</td>
<td>Gd$^{3+}$, SKF-6355, diethylstilbestrol (DES), 2-aminoethoxydiphenyl borate (2-APB; 50 $\mu$M).</td>
<td>High Ca$^{2+}$-selective channel activated following depletion of ER Ca$^{2+}$ stores. Activation of I$<em>{\text{CRAC}}$ participates in formation of sustained phase of metabolically induced Ca$^{2+}$ signals. Profound ER store depletion may cause persisting (tens of minutes) activation of I$</em>{\text{CRAC}}$-mediated store-operated Ca$^{2+}$ entry.</td>
<td>263, 655, 671, 914</td>
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<td><strong>Potassium channels</strong></td>
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<tr>
<td>Inward rectifier potassium channels</td>
<td></td>
<td>Ba$^{2+}$, Cs$^{+}$, TEA, quinine</td>
<td>Generally expressed in activated microglia; very low densities in resting microglia. 471, 572, 574, 657, 808</td>
<td></td>
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<tr>
<td>$I_{\text{Kir}2.1}$</td>
<td>Rat, mouse, human/culture, amoeboid microglia from acute juvenile corpus callosum slices/whole cell voltage clamp</td>
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<td>$I_{\text{Kir}<em>{R1}}$: K$</em>{\text{v},1.1}$, K$<em>{\text{v},1.2}$, K$</em>{\text{v},1.3}$, K$_{\text{v},1.5}$</td>
<td>Rat, mouse/cultured primary microglia; slices/whole cell voltage clamp/RT-PCR/immunocytochemistry</td>
<td>Cd$^{2+}$, Zn$^{2+}$, Ba$^{2+}$, TEA, 4-AP, CTX, KTX, NTX, MTX</td>
<td>The $I_{\text{Kir}<em>{R1}}$ is upregulated following microglial activation in both in vitro and in situ preparations. The K$</em>{\text{v},1.3}$ and K$<em>{\text{v},1.5}$ channels are required for $I</em>{\text{Kir}<em>{R1}}$ in adult activated microglia. The K$</em>{\text{v},1.1}$ and K$<em>{\text{v},1.2}$ channels are expressed in amoeboid postnatal microglia; hypoxic insults increase K$</em>{\text{v},1.2}$ expression in adult microglial cells. Both K$<em>{\text{v},1.3}$ and K$</em>{\text{v},1.5}$ channels are linked to various functional responses of activated microglia; from regulation of motility (K$<em>{\text{v},1.3}$) to release of NO (K$</em>{\text{v},1.5}$)</td>
<td>81, 134, 234, 235, 238, 239, 241, 288, 474, 540, 654, 656, 657, 688, 735</td>
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<tr>
<td>IK_{Ca}; K_{i.1}; K_{i.2}; K_{i.3}; K_{i.5}</td>
<td>Rat, mouse/cultured primary microglia; slices/whole cell voltage clamp/RT-PCR/immunocytochemistry</td>
<td>Ca^{2+}, Zn^{2+}, Ba^{2+}, TEA, 4-AP, CTX, KTX, NTX, MTX</td>
<td>The IK_{Ca} is upregulated following microglial activation in both in vitro and in situ preparations. The K_{i.1} and K_{i.5} channels are required for IK_{Ca} in adult activated microglia. The K_{i.1} and K_{i.2} channels are expressed in amoeboid postnatal microglia; hypoxic insults increase K_{i.2} expression in adult microglial cells. Both K_{i.1} and K_{i.5} channels are linked to various functional responses of activated microglia; from regulation of motility (K_{i.3}) to release of NO (K_{i.3}) to release of NO (K_{i.3}) to release of NO (K_{i.3})</td>
<td>81, 134, 234, 235, 238, 239, 241, 288, 474, 540, 654, 656, 657, 688, 735</td>
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<tr>
<td>Ca^{2+}-dependent potassium (K_{Ca}) channels</td>
<td>Calf, rat, mouse, human, adult, and newborn rat/primary cultures; slices/sections of striatum/whole cell voltage clamp/RT-PCR/immunohistochemistry</td>
<td>Apamin (KCNN2), clotrimazole (KCNN4),</td>
<td>The BK channels were identified based on their single-channel conductance (140–240 pS). The SK channels are linked to NO release, MAPK signaling and respiratory burst. The KCNN3/SK3 channels were predominantly expressed in both cultures and healthy striatum tissue; LPS treatment or ischemic insult increased the level of expression. Inhibition of KCNN3/SK3 affected microglial activation and reduced microglial neurotoxicity.</td>
<td>77, 436, 446, 572, 574, 802, 807</td>
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<tr>
<td>IK(Ca); BK/K_{Ca}1.1; KCNN1/SK1; KCNN3/SK2; KCNN4/SK4; G protein-activated K^{+} channels</td>
<td>Mouse/primary cultures/whole cell voltage clamp</td>
<td>4-AP, PTX</td>
<td>Currents were activated following stimulation of G protein-coupled metabotropic receptors.</td>
<td>401, 402</td>
</tr>
<tr>
<td>Volume-regulated Cl^{-} channels, Best family of channels</td>
<td>Mouse; microglial cell lines/primary cultures/whole cell voltage clamp/RT-PCR</td>
<td>Flufenamic acid; DIDS, SITS, DIOA, NPPB, IAA-94</td>
<td>Single-channel conductances 1–3.5 pS. The volume-activated channels are involved in setting the resting V_{m} regulation of microglia proliferation, phagocytosis and morphological phenotype. Single-channel conductance 6.5–8 pS. CLIC-1 channels are involved in microglia proliferation and ROS generation.</td>
<td>230, 242, 304, 638, 808, 1040</td>
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<tr>
<td>CLIC-1 chloride channels</td>
<td>Rat; microglial cell lines/primary cultures/whole cell voltage clamp</td>
<td>IAA-94</td>
<td></td>
<td>588, 662</td>
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<tr>
<td>Proton channels H_{i.1} voltage-activated H^{+} channels</td>
<td>Mouse, rat, human, microglial cell lines/primary cultures/whole cell voltage clamp</td>
<td>TEA, bi- and trivalent cations</td>
<td>Single-channel conductance ~ fS range. Highly sensitive to extracellular pH; probably are associated with generation of respiratory burst. Disruption of cytoskeleton leads to an ~50% reduction in H^{+} current amplitude, whereas cell swelling potentiates H^{+} currents.</td>
<td>237, 238, 460, 461, 574, 802, 905</td>
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Moreover, the individual recorded cells were not identified by cell-type specific markers. Furthermore, the cell population therefore could be obtained during surgery on patients suffering from brain tumors (658). This cell population therefore could be termed these cells microglia.

In activated microglia in autoimmune encephalomyelitis, generally employed as model for multiple sclerosis, the expression of a sodium channel Na$_{v}$,1.6 (both at mRNA and protein levels) was found to be increased (180). Furthermore, treatment of animals with Na$^{+}$ channel blocker phenytoin decreased the inflammatory cell infiltrate in this model by 75%, and TTX at micromolar concentrations inhibited the phagocytic capacity of microglia (180).

Each of these studies, however, has its weakness. 1) In the rat culture (471), only a subpopulation of microglial cells with a ramified morphology expressed $I_{Na}$ currents. It needs to be emphasized that the individually recorded cells in Korotzer’s study were not identified by microglial markers and could potentially also be NG2 cells. 2) Schmidtmaier (812) placed blood monocytes or spleen macrophages onto an astrocyte monolayer and termed these cells microglia. 3) In the study on human microglial cells, the cells were cultured from explants obtained during surgery on patients suffering from brain tumors (658). This cell population therefore could be potentially contaminated with blood monocytes. Furthermore, the individual recorded cells were not identified with cell-type specific markers. 4) The presence of a Na$^{+}$ channel protein as shown by Craner in a pathological model (180) does not necessarily indicate the presence of the functional Na$^{+}$ channel.

A large number of other labs found no evidence for the presence of $I_{Na}$ in a variety of different microglial preparations. Sodium channels were not detected in cultured rat or mouse microglia neither unstimulated nor activated with LPS or in cultured microglia from human fetuses obtained at 12–20 wk of gestation (574). Voltage-clamp experiments on ramified microglia in situ in acutely isolated brain slices failed to detect any signs of Na$^{+}$ currents (77, 81). The lack of sodium currents is also documented for microglia after pathology (81) or in amoeboid microglia during development (86). Finally, treatment with TTX (20–50 μM) does not affect the motility of microglial process in vivo (645).

### B. Calcium-permeable Channels

#### 1. Voltage-operated Ca$^{2+}$ channels

The evidence for microglial voltage-gated Ca$^{2+}$ channels is limited to a single report, in which voltage-clamp experiments revealed, in ~30% of the cells tested, a very small (if any) inward Ca$^{2+}$ current, which was somewhat enhanced by BAY K 8644 (168). Additional indirect evidence comes from the observation that treatment of cultured rat microglial cells with high K$^{+}$ (25 to 55 mM) solutions enhanced superoxide anion production induced by phorbol 12-myristate 13-acetate. This effect was blocked by the voltage-operated Ca$^{2+}$ channel (VOCC) antagonist nifedipine, whereas treatment with the VOCC-positive modulator BAY K 8644 enhanced superoxide anion production similarly to high-K$^{+}$ solutions (168). The incubation of cultured microglia with β-amyloid Aβ25–35 (836), prion protein PrP 106–126, HIV-1 regulatory protein Tat or CCR3 cytokine receptor agonist RANTES (367) triggered [Ca$^{2+}$], elevation that was sensitive to L-type Ca$^{2+}$ channels antagonists verapamil, nifedipine, and dil-tiazem, thus indicating a possible role for VOC channels (367, 836). The presence of VOCCs channels in microglial cells following LPS injection into substantia nigra.

### Table 2.—Continued

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<thead>
<tr>
<th>Channel Type/Subunit</th>
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<th>Pharmacology</th>
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</tr>
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<tr>
<td>Aquaporins AQP4</td>
<td>Rat/RT-PCR/Western blotting</td>
<td>?</td>
<td>Expression of AQP4 was observed in activated microglial cells following LPS injection into substantia nigra.</td>
<td>915</td>
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<tr>
<td>Connexins Cx43, Cx36, Cx32, Cx45</td>
<td>Rat, Human, mouse/RT-PCR, immunocytochemistry, imaging, whole cell voltage clamp</td>
<td>?</td>
<td>No indications for connexins in resting microglia in situ. Traumatic brain lesion resulted in an appearance of Cx43 immunoreactivity. Cx32, Cx6, Cx43, and Cx45 were identified in cultured microglia.</td>
<td>220, 255, 554, 898</td>
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See text for definitions.
was fully depleted (389). The CRAC channels are characterized by extremely low conductance (~1 fS range). Whole cell patch-clamp experiments on cultured microglia identified a current very similar to $I_{\text{CRAC}}$ (655). This current was activated by intracellular perfusion with 10 $\mu$M InsP$_3$, was very selective for Ca$^{2+}$, and was responsible for a Ca$^{2+}$ influx following store depletion with InsP$_3$ (655). Similarly to many other Ca$^{2+}$-selective channels (374, 796), the removal of extracellular divalent cations converted $I_{\text{CRAC}}$ channels into Na$^+$-permeable ones (344). The Na$^+$ currents through store-operated channels demonstrated very slow activation kinetics; the unitary channel conductance was 42.5 pS, and the channels were positively modulated by PKA and negatively by PKC (344). Likely, these currents reflected the activation of TRPM7 channels (671).

The currents through CRAC channels were measured from both resting and activated mouse microglial cells. In the resting conditions, $I_{\text{CRAC}}$ amplitude was $\sim$1.2–1.3 pA/pF, whereas LPS-induced activation significantly reduced the amplitude of the current (to $\sim$0.5 pA/pF in 48 h after exposure to LPS; Ref. 42).

Recently, the molecular identity of $I_{\text{CRAC}}$ was analyzed in cultured neonatal rat microglia. The qRT-PCR revealed expression of all three Orai mRNAs with maximal expression of Orai3 (671). Further electrophysiological and pharmacological investigations allowed the authors to suggest that highly Ca$^{2+}$-selective ($P_{\text{Ca}}/P_{\text{Na}} >1,000$) Orai1/CRAC channels are responsible for $I_{\text{CRAC}}$ generation in microglia (671).

3. **TRP channels**

TRP channels belong to an extended family of cationic ion channels that were originally identified as transient receptor potential (TRP)-generating channels in *Drosophila* photoreceptors (356, 591). Further experiments identified an extended superfamily of TRP channels, comprising seven major families of TRPC, TRPM, TRPV, TRPA, TRPP, TRPML, and TRPN channels (698). Many TRP channels are expressed in the nervous system, in both neurons and glial cells. The TRP channels are intimately involved in thermo- and chemosensitivity as well as in neural development and growth and may also play an important role in various types of neuropathologies (1, 643, 899).

There is evidence for the expression of several TRP channels in microglia. The qRT-PCR performed on neonatal cultured neonatal rat microglia revealed the following rank of mRNAs expression: TRPM7 > TRPC6 > TRPM2 > TRPC1 > TRPC3 > TRPC4 > TRPC7 > TRPC5 > TRPC2 (671). The TRPM7 expression was very high and comparable with expression of housekeeping gene HPRT-1 (671). A strong expression of TRPM2 mRNA was found in cultured rat microglia (286, 476); this expression was further confirmed by in situ hybridization in mouse brain (476). Exposure of cultured rat microglial cells to 0.1–5 mM H$_2$O$_2$ (which is known as TRPM2 activator, Ref. 703) triggered Ca$^{2+}$ influx and cationic currents that were significantly potentiated by LPS (476). Intracellular administration of ADP-ribose (which is also acting on TRPM2, Ref. 703) triggered large cationic currents in both naive and LPS-treated microglial cells (476). The hydrogen peroxide- and ADP-ribose induced currents and Ca$^{2+}$ influx were attributed to the activation of TRPM2 channels (476).

The [Ca$^{2+}$]$_i$-activated TRPM4-like currents were recently recorded from mouse microglial cells; they were half-activated at 1.2 $\mu$M of intracellular Ca$^{2+}$ (42). The TRPM7 transcripts were found in rat cultured microglia. The TRPM7-mediated currents were activated spontaneously after establishment of whole cell configuration and were inhibited by increase in intracellular Mg$^{2+}$ concentration and by tyrosine kinase inhibitors. Microglial activation did not affect TRPM7-mediated currents (425). Primary rat cultured microglial cells express TRPV1 channels as demonstrated by RT-PCR, Western blot analysis, and immunocytochemistry (453).

There is growing evidence that TRP channels control microglial functions. In the retina, elevated intraocular pressure triggers [Ca$^{2+}$]$_i$ elevation and IL-6 release from microglial cells; both Ca$^{2+}$ signaling and IL-6 release were partially inhibited by the TRPV1 specific antagonist indo-3 (655). TRPV1 antagonists capsaicin or RTX (453). Direct injections of capsaicin or TRPV1 endogenous agonist 12-hydroperoxyeicosatetraenoic acid to substantia nigra produced microglial death, which was prevented by the TRPV1 antagonists capsazepine and RTX. The cell death was due to TRPV1-mediated Ca$^{2+}$ influx with subsequent mitochondrial damage, release of cytochrome c, and upregulation of caspase-3 (453). Direct injections of capsaicin or TRPV1 endogenous agonist 12-hydroperoxyeicosatetraenoic acid to substantia nigra produced microglial death, presumably through TRPV1 activation (453). Inhibition of TRPV1 with La$^{3+}$, capsazepine, ruthenium red, or RTX reduced ROS production on activated cultured microglia (801). Ischemic insults produced by occlusion of cerebral artery induced an increase in the expression of TRPM2 mRNA in rat brain, which was suggested to be mainly microglial, but its functional role yet needs to be determined (286).

C. **Potassium Channels**

1. **Inward rectifier K$^+$ channels**

The inward rectifier K currents ($I_{\text{KIR}}$) can almost be considered as a marker for nonresting microglial cells. They are expressed in all microglial cells with the exception of resting microglia. The current was first described in cultured rat microglia, and a single-channel conduc-
tance of 30 pS was reported (442); subsequently, similar unitary currents were found in other in vitro microglial preparations (234, 572, 812, 965). A second inward rectifier channel with unitary conductance of 43 pS was also found in rat microglia cocultured with fibroblasts (812). The current showed a strong inward rectification and an increased inactivation with more negative potentials. The inward rectifier current can be recorded in all cultured microglial cells. Activation of cultured microglial cells by LPS results in a reduction of the current by 30% (728).

When recording from cells in slices from normal adult mouse or rat brain (see Fig. 6), the inward current was either small or undetectable (81, 82). It may even be possible that the small currents recorded in the acutely isolated slices are an early response to the slicing procedure, since there is usually a 2-h delay between slicing and recording, suggesting that resting microglia lack the inward rectifier entirely.

These channels can thus be considered as an early marker for activated microglia. Microglial cells in slices of the facial nucleus strongly increase the functional expression of this channel within 12 h after axotomy. The expression of inward rectifier returned to control levels 7 days after the lesion (81). This increase in the inward current correlates with a more negative membrane potential from −15 mV before axotomy to about −30 mV after axotomy. The presence of the Kir channels and its potential influence on the membrane potential could affect microglial Ca²⁺ signaling, since it increases electric driving force for capacitative Ca²⁺ entry. For example, the inhibition of Kir channels by Ba²⁺ significantly reduced the plateau phase of ATP-induced [Ca²⁺]i transients in cultured rat microglia (291).

In situ, the inward rectifier is present in microglial cells in a pathological context such as after ischemia (540) or facial nucleus axotomy (81). During postnatal development, invading microglial cells with amoeboid morphology can be studied in acutely isolated slices from 6- to 8-day-old mouse corpus callosum. The Ikir was the main component of K⁺ permeability of these invading microglia (86). The Ikir in these cells were inhibited by TEA (50 mM), but not by 4-AP, and were almost completely blocked by 2–5 mM Ba²⁺ (86).

In hippocampal slices acutely isolated from juvenile mice (P5–P9), 74% of ramified microglial cells exhibited relatively small (mean current density ≈3.6 pA/pF) Ikir; in activated microglia from the same organotypic slices cultured for 3–7 days, the current was expressed in 91% of cells and its density increased severalfold (to ≈9.6 pA/pF) (802).

2. Delayed (outward) rectifier K channels

In 1992, Norenberg et al. (656) found that the treatment of cultured rat microglial cells with bacterial lipopolysaccharide, IFN-γ, or their incubation in hydrophobic teflon bags (654, 657), which activate microglial cells in culture, induced the expression of a delayed rectifying outward K⁺ current. Since cycloheximide, a blocker of protein synthesis, prevented the development of this conductance, Norenberg et al. (656) concluded that the channel protein was newly expressed. Activation of microglia in situ resulted in prominent upregulation of delayed K⁺ currents in the facial nerve nucleus 24 h after axotomy (81) and 48 h after an ischemic insult (540).

Delayed rectifying K⁺ channels are mostly belonging to the large family of Kv channels (for review, see Ref. 341). Cultured rat microglia express transcripts for Kv1.2, Kv1.3, and Kv1.5 and LPS-induced activation triggers upregulation of Kv1.3 expression (288). In vivo analysis of Kdr channels expression has demonstrated that intracortical injection of 2 µg LPS triggered microglial activation as judged by OX42 immunostaining. The OX42-positive cells showed increased immunoreactivity for Kv1.5 but not for Kv1.3 channels (428). Incidentally, treatment of microglial cultures with “calming” signaling agent transforming growth factor-β (TGF-β) induced a fivefold increase in the Kv1.3 mRNA level and a sixfold increase in delayed rectifying K⁺ current density (805). The expression of Kv1.11 channels was identified in early postnatal (P1–P10) ameboid microglia; these channels disappeared by P14–P21 and were not detectable in ramified microglial cells (1003).

The expression of both Kv1.5 and Kv1.3 message is necessary for the presence of the outward currents as shown by two strategies to interfere with channel expression, a Kv1.5 knockout (Kv1.5−/−) mouse and an antisense oligonucleotide approach against Kv1.5 and Kv1.3. In the rat brain, Kv1.2 channels were detected in ameboid microglia at early postnatal (P1-P10) stages; these channels were still present in P14 ramified microglial cells but almost completely disappeared at P21. The exposure of postnatal rats brains to hypoxia significantly upregulated the expression of Kv1.2 (522).

There are a number of other agents besides LPS that trigger the activation of microglia and result in the activation of outward K⁺ currents. Norenberg et al. (656) reported that IFN-γ triggered the induction of the outward current. Treatment of microglial cultures from newborn mice and rats with pneumococcal cell walls (derived from Gram-positive Streptococcus pneumoniae) resulted in microglial activation and the upregulation of Kdr very similar to that observed after exposure to LPS and involved de novo synthesis of Kv1.3 channels (226). Interestingly, the embryonic microglia treated with pneumococcal cell walls despite showing all signs of activation (such as morphological shift, release of TNF-α, and downregulation of Ikir) did not demonstrate an upregulation of Kdr (226), suggesting a developmental remodeling of an ion channel-related activation program (226). β-Amyloid pro-
tein fragment 25–45 (Aβ25–45) as well as with full-length β-amyloid peptide (Aβ1–42) triggered the upregulation of K\(_{\text{DR}}\) and K\(_{\text{Cas}}\) channels in cultured rat microglial cells (161). The amplitudes of I\(_{\text{KDR}}\) (similar in their parameters to K\(_{\text{L3}}\)-generated currents) were -5.6 times larger in activated microglial cells from organotypically cultured (5–7 days) hippocampal slices compared with resting microglia in acutely prepared slices (802). In contrast, in rat cultured microglia subjected to oxygen/glucose deprivation, K\(_{\text{L3}}\) was downregulated. This downregulation was mediated via protein tyrosine kinases of the src family and was considered as a response to stress (134). The K\(_{\text{L3}}\)-mediated currents were increased in the microglia activated following experimentally induced (systemic kainate injection) status epilepticus (581).

The expression of K\(_{\text{Ca}}\) channels can be linked to microglial function. The LPS-induced NO release was reduced by the antisense deletion of K\(_{\text{L5}}\) and completely absent in the K\(_{\text{L5}}\)-deficient animals; the antisense deletion of K\(_{\text{L3}}\) had no effect (688). In contrast, proliferation was augmented with loss of both K\(_{\text{L3}}\) or K\(_{\text{L5}}\) channel expression. This is supported by the observation that the proliferation rate was higher in K\(_{\text{L5}}\)-deficient animals after a facial nerve lesion (688). Similarly, LPS treatment of cultured microglia induced de novo synthesis of K\(_{\text{L5}}\) channels; the inhibition of these channels with 4-AP suppressed the NO release (735). At the same time, the formation of peroxynitrite, which is formed from superoxide and nitric oxide, requires K\(_{\text{L3}}\) channel activity; K\(_{\text{L3}}\) channel blockers reduced the respiratory burst, but not NO production (288). Oxidative stress by itself, however, may downregulate microglial K\(_{\text{L3}}\) currents through src family protein tyrosine kinases-dependent phosphorylation; moreover, it was suggested that K\(_{\text{L3}}\) channel and the protein tyrosine kinases are linked into one functional complex through postsynaptic density protein 95 (PCD-95). Incidentally, the correlation between K\(_{\text{Ca}}\) channels expression and microglial proliferation (574). Mean open times of K\(_{\text{Cas}}\) were increased with patch depolarization due to an increase in both opening frequency and mean open time (572, 574). Microglia in hippocampal slices from patients who underwent surgery for pharmaco-resistant epilepsy showed a small component of a Ca\(^{2+}\)-sensitive outward current (77). The estimated single-channel conductances varied between 149 pS (in outside-out mode) and 187 pS (in cell-attached configuration), indicating the activation of high-conductance (BK) channels (77). The chemokine MIP1-α increased whole cell outward current amplitudes at +60 mV by a factor of 3.3 (77).

Rat cultured microglia also express high densities of small-conductance Ca\(^{2+}\)/calmodulin-activated K\(^{-}\) channels of KCNN4/KEC3.1/SK4/I\(_{\text{KCa}}\) type (436), although an expression of KCNN2/SK2 and KCNN3/SK3 mRNA was also reported (446). The inhibition of these channels by a selective antagonist triaryl methane-34 (TRAM-34) significantly decreased the neurotoxic potency of microglia in a mixed culture system (436). In addition, KCNN4/SK4 channels were involved in the control of microglia activation and contributed to the upregulation of iNOS and the production of NO and peroxynitrite, with these being directly implicated in neurotoxicity. On a signaling level, the KCNN4/SK4 channels were linked to the activation of mitogen-activated protein kinase (MAPK) cascade (436). As a consequence of these signaling functions, an injection of TRAM-34 into the rat optic nerve had significant neuroprotective impact (436). The K\(_{\text{Cas}}\) channels are also involved in controlling the microglial respiratory burst; the treatment of microglial cells with apamin (selective blocker of KCNN2/SK2 channels) or with clotrimazole (KCNN4/SK4 channels inhibitors) markedly inhibited the respiratory burst (446). The messages for KCNN1/SK1,
KCNN2/SK2, and KCNN3/SK3 channels were identified in rat cultured microglia; the KCNN3/SK3 showed predominant expression, which increased ~1.7 times following LPS stimulation (807). The immunoreactivity for KCNN3/SK3 was found in microglial cells in healthy adult rat striatum; this immunoreactivity substantially increased after ischemic lesion (807). Finally, inhibition of KCNN3/SK3 channels by 100 nM apamin or by 5 nM tamapin reduced microglial neurotoxicity and somewhat ameliorated microglial activation (807).

4. G protein-activated K⁺ channels

Activation of G proteins by either intracellular perfusion with guanosine 5′-O-(3-thiotriphosphate) (GTPγS) or by stimulation of metabotropic receptors (by ATP, C5a, TNF-α, or EGF) induced outward K⁺ currents. These currents were sensitive to 4-AP (~40% inhibition at 4 mM concentration) and did not show time-dependent inactivation (401, 402). The receptor-activated K⁺ currents disappeared after treatment of cultures with pertussis toxin (PTX).

D. Anion Channels

1. Volume-regulated Cl⁻ channels

Microglial cells express volume (or swelling)-regulated Cl⁻ currents. Conceptually, the volume-regulated Cl⁻ channels are implicated into a general cellular phenomenon of regulatory volume decrease, which follows a hypotonic shock and involves electroneutral extrusion of KCl (through Cl⁻ and K⁺ channels) with concomitant water loss needed to restore the cell volume (424).

Outwardly rectifying, voltage- and time-independent volume-sensitive Cl⁻ currents were detected in cultured murine microglial cells (808) where they were suggested to regulate proliferation (808) and to be involved in setting the resting membrane potential (638). The swelling-sensitive Cl⁻ currents are rapidly and reversibly activated by exposure to hypoosmotic (up to 55% normal osmolarity) extracellular solutions (230, 808). The selectivity of these channels to anions is similar to other anion channels being I⁻ > Br⁻ > Cl⁻ > F⁻ > aspartate ≥ glutamate (230). Incidentally, the swelling-activated Cl⁻ channels were also permeable to gluconate (P_{gluconate}/P_{Cl⁻} = 0.34; Ref. 808). Microglial volume-sensitive Cl⁻ channels are inhibited by flufenamic acid, 4-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB), 6,7-dichloro-2-cyclopentyl-2,3-dihydro-2-methyl-1-oxo-1H-inden-5-yl(oxy)acetic acid (IAA-94), and [(dihydroindenyl)oxy]acetic acid (DIOA) (230, 242, 808) as well as by DIDS and SITS in a voltage- and time-dependent manner (242).

The single-channel conductance of swelling-sensitive Cl⁻ channels determined indirectly by noise analysis was estimated to be very small, ranging between 1 and 3.5 pS (230), which is much lower than that reported for volume-sensitive Cl⁻ channels of other cell types (15–40 pS; Refs. 584, 980). Quantitative real-time PCR (and normalized vs. housekeeping gene HPRT-1) detected mRNAs for several Cl⁻ channel genes in purified rat microglial cultures; the order of expression being CLIC1 > CLIC3 > I_{Clin} ≥ CLIC2 > Best2 > Best1 ≥ Best3 > Best4 (230). Out of these genes, the family of Bestophine (Best) channels (359) is, according to its biophysical properties, the most suitable candidate for the microglial volume-regulated Cl⁻ channels (230).

In addition, Cl⁻ channels in murine microglial cells are involved in preserving the ramified morphology, as pharmacological inhibition of these channels prevented ramification (242). At the same time, Cl⁻ channels are also involved in phagocytosis and their inhibition by flufenamic acid (200 μM) or NPPB (200 μM) almost completely blocked phagocytic activity. Interestingly, SITS was less potent in respect to the inhibition of phagocytosis, whereas DIOA had no effect (304). Furthermore, the inhibition of swelling-activated Cl⁻ channels in primary cultured microglia by flufenamic acid (200 μM) or DIOA (10 μM) or the incubation of the cells in Cl⁻-free high K⁺ solution suppressed the formation of a lamellipodia (1040). The volume-sensitive Cl⁻ channel blockers NPPB, tamoxifen, and DIDS prevented the rapid process outgrowth of microglial processes initiated in response to brain tissue damage. In contrast, filopodia extension was resistant to Cl⁻ channel inhibitors, indicating that these motile processes have different cellular mechanisms (378).

2. CLIC-1 chloride channels

The chloride intracellular channel-1 (CLIC-1), active in both nuclear and plasma membranes, was found in several cell lines and was associated with activation of macrophages (916, 944).

The functional expression of CLIC-1 was identified in primary rat neonatal microglial cultures (662). The CLIC-1 channels were localized in the plasma membrane, and the single-channel currents were recorded in a cell-attached configuration. The single-channel conductances were ~6.5–8 pS. Treatment of microglia with Aβ1–42 or Aβ25–35 peptides potentiated expression of CLIC-1 proteins (as determined by Western blot) and increased CLIC-1 single-channel open probability and mean open time (662), with the latter effect being the result of Aβ-dependent promotion of CLIC-1 translocation from the cytosol to the plasma membrane (588). The activation of microglia with LPS or basic fibroblast growth factor affected neither the expression nor the functional properties of these Cl⁻ channels. Inhibition of CLIC-1 channels with the specific blocker IAA-94 (R(+)-1(6,7-dichloro-2-cyclopentyl-2,3-dihydro-2-
methyl-1-oxo-1H-inden-5yl-oxy]acetic acid) suppressed microglial proliferation and reduced neurotoxicity of Aβ-treated microglial cells. This reduced neurotoxicity was primarily due to the inhibition of the ROS generation following the inhibition of CLIC-1-mediated Cl\(^-\) conductance (588); this effect was present following the blockade of CLIC-1 by pharmacological agents or anti-CLIC-1 antibody, or by replacement of extracellular Cl\(^-\) with impermeable anions. Ultimately, the downregulation of CLIC-1 synthesis with siRNA reduced Aβ-potentiated release of TNF-α from microglial cells (662). Thus the CLIC-1 channels participate in various aspects of microglial activation during AD (588).

3. Other Cl\(^-\) channels

A yet unidentified Cl\(^-\) current was triggered by application of the chemokine CCL21. Local application of CCL21 for 30 s triggered a Cl\(^-\) conductance, which persisted for tens of minutes. It was identified as mediated by Cl\(^-\) channel by its sensitivity to the transmembrane Cl\(^-\) gradient and to the blockers DIDS and SITS (747).

High conductance (325 pS at symmetrical Cl\(^-\) concentrations) channels were detected in inside-out patches from cultured bovine microglial cells. These channels were activated in response to hyper- or depolarization of the patch membrane (572). In fetal human cultured microglia, the Cl\(^-\) channel with unitary conductance of 280 pS was also detected (574).

E. Proton Channels

Changes in extracellular pH accompany both physiological and pathological processes in the brain. Neuronal activity causes rapid alkalosis by a tenth of a pH unit, which is followed by longer lasting acidosis by about two-tenths of a pH unit for example in the cerebellum (477), in the cortex (942), in the hippocampus (431) or in the spinal cord (423)]. In the optic nerve, a white matter tract, the onset of neuronal activity only triggers an acid shift (191). In pathology, the pH shifts are even larger in an ischemic insult where pH can drop to 5.0.

Voltage-gated currents carried by protons were initially discovered in snail neurons and oocytes of axolotl (37, 911) and were subsequently detected in various cell types, including neurons and glia (see Ref. 237 for review). In microglia, H\(^+\) currents were discovered in primary cultures from mice, rats, and humans (239, 460, 461, 574, 965). Similarly to other cell types, microglial H\(^+\) current have an exceptionally high selectivity to protons (238). The voltage dependence of microglial H\(^+\) currents strongly depends on extracellular pH (pH\(_e\)), with an acidification shifting the activation threshold to more positive potentials thus attenuating the current (237). The single-channel conductance of proton channels is believed to be exceedingly small, being in a range of several fA, as estimated in noise analysis performed on human neutrophils (201).

Proton currents in microglial cells are inhibited by TEA (1 mM) and by micromolar concentrations of polyvalent metals with the order of potency Zn\(^{2+}\) > La\(^{3+}\) > Ni\(^{2+}\) > Cd\(^{2+}\) > Co\(^{2+}\) > Ba\(^{2+}\) (238). The inhibitory action of these cations is greatly potentiated by acidification (237).

The treatment of cultured microglia with either LPS or with an astrocyte-conditioning medium caused ~50% decrease in the H\(^+\) current amplitude and deceleration in their activation kinetic (461). Similar effects were caused by incubation of microglial cultures with cytoskeletal disruptive agents cytochalasin D (2 μM) or colchicine (1 μM, both for 24 h), whereas the stabilization of cytoskeleton by 20 μM phalloidin or 0.5 μM taxol did not affect proton currents (460, 461). Changes in cell volume are reported to potentiate H\(^+\) currents: for example, in cultured rat microglial cells exposed to lactoacidosis or to intracellular dialysis with acidic (pH 5.5–5.8) solutions (618). Acidosis-induced cell swelling potentiated H\(^+\) currents by shifting their activation curve to more negative potentials and by accelerating activation kinetics. This potentiation required intracellular ATP and was prevented by cytoskeleton-active agents phalloidin and cytochalasin D (618).

The functional role of microglial H\(^+\) currents is not known, although they may play an important role in the “respiratory burst” associated with phagocytosis (236, 368, 369, 610, 803), which represents rapid oxygen consumption because of the activation of NADPH oxidase with subsequent production of superoxide anion. As NADPH oxidase produced one proton per every superoxide ion the acidification of cytosol occurs; H\(^+\) channels may provide a pathway for protons efflux. Microglia, and especially activated microglia is known to produce superoxide (167), and therefore this role for H\(^+\) channels seems to be likely (237). The activation of H\(^+\) channels produced a substantial efflux of protons, which decreases extracellular pH and therefore reduces the H\(^+\) current during long-lasting activation (617).

F. Aquaporins

Aquaporins form transmembrane channels permeable to water and some other molecules such as, for example, glycerol and urea (8). In mammals, 11 types of aquaporins (designated as AQPO–10) are described. These channels are mainly responsible for water homeostasis, although they are also involved in the regulation of other cell functions, including cell migration (961). In the brain, aquaporins are important for maintaining the extracellular volume, and they play an important role in the pathogenesis of the brain oedema (1026). The AQP1,
AQP4, and AQP9 are expressed in the brain (463); the AQP4 and AQP9 are expressed predominantly in astrocytes (429, 838, 876), being the most abundant. An injection of LPS into substantia nigra of rats triggered an expression of AQP4 at both mRNA and protein level in the activated microglia, with the latter being identified by positive OX-6 staining (915). In the microglia from control specimens, the expression of AQP4 was almost undetectable. Hitherto, however, this finding did not receive independent confirmation, and the possible role of aquaporins in microglial cells remains unexplored.

G. Connexons

The intercellular channels, generally known as connexons, form gap junctions, which are responsible for cell-to-cell coupling important for spread of electrical (e.g., in the heart muscle) or metabolic (e.g., in astroglial syncytia or in the liver) signals (60, 257, 776, 843). Molecula- rly, every connexon is composed of six subunits, named connexins (i.e., a full intercellular channel is composed from 2 opposing connexons, which in turn are made up from 12 connexins). At least 20 different connexons have been identified molecularly; they are classified according to their molecular weigh as CxM.W., e.g., Cx26, Cx32, Cx43, etc. With the use of the appropriate knockout mice, the differential cell-type specific (Cx43 in astrocytes, ependymal, and vascular cells; Cx32 in oligodendrocytes; and Cx36 in neurons and microglia) expression of connexins in the brain was identified (396).

Importantly, connexons may function not only as fully assembled intercellular channels, but also as “hemichannels,” which may provide, for example, pathways for release of relatively big molecules (853).

Dye injection into resting or activated microglia in situ has not revealed any dye transfer to another cell, indicating that there is no evidence for intercellular coupling. Resting microglia in situ were not immunoreactive for Cx43, yet Cx43 immunoreactivity markedly increased after applying the stab wound to the rat brain. The Cx43 were mostly immunolocalized in aggregates of microglia around the insult area (255), and homocellular microglial gap junctions may be formed under various pathological conditions (448).

In vitro, in primary cultures, mouse microglial cells showed low levels of Cx43, as well as low levels of dye (Lucifer yellow) coupling. Treatment of cultured microglia with mixture of LPS and INF-γ or INF-γ and TNF-α triggered a significant increase of Cx43 expression, specific accumulation of Cx43 at the cell contact sites, and increase in the probability of dye coupling to ~60%. However, in the image which is representatively shown for microglial dye coupling, only three cells are visible (255). In cultured rat newborn microglia, expression of Cx43 was upregulated following treatment with Ca2+ ionophore 4-bromo-A23187 and downregulated by PKC activated with phorbol 12-myristate 13-acetate (554).

Another study indicates that microglial cells express Cx36. The Cx36 mRNA and protein were found in microglial cultures prepared from human and mouse, and Cx45 mRNA was found in mouse microglial cultures only. Functional coupling using electrophysiology was reported for one-third of human or mouse microglial pairs. Low-strength electrical coupling was also reported between cultured microglia and neurons. The expression of Cx36 was not affected by microglial activation (220). Cultured mouse microglial cells treated with TNF-α were also shown to significantly upregulate surface expression of Cx32; the latter reportedly function as hemichannels (898) and may mediate microglial release of glutamate following cytokine stimulation. Increased expression of Cx32 was detected in microglial cells deficient in MECP2 gene. The latter encodes the epigenetic factor methyl-CpG-binding protein-2, and MECP2 mutations are linked to Rett syndrome and various forms of autism. Increased expression of Cx32 was associated with elevated microglial release of glutamate that exhibited neurotoxic action (544). In conclusion, the expression pattern and physiological relevance of microglial connexons remain unresolved.

IX. MICROGLIAL RECEPTORS: CONCEPT OF “ON” AND “OFF” RECEPTOR-MEDIATED SIGNALING

Multiple signals converge on microglial cells to actively maintain or alter their functional state and to orchestrate the specific repertoire of microglial functions. Transitions between surveillance and activated states are triggered when microglia perceives the sudden appearance, abnormal concentration, or unusual molecular format of certain factors (72, 351). We can distinguish between two principles of signaling, termed the “on” and “off” receptor-mediated signaling (67, 351). The “on” signaling is quite obvious: a novel signaling molecule that appears in the brain is recognized by microglia and triggers activation. An “off signal” may, instead, maintain a persisting signaling to contain microglia. Much like a wire in a shopping window conducting a constant current, the constant signaling of “off signals” keeps the microglia in the default setting, and only its interruption represents the alarm.

The biochemical nature and the sources of “on” signals are diverse, as the variety of receptors expressed by microglia (see Tables 3–7 and sections X–XIV). Structures associated with bacterial cell walls, viral envelopes, or their respective DNAs and/or RNAs are typical examples for signals that are usually not present in the micro-
### TABLE 3. Neurotransmitter receptors in microglia

<table>
<thead>
<tr>
<th>Receptor Type</th>
<th>Experimental Preparation/Technique</th>
<th>Pharmacology</th>
<th>Properties and Functional Relevance</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Purinoceptors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1, A2A, A2B, A3 adenosine receptors</td>
<td>Rat, mouse/primary cultures/RT-PCR/immunohistochemistry/pharmacological assays</td>
<td>Agonists: 2-chloro-adenosine (A1); CGS 21680 (A2A); 2-chloro-N^6-(3-iodobenzyl-N^-methyl-5’-carbamoyladenosine, CI-IB-MECA (A3)</td>
<td>Adenosine receptors generally mediate trophic effects, regulating microglial proliferation, survival, secretion of cytokines, and expression of various genes.</td>
<td>282, 309, 347, 366, 484, 497, 506, 670, 795, 889, 947</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Antagonists: Caffeine, theophylline, 8-(p-sulphophenyl)-theophylline; N-[9-chloro-2-(2-furanyl)[1,2,4]triazolo[1,5-c]quinazolin-5-yl]benzeneacetamide, MRS1523 (A3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P2X1, P2X4, P2X7</td>
<td>Rat/brain sections/RT-PCR/immunohistochemistry</td>
<td>Inhibition by ATP-TNP; insensitive to PPADS; positively modulated by ivermectin</td>
<td>Expression of P2X7 receptors specifically increased after spinal cord lesions; pharmacological inhibition of P2X7 receptors alleviated symptoms of neuropathic pain in animal models</td>
<td>132, 1008, 44, 415–417, 633, 929, 930, 932</td>
</tr>
<tr>
<td>P2X4</td>
<td>Rat/whole brain, organotypic slices, spinal cord/immunocytochemistry, pharmacological assays, antisense expression inhibition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P2X7</td>
<td>Rat, mouse/primary cultures, corpus callosum slices/microglial cell lines/whole cell voltage-clamp/Ca^2+ imaging/RT-PCR/immunohistochemistry</td>
<td>Agonist: BzATP Inhibitors: oxATP, BBG</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Glutamate receptors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMPA receptors GluR1, GluR2, GluR3, GluR4</td>
<td>Rat/primary culture/microglial cell line/whole cell voltage-clamp/RT-PCR</td>
<td>Inhibitors: CNQX, LY300164 Positive modulators: PEPA, CTZ</td>
<td>AMPA receptors were identified in cultured cells only; activation of AMPA receptors is linked to TNF-α release, remodeling of cytoskeleton and glutamate-induced chemotaxis.</td>
<td>156, 343, 529, 652, 150, 218, 277, 279, 342, 377, 590, 787, 851</td>
</tr>
<tr>
<td>Metabotropic glutamate receptors Group I - mGluR5a</td>
<td>Rat/primary culture/Ca^2+ imaging/RT-PCR</td>
<td>Agonist: 1S,3R-ACPD</td>
<td>Stimulation of with 1S,3R-ACPD triggered [Ca^{2+}]_i transient.</td>
<td>66</td>
</tr>
</tbody>
</table>

Continued
glial environment and that are thus identified as signs of infection. The activation signals are represented not only by infectious agents but also by molecules delivered following tissue damage so that even the smallest lesions can cause rapid microglial responses (645).

Intracellular proteins or serum factors, which normally carry diverse physiological functions, can acquire microglia-activating features when they are further induced upon stress, when they leave their respective physiological compartments or when they become (bio)chemically altered (350, 512, 513, 536, 777). Both pathogen- and damage/danger-associated molecular patterns (PAMPs/DAMPs) can alert microglia in a quite similar, yet distinct way, and even employ overlapping receptors of the Toll-like receptor (TLR) family. By virtue of multiple receptors (see sects. X–XIV), microglia can also sense deviations from the physiological concentration of neurotransmitter molecules. Beyond critical levels, some neuro- and cotransmitters could indicate

<table>
<thead>
<tr>
<th>Receptor Type</th>
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<th>Properties and Functional Relevance</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group II - mGluR3, mGluR5</td>
<td>Rat/primary culture/RT-PCR</td>
<td>Agonist: 1S,3R-ACPD</td>
<td>Stimulation of group II receptors stimulated microglial activation, release of TNF-α, and neurotoxicity; stimulation of group III receptors reduced neurotoxicity.</td>
<td>906–908</td>
</tr>
<tr>
<td>Group III - mGlu4, mGlu6, and mGlu8</td>
<td>Rat, mice/primary cultures/acute slices/whole cell voltage-clamp/RT-PCR/immunocytochemistry/Western blot</td>
<td>Agonists: baclofen, SKF 97541</td>
<td>GABA receptors were identified at mRNA and protein levels as well as functionally by recording GABAergic-mediated whole cell currents. Axotomy of facial nerve triggered an increase in number of microglial cells in the facial nucleus that expressed GABA receptor.</td>
<td>142, 480</td>
</tr>
</tbody>
</table>

**GABA receptors**

| a7 nAChRs | Mouse, rat/RT-PCR, Western blot, immunohistochemistry/Ca²⁺ imaging | Agonist: methyllycaconitine | | |
| aβ3, aβ5, aβ4 nAChRs | Human embryo/RT-PCR | | | |
| α6 or β4 nAChR | Rhesus monkey/retina/immunohistochemistry | | | |
| α1a, α2a, β1, β2 | Rat/primary culture/RT-PCR/biochemical assays/Ca²⁺ imaging | Agonists: norepinephrine, terbutaline | Stimulation of b receptors increased cAMP level and induced Ca²⁺ release from the ER. Stimulation of adrenergic receptors modulated release of cytokines. | 302, 613, 727, 902, 990 |

**Cholinergic receptors**

| α1A, α2A, β1, β2 | Rat, mouse/primary culture, acute slices/whole cell patch clamp | Agonists: dihydrexidine (D₁ receptors), (-)-quinpirole (D₂ receptors) | Stimulation of dopamine receptors inhibited IKIR and activated outward potassium currents. Chronic stimulation enhanced migration and decreased NO release. In microglia from Parkinson disease brains, the expression of D₁ receptors was increased. | 267, 555 |

**Dopamine receptors**

See text for definitions.
impaired or excessive neuronal activity (82, 365), which are perceived by microglia as a sign of lesion.

The “off” receptor-mediated signaling is due to a loss of constitutive signaling. They may deliver a calming control in the normal CNS. Such a principle is indicated for the ligand-receptor pairs CD200-CD200R, CX3CL1-CX3CR1, and SIRPa(CD172a)-CD47 (33, 89, 123, 381, 1001). Here, disrupted signaling may set off the response.

### TABLE 4. Microglial receptors for neurohormones and neuromodulators

<table>
<thead>
<tr>
<th>Receptor Type</th>
<th>Experimental Preparation/Technique</th>
<th>Properties and Functional Relevance</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAF receptors</td>
<td>Rat, mouse, human/brain sections, primary cultures/in situ hybridization, Northern blot, Ca²⁺ imaging</td>
<td>In the brain, PAF receptors were predominantly expressed in microglia. Stimulation of PAF receptors triggers [Ca²⁺]ᵢ elevation, resulted from ER Ca²⁺ release and long-lasting activation of Ca²⁺ entry. The Ca²⁺ entry increases IL-6 expression in cultured microglia.</td>
<td>385, 614, 794, 982</td>
</tr>
<tr>
<td>Bradykinin receptors, B₁, B₂</td>
<td>Rat/primary cultures/RT-PCR, immunocytochemistry, whole cell voltage-clamp</td>
<td>Resting microglia express only B₂ receptors, LPS activation upregulates B₁ expression. Stimulation of B₁ receptors triggers IKCa following [Ca²⁺]ᵢ increase. B₁ receptors induce chemotaxis and activate NCX.</td>
<td>397, 648–650</td>
</tr>
<tr>
<td>Histamine receptors</td>
<td>Rat/primary cultures/Ca²⁺ imaging</td>
<td>Histamine triggers Ca²⁺ release from the ER.</td>
<td>29</td>
</tr>
<tr>
<td>Endothelin receptors, ETB</td>
<td>Mouse, human/primary cultures/single cell RT-PCR, Ca²⁺ imaging</td>
<td>Stimulation of microglia with ET-1 and ET-3 triggered Ca²⁺ release and SOCE in 13% of mouse and in 80% of human cells. Ca²⁺ responses were inhibited by ETB antagonist BQ788 and mimicked by ETB agonist BQ3020.</td>
<td>573, 603, 1017</td>
</tr>
<tr>
<td>Cannabinoid receptors, CB₁, CB₂</td>
<td>Rat, mouse, human/primary cultures/RT-PCR</td>
<td>Resting microglia express CB₂ receptors; high expression of CB₂ receptors found in mice with experimental autoimmune encephalomyelitis. Stimulation of CB receptors stimulates proliferation and reduces neurotoxicity.</td>
<td>114, 259, 549, 744, 766</td>
</tr>
<tr>
<td>Angiotensin II receptors, AT₁, AT₂</td>
<td>Rat embryo/primary cultures/RT-PCR, pharmacological assays</td>
<td>Unstimulated microglia express AT₂ receptors; LPS triggers upregulation of AT₁ receptor. Inhibition of AT₁ receptors by losartan suppresses microglial activation and reduces production of NO and IL-1β.</td>
<td>596</td>
</tr>
<tr>
<td>Somatostatin receptors, sst2, sst3, sst4</td>
<td>Rat/primary cultures/RT-PCR</td>
<td>Activation of sst receptors induced protein phosphorylation and inhibited microglial proliferation.</td>
<td>272</td>
</tr>
<tr>
<td>Glucocorticoid and mineralocorticoid receptors</td>
<td>Rat/primary cultures/binding assays/Western blot</td>
<td>Microglia express both glucocorticoid and mineralocorticoid receptors; stimulation of glucocorticoid receptors inhibited proliferation of cultured microglia and enhanced lysosomal formation.</td>
<td>900</td>
</tr>
<tr>
<td>Opioid receptors, KOR, MOR</td>
<td>Human embryo, cat, rat/primary cultures/RT-PCR, immunohistochemistry binding assay, pharmacological assay</td>
<td>Stimulation of MOR inhibits chemotaxis towards C5a, increases migration and upregulates P2X₄ expression in rat.</td>
<td>140, 221, 388</td>
</tr>
<tr>
<td>Neurokinin (substance P) receptors, NK-1, VIP receptors, VPAC₁</td>
<td>Human embryo, mouse/RT-PCR, immunocytochemistry</td>
<td>Stimulation of NK-1 receptors triggers activation of NF-αB. Stimulation of VPAC₁ receptors inhibits LPS-induced activation and secretion of proinflammatory factors TNF-α, IL-1β, and NO.</td>
<td>491, 749, 212–214, 330, 455</td>
</tr>
<tr>
<td>Neurotrophin receptors, Trk-B1</td>
<td>Rat/primary culture/RT-PCR; Ca²⁺ imaging</td>
<td>BDNF triggered sustained [Ca²⁺]ᵢ elevation resulting from PLC/InsP₃-mediated Ca²⁺ release followed by a long-lasting activation of SOCE. The Trk-B1 receptors were identified by RT-PCR.</td>
<td>597</td>
</tr>
</tbody>
</table>

See text for definitions.
Accordingly, triggers of microglial alert could be sorted by the mode of signaling as to “on” and “off” signals, independently of their chemical constitution (67, 351, 949). This distinction should not be mistaken as dividing “activating” and “inhibiting” influences. Certain factors, such as IL-10, have suppressive effects on microglia, as they have for other macrophages. These factors, however, themselves depend on induction and can then develop their influences. Overlap between principles of “off signals” and microglial suppressors certainly exist. Some receptor systems, such as adrenergic receptors, will probably prove to maintain some basal as well as inducible influences. However, the principle of “off signals” comes with another consequence for microglial recruitment. It allows microglia to react to unknown signs of danger.

Microbial structures are sensed by cells of the innate immunity through the pattern recognition receptors (PRR), such as the TLRs, which initiate first measures and also influence adaptive immune functions (350). Yet all of the typical “on signals” require the expression of cognate receptors to be identified as a sign of threat to the CNS homeostasis. In contrast, the “off signal,” when disruption of an input acts as a sign of alert, enables microglia to respond in situations where the actual source of disturbance is not known. Loss of input, rather than input per se, is the signal. Microglia may therefore recognize neuronal stress and loss of homeostasis independent of preformed receptors. The “on” and “off signaling” concept may thus relate to the detection of known and unknown indicators of danger (351). Upcoming research will demonstrate whether microglial containment by this mechanism plays a critical role in the steady-state functions and whether loosening of calming signals occurs with ageing (868).

Receptor equipment and signaling outcomes may differ among microglial populations, since CNS regions differ, e.g., by the neurotransmitter microenvironment, blood-brain barrier properties, or myelin content. The surveillance features and the interpretation of environmental cues could differ between microglial subsets that are probably predetermined for distinct reactive options (816). It will be in line with the differential induction of diverse reactive phenotypes to characterize such a preformed specialization also by anatomical divisions. Besides the mere density, the functional setup of microglia could decide on the role of these cells in CNS pathologies.

X. NEUROTRANSMITTER RECEPTORS

A. Purinoceptors

1. Purinergic signaling system

Purines and pyrimidines act as widespread extracellular signaling molecules. The purinergic signaling system has ancient evolutionary roots and is operative virtually in every type of cells and in all tissues (100, 103, 104, 107, 108). In the nervous system ATP (the principal purinergic signaling molecule) and its derivatives act both as a primary transmitter and as a cotransmitter in the brain, in the spinal cord, and in the peripheral nerves (5, 101, 107, 661). Neural cells release ATP through several mechanisms, which include exocytosis, diffusion through “maxi” plasmalemmal channels, through transporters and may be even by lysosomes (5, 686, 687, 851, 1034). Furthermore, massive ATP release invariably accompanies cell death and destruction, and therefore, ATP acts as a universal “danger” signal; the excess of ATP in the brain parenchyma is neurotoxic (108, 781). Importantly, ATP released from the cells is rapidly degraded by extracellular ectonucleotidases (5, 1041); ATP derivatives (ADP, AMP, and adenosine) thus produced also act as purinergic signaling molecules. Physiological effects of purines and pyrimidines are mediated through an extended family of purinoceptors, which are classified as metabotropic P1 adenosine receptors, metabotropic P2Y purinoceptors, and ionotropic P2X purinoceptors (102, 297, 660, 879), which are widely expressed in the majority of living cells (106) and are particularly abundant in glia (283, 457, 458, 493, 959).

The ATP-induced currents were, for the first time, identified in microglial cultures prepared from embryonic mouse or newborn rat brains (976). The cells were positively identified as microglia by specific staining with DiI-ac-LDL, as well as with Mac-1 and CD-45 antibodies. The application of 100 \( \mu \)M ATP evoked a biphasic current response comprising a fast cationic current (with reversal potential \( \sim 0 \) mV) and slower outward \( K^+ \) current. The fast inward current rapidly reached the peak of \( \sim 100 \) pA and desensitized in the presence of the agonist. Subsequent Ca\(^{2+}\)-imaging experiments have demonstrated that ATP also induced [Ca\(^{2+}\)]\(_{\text{cyt}}\) elevation, which was entirely produced by Ca\(^{2+}\) entry, most likely reflecting Ca\(^{2+}\) permeability of ionotropic purinoceptors (976). These initial findings were subsequently confirmed in LPS-treated rat cultured microglial cells (659) and in cultured human microglia (575). Pharmacological assays applied to these preparations demonstrated that the two components of the ATP-induced membrane current reflected activation of ionotropic P2X (nonselective currents) and metabotropic P2Y (modulation of \( K^+ \) channels) receptors (399, 575, 659). The very same pattern of the ATP current response was found in microglial cells voltage-clamped in acute slices from an adult mouse brain (82).
expression of various purinoceptors (283, 959). The damage to the brain is invariably associated with a massive release of ATP present in high concentrations in the cytosol of living cells. Experiments in vitro and in vivo have demonstrated that ATP and its analogs trigger rapid functional responses of microglial cells, comprising fast converging movement of microglial processes towards the lesion, membrane raffling, the outgrowth of microglial processes, and the release of various biologically active substances, such as cytokines and inflammatory proteins (see, e.g., Refs. 65, 190, 265, 277, 384, 645, 788, 997). The treatment of cultured rat microglia with 3 mM ATP triggered rapid (within 2 h) morphological activation and acquisition of the amoeboid phenotype (1009). In another study, however, ATP and adenosine (0.6–1 mM) had the opposite effect; when added to the medium, they induced the transformation of amoeboid microglia to a more ramified (and hence less activated) phenotype (997).

The effects of purines on microglia are mediated through purinoceptors of both metabotropic and ionotropic subtypes, which are abundantly expressed in microglial cells throughout the nervous system (265). Expression of purinoceptors in microglia can vary considerably depending on the activation status (604). Furthermore, microglia are endowed with ectonucleotidases, which degrade ATP and its derivatives. Microglial cells were shown to express nucleoside triphosphatase (NTPase), nucleoside diphosphatase (NDPase), 5′-nucleotidase (5′-Nase), and purine nucleoside phosphorylase (PNPase), with this expression being dependent on the activation and developmental stage (186). Microglial cells also express CD39 ectonucleotidase, which is critical for ATP-mediated signaling. In microglia deficient for CD39, migration cannot be stimulated by ATP (266). The application of ATP in concert with adenosine or the addition of exogenous soluble ectonucleotidase restored the ATP-stimulated migration, thus indicating that P1 and P2 receptor stimulation are required and that the ectonucleotidases can provide the substrate to P1 receptors (266).

3. Plasticity of the microglial purinoceptive system

Microglial cells, both in cultures and in brain slices, usually express several types of metabotropic and ionotropic purinoceptors. An analysis of ATP-induced Ca\(^{2+}\) transients in various microglial cultures demonstrated that, as a rule, more than one type of purinoceptor is involved in the regulation of Ca\(^{2+}\) influx and Ca\(^{2+}\) release (see, e.g., Refs. 527, 967, 984). Electrophysiological experiments in vitro and in situ also showed that several types of purinoceptors mediate ATP action (82). Even within the same culture dish microglial cells demonstrate a variability in pharmacological profiles of [Ca\(^{2+}\)]\(_i\) transients induced by different purinoceptor agonists (604). The expression pattern of microglial purinoceptors demonstrates activation-dependent plasticity. Microglial activation in vitro with LPS triggered significant remodeling of cells sensitivity to P1/P2 receptors agonists (604). Cultured rat microglia exposed to hypoxic conditions significantly upregulated both P2Y and P2X\(_{7}\) receptors (616). Treatment with LPS led to a significant increase in metabotropic ATP-induced Ca\(^{2+}\) responses, whereas the P2X\(_{7}\) ionotropic Ca\(^{2+}\) signaling was reduced (64).

Similar remodeling of purinoceptors expression was observed in situ in various pathological models. That is, epileptic seizures induced by kainate injections triggered an activation of microglia as seen in acute hippocampal slices (27). This activation resulted in the upregulation of the expression of mRNA specific for P2X\(_{1,4,7}\) and P2Y\(_{6,12,13}\) receptors. Functionally this upregulation was manifested by an increase in ATP-induced membrane currents and ATP-induced microglial motility (27). The upregulation of microglial P2X\(_{4}\), P2X\(_{7}\), and P2Y\(_{6}\) receptors was also observed in superoxide dismutase 1 mutant expressing animals (these being an accepted model for amyotrophic lateral sclerosis) (185). Changes in the P2X/P2Y mRNA expression were also observed in microglial cultures prepared from animals of different (1–12 mo) age (179).

Oxygen and glucose deprivation triggered an upregulation of P2X\(_{4}\) and P2X\(_{7}\) receptors in the hippocampal and cortical/striatal/subventricular zone organotypic slices (132, 133), which are linked to neuronal damage. Similarly the traumatic brain injury resulted in significant upregulation of microglial P2X\(_{4}\) receptors, which was suppressed by systemic treatment with 1 mg/kg dexamethasone (1033). Injections of kainate into rat hippocampus increased the expression of P2X\(_{6}\) receptors (467). The mechanical trauma of a rat nucleus accumbens rapidly triggered the appearance of immunoreactivity for P2X\(_{1,2,4,7}\) and P2Y\(_{1,2,4,6,12}\) receptors in microglial cells; in control preparations, the specific signal was completely absent (295). It was also found that an intramyocardial injection of formalin, which triggers acute heart ischemia, induced microglial activation in the locus coeruleus (which is intimately involved in pathogenesis of heart diseases); this microglial activation was paralleled with a significant increase in expression of P2X\(_{4}\) receptors (1030).

The P2Y\(_{12}\) receptor has been identified as an important control element for the movement of microglial processes as indicated by imaging studies in live animals (365, 384). This receptor is downregulated when microglia are activated following injury to the brain. The opposite was reported for another P2Y receptor, the P2Y\(_{6}\) receptors. After injury, these receptors are upregulated, and their activation triggers phagocytosis (467).

These plastic changes in the purinoceptors expression are important for orchestrating specific responses of microglia to purines and pyrimidines at different stages of neuropathology. Particularly robust changes in expres-
sion are observed for P2X$_4$, P2X$_7$, and several types of P2Y receptors, which are discussed in detail below.

4. Adenosine P1 receptors

Adenosine receptors of P1 type are represented by classical seven-transmembrane spanning G protein-coupled receptors and are subclassified into adenosine A$_1$, A$_2A$, A$_2B$, and A$_3$ receptors with distinct pharmacological and functional properties. The A$_1$ and A$_3$ receptors are coupled to G$_i/o$ proteins, whereas A$_2A$ and A$_2B$ receptors usually act through G$_s$ proteins (297, 298).

All four types of adenosine receptors were identified at the mRNA level in cultured rat microglia (282). Functional P1 receptors were also identified in cultured microglial cells, where they exert several metabotropic effects. The application of adenosine in concentrations 0.001–100 μM triggered an outward current in cultured rat microglia voltage-clamped at 0 mV. This current had a reversal potential at −70 mV and was produced by the activation of K$^+$ channels. The effects of adenosine were antagonized by an incubation with PTX and inhibited by the broad P1 receptors antagonist 8-(p-sulfophenyl)theophylline (497). A proliferative response of cultured rat microglia required the simultaneous expression of A$_1$ and A$_2$ receptors (309). The A$_1$ adenosine receptors were particularly concentrated in microglial cells surrounding glioblastomas, and their activation suppressed tumor growth by a yet unknown mechanism (889). There is also evidence for a protective role of microglial A$_1$ receptors following acute brain trauma (360). The activation of A$_2A$ receptors stimulated the expression of NGF mRNA as well as the release of NGF in rat cultured microglial cells (366) and potentiated release of NO from LPS-activated microglia (795). The same A$_2A$ receptors also regulated the expression of COX-2 and synthesis and the release of NO from LPS-activated microglia (795). The same A$_2A$ receptors also regulated the expression of COX-2 and synthesis and the release of prostaglandin E$_2$ from cultured rat microglial cells (282).

In addition, the A$_2A$ receptors control retraction of microglial processes under conditions of chronic neuroinflammation (678). Selective stimulation of the A$_2A$ receptor in primary cultured rat microglia by 6 h of incubation with the specific agonist CGS 21680 increased expression of mRNA for K$_{1.3}$ channels and renal epithelial K$^+$ channel ROMK1 (K$_{i.1.1}$) and increased levels of K$_{1.3}$ protein (484). A$_2A$ receptor-induced upregulation of K$_{1.3}$ channels was mediated through a cAMP-dependent pathway, whereas upregulation of ROMK1 channels involved PKC (484). Finally, A$_2A$ receptors may be involved in microglial activation in neuropathic pain, and intrathecal injection of A$_2A$ antagonists prevented the development of mechanical allodynia and thermal hyperalgesia in rats with chronic constriction injury neuropathic pain model (534).

Pharmacological assays have also identified functional A$_3$ receptors in primary mouse microglial cultures. The activation of A$_3$ receptors by selective agonist 2-chloro-N$^6$-(3-iodobenzyl-N-methyl-5′-carbamoyladenosine) (Cl-IB-MECA) affected the phosphorylation of the extracellular signal-regulated protein kinase 1/2 (ERK1/2). These effects were absent in cells obtained from transgenic mice with deleted A$_3$ receptor gene (347). The dynamic balance between A$_2A$ and A$_3$ receptors controlled the TLR-dependent activation of microglia and secretion of cytokines (947).

Finally, the treatment of cultured rat microglial cells with broad A$_1$ agonist 2-chloro-adenosine induced programmed cell death, which was not prevented by specific adenosine receptors antagonists, suggesting the existence of atypical adenosine regulated signaling pathway (670).

5. P2X receptors

Ionotropic ATP receptors, named P2X in 1985 (105), are classic ligand-gated cationic (Na$^+$,K$^+$/Ca$^{2+}$) channels, which are assembled from different subunits into homomeric or heterotrimers (444, 642, 761). These trimers form a chalice-shaped structure as revealed by their crystal structure (439). The P2X subunits are encoded by distinct genes, which, depending on the subunit composition, may vary (P$_{ca}$/P$_{monovalent}$) between 1 and >10 (684, 685) and could be even greater for the pore-forming P2X$_7$ receptors (243).

The P2X-mediated currents were identified in microglial cultures prepared from human and rodent brains (976, 650, 575) and in resting microglial cells from adult mouse acute brain slices (82). The P2X-mediated [Ca$^{2+}$]$_i$ elevation was also described in cultured microglia from retina; the ATP/Bz-ATP-induced Ca$^{2+}$ entry was potentiated after keeping the cultured cells with 30 mM glucose for 7 days. This increased Ca$^{2+}$ influx was not inhibited by Brilliant Blue G, thus ruling out the participation of P2X$_7$ receptors (702).

Using single- and double-labeling immunofluorescence and RT-PCR techniques, Yang and Burnstock (1008) characterized developmental changes in the P2X subunits expression in the rat brain. The majority of microglial cells stained positively for the marker ED1 at embryonic day 16 expressed P2X$_1$ and P2X$_4$ subunits, whereas only 30% of these cells expressed P2X$_7$ receptors. From postnatal day 7, the P2X$_7$-positive microglia concentrated around blood vessels. At postnatal day 30, the cells expressing P2X$_7$ receptors virtually disappeared; the P2X$_7$-positive cells were distributed evenly through the forebrain, whereas cells bearing P2X$_4$ receptors outlined blood vessels and subarachnoid space (1008). In organotypic slices from the cortical/striatal/subventricular zone, the immunostaining for P2X$_4$ and P2X$_7$ receptors showed the colocalization with microglial marker OX42 (132).
6. P2X4 receptors and neuropathic pain

Neuropathic (i.e., chronic and malignant) pain develops following peripheral nerve damage of multiple aetiology (e.g., acute trauma, diabetic neuropathy, cancer, or surgery) and represents a severe medical problem (999). Experiments of the recent decade demonstrated the primary role of microglial purinergic signaling in the pathogenesis of neuropathic pain (see, e.g., Refs. 408, 409, 411, 416, 921 for detailed reviews). In particular, two types of purinoceptors, the P2X4 and P2X12 receptors, are critical for triggering neuropathic remodeling in the spinal cord.

Initially, it was found that peripheral nerve injury triggers rapid activation of microglial cells in the spinal dorsal horn. The first signs of activation (rounding of the soma, retraction of processes, and increase in immunoreactivity for OX42) became obvious 24 h after lesioning the peripheral nerve (252, 887). In parallel, peripheral nerve injury induced microglial proliferation, which peaked within 2–7 days after the lesion (311, 632). Microglial activation paralleled the development of tactile allodynia (178), the latter being a primary symptom of the neuropathic pain. The activation of microglia was identified also in animal models of bone cancer (1032), experimental autoimmune neuritis (44), and diabetes (934). Incidentally, activated microglia were also found in dorsal root ganglia following sciatic nerve lesion (696).

Initial evidence for the role of purinoceptors in mediating microglial activation in neuropathic pain was obtained by Tsuda et al. in 2003 (930), who used the rat spinal nerve injury model in which L5 spinal nerve was surgically severed. Tsuda et al. found that intrathecal injection of 2’,3’-O-(2,4,6-trinitrophenyl)adenosine 5-triphosphate (TNP-ATP) reversed the decrease of the paw withdrawal threshold (the latter being a readout of the tactile allodynia). In contrast, an injection of pyridoxal phosphate-6-azophenyl-2’,4’-disulfonic acid (PPADS) had no effect. This peculiar sensivity of tactile allodynia to P2X4 receptors whereas PPADS blocks all P2X receptors but P2X4 (Refs. 98, 850) led them to suggest the specific role of P2X4 receptors in lysosomes; the exocytosis of the latter (following, e.g., Ca2+ signaling) may very rapidly increase the pool of plasmalemmal functional receptors and contribute to an increase in P2X4 currents (741).

An increase in P2X4 expression was further corroborated by immunostaining, which demonstrated a significant increase in P2X4 reactivity in the spinal cord ipsilateral to the site of nerve injury. Most importantly, increased P2X4 immunoreactivity was detected exclusively in OX42 positive microglial cells (930). The inhibition of P2X4 receptors expression by antisense prevented the development of tactile allodynia following nerve injury, whereas an intrathecal injection of activated cultured microglia expressing P2X4 receptors triggered allodynia without cutting the nerve (930). These results have demonstrated that P2X4 receptors are critically important for the initiation of neuropathic pain in the animal model (417, 929, 930).

The mechanisms of how activated microglia cause neuropathic pain are probably many. Electrical substrate of neuropathic pain is believed to be represented by long-term potentiation of C-fiber-evoked field potentials in the spinal dorsal horn, which occurs in response to injury and inflammation (785). Stimulation of P2X4 receptors of spinal cord microglia induces long-term potentiation of C-fibers, by yet poorly understood mechanisms, which may
possibly involve p38 signaling cascades and release of TNF-α or IL-1 from microglial cells (329). In addition activated microglia can cause neuronal hyperexcitability by an increase in Cl⁻ concentration in spinal neurons, thus turning GABA-mediated responses from hyperpolarizing to depolarizing (176). Such a shift of Cl⁻ equilibrium potential ($E_{Cl}$) in lamina I spinal neurons was shown to be caused by ATP-stimulated release of brain-derived neurotrophic factor (BDNF) from activated microglia; BDNF in turn affected transmembrane distribution of chloride ions by the activity of the potassium/chloride exporter (KCC-2), responsible for maintaining low intraneuronal Cl⁻ concentration (175, 1027). The inhibition of BDNF synthesis and the release by interfering RNA or blockade of signaling via TrkB receptors (by using function-blocking antibody against the TrkB receptor or BDNF-sequestering fusion protein) as well as the inhibition of P2X receptors with TNP-ATP prevented both the $E_{Cl}$ shift and development of tactile allodynia (175). Furthermore, BDNF secreted following stimulation of microglial P2X₄ receptors may induce phosphorylation of NR1 subunit of NMDA receptors of dorsal horn neurons, which could also contribute to neuronal hyperexcitability (940). The release of BDNF triggered by activation of P2X₇ receptors takes place in two waves; the early peak of release occurs 5 min after ATP stimulation, whereas the second peak of release is attained ~60 min later (922). This biphasic kinetic reflects the rapid release of an already existing pool of BDNF, followed by an increase in BDNF synthesis. The BDNF release occurs through Ca²⁺-regulated exocytosis, as both elimination of Ca²⁺ influx and inhibition of soluble N-ethylmaleimide-sensitive factor attachment protein receptor, SNARE, inhibits BDNF secretion, and regulation of BDNF synthesis involves p38-MAPK signaling pathway (922).

7. P2X₇ receptors and microglial function

First indications for ATP opening large plasmamembrane pores were found in cell cultures, in which brief application of ATP made cellular membranes permeable to inorganic phosphates (163, 775). Subsequently, the underlying ATP-gated ion channel producing transmembrane pore upon activation was biophysically characterized and named P2X₂ receptor (332).

This P₂ₓ receptor became P₂X₇ receptor, when it was cloned and molecularly characterized in 1996 (880). These receptors share the least homology with other members of P₂X family and have several unique functional characteristics. The P₂X₂ receptors demonstrate an exceptionally low sensitivity to ATP (their activation requires millimolar concentrations of ATP); in all probability, it is the tetra-anionic form of ATP (ATP₄⁻) that acts as a true agonist for P₂X₇ receptors (although this was never proved experimentally). The P₂X₇ receptors show little desensitization in the presence of the agonist, and upon intense stimulation, they produce large transmembrane pores (699, 852, 879); the phenomenon initially described as an “ATP-dependent plasma membrane permeabilization” (163). The mechanism of the pore formation remains unclear (227); the pore formation may result from the dilatation of the channel or from the activation of other pore-forming proteins (699, 700) associated with P₂X₇ receptors. Several relatively specific pharmacological tools for probing for P₂X₇ receptors are available. They include an agonist 2’,3’-(benzoyl-4-benzoyl)-ATP (BzATP) which is ~30 times more potent than ATP at P₂X₇ receptors (although BzATP can activate other P₂X receptors and can be degraded to Bz adenosine, which stimulates P₁ receptors; Ref. 481), and several antagonists such as Brilliant Blue G (BBG) which blocks P₂X₇ receptors at 10–200 nM concentrations and oxidized ATP (oxATP). The latter, however, also inhibits P₂X₁ and P₂X₂ receptors (660).

P₂X₇ receptors are ubiquitously expressed in the cells of the immune system (106, 147) and are believed to be responsible for numerous immune reactions, including the processing and the release of various cytokines (278, 700). Furthermore, intensive stimulation of P₂X₇ receptors associated with pore formation is involved in initiating apoptosis and cells death (935).

Functional P₂X₇ receptors (which, at that time, were referred to as P₂X₇ receptors) were identified in microglial cells in 1996, both in situ, in amoeboid microglia (342) and in vitro, in freshly isolated mouse microglia (279). In cultured microglia, the existence of P₂X₇ receptors was deduced based on 1) detecting massive Ca²⁺ influx following exposure to ATP and Bz-ATP, 2) ATP-induced uptake of ethidium bromide or Lucifer yellow, 3) ATP-induced release of cytoplasmic markers such as lactate dehydrogenase, and 4) identification of P₂X₇ protein (218, 276, 279). The treatment of microglial cell lines with high concentrations of ATP also induced rapid excitotoxicity associated most likely with plasmalemma permeabilization (276). All these effects were blocked by the P₂X₇ receptor antagonist oxidized ATP (218, 279).

In the amoeboid microglia collected from the surface of corpus callosum slices of 5- to 7-day-old mice, the P₂X₇-mediated currents were recorded directly, using a whole cell patch-clamp technique (Fig. 9). The P₂X₇ currents in amoeboid microglia were activated by 1 mM ATP in normal extracellular solution, or by 100 μM ATP after removal of extracellular divalent cations (342). Subsequently, P₂X₇ receptor-mediated currents were detected in ramified microglia in acute slices from adult (6–8 wk old) mice, as indicated by their sensitivity to BzATP and current potentiation in Ca²⁺/Mg²⁺-free extracellular solution (Fig. 9; Ref. 82).

In the undisturbed brain, microglial cells expressing P₂X₇ receptors are diffusely scattered throughout virtu-
ally all areas (1025). In depth analysis of the expression of various P2X receptor subunits have found prominent induction of P2X7 receptor expression in microglial cells following brain damage (165). This initial observation was subsequently confirmed in many in vitro and in vivo neuropathological models, and the link between microglial activation and P2X7 receptors expression was established (851, 852). Upregulation of P2X7 receptors is generally induced by ischemic and traumatic insults (294, 580).

The MCAO triggered a pronounced increase in P2X7 expression in microglia in striatum and a frontoparietal cortex in both infarcted and surrounding areas (the P2X7 immunoreactivity was absent in microglia from control samples); the P2X7 immunoreactivity was also detected in microglia in ipsi- and contralateral cingulate and medial frontal cortex (580). Injections of broad P2X antagonist Reactive Blue 2 reduced the size of ischemic damage and improved neurological deficit (294, 580). Upregulation of P2X7 receptors is generally induced by ischemic and traumatic insults (294, 580).

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**Fig. 9.** P2X\textsubscript{7} receptor-mediated currents in microglial cells in situ. A: ATP-induced membrane currents measured from amoeboid microglial cells collected from the acute young mouse corpus callosum slices (see also Fig. 7) in control conditions and \(\text{Ca}^{2+}/\text{Mg}^{2+}\)-free bath solution. B: membrane currents were recorded from microglial cells in the acute mouse forebrain slices when clamping the membrane potential to \(-20\text{ mV}\). The membrane was repetitively clamped for 100 ms to a series of \(\pm 0, 20, 40,\) and \(60\text{ mV}\) potentials. The series of voltage steps were performed every 5 s in the condensed time scale on each series of voltage jumps appears as a line. BzATP (□) and ATP (○) were applied in \(\text{Ca}^{2+}/\text{Mg}^{2+}\)-free bath solution. Subsequently, ATP was applied in normal bath solution (○). The current-voltage curves were constructed from series of voltage jumps as indicated by the different symbols and relate to the current component induced by the agonist application (after subtraction of currents without agonist). [A modified from Haas et al. (342); B modified from Bocusein et al. (82).]
P2X7 receptors are also involved in regulation of microglial activation in response to LPS stimulation. The stimulation of P2X7 receptors in primary cultured microglia triggers cell death (90, 276), which was absent in P2X7 knockout animals (90). Activation of P2X7 receptors in rat cultured microglia also induced the release of superoxide, which may mediate microglial cytotoxicity (694). Prominent microglial cell death was also observed in organotypic rat hippocampal slices treated with a combination of LPS and ATP (1 mM) or BzATP (100 μM); this cell death was prevented by pharmacological blockade of P2X7 receptors (49). The P2X7 receptors are also involved in regulation of microglial autophagy through the release of autolysosomes (893, 895).

At the same time, many experiments demonstrated a prominent trophic role of microglial P2X7 receptors. In experiments in rat hippocampal cultures or in microglia-enriched cultures, the sole overexpression of P2X7 receptors (without any additional stimulation) was found to launch microglial activation (the latter being assessed morphologically and through expression of specific markers isolecitin GS-IB4 and CD68; Ref. 607). This microglial activation was sensitive to oxATP, thus confirming the specific role for P2X7 receptors. Surprisingly, the initiation of the activation program required a P2X7 receptor-associated pore formation; in microglial cells transfected with a point mutant P2X7-RG345Y (this mutant protein retains the channel function but cannot form the pore), microglial activation was significantly reduced (607). The P2X7 receptors were also obligatory in mediating microglial activation in response to an intrahippocampal injection of Aβ protein (787); considering the pivotal role of microglial activation in AD-associated neuroinflammation (115, 370, 767), the P2X7 receptors may be considered as important therapeutical targets.

The P2X7 receptors are linked to various intracellular transcription factors. The activation of P2X7 receptors is linked to phosphorylation of another transcription factor, CREB (724). The P2X7 receptors play a central role in positive modulation of IFN-γ induced upregulation of iNOS production and NO synthesis and RK1/2 phosphorylation (314). The activation of P2X7 promotes release of cytokines IL-1α and IL-1β from activated cultured microglial cells (90, 277), which may occur through membrane vesicle formation and shedding (65). Similarly, in vivo in P2X7 knockout (KO) mice, the systemic treatment with LPS induced smaller increases in the brain IL-1β and TNF-α compared with the wild-type controls (590). In microglial cultures prepared from P2X7 KO animals, the P2X7 receptors were demonstrated to be selectively coupled to the release of IL-1β (590). Pretreatment of cultured microglia with LPS or with Aβ1-42 significantly (1.5- to 4-fold) increased P2X7-induced secretion of IL-1α and IL-1β (745).

The brief conditioning of microglial cells with ATP or BzATP sensitized microglial P2X7 receptors to ADP and AMP, so the latter acquire an ability to activate the receptors (albeit without pore formation) and stimulate the release of IL-1β (136). Treatment of primary microglial cultures with an inflammatory phospholipid lysophosphatidylcholine potentiated P2X7-dependent Ca2+ influx and pore formation (896). The P2X7-mediated signaling is involved in LPS-stimulated release of interleukins, as LPS (as well as other bacterial endotoxins; Refs. 276, 277) triggers the release of ATP from microglia, which, in turn activates purinoceptors in autocrine/paracrine fashion (277). Similarly, the stimulation of P2X7 receptors with 1 mM ATP or BzATP triggered the release of TNF-α from primary cultured rat microglia (377), this release being controlled by P2X7-mediated sustained [Ca2+]i elevation and ERK/p38 signaling pathway. The TNF-α release following stimulation of microglial P2X7 receptors can protect neurons against glutamate-induced neurotoxicity (883). The stimulation of P2X7 receptors in primary microglia upregulated synthesis and induced release of CC-chemokine ligand 3 (CCL3)/macrophage inflammatory protein-1α; this release was blocked by P2X7 receptor antagonists and selective inhibition of nuclear factor activated T cells (NFAT) (435). Similarly, P2X7 receptor activation increases expression and induces the release of CXCL2 chemokine from rat cultured microglia via NFAT and MAPKs (p38, ERK, and JNK) signaling cascades (830). P2X7 receptor-mediated Ca2+ influx was shown to suppress the synthesis of microglial response factor-1 gene (mrf-1; the latter being the part of microglial activation induced by neuronal death in vitro and in vivo) at the transcriptional level (440). Another group, however, found that P2X7-mediated Ca2+ influx significantly (~10 times) increases the expression and release of MRF-1 protein from cultured rat microglia (904). Calcium influx through P2X7 receptors is instrumental in triggering the release of plasminogen from cultured rat microglia (413). The release of plasminogen was inhibited either by clamping [Ca2+]i with BAPTA-AM or by removing extracellular Ca2+ or by inhibiting P2X7 receptors with oxATP. The specific P2X7 receptor agonist BzATP was much more potent than ATP in triggering plasminogen release (413). Sustained calcium influx through P2X7 receptors was also instrumental in increasing microglial production of endocannabinoid 2-arachidonoylglycerol (2-AG) through direct Ca2+-dependent activation of diacylglycerol lipase and the simultaneous inhibition of monoacylglycerol lipase, the enzyme that degrades 2-AG (996). Importantly, the stimulation of microglial P2X7 receptors can occur...
following ATP release in astrocytes, with this release being potentiated by IFN-γ (953).

The stimulation of microglial P2X7 receptors can induce neurotoxicity. In neuronal-microglial cocultures, treatment with ATP and BzATP promoted neuronal cell death, which was prevented by P2X7 inhibition with ox-ATP or Brilliant Blue G (841). This type of P2X7-dependent neurotoxicity was mediated through microglial release of superoxide and NO (694, 841), and treatment of cocultures with superoxide dismutase mimetic or with a peroxynitrite decomposition catalyst was neuroprotective. When microglia for cocultures was prepared from P2X7 KO mice, BzATP did not trigger neurotoxicity, further corroborating the role for P2X7 receptors (841). The P2X7-mediated IL-1 release from microglia was also implicated in neurotoxic effects in organotypic hippocampal slices (49).

Under certain conditions, however, microglial P2X7 receptors may acquire neuroprotective potential, as for example was suggested by studying ischemic insults following the middle cerebral artery occlusion in rats. In these experiments the activation of P2X7 receptors by injection of BzATP alleviated, whereas the inhibition of P2X7 receptors by oxATP exacerbated brain damage (1018). Incidentally, the microglial P2X7 receptors may also be somehow linked to the development of mood disorders. Behavioral analysis of P2X7 knockout mice found that they were more resistant to induction of depression and more sensitive to antidepressant drugs (39).

8. P2Y receptors

Metabotropic P2Y purinoceptors belong to archetypical seven-transmembrane domain G protein-coupled receptors (5, 284). They are generally divided into the P2Y1,2,4,6,11 and P2Y12,13,14 groups based on phylogenetic similarity and G protein preference (4). The P2Y1,2,4,6,11 are linked with Gq/G11 proteins and regulate cytosolic Ca2+ mobilization through PLC/InsP3/ER Ca2+ release signaling cascade. The P2Y12,13,14 receptors are coupled to Gαi proteins and inhibit adenylyl cyclase or modulate ion channels activity (4). In addition, P2Y receptors are differentially activated by various nucleotides: the P2Y1,11,12,13,14 are sensitive to adenine nucleotides ATP/ADP, the P2Y4,6 are activated by uracil nucleotides UTP/UDP, the P2Y14 receptor is specifically sensitive to UDP-glucose, and finally P2Y2 receptors are activated by both adenine and uracil nucleotides (284).

In cultured mouse microglia, the real-time semi-quantitative RT-PCR analysis revealed the expression of P2Y6, P2Y12, P2Y13 receptors; the activation of these receptors participated in [Ca2+]i elevation evoked by various P2 receptors agonists (527, 568). In rat cultured microglia, nucleotide-induced Ca2+ signaling and [Ca2+]i oscillations are the consequence of activation of P2Y2, P2Y6, and P2Y12 receptors (966). The activation of P2Y receptor-mediated ER Ca2+ release often triggers secondary store-operated Ca2+ entry; incidentally, the store-operated Ca2+ influx is negatively regulated by concomitant activation of P2X receptors, because depolarization produced by stimulation of the latter reduces electro-driving force for Ca2+ (985). In ramified microglial cells from acute adult mouse slices, activation of P2Y receptors triggers an activation of outward-rectifying K+ conductance (82). The activation of P2Y receptors can also affect microglial secretion of cytokines and other biologically active substances. The P2Y receptors, for example, were shown to inhibit release of TNF-α, IL-1β, IL-6, and IL-12 in LPS-activated cultured rat and mice microglia (82, 669). In contrast, the activation of P2Y4/P2Y11 receptors increased IL-10 expression and release in LPS-activated rat microglia (820, 821). The activation of P2Y receptors in cultured microglia also resulted in rapid accumulation of immediate early genes c-fos, junB, c-jun, and TIS11 (725). In the retina, stimulation of P2Y1 receptors induced morphological remodeling of microglia represented by the decrease in the cell soma size and retraction of processes, without affecting microglial cell density (938).

Cultured rat microglia were also shown to express UDP-preferring P2X6 receptors at both mRNA and protein levels (467). These P2Y6 receptors are implicated in pyrimidine-dependent activation of microglial phagocytosis (467). Stimulation of microglia with UDP triggered [Ca2+]i transients through activation of InsP3-mediated Ca2+ release from the ER and induced phagocytosis of fluorescent zymosan particles and latex beads. The P2Y6 agonist reactive blue 2 inhibited both Ca2+ signals and phagocytosis induced by UDP (467). The P2X6 receptors were also involved in regulation of phagocytosis in vivo in kainate-lesioned CA1 and CA3 hippocampal areas (467).

The ADP-preferring P2Y12 receptors represent an important target for purines in early microglial responses to injury in the CNS (365, 410). The P2Y12 receptors are predominantly expressed in the brain, and within the brain they are predominantly expressed in microglial cells as was shown by in situ hybridization and immunohistochemistry (792). This specific expression was further corroborated in experiments using a specific antibody against the COOH terminus of mouse P2Y12 receptors. The labeling with this antibody (in both gray and white matter) colocalized with microglial-specific markers (EGFP expressed under control of microglia specific fractalkine receptor promoter or with integrin CD11b antibodies; Ref. 365). The P2Y12 receptors were localized on the processes and somatic membrane of resting microglial cells (365). The activation of microglial cells following brain injury induced a very significant decrease in P2Y12 receptors expression, which became almost absent 24 h after the isolation of hippocampal slices (365). Similarly,
when microglia were activated in vivo by LPS injection into the striatum, the P2Y12 receptors expression decreased to nondetectable levels 4 days after the insult (365).

At the very same time P2Y12 receptors expressed in resting microglia appeared to be critical for inducing the morphological activation and chemotaxis following brain injury. ATP/ADP induced membrane raffling and microglial chemotaxis in vitro are sensitive to pharmacological inhibition of P2Y12 receptors with a specific antagonist AR-C69931MX (384) and are severely impaired in P2Y12 KO mice (365). Genetic deletion of the P2Y12 gene severely affects the ability of microglial cells to migrate, proliferate, and extend processes towards a mechanical lesion as was demonstrated both in vitro and in vivo (365). The P2Y12-mediated microglial chemotaxis requires intracellular Ca\(^{2+}\) signaling (mediated through PLC-InsP\(_3\)-induced Ca\(^{2+}\) release) and the activation of phosphatidylinositol 3′-kinase (PI3K) and Akt cascades (419, 673). The P2Y12 receptor activation engages integrin-β1 signaling cascade, which controls extension of microglial processes (674). There are also some indications that microglial chemotaxis involves activation of P2Y-regulated K\(^+\) channels (1004).

In the spinal cord, the P2Y12 receptors are also confined exclusively to microglia and are critically involved in the genesis of neuropathic pain (414). In contrast to the CNS, however, spinal nerve injury triggers a significant upregulation of P2Y12 receptors at both mRNA and protein levels (464, 920). The activation of these receptors (in parallel with activation of P2X\(_3\) receptors; see above) is instrumental in inducing tactile allodynia and thermal hyperalgesia (464, 920). In P2Y12 KO mice, nerve injury failed to produce allodynia, although the basic mechanosensitivity was not affected (920). Furthermore, an intrathecal injection of P2Y12 receptor blocker AR-C69931MX or oral administration of P2Y12 receptor antagonist clopidogrel markedly reduced allodynia in rats, which underwent surgical nerve injury (920). Direct injection of P2Y12 agonist 2-(methylthio)adenosine 5′-diphosphate trisodium salt (2Me-SADP) mimicked nerve injury and increased neuropathic pain symptoms (464). Interestingly, the genetic ablation of P2Y12 receptors did not affect microglial activation, yet it definitely determined the development of neuropathic pain (920). Effects of P2Y12 receptor activation involve p38 mitogen-activated protein kinase (p38 MAPK) signaling pathway (464).

Recently, microglial cells were shown to express the new P2Y-like receptor GRP17, which is activated by UDP, UDP-glucose, and UDP-galactose as well as by cysteinyl-leukotrienes LTD4 and LTC4; microglial expression of this receptor appeared after brain injury (502).

## B. Glutamate Receptors

### 1. Ionotropic receptors

#### A) AMPA receptors. In cultured rat microglia, the administration of both glutamate and kainate triggered cationic currents (343, 652). These currents were completely inhibited by selective AMPA receptor blocker CNQX, and potentiated by allosteric modulators of AMPA receptors 4-[2-(phenylsulfonylamino)ethylthio]-2,6-difluoro-phenoxycetamide (PEPA) and cyclohexazole (CTZ). The AMPA-mediated currents in cultured microglia showed negligible Ca\(^{2+}\) permeability (652). Transcription analysis using PCR revealed microglial expression of GluR2, GluR3, and GluR4 (652). In another study of rat cultured microglia, the expression of GluR1, GluR2, and GluR3 mainly in flip form was reported (343). Activation of microglial AMPA receptors by glutamate or kainate induced release of TNF-α (343, 652), which was, rather surprisingly, blocked by CTZ and PEPA, prompting authors (343) to suggest that potentiation of AMPA receptors produces negative feedback to TNF-α release. The AMPA receptors are also implicated (together with unidentified metabotropic glutamate receptors) in glutamate-induced microglial chemotaxis, which was found in microglial cultures and in the spinal cord slices (529).

Activation of microglial AMPA receptors with 0.1–1 mM of kainate led to a rapid and substantial remodeling of the cytoskeleton manifested by condensation of cytoplasmic actin filaments, rapid de- and repolymerization, and cytoplasmic redistribution of condensed actin bundles. These changes may play a role in the regulation of motility and phagocytosis of activated microglial cells (156).

Stimulation of cultured rat microglia with 500 μM glutamate NMDA, AMPA, kainate, or mGluR agonist (RS)-3,5-dihydroxyphenylglycine (DHPG) markedly (23-fold) and transiently upregulated c-fos gene expression (on both mRNA and protein levels); this stimulation required [Ca\(^{2+}\)]\(_i\) elevation (being blocked by BAPTA-AM) and involved the activation of glutamate receptors sensitive to specific antagonists; treatment of microglia with CNQX, MK-801, or AIDA (group I mGluRs antagonist) completely inhibited the increase in expression of immediately early genes (256). Such a broad sensitivity of gene expression to very different glutamate receptors antagonists remains inexplicable, and these data were never independently confirmed.

#### B) KAINATE RECEPTORS. There is no direct evidence for the functional expression of kainate receptors in microglial cells. Despite the identification of mRNA for GluR5 kainate receptors in cultured rat microglial cells, electrophysiological analysis demonstrated that kainate-induced currents were carried through AMPA receptors (652). Treatment of primary cultured rat microglia with 100 μM
kainite was reported to initiate their activation and stimulate release of TNF-α (1038).

C) NMDA Receptors. The expression of NMDA receptors in microglia remains doubtful; only indirect evidence is available. An injection of 50 nM NMDA into the sensorimotor cortex of 6-day-old rats triggered transient activation of microglia with the appearance of pseudopod morphology (7), which however is not necessarily indicative for microglial receptor. Treatment of rat microglial primary cultures with MK-801 [a use-dependent (393) inhibitor of NMDA receptors] induced cytotoxicity, which was decreased by the addition of glutamate, NMDA, and (surprisingly) kainite, thus prompting authors to suggest an expression of functional NMDA receptors (380). Both LPS- and neurotoxic Tat HIV protein-induced activation of cultured mouse microglia were suppressed by MK-801 and another NMDA receptor inhibitor dextromethorphan (910). Similarly, systemic administration of MK-801 prevented rapid microglial activation in the hippocampus following ischemic insult (871). Finally, another set of data suggested that the activation of NMDA receptors induced MAPK phosphorylation in spinal cord microglia from rats with streptozotocin-induced diabetes, which may be somehow involved in diabetic hyperalgesia (189). Thus all the evidence remains indirect.

2. Metabotropic glutamate receptors

In rat cultured microglial cells, expression of mGluR5a (group I members) receptors was identified at mRNA level; subsequent [Ca\(^{2+}\)]\(_i\) recordings confirmed their functional expression by demonstrating Ca\(^{2+}\) signals in response to mGluR antagonist trans-(1S,3R)-1-amino-1,3-cyclopentanedicarboxylic acid (1S,3R-ACPD) (66). There is some evidence that stimulation of group I mGluRs may regulate LPS-induced microglial activation in primary cultures (269). The stimulation of group II mGluRs by glutamate released from oxygen-glucose-deprivation-stressed embryonic rat neurons cocultured with rat microglia or by endogenously applied glutamate or (2S,2’R,3’R)-2-(2’3’-dicarboxycyclopropyl)glycine (agonist of group II mGluRs) triggered the activation of microglial cells and induced neurotoxicity mediated through microglial release of TNF-α (437). The mGluR-dependent microglial activation involved NF-κB signaling. In contrast to LPS-induced microglial activation, this mGluR-dependent pathway does not involve MAPK signaling; neither does it promote NO production and release (437). Further analysis indicated that cultured rat microglia express mRNA and proteins of both group II (mGluR2 and mGluR3 receptors; Ref. 906) and group III (mGluR4, mGluR6, and mGluR8, but not mGluR7; Ref. 907). Activation of group II receptors triggered microglial activation and neurotoxicity, promoted the release of TNF-α (908), and was also suggested to participate in microglia activation induced by chromogranin A, a secretory peptide present in neuritic plaques in Alzheimer’s disease (906). In contrast, activation of group III receptors reduced microglial neurotoxicity following treatment with LPS or chromogranin A. On a molecular level, group III receptors inhibited activity of adenylate cyclase (907).

C. GABA Receptors

The main CNS inhibitory neurotransmitter GABA has a well-documented neuroprotective effect (273), and GABA levels were reported to increase in patients with cerebral ischemia (395).

Functional GABA\(_B\) receptors were identified in a subpopulation of microglial cells in culture (Fig. 10) and in ~50% of tomato-lectin positive cells in brain slices (480). Stimulation of GABA\(_B\) receptors in microglia activated an outwardly rectifying K\(^+\) current and triggered [Ca\(^{2+}\)]\(_i\) transients. In addition, stimulation of GABA\(_B\) receptors reduced the release of IL-6 and IL-12p40 from LPS-stimulated cultured microglia (480). Both GABA\(_B\) subunits 1 and 2 were determined in a majority (>90%) of cultured microglial cells by immunostaining, and both proteins were detected by immunoprecipitation, with GABA\(_B\) rece-
ceptor subunit 1 being represented by three splice variants GABAB(1α), GABAB(1β), and GABAB(1ε) (142, 480). Immunostaining indicated also that GABA_B receptors were preferentially concentrated in microglial lamellipodia (480). Facial axotomy increased the population of GABA_B receptors-expressing microglia in the facial nucleus (480).

At the same time, microglial cells may detect GABAergic activity in their immediate environment indirectly, as for example was found for amoeboid microglia in postnatal (6–8 days) mouse corpus callosum slices (151). The application of GABA_A agonist muscimol triggered a transient increase in IKIR in these amoeboid microglia; this increase, however, completely disappeared when cells were removed from the slice surface (Fig. 11). It turned out that microglial responses to muscimol resulted from the transient elevation of extracellular K⁺ concentration following stimulation of neuronal or macroglial GABA_A receptors. This indirect mechanism had some functional consequences, however, because activation of IKIR stimulated release of chemokine macrophage inflammatory protein-1α (MIP1-α) from microglia (151). Inhibition of GABA signaling by the GABA_A receptors blocker bicuculline increased the resting motility and volume sampling by resting microglia in vivo (645). This is most likely also an indirect effect mediated by neuronal or macroglial activity.

D. Cholinergic Receptors

Cholinergic pathways control global anti-inflammatory reactions in the brain, which are believed to be mainly mediated through α7 nicotinic receptors, α7nAChRs (978). Importantly, cholinergic systems are impaired by numerous neurodegenerative diseases, such as for example AD and Parkinson disease; the weakness of cholinergic transmission may facilitate the development of neuroinflammation particularly through decreasing the ACh input to microglial cells (124).

The expression of neuronal α7 nicotinic receptors was initially found in cultured mouse microglia through...
the combination of RT-PCR, western blot, immunofluorescence, and immunohistochemistry analyses (198, 833). In microglial cells from the retina of a rhesus monkey, only α6 or β4 nAChR subunits were identified by immunostaining (530). In human embryonic microglial cell cultures, RT-PCR revealed expression of mRNAs specific for α3, α5, α7, and β4 subunits (765). A rather unusual signaling pathway associated with α7nAChRs was identified in primary rat cultured microglia. The treatment of these cells with nicotine induced [Ca^{2+}], transients, which were blocked by the specific α7nAChRs blocker methyllycaconitine (MLA; 10 nM), but they were also blocked by the PLC inhibitor U73122 and the presumed InsP3 receptor blocker xestospongin C (884). Furthermore, nicotine-induced [Ca^{2+}]i transients persisted in Ca^{2+}-free extracellular media, and nicotine failed to induce any currents in voltage-clamped microglial cells. These results suggested that α7nAChRs in microglia could be linked to the PLC/InsP3/Ca^{2+} release signaling pathway (884). The nicotine-induced Ca^{2+} signals modulated the release of TNF-α in response to either activation of P2X7 receptors (positive modulation) or LPS (negative modulation) (884).

The activation of nACh receptors generally inhibits the immune response of microglial cells, thus representing endogenous "cholinergic anti-inflammatory pathway" (833). Nicotine was shown to inhibit ATP/P2X7-mediated ROS production in Aβ1-42 stimulated cultured microglial cells, with this effect being antagonized by α-bungarotoxin, thus indicating specific involvement of α7nAChRs (608). Long-term (15 days) incubation of corticostrital organotypic slices with nicotine (3–30 μM) reduced the thrombin-dependent activation of microglia (672).

The activation of nAChRs in human embryonic microglial cultures by treatment with nicotine (15 min to 24 h in concentrations ranging between 3 and 300 μM) increased the expression of human immunodeficiency virus HIV-1 and modified the gene expression profile in HIV-1 infected cells (765). At the same time, activation of nAChRs was reported to somewhat suppress microglial activation in response to IFN-γ and the HIV-1 coat glycoprotein gp120 (322).

In cultured microglial cells from the human brain, application of carbachol triggered cytosolic [Ca^{2+}], transients, which were blocked by atropine and originated from Ca^{2+} release from the ER, thus suggesting functional expression of muscarinic cholinoreceptors (990, 1031).

E. Adrenergic Receptors

Accumulation of intracellular cAMP following stimulation of β2 receptors provided the first evidence for the expression of adrenergic receptors in microglia (727). While β2 receptor stimulation suppressed LPS-induced release of IL-12p40 in cultured microglia (727), stimulation of β1 receptors increased expression of IL-1β mRNA (902). RT-PCR analysis of cultured rat microglial cells revealed expression of mRNAs for α1A, α2A, β1, and β2 receptors (613, 902). Stimulation of β2 receptors with terbutaline and norepinephrine increased cytosolic cAMP levels (902). Norepinephrine also induced [Ca^{2+}], transients originating from ER Ca^{2+} release in cultured rat microglial cells (990). Norepinephrine attenuated the LPS-induced release of NO, TNF-α, and IL-6 (267). Selective stimulation of α1 or β1 or β2 receptors suppressed the expressions of IL-6 and TNF-α at transcriptional level (613). Activation of β2 ARs suppressed proliferation of microglia through cAMP-dependent mechanism (302). Norepinephrine, acting through β1/2 receptors, controlled p38 MAPK signaling cascades via both cAMP and PKA; this inhibited ATP-induced release of TNF-α (620). Stimulation of β adrenoreceptors was shown to be involved in pro-inflammatory cytokine production in microglial cells following surgical trauma (979).

In mesencephalic neuronal-glia cultures, the selective β2 receptor agonist salmeterol induced ERK phosphorylation and microglial activation. Activated microglia upregulated the production of reactive oxygen species (ROS) by NADPH oxidase; the ROS in turn induced neurotoxicity (736).

Adrenoreceptors can be also instrumental in regulation of microglial migration and phagocytosis. Specifically, this can be relevant for pathogenesis of AD, where early degeneration of locus ceruleus and depletion of adrenergic input to the brain affects the ability of microglia to provide for effective clearance of β-amyloid (371).

F. Dopamine Receptors

Functional dopamine receptors have been identified in mouse and rat microglia, in culture, and in brain slices (267). With the use of subtype-specific ligands, D1- and D2-like dopamine receptor-mediated membrane currents were recorded in voltage-clamp experiments. Dopamine triggered the inhibition of the constitutive potassium inward rectifier currents and activated potassium outward currents in a subpopulation of microglia (267). Chronic dopamine receptor stimulation enhanced migratory activity and attenuated the LPS-induced NO release similar to stimulation of adrenergic receptors. While, however, norepinephrine attenuated the LPS-induced release of TNF-α and IL-6, dopamine was ineffective in modulating this response. This indicates that dopamine does not mediate its effect by triggering adrenergic receptors, but provides evidence for microglial dopamine receptors expression (267). The dopamine-induced chemotaxis was confirmed also for cultured elderly human microglia (555). Indeed, the D1,2,3,4 (but not D5) receptors were identified on trans-
The role of dopamine receptors in the substantia nigra (the brain region primarily affected in PD) is the highest in the brain: in mouse microglia amount to ~12% of total cells number in this region, compared with ~5% in the cortex and in the corpus callosum (498). In Parkinson’s disease, microglial cells in substantia nigra are activated and concentrated around dystrophic dopaminergic neurons (768); the role of dopamine receptors in this specific activation and migration of microglia need to be clarified further. The inhibition of D4 receptors by this specific activation and migration of microglia, for example, the removal of extracellular Ca2+ from cultured microglia (614). Similarly, entry following PAF stimulation produced release of arachidonic acid from cultured microglia (614). Bradykinin receptors were initially discovered in primary cultured rat microglia (648, 649). In these mixed cerebrocortical cultures from neonatal (P3) Wistar rats, the exclusive expression of B2 receptors at both transcriptional and protein levels was demonstrated (648). The expression of B1 receptors in resting (or not fully activated) microglia was very low (648). The stimulation of cultured microglia with 100–200 nM bradykinin triggered an outward current in 14% of cells studied; these currents were due to an activation of Kca channels following an increase in [Ca2+]i, stimulated via B2/InsP3 cascade (648). Similar currents were also observed in amoeboïd microglia in situ in forebrain slices of young mice (650). Activation of cultured microglia with LPS or with 24 h treatment with 300 nM of bradykinin induced a strong elevation in expression of both B1 and B2 receptors (650). These two types of receptors had a neuroprotective potential by attenuating cytokines release from microglia (650). Bradykinin was also found to induce chemotaxis in cultured microglia (397). This was mediated by activation of B1 receptors (as judged by their agonist/antagonist sensitivity and complete disappearance in cells from B1−/− but not B2−/− mice). Action of bradykinin was mediated through PKC and phosphoinositide 3-kinase, which activated the Na+/Ca2+ exchanger (NCX) and intermediate-conductance Kca channels (Fig. 12); in NCX−/− heterozygotes, the effects of bradykinin were substantially decreased (397).

C. Histamine Receptors

Histamine induced [Ca2+]i increase in ~30% of cultured rat microglia; this [Ca2+]i increase originated from InsP3-induced Ca2+ release from the ER (29).

D. Endothelin Receptors

Endothelins represent a family of peptides initially discovered as potent vasoactive compounds (1019). Two types of endothelin receptors, the ETa and ETb, were discovered in neural cells; the stimulation of these receptors triggers Ca2+ mobilization via InsP3-induced intracellular Ca2+ release (621, 811, 937). Transcripts of ETb endothelin receptors were found in purified cultured mouse microglial cells and in the individual cells (using single-cell PCR) from the same
cultures. The functional expression of ETB receptors was further confirmed by Ca\(^{2+}\)/H\(_{11001}\) imaging. The stimulation of mouse cultured microglia with ET-1 or ET-3 triggered \([\text{Ca}\(^{2+}\)/H\(_{11001}\)]\) transients in a small (~13%) subpopulation of cells. These Ca\(^{2+}\) responses were mimicked by specific agonist BQ3020 and blocked by selective antagonist BQ788. The ETB-mediated Ca\(^{2+}\) signals had a complex nature involving both Ca\(^{2+}\) release and store-operated plasmalemmal Ca\(^{2+}\) entry (603). In cultured human microglia, ET-1 and ET-3 induced \([\text{Ca}\(^{2+}\)/H\(_{11001}\)]\) transients in ~80% of cells. These Ca\(^{2+}\) signals were also mediated by ETB endothelin receptors (as judged by their inhibition by selective antagonist BQ788) and were dependent on both intracellular Ca\(^{2+}\) release and plasmalemmal Ca\(^{2+}\) influx (573).

Expression of both endothelins and ETB receptors was detected in the amoeboid invading microglia in the corpus callosum of neonatal rats (1002). This expression diminished with age and almost completely disappeared in the mature brains.

Significant increase in ETB receptors expression in hippocampal microglia was found following brain ischemia induced by a 10-min bilateral carotid occlusion and reperfusion (1017). Similarly, an increase in expression of endothelins and ETB receptors was reported in ischemic cerebral cortex of adult rats subjected to MCAO (523).

### E. Cannabinoid Receptors

The cannabinoid signaling system comprises the endogenous cannabinoids, cannabinoid receptors (CB\(_1\), CB\(_2\), orphan receptor GPR55 and 2 additional pharmacologically identified receptors, which await cloning; Refs. 433, 858, 859), and cannabinoid degrading enzymes. Cannabinoids may also modulate the activity of TRPV1, TRPA1, and TRPM8 channels by a yet unknown mechanism (196). Rodent microglial cells in vitro express CB\(_1\) and CB\(_2\) cannabinoid receptors as well as the third, not yet cloned, receptor (114, 259, 744, 766). The constitutive expression of cannabinoid receptors in the resting microglia is very low, if at all existing. The immunochemistry did not visualize CB\(_1\) receptors in resting microglia; neither CB\(_2\) mRNA is detectable in the healthy brain tissue (114, 858, 859). The specific expression of CB\(_2\) receptors was found in perivascular microglial cells in white matter of human cerebellum (663), although this study relied solely on immunostaining with CB\(_2\)-specific antibodies, which can have non-
specific binding (859). Microglial activation, however, results in rapid upregulation of cannabinoid receptors expression (858).

Microglial CB1 and CB2 receptors are coupled to G_{i/o} and G_{i} proteins, respectively (859). The activation of cannabinoid receptors reduced NO production by fully activated microglia and may inhibit production of cytokines (114). The stimulation of CB2 receptors in cultured microglia by selective agonist JWH-015 suppressed microglia activation by inhibiting CD40 signaling pathway, inhibited phosphorylation of JAK/STAT1 and production of NO and TNF-α (244). The treatment of rat cultured microglia with endogenous and synthetic cannabinoids suppresses LPS-induced TNF-α release (259); this action was mediated by one of uncloned CB receptors.

A very high expression of CB2 receptors was found in the CNS of mice with experimental autoimmune encephalomyelitis (549). The upregulation of CB2 receptors was due to synergistic action of IFN-γ and GM-CSF, which both are expressed in experimental autoimmune encephalomyelitis brains (549). Similarly, CB2 receptors are also prominently expressed in microglial cells from the brains of patients with AD, ALS, and AIDS-associated dementia (45–47, 1022). Incidentally, acute treatment with 3,4-methylenedioxymethamphetamine (popularly known as “ecstasy”) increased microglial expression of CB2 receptors in the rat in vivo (917).

Generally, the activation of cannabinoid receptors increases microglial proliferation (127) and reduces microglial neurotoxicity (859). The cannabinoid receptors are therefore potentially neuroprotective, and indeed, activation of CB2 receptors reduced neurotoxicity, neuroinflammation, brain edema, and death of striatal neurons, simultaneously improving motor symptoms in the animal model of Huntington disease (682). The activation of CB2 receptors facilitated microglial uptake of Aβ1–42 peptide (244). The stimulation of cannabinoid receptors inhibited microglial activation by β-amyloid and reduced microglia-dependent neurotoxicity (744). Massive release of anandamide, observed upon brain lesions, was shown to exert neuroprotection through activating microglial CB1 and CB2 receptors with subsequent induction of mitogen-activated protein kinase phosphatase-1 (MKP-1). The latter terminated the MAPK signaling transduction cascade, limiting thus TLR4-dependent activation of microglia (246, 760). Similarly, the activation of CB2 receptors expressed in microglial cells in the spinal cord may limit inflammatory and pain reactions after transaction of peripheral nerve (770). Finally, CB2 receptors reduce HIV-1 expression in microglia possibly through down-regulation of chemokine receptor CCR5, which is involved in virus entry into microglial cells (716).

**F. Angiotensin II Receptors**

The functional expression of angiotensin II receptors type 1 and 2 (AT1/AT2) was suggested based on RT-PCR and pharmacological studies of cultured microglial cells from embryonic rat brain. It was found that unstimulated microglia expressed mRNA for AT2 and angiotensinogen but not for AT1. Treatments of cultures for 6 h with LPS induced additional expression of AT1 specific mRNA. The exposure of cultures to specific AT1 receptor antagonist losartan (0.1–10 μM) suppressed morphological activation of microglia and reduced production of NO and IL-1β. Furthermore, losartan almost completely inhibited LPS-induced activation of NF-κB (596).

**G. Somatostatin Receptors**

The RT-PCR of material obtained from cultured rat microglial cells revealed an expression of sst2, sst3, and sst4 (but not sst1 and sst5) subtypes of somatostatin receptor. The activation of these receptors by somatostatin per se or by metabolically stable analog octreotide (SMS 201–995, selective agonist of sst2, sst3, and sst5 receptors) induced protein phosphorylation and inhibited microglial proliferation (272).

**H. Glucocorticoid and Mineralocorticoid Receptors**

Binding studies suggested the functional expression of both glucocorticoid and mineralocorticoid receptors (GR and MR, respectively) in cultured microglia from the forebrain of newborn rats. The presence of GR was also confirmed with immunoblotting using specific antibody. Activation of GR inhibited proliferation of cultured microglia and enhanced lysosomal formation (900).

**I. Opioid Receptors**

Kappa opioid receptors were identified in human fetal microglia using RT-PCR, immunohistochemistry, and ligand binding assay (140); these receptors may regulate microglial immune responses particularly in HIV-1-dependent encephalopathies and HIV-1-associated dementia (140). The activation of kappa-opioid receptors by cocaine is somehow involved in potentiation of HIV-1 expression and facilitation of HIV-1 associated dementia (313). Similarly, the RT-PCR analysis demonstrated the constitutive expression of mu-opioid receptor mRNA in human microglia. The activation of these receptors in cultured microglial cells strongly inhibited their chemotaxis towards C5a with an IC_{50} ~1 fM (141).

Exposure of feline cultured microglia to morphine and endorphin triggered morphological activation, sup-
posedly mediated by mu3 (opiate alkaloid-selective) receptors, as suggested because of inhibitory effects of naloxone (221). Microglial expression of mu3 receptors appears very early in evolution; the stimulation of mu3 receptors by morphine promotes NO release and induces activation of microglial cells in invertebrate, the mussel *Mytilus edulis* (531). The mu-opioid receptors increase migration and upregulate expression of P2X4 purinoceptors in cultured rat microglia (388). On the contrary, in neuronal-glial cultures prepared from rat mesencephalon, morphine significantly reduced microglial neurotoxicity and LPS-induced microglial activation, albeit through an unknown pathway that, most likely, did not involve opioid receptors (737). Reportedly morphine can also cooperate with HIV-1 transactivating protein Tat in stimulating microglial activation and secretion of proinflammatory factors (76).

**J. Neurokinin (Substance P) Receptors**

Neurokinin-1 (NK-1 or substance P) receptors were detected in primary cultured murine and fetal human microglia at mRNA and protein levels (491, 749). Moreover, human microglia was shown to produce significant amounts of substance P (491). Activation of NK-1 receptors by nanomolar concentrations of substance P triggered the activation of transcriptional factor NF-κB (749). Furthermore, the NK-1 receptors may regulate production of cytokines in microglial cultures and in microglia in vivo following stimulation with bacterial pathogens, *Neisseria meningitidis* and *Borrelia burgdorferi* (144).

In rat cultured microglia, substance P, when administered together with LPS, synergistically increased IL-1 production (which was 4 times greater than after treatment with LPS alone; substance P when added on its own did not affect IL-1 synthesis), with this action possibly involving “nonclassical” NK-1 receptors (553).

Activation of NK-1 receptors can enhance inflammatory responses induced by bacterial infections of CNS (750). At the same time, the pharmacological inhibition of NK-1 receptors in cultured microglia by aprepitant (1 nM to 10 μM) suppressed HIV-1 infection by reducing virus replication (983).

**K. Vasoactive Intestinal Polypeptide Receptors**

The vasoactive intestinal polypeptide (VIP) exerts its action through the activation of G protein-coupled receptors of VPAC (VIP/pituitary adenylate cyclase-activating polypeptide) receptors 1 and 2 (VPAC1/VPAC2; Ref. 496). The activation of these receptors is known to trigger anti-inflammatory and immunodepressive responses in various peripheral tissues (e.g., Refs. 23, 503). The VPAC1 receptor mRNA was identified in rat microglia in vitro, and stimulation of these receptors with VIP (0.1 μM) or with its functional/structural analog pituitary adenyl cyclase-activating polypeptide (PACAP, 0.1 μM) strongly inhibited TNF-α production in LPS-activated cultured rat microglia (455). These effects of VPAC1 receptor activation were mediated through cAMP-dependent pathway (455). The exposure to VIP inhibited COX-2 expression and the production of prostaglandin E2 (PGE2) in activated cultured mouse microglia (330). Similarly, VIP and PACAP inhibited LPS-induced activation of and secretion of proinflammatory factors TNF-α, IL-1β, and NO from cultured microglial cells (212–214). In addition, VIP suppressed microglial activation in vivo, after intraventricular LPS injection or mechanical brain trauma (212).

Stimulation of VPAC1 receptors was also reported to inhibit the activation and neurotoxic potential of cultured microglial cells exposed to Aβ1-42. In this study, the action of VPAC1 receptors was mediated through several intracellular signaling cascades that included p38 MAPK, p42/p44 MAPK, and NF-κB pathways (211, 215).

**L. Neurotrophin Receptors**

The neurotrophins [nerve growth factor (NGF), brain-derived growth factor (BDNF), and neurotrophins 3 and 4 (NT-3, -4)] exert their biological action through activation of two classes of receptors, the Trk tyrosine kinase receptors and the p75 neurotrophin receptor (p75NTR; for review, see Refs. 434, 695). The subclass of Trk receptors, the truncated tropomyosin-related kinase B-T1 (Trk-B1) receptors, were identified (at the mRNA level) in cultured rat microglia. Activation of these receptors by BDNF resulted in sustained [Ca2+]i elevation, which was mediated by an initial PLC/InsP3-driven Ca2+ release from the ER that followed by a long-lasting activation of the SOCE. Incubation with BDNF also decreased release of NO from the activated microglia (597). LPS-induced activation of microglia can trigger the expression of NGF in cultured microglial cells, which can also be induced by stimulation of A2 adenosine receptors (366).

**XII. CYTOKINE AND CHEMOKINE RECEPTORS**

**A. Chemokine Receptors**

Chemokines comprise a growing family of chemotactic cytokines with pivotal functions in cell migration under both physiological and pathophysiological conditions (25, 30, 310, 623, 783). Since the first description of IL-8 as a neutrophil attractant in 1987, the list of identified chemokines exceeded more than 50. Characterized by
presence of up to four conserved cysteine residues in their sequences, these small proteins of 8–12 kDa fall into four subgroups, i.e., the C, CC, CXC, and CX3C chemokines (275, 492, 623). Most of the chemokines act upon release as soluble messengers that can create chemotactic gradients for cell migration, while especially CX3CCL1 (also known as fractalkine) occurs also in surface-associated format. In addition, chemokines may associate with proteoglycans on the vascular endothelium or in the extracellular matrix, thus providing also immobilized cues. A distinction as to inflammatory and homeostatic chemokines is based on the inducible versus constitutive expression, with overlap occurring as well (623). Initially the chemokines were believed to participate in the migration and maturation of immune cell populations within and between immune organs as well as in the recruitment and guidance of immune cells to inflammatory sites. Subsequent experiments also described chemokines as important messengers in other tissues, which probably serve a multitude of functions beyond leukocyte attraction (62, 69, 258). These other roles of chemokines may range from neovascularization, control of cell adhesion or regulation of apoptosis and phagocytosis, to contribution to various signaling cascades (784, 927, 943).

The chemokines are expressed by neurons and microglial cells (69, 194) as well as by microglia in the human and mouse CNS (69, 195, 219, 323, 349, 351, 363, 454, 509, 950). Basal and inducible levels may vary between species (74, 285), anatomical regions (193), ages (834), and even between human individuals (579), with the latter variability being likely a consequences of disease history. Microglial expression at transcriptional and/or protein level has been documented for rodent CCR1, CCR2, CCR7, and CCR5 (74, 219), the latter being most abundant, as well as for human CXCR1, CXCR3, and CCR3, whereas CCR4, CCR5, CCR6, CXCR2, CXCR4, and CXCR5 were expressed at much lower levels (285). Expression of inducible chemokines in microglia may be dynamically regulated in the course of an activation process. Feedback signals from leukocyte populations attracted, in the first place, by microglial chemokines may subsequently reorganize the microglial chemoattractive cocktail. This can result in a phenomenon of “self-limitation” as well as in a shift of the chemoattractive call towards other cell populations, as indicated for the Th1 cytokine IFNγ and its impact on Th1 attraction (281, 363).

The cellular effects of chemokines are mediated through families of seven-transmembrane domain GPCRs, which are now classified in accordance to the systematic nomenclature of their ligands (e.g., CCR, CXCR, or CX3CR). They are linked to numerous intracellular signaling cascades (69), which include adenylate cyclase, phospholipases, GTPases (Rho, Rac, and Cdc42), and some kinases such as MAPK or phosphatidylinositol 3-kinase (PI3-K) (30). Besides the link to G protein signaling, there is also another property of the chemokine system that is not shared by other cytokines. Ligands and receptors reveal some promiscuity in their interactions, allowing individual ligands to bind more than a single receptor, and vice versa. Considering the expression of several chemokine receptors by a given cell and the simultaneous presence of different chemokines in their environment, the movements and functions of cells could be orchestrated in a rather complex fashion. Not only the direct access to diverse signaling pathways including [Ca2+]i (30, 69, 74), but also physical interaction with other receptor/effector systems, such as TLRs (927), further increase the options of chemokine receptor-mediated control over cellular functions.

The stimulation of cultured microglia with β-chemokines MCP-1/CCL2 (agonist of CCR2 receptors) or MIP-1α/ CCL3 and RANTES/CCL5 (agonists of CCR5 receptors) triggered rapid [Ca2+]i transients in distinct subpopulations of control and LPS-treated microglia; LPS-induced microglial activation resulted in an increase of Ca2+ responses. Both types of Ca2+ responses required a functional Ca2+ store (as indicated by inhibition by thapsigargin), although MCP-1 responses were also dependent on extracellular Ca2+ and were blocked by ryanodine (74), thus indicating a combination of initial Ca2+ influx and subsequent Ca2+-induced Ca2+ release. The CCR5-dependent MIP-1α/CCL3 and RANTES/CCL5-induced Ca2+ signals most likely involved the InsP3-dependent Ca2+ release. Ultimately, both types of [Ca2+]i transients were inhibited by PTX, reflecting the role of Goa and Goi proteins (74). The role for PI3-K, Bruton’s tyrosine kinase (Btk), and stimulation of cADPR production with possible activation of RyRs was also suggested (826).

In human microglial cells from hippocampal slices, 15-s application of MIP-1α/CCL3 (2 mg/ml) trebled the amplitude of Ca2+-dependent K+ currents by a yet uncharacterized mechanism (77), indicating possible role of Kca channels in regulation of microglia migratory activity. Activation of CXCR3 receptor by secondary lymphoid tissue chemokine CCL21 induced the activation of microglial volume-regulated Cl− channels, which was linked to microglial migration (747).

Microglial activation following neuropathological challenges affects the expression of chemokine receptors (478). Recruitment of microglia to a site of infection, lesion, or degeneration may include several factors including chemokines released from local cell populations. Monocyte/macrophage-attracting CCL2, for example, can be produced by activated microglia (such as upon TLR stimulations), while representing itself a microglial chemoattractant. Thus microglia act as a source and a target of chemokine actions, also in auto/paracrine fashion. The CCL2/CCR2 system seems to be crucial for the recruitment of myeloid cells to the CNS where they can acquire microglia-like features (192, 586, 729). Distinct circulating
monocyte subsets have been discussed as giving rise to populations replenishing tissue resident macrophages or to comprise the infiltrates invading during pathological conditions. Expression of CCR identifies the respective subsets, and this is functionally underlined by its role as the receptor for CCL2. The CX3CR1, the receptor for CX3CL1 (fractalkine), represents another key molecule in this CNS-relevant monocyte subclassification (729). Microglial expression of CX3CR1 receptor mRNA was suppressed by treating cultures with LPS (73). The application of 0.1–1 nM of fractalkine to cultured microglia triggered [Ca2+]i transients; whereas at 10 nM it triggered [Ca2+]i oscillations (73). These fractalkine-induced Ca2+ signals originated from ER Ca2+ release. The genetic deletion of CX3CR1 enhanced microglial neurotoxicity in several pathological models, including systemic inflammation induced by an intraperitoneal injection of LPS, MPTP-induced model of Parkinson disease, and in a SOD1 over-expressing mouse used as a model for ALS (123).

The expression of CCR5 receptors (on transcriptional level) was increased following hypoxic-ischemic insults and nerve injury (177, 307). LPS-induced expression of mRNAs for inflammatory cytokines (IL-1β, IL-6, TNF-α) and iNOS in microglia were all suppressed by RANTES/CCL5-induced activation of CCR5, and nerve injury-induced motor neuron death seen in wild-type C56BL/6J mice was accelerated in CCR5 knock-out C57BL/6J, suggesting that CCR5-mediated neuron-glial signaling protects neurons by suppressing microglia toxicity (307).

The chemokines including CCL2, CCL21, or CX3CL1 may serve as signals from endangered neurons to microglia (69). The CX3CL1 expressed in neurons may provide a constitutive calming influence on CX3CR1-expressing microglia, thus representing a neuron-to-microglia signaling system similarly to already described for CD200/CD200R or CD47/SIRP-1α. Interruption of this influence may release microglia from a control to allow activation or enhanced responses to activating signals. Indeed, deficiency in this chemokine signaling can lead to enhanced severity of CNS damage in several disease models (123, 729). The CX3CR1/CX3CL1 system reveals some complexity as the protein expression may not be cell-specific, as surface-attached and soluble ligands may differ functionally and as the system may have distinct importance in different regions of the brain and spinal cord or impact differently depending on the disease scenario (75, 216, 301).

There is increasing evidence for altered chemokine signaling in diverse CNS diseases such as AD or multiple sclerosis (310, 924) which may involve microglia activation and “sterile inflammation” (864). Microglial cells from AD brains may have elevated levels of CCR3 and CCR5 receptors (310). Increased levels of CCR5 receptors were also found in biopsic material from patients with early stages of multiple sclerosis; the CCR5 receptors were suggested to be somehow linked to microglia-dependent initiation of remyelination (924). Chemokine receptor functions in the CNS in general and in microglia in particular reveal broad implications with remarkable mechanisms and consequences. Activation of CCL21 receptors was reported to mediate microglial activation in the thalamus, which was related to neuropathic pain responses following spinal cord injury (1036). CCL2, its receptor CCR2, and other chemokines in the hippocampi of patients and laboratory animals have been linked to seizures and epilepsy (258).

CXCL10 and its receptor CXCR3 have been linked to various CNS pathologies (951). Studying the mechanisms by which this system mediates NMDA-induced neuronal toxicity in the hippocampus, the authors demonstrated that astrocytes and microglia cooperate to deliver the effect and that the deficiency in either the ligand or the receptor diminished or enhanced cell death depending on the tissue subregion and that microglia was the responsible cellular element by which this difference in neuronal vulnerability is organized. This report thus documented not only regional diversity in signaling consequences for the CXCL10/CXCR3 system per se, but an intimate link of this phenomenon to microglia, in this regard demonstrating the surprisingly refined organization of microglial chemokine physiology within anatomically and functionally circumscribed CNS divisions.

A similarly intriguing mechanism involving microglia, astrocytes, and a chemokine system had been unraveled earlier when studying astroglial contributions to glutamate toxicity (62). Binding of CXCL12 (also known as stromal cell-derived factor, SDF-1α) to its receptor CXCR4 in astrocytes causes InsP3 production, [Ca2+]i increases, and release/shedding of TNF-α. The binding of TNF-α to its receptor triggers a second wave of signaling (either in the same or in another astrocyte, through autocrine or propagating mechanism) that causes PGE2 production. The PGE2, in turn, induces the release of glutamate, which may serve in glial or glia-neuron communication, but which can also initiate neurotoxicity. In the latter situation, SDF-1α would also act on microglia, thus driving enhanced TNF-α release from both glial populations and ultimately causing massive glutamate release. Chemokines may thus reveal even more important roles in microglial activities as originally anticipated from their chemoattractive potential.

**B. TNF-α Receptors**

The action of TNF-α is mediated through two types of receptors, TNFR1 and TNFR2 (543). Stimulation of TNF-α receptors enhanced microglial phagocytic activity (971). Activation of microglial cells induced by brain lesions was
significantly reduced in transgenic mice lacking TNF-α receptors (95). Cultured mouse microglial cells release TNF-α following stimulation of TNF-α receptors 1, thus producing a positive autocrine loop, which can participate in microglial activation (483). In mice deficient in TNF-α receptors, microglial activation was very much reduced in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of Parkinson’s disease (854).

C. Interleukin Receptors

The IL-1 receptors are represented by IL-1 type I receptor (IL-1RI), IL-1 type II receptor (IL-1RII), and IL-1 receptor accessory protein (IL-1RAcP). A transcriptional analysis of purified cultured microglia showed weak expression of IL-1RI and stronger expression of IL-1RII, whereas the treatment with LPS significantly increased expression of IL-1RI, IL-1RII, and IL-1RAcP. Cultured rat microglial cells also expressed mRNA for IL-10 receptor (IL-10R1), which was upregulated following LPS stimulation; interestingly, IL-10 released by activated microglia or microglial cells also expressed mRNA for IL-10 receptor accessory protein (IL-1RAcP). A transcriptional analysis of purified cultured microglia showed weak expression of IL-10R1 by auto- or paracrine loops (504). In human microglial cells, mRNA transcripts for multiple interleukin receptors (IL-1RI, IL-1RII, IL-5R, IL-6R, IL-8R, IL-9R, IL-10R, IL-12R, IL-13R, and IL-15R) were detected (509).

It was suggested that IL-RII specifically regulates effects of IL-1β on microglial cells by binding the excess levels of this cytokine (721). The stimulation of IL-1 receptors in cultured human microglial cells by IL-1β (2 ng/ml) induced a slow increase in [Ca²⁺]i through an uncharacterized mechanism, which potentially involved both Ca²⁺ entry and intracellular Ca²⁺ release (326).

The activation of cultured mouse microglia with LPS induced expression of IL-2 receptors, which were absent in untreated cells. These newly acquired IL-2 receptors mediated effects of IL-2 on activated microglia by enhancing growth and viability. These effects were suppressed by treating cultures with anti-mouse IL-2 receptor beta-chain antibodies (797). The IL-2 receptors, coupled to Janus kinase 1 signaling pathway, were also shown to mediate effects of IL-15 on cultured microglia.

Spinal cord microglia showed sensitivity to IL-6, connected with a signal transducer and activator of transcription (STAT) pathway. This system was reported to be activated after a peripheral nerve injury and may contribute to development of mechanical allodynia and neuropathic pain (223).

XIII. PATTERN-RECOGNITION RECEPTORS

As the principal resident immune cells of the CNS, microglia serve at the first line of defense against exogenous threats, such as viruses and bacteria. The pattern recognition receptors (PRRs) abundantly expressed in microglia had evolved to detect invading infectious agents and to assist in the control of the adaptive immunity and govern the cooperative activities of effector cells (59, 350, 681). In mammals, host defense is based on innate and adaptive immunity. Adaptive (also acquired or specific) immunity occurred in jawed vertebrates and is carried by specialized helper, effector, and regulatory T-cell populations as well as antibody-producing B lymphocytes. The innate (also natural or native) immunity, the much older defense system, is based on physical and chemical barriers (e.g., epithelia), phagocytotic (e.g., macrophages) and natural killer (NK) cells, as well as a range of proteins with signal and effector functions, such as the complement system.

The repertoire of germline-encoded nonclonal receptors for detection of pathogen-associated molecular patterns (PAMPs) includes 1) lectin-type, mannose and β-glucan receptors (e.g., dectin-1); 2) nucleotide binding and oligomerization domain (NOD)-like receptors; 3) receptors characterized by a RNA helicase domain and two caspase-recruitment domains (CARD), collectively known now as RIG-I-like receptors (RLR); and 4) the Toll-like receptors (TLR) (see Refs. 299, 683, 786, 991 for review).

Reaching beyond pathogen detection, several PRRs, and specifically members of the TLR family claim a dual function by also detecting endogenous molecules that are generated, released, or modified upon tissue injury. These molecules are classified as damage- or danger-associated molecular patterns (DAMPs), or alarmins (63). Sharing receptors and inducing overlapping sets of genes, PAMPs identify strangers and DAMPs spell danger (470). Ultimately, both inform about disturbed homeostasis and initiate appropriate reactions in dangerous situations (561).

A. Toll-like Receptors

The Toll receptors were initially discovered in Drosophila, where they are important for embryogenesis, being involved in controlling dorsoventral polarity of the fly (17). Further studies demonstrated the role of Toll proteins in the immune defense of Drosophila (515). The mammalian analog, the Toll-like receptor, was first cloned in 1997 (578), and by now at least 11 human and 13 mouse TLRs have been described (519, 676). Toll-like receptors are coupled to a complex signaling pathways (which, for most of TLRs except TRL3 involves adaptor protein MyD88) that end at the transcription factor AP-1 and/or in the nuclear translocation of another transcription factor NF-κB (355, 426).

2The name “Toll receptors” was coined in the course of analyzing the data by the discoverer Christiane Nüsslein-Volhard, who exclaimed “Das war ja toll!” (355).
TLRs are type I integral membrane glycoproteins. TLR1/2, -6/2, -4, and -5 are located on the cell surface, whereas TLR3, -7, -8, and -9 are confined to endosomal compartments. They relate to the interleukin-1 receptor (IL-1R) family, due to homology in cytoplasmic Toll/IL-1R (TIR) domains (11, 840). TLRs are characterized by leucine-rich repeat motifs (for TLR4, e.g., within the extracellular portion), forming a horseshoe-like structure with its concave surface likely interacting with the agonists. TLRs are widely expressed in cells of the innate as well as adaptive immune system, but also in nonimmune cells and organs traditionally not considered as immunologically active (799).

In the brain, Toll-like receptors are mainly expressed in glia, although some of them can be detected in neurons (see Refs. 22, 125, 350, 469, 676 for review). The Toll-like receptors are widely expressed in microglia. Structural (mRNA and protein detection) and functional (responses to agonists) evidence was presented for TLR1 to 9 receptors, for coreceptors, like CD14 and for TLR signaling components, such as MyD88 in the mouse and human system by both in vitro and in vivo approaches (56, 96, 146, 226, 232, 233, 253, 340, 353, 422, 432, 508, 510, 677, 728, 738, 903, 918).

TLRs are essential for mounting an immune response against infection, with different receptors being sensitive to specific agents. TLR2 in heteromeric associations with TLR1 or TLR6 conveys signals to bacterial tri- and diacyl lipopeptides, lipoteichoic acid, and peptidoglycan. TLR3 recognizes virus-specific double-stranded RNA. TLR4 is activated by LPS, a major cell wall component of gram-negative bacterial strains. TLR5 responds to bacterial flagellin. TLR7 and TLR8 are sensors of single-stranded (viral) RNA, while TLR9 is sensitive to bacterial and viral unmethylated CpG DNA (11, 22, 125, 489, 677, 928). For several TLRs, such as TLR10 or -11, ligand specificities have not clearly been unravelled, and species differences exist for the functional expression of certain members, such as TLR7/8. How the recognition of structurally diverse ligands by a given TLR is organized cannot yet be explained in all cases, and surprising cross-recognition of apparently unrelated motifs can be noticed (928).

Numerous reports demonstrated the importance of TLRs in various CNS diseases including infection, trauma, stroke, neurodegeneration, and autoimmunity (28, 131, 511, 634, 641). The stimulation of TLRs triggers various programs of microglial activation and activates secretion of cytokines and chemokines (22, 676). The stimulation of TLR3, for example, induces the release of IL-6, IL-12, chemokine ligand 10, TNF-α, and IFN-β (13, 422); TLR2 controls the secretion of IL-6 and IL-10 (422); TLR9 regulates production of NO and TNF-α (398), whereas stimulation of TLR4 triggers release of IL-6 and TNF-α (676). Incidentally, TLR4 receptors are also involved in microglial activation following exposure to ethanol (274).

The Toll-like receptors directly control microglial activation. TLR2, for example, is important for microglial activation following damage to sensory neurons, and TLR2−/− mice showed reduced activation in the spinal cord following peripheral nerve injury (451). Intrathecal injection of antisense mRNA to TLR3 also reduced spinal cord microglia activation and suppressed tactile allodynia following spinal nerve ligation in rats (667). Similarly, tactile and thermal hypersensitivity following spinal nerve injury was attenuated in TLR4−/− mice and in mice and rats receiving TLR4 antisense oligodeoxynucleotides intrathecally (905). Alzheimer's disease is associated with significant elevation in TLR expression in the brain (518, 974). In experiments in vitro in primary mouse microglial cultures, treatment with β1–40 potentiated TLR2 and TLR4-mediated responses, while inhibiting the TLR9 (535). At the same time, all three receptors (TLR2, TLR4, and TLR9) stimulated the uptake of amyloid β-protein by microglial cells (890). The role of TLR2 in Aβ uptake was mediated through the induction of expression of formyl peptide receptor mFPR2 (mouse homolog of human GPCR formyl peptide receptor-like 1, FPR1L) (146). The levels of Toll-like receptors in CNS are generally upregulated in many other neurodegenerative diseases, including multiple sclerosis, Parkinson's disease, and ALS (see Ref. 676 for review), however the specific role of microglial TLRs in these forms of pathology remains unknown.

Microglial activation, in turn, upregulates the synthesis of Toll-like receptors (449, 566); similarly, the levels of TLRs in microglial cells are increased following hypoxia (668) and focal cerebral ischemia (in the latter case the highest increase was found for TLR2; Ref. 1039). The expression of Toll-like receptors can also be regulated by inflammatory factors; for example, TNF-α (which may act in both para- and autocrine manner) stimulates expression of TLR2 in cultured mouse microglia (888).

The Toll-like receptors also regulate microglial death following pathological activation. The TLR4 triggers microglial apoptosis via autocrine production of IFN-γ, whereas TLR2 is coupled to caspase-8-dependent apoptotic pathways (514). Similarly, TLR2 receptors were instrumental for microglial apoptosis following HIV-1 infection (20, 21). Conceptually, TLR-controlled microglial cell death can represent an intrinsic mechanism preventing microglial over-activation (676).

The TLRs are also involved in DAMP-induced signaling. Heat shock protein 60 (hsp60) was recently shown to drive neurodegeneration (513). Released from CNS cells undergoing necrosis or apoptosis hsp60 was found to drive a TLR4- and MyD88-dependent microglial activation, with NO synthesis as well as associated
### TABLE 5. Cytokines and chemokine receptors in microglia

<table>
<thead>
<tr>
<th>Receptor Type</th>
<th>Experimental Preparation/Technique</th>
<th>Pharmacology</th>
<th>Properties and Functional Relevance</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemokine receptors</strong></td>
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<tr>
<td>CCR1, CCR2, CCR5</td>
<td>Rat/primary culture/RT-PCR, Ca(^{2+}) imaging</td>
<td>Agonists: CCR2-MCP-1, CCR5-MIP-1α, RANTES</td>
<td>Stimulation of CCR2 and CCR5 triggered ([\text{Ca}^{2+}]_i) transients; activation of microglia by LPS increased these ([\text{Ca}^{2+}]_i) responses.</td>
<td>73, 74</td>
</tr>
<tr>
<td>CCR1, CXCR3, and CCR3</td>
<td>Human/primary culture/RT-PCR, immunocytochemistry</td>
<td></td>
<td>Receptor expression was increased following treatments with TNF-α and IFN-γ.</td>
<td>285</td>
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<tr>
<td>CCR5</td>
<td>Neonatal rat forebrain/RT-PCR</td>
<td></td>
<td>Hypoxia/ischemia increased CCR5 mRNA expression.</td>
<td>177</td>
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<tr>
<td>CCR5</td>
<td>Human hippocampus/acute slices/whole cell voltage-clamp</td>
<td></td>
<td>Short application of MIP-1α increased the amplitude of K(^+) currents 3-fold.</td>
<td>77</td>
</tr>
<tr>
<td>CXCR3</td>
<td>Mouse/primary cultures/whole cell voltage-clamp</td>
<td>Agonist: CCL21T</td>
<td>Activation of CXCR3 receptors activated volume-regulated Cl(^-) channels.</td>
<td>747</td>
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<td><strong>TNF-α receptors</strong></td>
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<tr>
<td>TNFR1 and TNFR2</td>
<td>Mouse/knockout and disease models/primary culture, isolated cells/protein detection, also of soluble TNFR</td>
<td>Agonist: TNF-α</td>
<td>Reduced microglial activation along with increased neuronal damage in receptor-deficient mice challenged by ischemia and seizures, upregulation of TLR2.</td>
<td>95, 353, 357, 888</td>
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<td><strong>Interferon (IFN) receptors</strong></td>
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<tr>
<td>IFN(\gamma)R</td>
<td>Mouse/primary culture, slice preparations</td>
<td>IFN-γ</td>
<td>Regulation and massive reorganization of induced cytokine and chemokine production, upregulation of MHC II, induction of immunoproteasome, induction of neuronal markers and a neuroprotective phenotype, instruction of a M1-like reactive phenotype.</td>
<td>110, 112, 349, 363, 585, 866, 950</td>
</tr>
<tr>
<td>IFNAR, type I IFN receptor</td>
<td>Mouse/primary culture, knockout in vivo</td>
<td>IFN-β</td>
<td>Suppression of glutamate and superoxide production, regulation of gene expression, involvement in the control of microglial functions in vivo and ex vivo.</td>
<td>349, 730</td>
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<tr>
<td><strong>Interleukin receptors</strong></td>
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<tr>
<td>IL-1R1/IL-1R2, and related molecules</td>
<td>Human, mouse, rat/primary cultures, tissues/mRNA detection, antibody blockade</td>
<td>IL-1β</td>
<td>Induction of inflammatory mediators, like PGE(_2) and IL-6, activation of NF-κB, p38, JNK, and ERK1/2.</td>
<td>19, 184, 509, 576, 721</td>
</tr>
<tr>
<td>IL-2Ra/β/γ ((\gamma_c))</td>
<td>Mouse, rat/primary cultures/ mRNA, partially protein detection</td>
<td>IL-2 as agonist, blocking antibodies against receptor components</td>
<td>Augmented NO production, increased growth and viability, indirect evidence for recruitment of MHC II(^+) and CD11b(^+) microglia to lesions.</td>
<td>349, 352, 717, 782, 707</td>
</tr>
<tr>
<td>IL-4R</td>
<td>Mouse, rat/primary cultures, brain and nerve tissue/gene profiling, detection of protein</td>
<td>IL-4</td>
<td>Induction of cytokines and chemokines with M2-like profile, enhanced amyloid β clearance, regulation of scavenger receptor expression, oligodendrocyte markers and dendritic cell (DC) marker, CD11c, downregulation of proinflammatory cytokines, induction of a M2-alternatively activated phenotype.</td>
<td>110, 112, 349, 585, 829, 862, 950</td>
</tr>
<tr>
<td>IL-10R</td>
<td>Human, rat/primary cultures, brain tissue/mRNA, gene profiling, protein detection</td>
<td>IL-10</td>
<td>Induction of a M2-deactivated phenotype.</td>
<td>331, 349, 509, 585</td>
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*Continued*
axon loss and neuronal death, consequences which again would deliver hsp for a vicious cycle. Such a mechanism could apply to various types of CNS damage where innate immunity participates by TLR4-mediated recruitment and TLR4-organized release of toxic compounds (513). Since it can bind LPS, hsp60 could additionally lower the threshold for triggering responses to the PAMP in an infectious setting (680), with this probably being the beneficial facet of its TLR4 interaction. TLR4 binding as well as cotrafficking properties and roles in the initiation of an immune response have been assigned also to hsp22, hsp70, hsp72, hsp90, gp96 (hsp90b1), chlamydial hsp60, and Francisella tularensis hsp DnaK (24, 26, 43, 251, 470, 793, 925, 926).

Similarly, the high-mobility group box 1 protein (HMGB1, HMG1, amphoterin) was found to inhibit amyloid β/α252 uptake (891) and to amplify inflammatory responses in multiple sclerosis and its animal model, experimental autoimmune encephalomyelitis (18). A direct link of neurodegenerative processes in AD to microglial TLR4 became obvious when amyloid β assembly was found to bind to CD14, the prominent coreceptor for LPS signaling via TLR4 (270). CD14 and TLR-dependent mechanism may promote phagocytotic clearance of Aβ-containing plaque material and/or participate in inflammatory responses of microglia (270, 494, 751, 890). Pronounced CD14 immunoreactivity was, indeed, observed for microglia in vicinity to AD lesion sites in sections from AD brains (532). Importantly, a microglial CD36-TLR4-TLR6 complex has now been identified to promote sterile inflammation in response to amyloid-β (864).

Finally, it has to be emphasized that TLR signaling can be neuroprotective, not only by driving clearance of infectious agents, but by organizing CNS-intrinsic as well as immune system-mediated (T cell-involving) support of neural cell survival, tissue preservation, and CNS functioning (325, 350). The critical question regards the mechanisms by which TLRs could engage detrimental or beneficial programs.

XIV. OTHER RECEPTOR SYSTEMS

A. Calcium Receptors

The plasmalemmal calcium receptor (CaR), identified in 1993 by Brown et al. (91), is a member of a G protein-coupled receptors of seven-transmembrane domain topology, which is activated by relatively subtle (though physiological) changes in extracellular Ca2+ concentration, and plays a critical role in the regulation of serum Ca2+ homeostasis (92). The activation of CaR, depending on cellular context, triggers multiple signaling cascades, with the most common being InsP3 production with subsequent InsP3-induced Ca2+ release or regulation of plasmalemmal channels activity (987, 1020).

The expression of CaR in cultured rat microglial cells was demonstrated at both mRNA and protein levels (143). The activation of these receptors either by an increase in [Ca2+]o from 0.75 to 3 mM or by CaR agonists (neomycin or “CaR activator” R-467) increased the open probability of 84 pS Ca2+-dependent K+ channels (143). The CaR receptor may also be involved in the regulation of IKR, because fluctuations in [Ca2+]i are known to modulate the activity of IKR channels in microglia (402). Incidentally, CaR in neurons may also be activated by β-amyloid peptides (1021), whether this may also be the case for microglial CaRs remains unknown.

B. Leukotriene Receptors

Cultured rat microglia expressed mRNAs for cysteinyl leukotrienes (CysLTs) receptors of CysLT1 and CysLT2 types, and stimulation of these cells by CysLT triggered a [Ca2+]i rise, which in turn triggered the release of purines (mostly ATP) and CysLT (32).
C. Notch Receptors

Notch-1 receptors were identified in amoeboid microglial cells in the early postnatal brain; the maximal expression was found in the corpus callosum, which contains the highest concentration of invading microglia (119). Expression of Notch-1 receptor was downregulated with age and can be stimulated by LPS. The notch-1-
TABLE 7. Other receptor systems

<table>
<thead>
<tr>
<th>Receptor Type</th>
<th>Experimental Preparation/Technique</th>
<th>Properties and Functional Relevance</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium receptors, CaR</td>
<td>Rat/primary cultures/RT-PCR, whole cell voltage clamp</td>
<td>Stimulation of CaR by activators (neomycin or a R-467) or by an increase of Ca(^{2+}) o from 0.75 to 3 mM increased the open state probability of 84 pS Ca(^{2+})-activated K(^{+}) channel.</td>
<td>143</td>
</tr>
<tr>
<td>Leukotriene receptors, CysLT1 and CysLT2</td>
<td>Rat/primary cultures/Ca(^{2+}) imaging</td>
<td>Stimulation of microglia with cysteinyl leukotrienes triggered [Ca(^{2+})](_i) rise.</td>
<td>32</td>
</tr>
<tr>
<td>Notch-1 receptor</td>
<td>Rat/corpus callosum/immunocytochemistry</td>
<td>Notch-1 receptors were identified in amoeboid invading microglia in corpus callosum. Notch-1 receptors are suggested to regulate production and secretion of NO and cytokines in activated microglia.</td>
<td>119, 335</td>
</tr>
<tr>
<td>Complement receptors, C3a, C5a</td>
<td>Human/mouse/primary cultures/RT-PCR, immunocytochemistry/Ca(^{2+}) imaging</td>
<td>Activation of C5a and C3a receptors induced Ca(^{2+}) signaling via intracellular Ca(^{2+}) release. The C5a receptors also control microglial motility.</td>
<td>486, 605, 653</td>
</tr>
<tr>
<td>Thrombin receptors, PAR1,3,4</td>
<td>Rat, mouse/primary cultures/Ca(^{2+}) imaging</td>
<td>Thrombin triggered Ca(^{2+}) signals via activation of Ca(^{2+}) release from the ER.</td>
<td>31, 602</td>
</tr>
<tr>
<td>Macrophage colony-stimulating factor receptors (M-CSFRs)</td>
<td>Mouse/primary culture</td>
<td>Activation of M-CSFR stimulates release of NO and cytokines.</td>
<td>503–505</td>
</tr>
<tr>
<td>Epidermal growth factor receptors (EGFRs)</td>
<td>Mouse/primary culture/whole cell voltage clamp</td>
<td>Stimulation of microglial cells with EGF increase the amplitude of IK(_{\text{on}}) via PTX-sensitive G protein cascade.</td>
<td>401</td>
</tr>
<tr>
<td>CD200 receptors</td>
<td>Rat, mouse/brain sections, primary culture/RT-PCR</td>
<td>CD200 receptors downregulate microglial activation.</td>
<td>986a, 1000</td>
</tr>
<tr>
<td>Lysophosphatidic acid receptors, LPA(_1), LPA(_3)</td>
<td>Rat, mouse/primary culture/RT-PCR, Ca(^{2+}) imaging</td>
<td>Mouse microglia expressed LPA1 receptors; rat LPA3 receptors. Exposure of microglial cells to LPA triggered [Ca(^{2+})](_i) transients associated with ER Ca(^{2+}) release.</td>
<td>303, 601</td>
</tr>
<tr>
<td>Formyl peptide receptors, FPR1, FPR2</td>
<td>Mouse/primary culture/Ca(^{2+}) imaging</td>
<td>Activation of microglia increases expression of FPR1/2; activation of receptors triggers [Ca(^{2+})](_i) elevation.</td>
<td>182, 913</td>
</tr>
<tr>
<td>Sigma-1 receptors</td>
<td>Human, rat/primary culture/Ca(^{2+}) imaging, migration assays, pharmacological assays</td>
<td>Activation of sigma-1 receptors inhibits ATP-mediated Ca(^{2+}) signals and suppresses microglial inflammatory and migratory responses.</td>
<td>312, 346</td>
</tr>
</tbody>
</table>

See text for definitions.

dependent signaling was suggested to be involved in regulation of cytokines and NO production (119). The stimulation of Notch receptors in activated microglia decreases NO production and secretion of inflammatory cytokines (335).

D. Complement Receptors

The complement fragments C3a and C5a are anaphylotoxins associated with the activation of complement system, which represents an important part of the immunological response (328). Receptors for C5a and C3a fragments are classic seven-transmembrane domain G protein-coupled metabotropic receptors linked to several intracellular signaling cascades, including PLC/InsP\(_3\)-dependent pathway (315, 383).

C5a receptors were identified at both transcriptional and protein levels in cultured human microglia (486). The expression of complement C5a receptors is greatly potentiated in the inflamed brain, although its expression in healthy CNS seems to be minimal (308). Expression of C5a receptors in spinal cord microglia was significantly increased after peripheral nerve injury (337).

The C5a receptors are implicated in the control of microglial motility. In murine microglial cells in culture, the application of C5a rapidly (within seconds) induced a ruffling of microglial membranes, which was followed by the extension of lamellipodia and the rearrangement of the actin cytoskeleton (653). The motility reactions were independent from [Ca\(^{2+}\)]\(_i\) but were blocked by PTX and cytochalasin B, indicating a critical role for G proteins and cytoskeleton (653). Both C5a and C3a receptors triggered Ca\(^{2+}\) signals in cultured mouse microglia (605); these signals demonstrated biphasic kinetics, which represented an initial InsP\(_3\)-induced Ca\(^{2+}\) release (sensitive to thapsigargin) from the ER store and the subsequent store-operated Ca\(^{2+}\) entry (sensitive to Ca\(^{2+}\) removal from extracellular solution). The action of both receptors...
The newly formed NH2-terminal sequence stretch of C5a receptors are also coupled to K+ channels. The stimulation of C5a receptors triggered rapid (in ~20 s) and transient upregulation of K+ conductance in primary cultured mouse microglia (401) that was blocked after incubation with 1 μg/ml PTX for 30 min prior to the recording. The complement-induced stimulation of K+ currents, however, was not linked to regulation of microglial motility (401).

The C5a receptors participate in microglial-related pathogenesis of neuropathic pain. The complement fragment C5a applied intrathecally induced cold pain, whereas an injection of C5a antagonist (synthetic cyclic AcF-[OPdChaWR] peptide) reduced cold allodynia in animals with peripheral nerve injury (337).

C1q, the recognition subcomponent of the classical complement activation pathway, in the CNS is produced in rat brain microglia (but not in astrocytes or neurons) within 24 h after an ischemic insult (798). C1q acts in an autocrine fashion by triggering the release of IL-6, TNF-α, and NO as well as by inducing the oxidative burst in rat primary microglial cells. These data indicate that 1) extrinsic plasma C1q is involved in the initiation of microglial activation in the course of CNS diseases with blood-brain barrier impairment and 2) C1q synthetized and released by activated microglia is likely to contribute in an autocrine/paracrine way to maintain and balance microglial activation in the diseased CNS tissue (262).

E. Thrombin Receptors

Thrombin (factor IIa) is best known for cleaving fibrinogen as a central event in the coagulation cascade (495). Thrombin is suspected to cause severe damage to neural cells (318, 646, 977) and to act as a link between injury, hemostasis, and inflammation (174, 646, 877). Upon blood-brain barrier disruption, plasma components can develop a significant impact on glial and neuronal cells (317, 318, 969).

Search for the direct molecular target of thrombin on the surface of platelets and other cells resulted in the discovery of the proteinase-activated receptors (PARs). The protease cleaves the extracellular portion of a PAR (832). The newly formed NH2-terminal sequence stretch subsequently acts as an intramolecular (“tethered”) ligand that binds to an extracellular receptor domain to cause cytosolic events, largely through G proteins (679, 857). Four PARs have been identified thus far. Thrombin was shown to act on PAR1, PAR3, and PAR4, whereas PAR2 accepts trypsin or proteases with trypsin-like substrate preference (85, 164, 173, 665).

Microglial cells respond to thrombin preparations with the release of NO, various cytokines, and chemokines, including TNF-α, IL, IL-12(p40), KC, the mouse equivalent of GROα (CXCL1), monocyte chemoattractant protein 1 (MCP-1, CCL2), macrophage inflammatory protein 1α and 1β (MIP-1α/1β, CCL3/CCL4) or “regulated upon activation, normal T-cell expressed and secreted” (RANTES, CCL5) (452, 602, 779, 878). Thrombin triggers Ca2+ signals in microglial cells; these Ca2+ responses have PAR-typical features (fast kinetics, sensitivity to hirudin, and use-dependent destruction), suggesting functional expression of the respective receptors. All four PARs were also found in rodent microglia at both mRNA and protein levels (31, 602). Toxic consequences of thrombin administration in vivo and association with CNS lesions support the concept that the protease may account for neuronal impairment and that microglial mediators could play a critical role (126, 224, 225, 317, 318, 348, 507, 675, 874, 1012). The Par-1 receptors activation was suggested to be involved in microglial inflammatory response in Parkinson’s disease. However, not all of the microglial activities assigned to thrombin may actually relate to the proteolytic activation of PARs (354, 606, 989).

F. Macrophage Colony-Stimulating Factor Receptors

The M-CSF is a hematopoietic cytokine, which is present in both neurons and glia. The corresponding receptor, the M-CSF receptor (M-CSFR, encoded by proto-oncogene c-fms), is expressed in activated microglial cells surrounding neuritic plaques in AD and also in microglia after traumatic or ischemic brain insults (593–595). The activation of M-CSFR stimulates release of NO and proinflammatory cytokines and also promotes phagocytosis and the uptake of Aβ (593–595). Overexpression of M-CSFR in microglia had a significant neuroprotective effect in organotypic hippocampal slices subjected to excitotoxic stress (592).

G. Epidermal Growth Factor Receptors

Epidermal growth factor receptors (EGFRs) belong to the ErbB family of receptor protein kinases (97). The functional expression of these receptors was demonstrated in cultured mouse microglial cells; the exposure of these cells to EGF resulted in rapid increase in the amplitude of K+ conductance (401). The EGF-induced modulation of K+ channels was mediated through PTX-sensitive G proteins; similar signaling cascades activated by EGF were reported for fibroblasts (181) and hepatocytes (567).

H. CD200 Receptors

CD200 receptors (CD200Rs), activated by surface glycoprotein CD200 (or OX2), are expressed in various
types of myeloid cells and are involved in the regulation of phagocytic activity (1000). The CD200Rs were also identified in rodent microglial cells and are generally involved in downregulation of microglial activation (986a).

I. Lysophosphatidic Acid Receptors

The actions of signaling phospholipids lysophosphatidic acid (LPA), sphingosine-1-phosphate (S1P), and sphingosylphosphorylcholine (SPC) are mediated through an extended family of G protein-coupled receptors (158, 171, 693). The LPA receptors include three molecularly distinct types, LPA₁/Edg2, LPA₂/Edg4, and LPA₃/Edg7. Rat primary cultured microglia expressed LPA₃ mRNA receptors, whereas cultured mouse cells expressed mRNA specific for LPA₁ receptor (303, 601). Treatment of both cultures with LPA resulted in [Ca²⁺]i elevation, which was mostly produced by Ca²⁺ influx in rat microglia, whereas in mouse microglia, the dominant pathway was associated with intracellular Ca²⁺ release (600, 601). Activation of LPA₃ receptors in rat cultured microglia also caused the release of ATP; the latter in turn induced membrane ruffling via activation of P2Y₁₂ receptors (303).

J. Formyl Peptide Receptors

There are two subtypes of receptors to bacterial N-formylpeptides: the high-affinity receptors FPR1 and the low-affinity receptor FPR2 (500, 600). Both receptors belong to GPRC class, and the expression of both (albeit at low levels) was found in primary mouse microglia (182). Treatment of microglial cells with LPS or Aβ markedly upregulated expression of FRP1/FRP2; in the stimulated cells, activation of formyl peptide receptors triggered [Ca²⁺]i elevation (182). The FPR2 expression is also significantly upregulated by the exposure of primary microglial cultures to TNF-α and IL-10 (418). This was correlated with an increased chemotaxis to β₂₄ and further FPR2-mediated endocytosis of amyloid β-protein (418).

The FPRs may also act as receptors to prion protein peptides (501). The microglial cells respond to PrP₁₀₆₋₁₂₆ by generation of slow [Ca²⁺]i elevation (372, 836), which was suggested to involve voltage-gated Ca²⁺ channels (see sect. VIII and Ref. 836), yet the role of FPRs has to be explored further.

K. Sigma Receptors

Sigma receptors of two subtypes, sigma-1 and sigma-2, are abundantly expressed in the brain; they are regulating Ca²⁺ release from the ER (364). Sigma-1 receptors were identified in primary microglial cultures prepared from embryonic human (312) and neonatal rat (346) brains. Activation of sigma-1 receptors suppresses ATP-induced Ca²⁺ signaling, membrane ruffling, as well as inflammatory and migratory responses in cultured microglial cells (346).

XV. MICROGLIAL PLASMALEMMAL TRANSPORTERS

A. Xc Transporters

Microglia, activated by various stimuli [e.g., by culture conditions (718), or by secreted amyloid precursor protein (35) as well as by aggregated β-amyloid protein₁₋₄₀ (740), or by LPS (36)], release substantial amounts of glutamate, which may contribute to the excitotoxic damage of the brain. Microglial release of glutamate occurs almost exclusively through glutamate-cystine antiporter Xc, which exchanges glutamate for cystine according to the respective concentration gradients; as a consequence, removal of extracellular cystine completely blocks microglial glutamate release (35). The abnormal activation of Xc antiporter in reactive microglia may be linked to massive demand for cysteine/cysteine for replenishing glutathione, the latter being driven down by oxidative bursts accompanying microglial activation and brain insults (34, 36).

The release of glutamate from microglia via Xc transporter was implicated in oligodendrocytes damage and death; the activation of microglia by LPS triggered prominent oligodendrocytes extinction in the whole-mount rat optic nerve. This death was prevented by inhibition of Xc transporter or blockade of AMPA/KA receptors (222).

B. Glutamate Transporters

The overall involvement of microglial glutamate transport in glutamate homeostasis in the brain is relatively minor as it accounts for <10% of astroglial glutamate uptake (710). Baseline expression of GLT-1 and GLAST in naive animals is primarily localized in astrocytes (824, 1010). At the same time, insults to the CNS may upregulate microglial glutamate transporters, thus increasing their ability to accumulate glutamate in the regions of injury. In post mortem human tissue, microglia activated following stroke expressed EAAT-1/GLAST (as determined by immunohistochemistry) during the first week after the insult; this expression was detected in both ramified and amoeboid microglial cells, and was down-regulated at later stages (57). The expression of EAAT-1/GLAST transporters was limited to microglia, as peripheral macrophages did not have the glutamate transporter. Similarly, the expression of the EAAT-1/GLAST transporters was transiently upregulated in microglial cells at early
activated stages following traumatic brain injury in humans, which may indicate their neuroprotective potential (55). A rapid de novo expression of both GLAST and GLT-1 transporters was identified in rat microglia following controlled cortical impact injury; the expression starts at 4 h and reaches the steady-state in 48 h after the injury (948). The EAAT-1 transporters were also found in microglial cells of HIV-1-positive patients (945).

An increase in EAAT-1 expression was also observed in microglial cells in samples from the cerebral cortex, striatum, thalamus, and cerebellum from 14 patients with prion disease (Creutzfeldt-Jakob disease and fatal familial insomnia), which may indicate a certain neuroprotective adaptation of microglia in chronic prion infections (155).

The GLT-1/EAAAT-2 receptors were found in activated microglia in cultures and in situ in facial nuclei of adult rats, which underwent unilateral facial nerve axotomy (533). Similarly, EAAT-2/GLT-1 (but not EAAT-1/GLAST) transporters were identified in primary cultured rat microglial cells, which also demonstrated the $^{[14]}$C-glutamate uptake (627), which was blocked by dihydrokainate. Microglial $^{[14]}$C-glutamate uptake was stimulated by incubation of cultured microglia with a neuronal conditioned medium, suggesting some degree of neuronal control over glial glutamate transporters (628). Both EAAT-1/GLAST and EAAT-2/GLT-1 were detected in rat cultured spinal microglia, and their activity was inhibited by activation of P2X$_7$ receptors (619). In brain autopsy tissue specimens of HIV-1-infected patients, the expression of EAAT-2 by activated microglia suggests a compensatory effect that protects neurons from glutamate neurotoxicity (1011).

The glutamate uptake systems in microglial cells can also have a self-defense function. The GLT-1 transporter is upregulated in microglial cells exposed to the Herpes simplex virus of both HSV-1 (encephalitis) and HSV-2 (meningitis) types (708). Microglial cells also demonstrated a higher resistance to HSV viruses compared with neurons and astrocytes (708). This higher resistance can result from upregulated GLT-1 transporters, which in turn are instrumental in increasing microglial glutathione content, as the microglial glutamate uptake was shown to be directly coupled to glutathione synthesis (710). The complement-derived anaphylatoxin C5a also increases microglial GLT-1 expression and glutamate uptake in a TNF-α-independent manner (709).

The main functional role of the glutamate transporters, especially in glial cells, is to maintain low glutamate concentration in the extracellular space of the CNS (188, 773). However, the reverse glutamate transporter has been suggested as the mechanism of the neurotoxic rise in extracellular glutamate concentration during brain anoxia (524). Transport of glutamate is driven by transmembrane ion gradients, and translocation of a single glutamate molecule requires influx of three Na$^{+}$ and one H$^{+}$ coupled with efflux of one K$^{+}$ (1028), thus producing a net inward current (456). Since glutamate release can be monitored electrophysiologically, a possible modulatory effect of a synthetic 11-amino-acid analog of amyloid-β peptide (Aβ$_{25–35}$) on electrogenic glutamate transport in primary cultured rat microglia was investigated in vitro (651). It was found that Aβ enhanced glutamate release from primary cultured rat microglia via the Na$^{+}$-dependent glutamate transporter, which was also activated by extracellular K$^{+}$.

C. Glucose Transporters

The exclusive expression of glucose transporter 5 (GLUT5) was demonstrated in microglial cells from sections of human and rat brains (697). Expression of these transporters was even suggested to be a specific marker of microglial cells in situ (791). The primary isoforms in brain are GLUT1, detected at high concentrations as a highly glycosylated form (55 kDa) in blood-brain barrier, and also as a less glycosylated, 45-kDa form, present in parenchyma, predominantly in the glia; GLUT3 in neurons; and GLUT5 in microglia (952). It was also shown that the 45-kDa form of glucose transporter 1 (GLUT1) is localized in oligodendrocytes and astrocytes but not in microglia in the rat brain (1023).

D. Monocarboxylate Transporters

An extended family (14 members) of monocarboxylate transporters (MCTs) are represented by proton-dependent transporters, which provide for transmembrane transport of various energy substrates including lactate (345). Three members of the MCT family, the MCT1, MCT2, and MCT4, are found in the central nervous system: the MCT1 in endothelial cells and in astrocytes, MCT2 in neurons, and MCT4 in astroglia (720). Microglia activated following compression-induced ischemia demonstrated significant expression of MCT1 and MCT2, with the latter possibly involved in supply of microglial cells with energy substrates (609).

E. ATP-Binding Cassette Transporters

The ATP-binding cassette (ABC) transporters belong to an extended superfamily, which comprises seven subtypes (designated as ABCA to ABCG transporters; Ref. 427). The expression of ABC transporters was reported in cultured rat microglia, where they can be involved in the release of purines and cysteinyl leukotrienes (32). An expression of ABCA1 transporters was found to be upregulated in cultured microglia treated with mutant (Swedish) amyloid precursor protein (468). High levels of expression of ABC transporter G4 (ABCG4) protein was
found in microglial cells associated with neuritic plaques in the AD human brains (939). The ABCG4 is responsible for cholesterol transport and may be involved in AD-associated alterations of lipid metabolism.

F. Chloride Transporters

Chloride channels and transporters were characterized by whole cell recording and RT-PCR (1040). The repertoire of chloride-conducting pathways in murine primary microglial cells includes the K-Cl cotransporters, KCC1, KCC2, KCC3, and KCC4, as well as swelling-activated chloride channels. KCl cotransporters induce K+ and Cl− efflux when swelling-activated chloride channels are activated.

G. Bicarbonate Transporters

The presence of operational Na+/HCO3− cotransporter and/or Na+-dependent Cl−/HCO3− exchanger was found in cultured microglia (260). Anion exchange-mediated chloride/bicarbonate transporter is a major component in the regulation of intracellular pH. The functional consequences of changes in anion exchange structure may range from acidosis, disturbance of cytoskeleton integrity, and untimely or impaired recognition of cells by components of the immune system, such as microglia. Microglial cells express a distinct set of pH regulatory carriers that control for a defined level of intracellular pH (80).

H. H+/K+ pump

When monitoring K+ concentration in cultured rat microglia with ion-selective microelectrodes, Shirihai et al. (831) found that the H+/K+ ATPase plays the leading role in regulation of potassium and proton gradients. The Na+/K+ ATPase, in contrast, was not functionally active. The K1/2 for microglial H+/K+ pump was ~3.7 mM, thus being in the physiological range of extracellular K+ concentrations (831).

I. Proton Pump

The proton pump was suggested to participate in volume regulation in microglia, macrophages, and T lymphocytes (538). Proton pump inhibitors possess anti-inflammatory effects and can decrease human microglial and monocytic neurotoxicity. In addition, proton pump inhibition combined with nonsteroidal anti-inflammatory drugs may be effective in the treatment of a broad spectrum of neurodegenerative diseases associated with activation of microglia (361).

XVI. MIGRATION AND MOTILITY

Cell migration plays a central role in many physiological and pathophysiological processes, including immune defense and wound healing. Microglial cells exhibit two types of movement activity: in the ramified form, they actively move their processes without translocation of the cell body. In the amoeboid form, they also move their processes, but in addition the entire cell can migrate within brain tissue including translocation of their soma. Migration either occurs during development, when cells of monocytic origin migrate into the brain or after a pathological insult when ramified microglia transform into the amoeboid form and migrate to the site of injury. Most studies were performed in cell cultures from postnatal mice and rats. These cells might therefore resemble the amoeboid microglia early in postnatal development. It is not yet explored whether these forms of motility, migration, and process movement are distinctly regulated. Moreover, there is also no a systematic study comparing the migratory behavior of microglia during development and in response to pathology. There are many candidate molecules that serve as signals for pathological events to microglia including ATP (190, 384), cannabinoids (973), morphine (892), the chemokine CCL21 (747), lysophosphatidic acid (806), and bradykinin (397). In addition, ion channels and transporters play an important role in cell migration (Fig. 13), such as K+ channels, Cl− channels, Na+/H+ exchanger, Cl−/HCO3− exchanger, and Na+/HCO3− cotransporter, which all are linked to actin cytoskeleton (813, 814). Although the roles of these mechanisms in microglial migration were not analyzed in detail, there is evidence for the contribution of Cl− and K+ channels, as well as the Na+/Ca2+ exchanger and Ca2+-permeable stretch-activated cation channels (397).

A. Control of Processes Motility In Vivo

Microglial processes are not static, but rapidly move within the brain parenchyma as shown by real-time imaging using two-photon laser microscopy. Process movements are highly random in direction. At rest, however, these process movements do not result in overt cellular migration (190, 645). Processes rapidly move towards a lesion as experimentally triggered by a laser. This rapid chemotactic response can be mimicked by local injection of ATP and can be inhibited by the ATP-hydrolysing enzyme apyrase or by blockers of G protein-coupled purinergic receptors and connexin channels, which are highly expressed in astrocytes. It was speculated that ATP acts first on astrocytes, which then release ATP and trigger the microglial response (190). The responsible receptor subtype is P2Y12 (see sect. X). Microglia in mice deficient for P2Y12 show significantly diminished directional branch
extension toward sites of damage. Moreover, P2Y_{12} expression is robust in the “resting” state, but reduced after microglial activation.

**B. Control of Microglial Migration**

Under pathological conditions such as lesion, stroke, neurodegenerative disorders, and tumor invasion, activated microglia migrate to the site of injury. Using time-lapse confocal microscopy in acutely isolated living slices from adult brain-injured mice, extensive migration of perilesional microglia was apparent by 24 h after injury and peaked at 3 days with an average migration speed of ~5 μm/min and peak speeds >10 μm/min. This was not a collective, directed migration towards the lesion edge as might be expected in the presence of chemoattractive gradients, but rather a random walk migration (121, 122). In the ex vivo retina, focal laser injury leads to a transition of microglia from a fixed to a migratory phenotype while retaining their ramified morphology (505). There are a number of receptor systems that control the migration of microglial cells. Most studies were performed in culture using migration assays based on twin chambers separated by a membrane. It is important also to distinguish between random migration and chemotaxis, i.e., directed migration along a chemical gradient.

**1. Neurotransmitters**

ATP had been reported to be a chemoattractant for microglial cells (384, 419, 673). It was reported that ATP-induced microglial migration was inhibited by PTX, suggesting that activation of G_{16} proteins induced by P2Y receptors is part of the intracellular signaling cascade controlling microglial migration (384). Extracellular ATP induces membrane ruffling and chemotaxis of microglia mediated by the G_{16} protein-coupled P2Y_{12} receptor. Thus P2Y_{12} receptors control both chemotaxis of amoeboid microglia and process motility of ramified microglia as described above. P2Y_{12} receptor-mediated activation of the PI3K pathway and increased Akt phosphorylation are required for microglial chemotaxis in response to ATP (419). Akt phosphorylation was reduced upon chelation of extracellular calcium, suggesting that ionotropic P2X receptors are also involved in microglial chemotaxis acting through the PI3K pathway. Pharmacological blockade or knockdown of the P2X_{4} receptor in microglia by RNA interference suppressed the microglial chemotaxis, suggesting that P2X_{4} as well as P2Y_{12} receptors are involved in ATP-induced microglial chemotaxis (673). As for ADP-induced chemotaxis of microglia, the role for P2Y_{12/13} receptors and the β-integrin was reported (633). Activation of purinergic receptors triggers a K+ outward conductance (82). The induction of P2Y-associated outward potassium current in microglia is required for the ATP-induced chemotactic response and baseline motility (1004).

The CD39 and CD73 are the dominant cellular ectonucleotidases of microglial cells that degrade nucleotides to nucleosides, including adenosine. These ectonucleotidases play an important role in the ATP-induced chemotaxis. ATP failed to stimulate P2 receptor-mediated migration in CD39-deficient microglia. However, the effects of ATP on migration in CD39-deficient microglia were restored by costimulation with adenosine or by addition of a soluble ectonucleotidase. This indicates that costimulation of purinergic and adenosine receptors is a requirement for microglial migration and that the expression of CD39 controls the ATP/adenosine balance. CD39 deletion has also an impact in a pathological context, since the accumulation of microglia/macrophages in a model of ischemia was markedly decreased in CD39-deficient animals (266).
As described above, microglial cells express AMPA-type and metabotropic glutamate receptors. It was recently demonstrated that glutamate triggers microglial membrane ruffling and migration to a source of glutamate in cell culture and in spinal cord slices. This chemotaxis event is mediated by both AMPA and metabotropic glutamate receptors (529). Dopamine and epinephrine also enhanced migratory activity (267).

2. Chemokines

As mentioned previously, chemokines are expressed by neurons and macroglia as well as microglia and, when released after cellular damage, may act as major chemoattractants for microglia. Damaged neurons express the chemokine CCL21, which acts as a chemotactic substrate for microglial cells (68). A local application of CCL21 for 30 s to microglial cells in culture and in brain slices triggered a Cl− conductance with lasted for tens of minutes, and the chemotaxis response was sensitive to Cl−-channel blockers. The response is mediated by CXCR3 receptors as revealed by studies involving CXCR3 and CCR7 receptor-deficient animals. Therefore, the CCL21-induced Cl− current is a prerequisite for the chemotaxis response mediated by the activation of CXCR3 but not CCR7 receptors, indicating that in brain CCL21 acts via a different receptor system than in lymphoid organs (747).

Fractalkine or CX3CL1 is released by both neurons and astrocytes, while the CX3CL1 receptor (CX3CR1) is predominantly expressed in microglial cells. Both the movement of processes under resting conditions and in response to tissue injury as well as the migration of microglia in response to injury was slowed down in CX3CR1-deficient retinal explants (525).

Microglial migration can be induced by stromal cell-derived factor-1α (SDF-1α), acting through CXC chemokine receptor 4 (CXCR4). Migration triggered by CXCR4-specific ligands is accelerated by hypoxia. This effect is due to the activation of hypoxia inducible factor-1α (HIF-1α) and P3K/Akt signaling pathway, which leads to the enhancement of CXCR4 expression (986). Pharmacologically blockade of the cysteine-cysteine chemokine receptor 5 reduced migration velocity in acutely isolated living slices from adult brain-injured mice (121). MCP-1, a member of β-chemokine subfamily, regulates the migration of microglia, monocytes, and lymphocytes to the inflammatory site in the CNS (839). Following a hypoxic insult, amoeboid microglial cells in the neonatal rats increase MCP-1 production via NF-κB signaling pathway. This induces further migration and accumulation of amoeboid microglial cells from the neighboring areas to the periventricular white matter. Ameoboid microglial cells are the main source of MCP-1, and this may offer an explanation for the periventricular white matter being susceptible to hypoxic damage in newborn brain (217).

3. Cannabinoids and related compounds

Microglial cannabinoid receptor of CB2 type and abnormal cannabinoid-sensitive receptors stimulate microglial migration (973). Cannabinoid stimulation triggers the activation of the extracellular signal-regulated kinase 1/2 signal transduction pathway. Cannabinoids are released in a pathological condition, since excessive stimulation of neurons by glutamate together with carbachol dramatically increases the production of the endocannabinoid 2-arachidonyl glycerol. Similarly, pathological stimulation of microglial cells with ATP also increases the production of 2-arachidonyl glycerol (973). In LPS-stimulated primary cultured microglial cells, a CB2 agonist inhibits microglial migration (771).

Focal cerebral ischemia induces rapid neuronal death in the ischemic core, which gradually expands toward the penumbra, partly as the result of a neuroinflammatory response. It is known that propagation of neuroinflammation involves migration of microglial cells. Endocannabinoids are an important factor that increased microglial cell motility after the ischemic insult (296). It was found that in mouse cerebral cortex focal ischemia greatly increases palmitoylethanolamide (PEA), moderately increases anandamide (arachidonylethanolamide, AEA), and does not affect the level of 2-arachidonyl glycerol. It was further demonstrated that PEA potentiates AEA-induced microglial cell migration, without affecting other parameters of microglial activation, such as proliferation, particle engulfment, and NO production. This potentiation of microglial cell migration by PEA involves reduction in cAMP levels. In line with this, evidence was provided that PEA acts through G protein-coupled receptors. Interestingly, the receptors engaged by PEA are pharmacologically distinct from CB1 and CB2 cannabinoid receptors, as well as from the WIN and abn-CBD (abnormal cannabidiol) receptors. The results show that PEA and AEA increase after focal cerebral ischemia and synergistically enhance microglial cell motility. Because such a response could participate in the propagation of the focal cerebral ischemia-induced neuroinflammation within the CNS, and because PEA is likely to act through its own receptor, a better understanding of the receptor engaged by PEA may help the search for improved therapies against neuroinflammation.

4. Lysophosphatidic acid

Lysophosphatidic acid (LPA) enhances chemokinetically migration of murine microglial cells. In the presence of 1 μM LPA, the mean migration rate of microglial cells was increased 3.8-fold. In patch-clamp studies, LPA induces activation of a Ca2+-activated K+ current. The LPA-stimulated migration of microglial cells was inhibited by specific inhibitor of I KCa1, Ca2+-activated K+ channels charybdoxin, which decreased the migration rate by ~60%.
Therefore, it was concluded that $I_{K,Ca}^{11}$ Ca$^{2+}$-activated K$^+$ channels are required for LPA-stimulated migration of microglial cells (806).

5. Morphine

Morphine, in a relatively high concentration (1 $\mu$M), induced morphological changes and ruffling in cultured microglia and triggered chemotaxis as assayed by Boyden chamber analysis (892). Addition of morphine to microglia stimulated gene expression of BDNF as early as in 1 h, which persisted for >12 h; in parallel, ERK1/2 (extracellular signal-regulated kinase 1/2) phosphorylation was also induced. Activation of G$\beta$$\gamma$, PI3-K gamma, and Rac are involved in chemotaxis, whereas indirect pathways through ERK1/2 phosphorylation induced by unknown growth factors generated through a metalloprotease activation are linked to the enhanced BDNF gene expression (892).

Morphine increased microglial migration via a specific interaction between $\mu$-opioid and P2X$_3$ receptors mediated through PI3-K/Akt pathway activation (388). The action of morphine is accomplished in two phases. The initial phase develops in minutes, involves PI3-K/Akt pathway activation, and leads to acutely enhanced migration. The longer-term phase occurs within hours and involves increased expression of Iba1 and P2X$_3$ receptors that facilitates a promigratory phenotype (388).

6. Bradykinin

Bradykinin can attract microglia to a lesion or inflammation sites where bradykinin is upregulated. This involves inducible bradykinin receptors of B$\beta$ type. Bradykinin-induced microglial migration is PTX insensitive, suggesting that the intracellular signaling is G$\beta$$\gamma$ independent and distinct from ATP-induced microglial migration or chemotaxis (397). Downstream signaling cascade includes activation of PI3-K and PKC, followed by subsequent phosphorylation and activation of NCX in reverse mode as well as activation of K$\text{Ca}$ channels. From pharmacological study, intermediate-conductance Ca$^{2+}$-dependent K$^+$ channels are supposed to be activated (397); these channels are distinct from those activated by purines.

7. Growth factors

NGF induced chemotaxis of microglial cells through the activation of TrkA receptor. In addition, the NGF-induced chemotactic activity was increased in the presence of low concentrations (0.2 ng/ml) of TGF-$\beta$, which at this concentration is also chemotactic per se. On the contrary, NGF-induced microglial migration was reduced in the presence of chemokinetic concentration of TGF-$\beta$ (0.2 ng/ml). It was concluded that both NGF and TGF-$\beta$ contribute to microglial recruitment (199).

Hepatocyte growth factor-like protein (HLP)/macrophage stimulating protein (MSP), which was reported to be involved in the regulation of peripheral macrophage activation, promoted microglial migration without affecting cell survival and proliferation. Ron, the receptor for HLP, is expressed in primary microglia, and HLP greatly increased the mRNA levels of inflammatory cytokines, including IL-6 and GM-CSF, and iNOS (885).

8. $\beta$-Amyloid

It is well established that microglial cells accumulate at senile plaques in AD (370, 767). The pattern recognition receptor CD36 initiates a signaling cascade that promotes microglial activation and chemotaxis to $\beta$-amyloid deposits in the brain (875). Downstream of CD36 are the focal adhesion-associated proteins p130Cas, Pyk2, and paxillin representing novel members of the tyrosine kinase signaling pathway; assembly of this complex is essential for microglial migration (875). Chemokines may be involved in the recruitment of microglia to senile plaques, because neutralizing antibodies against chemokines significantly attenuated $\beta$-induced microglial clustering and the enlargement of $\beta$ aggregates (391). A recent study, however, did not find that the presence of microglia in a mouse model of AD has an impact on plaque load (336).

C. Inhibitors of Microglial Migration

1. LPS/IFN-$\gamma$

Normally, cultured microglia are nonmigratory and have amoeboid cell morphology, no polarity, many short processes that extend into lamellipodia in opposing directions, and undulating cell membrane projections. After addition of LPS, some cells acquire polarity by forming a large lamellipodium and begin to migrate, although this migration ceases in 2 h (6). Proinflammatory stimulation with LPS/IFN-$\gamma$ induces an IL-10-mediated downregulation of cell surface antigen expression and loss of migratory and phagocytic activity (88). In retinal explants, microglia readily migrate and are negative for MHC class II, and iNOS, while producing IL-12. In response to LPS/IFN-$\gamma$, microglia produce IL-10, which inhibits both their migration and activation (129).

2. Minocycline

Minocycline, a semisynthetic tetracycline derivative with anti-inflammatory and neuroprotective effects unrelated to its antimicrobial action, significantly reduced microglial migration to cellular debris, astrocyte-conditioned medium, ADP, and algesic mediators and inhibited the expression of CD29 ($\beta_1$-integrin) but not CD18 ($\beta_2$-
integran. Minocycline reduced the effect of extracellular potassium and later decreased microglial K_1.3 expression (664).

3. Wogonin

Microglial migration toward MCP-1 was inhibited by wogonin, an active component from the root of Scutellaria baicalensis Georgi and had neuroprotective effect, via suppression of NF-κB activity (719).

4. Dexamethasone

MCP-1 production is suppressed by dexamethasone, an anti-inflammatory and immunosuppressive drug, resulting in the inhibition of subsequent microglial cell migration to the inflammatory site by regulating MKP-1 expression and the p38 and JNK MAPK pathways (1037).

D. Microglial Residence in the Brain

Exogenous microglia enter the brain and migrate into ischemic hippocampal lesions (403–405, 882). The entry of intra-arterially injected microglia and macrophages into the brain using a rat muscle graft model was tested to compare their respective abilities to invade the brain parenchyma. Isolated microglia without any activation treatment entered into the brain with or without the muscle graft, while macrophages activated by phorbol 12-myristate 13-acetate entered the brain only in the presence of the muscle graft, suggesting that microglia have a higher affinity for the brain than macrophages (403). Microglia isolated from a mixed glial culture drawn from neonatal Mongolian gerbils produced high amounts of BDNF and glial cell line-derived neurotrophic factor (GDNF). The gerbil microglia retained the capability to migrate into the brain parenchyma after intra-arterial injection. After invasion, microglia retained their BDNF and GDNF productive ability and expressed large amounts of BDNF and GDNF in damaged brain areas, which suggests that microglia protect damaged neurons (882). In addition, GDNF was reported to activate transmembrane receptor tyrosine kinase Ret in microglial cells in CA1/CA3 hippocampal slices (78).

XVII. PHAGOCYTOSIS

Microglial cells are the professional phagocytes of the CNS tissue (Fig. 14 and supplementary video 2). This function is important for the normal brain, during brain development, and in pathology and regeneration (for review, see Refs. 630, 637).

During development, microglial cells play a specific role in removing apoptotic cells. In the developing cerebellum they phagocytose Purkinje neurons, which underwent caspase-3-mediated cell death. In the absence of microglia, these neurons can survive, indicating that apoptotic cells deliver an “eat me” signal to the microglial cells (550). Microglial cells are also considered to be involved in synapse removal during development (863) and potentially in pruning synapses in the postnatal brain.

The role of microglia phagocytosis in neurodegeneration is established in many experimental paradigms. For example, microglial cells are instrumental for removing the dendritic trees of the parvalbumin-positive interneurons in the dentate gyrus following entorhinal cortex lesion (746). In response to the lesion, microglial cells accumulate at the molecular layer in the dentate gyrus, the location of the synaptic contacts of entorhinal fibers and dendrites of the interneurons. This accumulation is mediated by signaling through CXCR3 receptors. When microglial attraction to the lesion site in the molecular layer is impaired by deletion of the chemokine receptor CXCR3, the dendritic trees of the interneurons are preserved. Thus microglial cells also recognize signals which tell them to remove parts of a cell.

In addition to cells and parts of cells, microglia are also known to phagocytose molecules and debris such as myelin or amyloid deposits. There are several studies that have established that β-amyloid is taken up by microglia in culture. For example, fibrous αβ in vitro induces phagocytosis through a recently characterized αβ cell surface receptor complex comprising the B-class scavenger receptor CD36, αβ1 integrin, and CD47 (integrin-associated protein), this complex therefore being distinct from that used by the classical phagocytic receptors, the immunoglobulin, or complement receptors (465). In contrast, soluble αβ is taken up by fluid-phase macropinocytosis, a mechanism distinct from phagocytosis and receptor-mediated endocytosis (548). Recently, the role of signal regulatory protein-beta1 (SIRPβ1) in regulation of αβ phagocytosis was identified in microglial cells isolated from amyloid precursor protein J20 transgenic mice and in AD patients (305).

While microbes and related particles are recognized by the family of Toll-like receptors, the apoptotic neurons are recognized by different receptor systems. These include asialoglycoprotein-like-, vitronectin-, and phosphatidylserine-mediated receptors (995).

There are multiple factors, which can regulate the phagocytic activity. The intracellular chloride channel (Cl/C1) seems to be important for microglial phagocytic activity. Pharmacological inhibition of this channel or downregulation of its expression by small interfering RNA impairs phagocytic activity. The growth factor ciliary neurotrophic factor increased microglial phagocytosis through a Ca^{2+}-mediated pathway (509a). Similarly, GDNF and M-CSF increased the phagocytic capability of the microglia (139, 594). Substrate-bound complement component Clq was shown to enhance both FcR-and
CR1-mediated phagocytosis two- to fourfold (988). Conversely, the prostanoid receptor subtype 2 (EP2), down-regulates phagocytosis, because ablation of EP2 enhanced microglial Aβ accumulation in cell culture (526, 827, 828). As mentioned above, ATP is an important signaling molecule for microglia. The metabotropic P2Y6 receptor controls microglial phagocytosis (412). Increased levels of mRNA encoding P2Y6 receptors were correlated to the activation state. Thus the P2Y6 receptor is upregulated when neurons are damaged and could function as a sensor/trigger for phagocytosis (467). At the same time, activation of P2X7 receptors in cultured rat microglia suppressed phagocytosis in a Ca2+ -independent manner; inhibition of P2X7 expression with lentiviral-mediated shRNA interference or with oxATP/BBG restored the phagocytic activity (261).

**XVIII. NEURONAL-MICROGLIAL INTERACTION: POSSIBLE ROLE IN NEURAL PLASTICITY?**

Do microglial cells play any physiological functions in the undisturbed brain? This question, although fundamentally important, remains largely without an answer. Without doubt microglial cells have the capacity to sense synaptic activity through their extended complement of neurotransmitter receptors. Can this interaction bear functional consequences? Recent in vivo imaging experiments have demonstrated that microglia in their surveillance state constantly scans the synaptic contacts dwelling in its territorial domain (972) through establishing brief (4–5 min) contacts with synaptic structures. Importantly, in the conditions of ischemia, these contacts were much more persisting; they lasted for an hour or more (972). Even more importantly, these events of prolonged liaisons between microglial processes and synapses often caused the disappearance of the latter. This behavior may indicate the constant monitoring of the synaptic status by resting microglia. A question remaining is the molecular instruction by which microglia understands the (activity) status of a synaptic structure. It could be the very local level of neurotransmitter, which would allow for the evaluation of an individual synapse; rather than an averaged transmitter concentration. On the other hand, short interaction via adhesion molecules could help the transient contact establishment between neuronal and microglial...
processes. NCAM, integrins, preferentially structures with a signaling component in both directions could be candidates.

Any abnormalities in synaptic strength/performance may trigger local responses of microglial cells, which although not inducing the full activation process can initiate remodeling of synaptic architecture. Indeed, the role of microglia in removing synapses was shown long ago, when it was shown that microglia are responsible for removing the synapses from axotomized motoneurons (71), the process that became known as “synaptic stripping.” Interestingly, the actual removal of synapses preceded by decrease in the synaptic strength, which could be mediated by ATP/adenosine release from microglial cells at the early activation stages (1016). A similar process of synaptic stripping was observed in the cortex in response to focal inflammation (923). The “stripping” process is specific, as it predominantly removes excitatory glutamatergic synapses leaving inhibitory inputs operational, thus limiting neuronal excitability and glutamate toxicity (528). This specificity of action is associated with the MHC class F receptors, which are present in both neurons and microglia (183). Of course, these examples are coming from pathological realm, yet they indicate that microglial cells in principle are able to remove or remodel synapses operating in their territorial domains. An initial drop in synaptic efficacy could trigger the “microsurgical” elimination by microglia, which would thereby interfere with neuronal communication. This microglia-to-neuron interaction would reach much further than the previous modes of neurotrophin supply or the passive listening to broadcasted signals.

At the same time, microglia can potentially be involved in the opposite process of creating new synapses. Several lines of evidence indicate the involvement of microglia in regulation of synaptogenesis in the early postnatal brain. Microglia can stimulate synaptogenesis by secreting thrombospondins (TSPs) (137, 599); the latter belong to extracellular matrix proteins critically important for synaptic formation and are also produced by astrocytes (157). TSP1 interacts with the integrin-associated protein CD47, the latter accepting also signal regulatory protein (SIRP)α, a transmembrane protein expressed in neurons and macrophages (558). The SIRPα-CD47 system is involved in the regulation of migration and phagocytosis, immune homeostasis, and neuronal networks. CD47 and its two ligands, SIRPα and TSP1, may thus play not only homeostatic roles in the immune system, but participate in synaptic patterning involving the innate immune cell of the CNS, i.e., the microglia (941). Indeed, thrombin, the blood coagulation factor II(α) and name-giving inducer of TSP in platelets, which has been associated with synapse elimination and plasticity (936, 1043), has its receptors expressed on microglia (31). There is probably not only shared use of critical pro- teases, substrates, and receptors in the hemostatic, immune, and nervous systems upon injury, but some still not fully understood interactions between molecular and cellular players and targets in the control of neuronal circuits. Dynamic neuronal-microglial interactions were also reported to participate in postnatal remodeling of the visual cortex. Importantly, the microglial phenotype was regulated by visual experience with a faster ramification in animals that were rendered monocular from the moment of birth (764).

Microglia appear as an important player in the restoration of neuronal connectivity by controlling the reactive synaptogenesis following the insult (58). The regenerative potential of microglia is well documented; the importance of microglia for recovery of neuronal connectivity and synaptic repair has been repeatedly demonstrated (40, 94, 599). Furthermore, transplantation of microglia/macrophages into lesioned optic nerve or spinal cord significantly improved posttraumatic regeneration (499, 742). Contributions of microglia, in particular certain phenotypes, to the plasticity of the CNS may include support of neurogenesis as well (113, 577). Recent studies have found T cells and microglia to be important to maintain both neurogenesis in the hippocampus and spatial learning abilities. Apparently, contributions of the adaptive immune system and the local innate immunity are required to keep the capacity for cellular renewal as well as to support higher CNS functions (1042). It was also suggested that TLRs modulate adult hippocampal neurogenesis. TLR2 and TLR4 were identified in neural stem/progenitor cells and were found to exert opposite effects on their proliferation and differentiation (769). Agonists of these TLRs would simultaneously affect microglia. The importance of a normal microglial function has been demonstrated in mice with a homozygous loss of function mutation in Hoxb8 (149). These animals suffer from compulsive grooming and hair removal, a syndrome resembling an obsessive-compulsive disorder in humans. The Hoxb8 lineage was found to associate in the CNS with a microglial subpopulation, and wild-type bone marrow transplantation was sufficient to rescue the mutant-carrying animals from their abnormal behavior (149). So, both synaptic contacts and neuronal elements themselves could depend on or benefit from the active microglia engagement. Furthermore, recent studies revealed that microglia may play an active (or at least supportive) role in the functional integrity of the CNS and its normal physiological performance even at the level of learning and behavior. Links between immunological and neuropsychiatric disorders could thus also involve disrupted microglia activity.

Microglial-derived factors may also affect synaptic transmission directly. This, for example, was observed in the spinal cord, where stimulation of microglia with ATP triggers release of BDNF, which in turn affects Cl−
distribution in neurons, thus turning GABA- and glycine-mediated postsynaptic responses from inhibitory to excitatory (175). This type of microglia-neuronal interactions has been discussed already in the context of neuropathic pain (see section IX), although similar mechanisms may be in operation in the absence of pathological insults. Direct action of microglia on synaptic plasticity was also observed in hippocampal slices treated with Aβ1–42. This treatment inhibited induction of LTP, which in turn was mediated through NO released by activated microglia (981). Microglial cells have the potential to influence homeostatic synaptic scaling, which entails uniform adjustments in the strength of all synapses on a cell mechanism distinct from long-term synaptic potentiation or depression. Stellwagen and Malenka (860) found that synaptic scaling in response to prolonged blockade of activity is mediated by the proinflammatory cytokine TNF-α. They demonstrated that glia are the source of the TNF-α, while they did not distinguish between astrocytes and microglia. They conclude that glia actively participate in the homeostatic activity-dependent regulation of synaptic connectivity by modulating TNF-α levels (860). At the same time however, induction of LTP by strong tetanic stimulation of Schaffer collaterals did not affect motility of microglial processes in acute hippocampal slices; neither did it induce any current responses from neighboring microglial cells (1005).

NO is not only the poisonous gas used by immune cells for cytotoxic attack. In the CNS it is, first of all, a neuromodulatory factor. The NO contribution for the neuroendocrine axis lies in the control of releasing factors and hormones (756); this ability may, at least partially, allow microglia to exert certain influences, especially under immune-activating conditions. Microglial NO release could thus integrate activities of the endocrine system in responses to infection and injury, when the NOS is induced as part of a respective reactive phenotype profile.

Microglial cells can also have a trophic role, indicated by distinct neurotrophin expression (247). There is some early evidence that secretory proteases secreted by microglia may provide for trophic regulation of neuronal circuits (466), affecting their growth, differentiation, and circuitry formation. Conceptually, depending on the status, microglial cells are able to release a multitude of factors with powerful neurotrophic action (611). It would still require a systematic approach, however, to determine the regional and temporal patterns of neurotrophin production by microglia. This production may occur during development versus induction upon request by endangered neurons. Keeping a broader perspective on developmental potential, a number of cytokines, as being more typically associated with the inflammatory response, may have roles in the maturing CNS. The astroglial growth factor capacity of IL-1 is one example (321). In the adult CNS, the very same cytokine as produced by microglia then drives astrogial proliferation in response to injury.

Microglial cells are also intimately involved in the development of the nervous system, which relies upon tightly controlled balance between neurogenesis and neuronal death. Microglia have dual action on neurogenesis, as both detrimental and supportive effects were reported (245). The underlying mechanisms of this dichotomy are still obscure, although most likely they depend on the differences in the activation state of microglia, and hence differential secretion of cytotoxic or protective/instructive factors (816). Differentiation of neural precursors isolated from supraventricular zone in culture, for example, required the presence of microglia or microglia-conditioned media (629, 975). Furthermore, microglia-derived factors are involved in directing migration and final instruction of newly born neural cells (2). As to whether microglial cells can ever give rise to other cells, including those with neuronal phenotype, is an intriguing question (110). Similar reports have been published even earlier, but remained of little resonance (351). Even though microglia have been shown to express some stem cell markers and have therefore been considered as a kind of immature cell (see sect. III), trans-differentiations of that kind, i.e., shift towards neuronal orientation, would challenge much of the concept of a mesodermal origin of microglia.

The role of microglia in controlling neuronal extinction during periods of developmentally associated programmed cell death was demonstrated in several brain regions. In the retina, for example, the massive neuronal programmed death follows the early invasion of macrophages (135). Dissection of the retina prior to macrophages invasion prevents neuronal demise, whereas further addition of microglia to retinal preparation induces cell death. The pro-apoptotic action of microglia is mediated through NGF, exemplifying the other side of a neurotrophic factor supply (290). Similarly microglial cells induce apoptotic death of Purkinje neurons in organotypic slices mediated through microglia-produced superoxide ions (550). In the embryonic (E12-E13) spinal cord, invading microglia control the apoptotic death of motoneurons via secretion of TNF-α (818); incidentally, motoneurons transiently express TNF-α receptor 1 (TNFR1) precisely at that stage (818).

To conclude, microglial cells do have the potential to regulate the development, structuring, and function of neuronal networks. They constantly monitor the status of synaptic contacts and receive information from neuronal networks. Potentially, at least, microglial cells are also able to remodel neuronal connectivity and thus participate in physiological processes within neural networks. Multiple activation states of microglial cells may allow the existence of “resting active” microglia, or even “active” compartments in the processes of surveillant microglia,
which dynamically interact with neural circuitry and provide it with additional plastic capabilities.

Commitment to distinct reactive phenotypes as instructed by specific activating signals may then have a rather variable impact on the neur(on)al community in the affected (infected, injured, degenerating) tissue region (351, 816). While such distinct and overlapping functional orientations have been studied intensively in extraneural macrophages, still much has to be addressed with regard to microglia (166, 622). It will be important to identify the nature and sources of such instructing signals as they govern functional orientations of microglia and their shifts along an activation episode. Equally important, little is known about the heterogeneity of microglia, i.e., the differences in functional capacities of individual microglial populations between and within CNS regions. Finally, in pathological situations with blood-derived monocytes/macrophages infiltrating the CNS, features and functions of resident microglia and the invading cells may overlap or complement each other, with both detrimental and beneficial consequences (823, 837). Why is there a need for recruiting peripheral macrophages when the microglia could fulfill all tasks? Answers are awaited for understanding microglia in health and disease and to evaluate the therapeutic potential of correcting their dysregulation.

XIX. OUTLOOK AND FUTURE DIRECTIONS

Brain pathology is correlated with the activation of microglial cells as recognized by the morphological transition from a ramified to an amoeboid form. In the last two decades, microglial cells have emerged as an essential cellular component for understanding brain diseases. The development of cell culture models to isolate and study microglial cells has led to a bulk of information on their functional properties. In addition to the previous purely morphological studies of the past, it became evident that microglial cells can release a battery of signal molecules that serve in the cross-talk with brain cells, i.e., macroglial cells and neurons, as well as with infiltrating immune cells, such as T lymphocytes. Very recently, functional studies have been extended to acutely isolated tissue slices or even to in vivo mouse models. In these studies, the focus was on cell motility and led to the conclusion that microglial cells are active even in the normal brain. It becomes evident that more in vivo approaches for determining microglial functions in the normal and diseased brain need to be developed. Such future studies will likely produce a more heterogeneous picture of microglial cell diversity depending on the development, brain region, or pathological state. It remains also an intriguing and unresolved question under which conditions and by which mechanisms microglial cells either prevent and dampen or facilitate the progression of a given brain disease.

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