Ion channels in microglia (brain macrophages)

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Microglia are immunocompetent cells in the brain that have many similarities with macrophages of peripheral tissues. In normal adult brain, microglial cells are in a resting state, but they become activated during inflammation of the central nervous system, after neuronal injury, and in several neurological diseases. Patch-clamp studies of microglial cells in cell culture and in tissue slices demonstrate that microglia express a wide variety of ion channels. Six different types of K⁺ channels have been identified in microglia, namely, inward rectifier, delayed rectifier, HERG-like, G protein-activated, as well as voltage-dependent and voltage-independent Ca²⁺-activated K⁺ channels. Moreover, microglia express H⁺ channels, Na⁺ channels, voltage-gated Ca²⁺ channels, Ca²⁺-release activated Ca²⁺ channels, and voltage-dependent and voltage-independent Cl⁻ channels. With respect to their kinetic and pharmacological properties, most microglial ion channels closely resemble ion channels characterized in other macrophage preparations. Expression patterns of ion channels in microglia depend on the functional state of the cells. Microglial ion channels can be modulated by exposure to lipopolysaccharide or various cytokines, by activation of protein kinase C or G proteins, by factors released from astrocytes, by changes in the concentration of internal free Ca²⁺, and by variations of the internal or external pH. There is evidence suggesting that ion channels in microglia are involved in maintaining the membrane potential and are also involved in proliferation, ramification, and the respiratory burst. Further possible functional roles of microglial ion channels are discussed.

voltage- and calcium-activated potassium channels; proton current; fast-inactivating sodium current; calcium channels; chloride conductances

IT IS NOW GENERALLY ACCEPTED that microglia are a macrophage population in the brain. Microglial cells express surface markers similar to those expressed by macrophages in peripheral tissues, and they share many functions of macrophages, including phagocytosis, antigen presentation, and release of various cytokines and cytotoxins (7, 8, 57, 136). Microglia play important roles in the normal and pathological brain, and they can have both neurotrophic and neurotoxic properties (88, 101, 131). During brain maturation, microglia of an ameboid morphology phagocytose cellular debris resulting from spontaneous degeneration or remodeling of nerve fibers (92, 101). In the adult brain, microglial cells that exhibit a ramified morphology are thought to be resident macrophages (88, 131). Resting microglia become activated under several pathological conditions: transformation of microglia from a resting to an active state is characterized by proliferation and by several immunophenotypical and morphological changes of the cells, such as an upregulation of major histocompatibility complex (MHC) molecules, expression of various macrophage surface markers, hypertrophy, and transformation to an ameboid morphology. Furthermore, activated microglia become phagocytic and secrete several cytotoxic substances such as free oxygen intermediates, nitric oxide, proteases, arachidonic acid derivatives, excitatory amino acids, quinolinic acid, and cytokines (56, 88, 131, 145). Activation of microglia has been observed during infection, inflammation, physical injury, trauma, ischemia, and in a number of neurodegenerative disorders such as Alzheimer’s disease, Parkinson’s disease, amyotrophic lateral sclerosis, multiple sclerosis, and prion disease (7, 14, 56, 88, 99, 101, 131).

So far most studies have been performed on microglia using different histochemical staining techniques to visualize morphological and phenotypical changes of the cells during development and at various pathologies of the brain. Only recently a few groups of researchers began to investigate physiological properties of microglial cells.
Since the first study on microglial ion channels published in 1990 (83), a wide variety of ion channels has been identified and characterized in microglia. To investigate microglia in isolation, the majority of patch-clamp studies has been performed on cultured primary cells or on cell lines. A few electrophysiological studies of microglia have been done in brain slices. Microglial cells are capable of expressing ion channels that selectively conduct K\(^+\), H\(^+\), Na\(^+\), Ca\(^{2+}\), or Cl\(^-\) (Table 1). The expression level of each of these ion channels strongly depends on the functional state of the microglial cells.

The aim of this review is to summarize the existing literature documenting properties, regulation, and functional role of microglial ion channels. The channels described in microglia are compared with those characterized in blood and other tissue macrophages (for reviews, see Refs. 30 and 51).

Several authors also demonstrated the existence of distinct ATP receptors in microglia with the use of patch-clamp and video-imaging techniques. Because properties of ion currents induced by ATP receptor stimulation (72), molecular identification of microglial ATP receptors (114), and their functional importance (33) have been reviewed recently, they are not a topic of this review.

**PROPERTIES, EXPRESSION, AND REGULATION OF ION CHANNELS IN MICROGlia**

**Inward Rectifier K\(^+\)** Channels: Properties and Expression

Inward rectifier (IR) channels have been described in cultured rat (83, 87, 109, 111, 112, 121, 122, 126, 134, 135), murine (36, 45, 73, 74), bovine (97), and human (98, 142) microglia. IR channels were also detected in the microglial cell line BV-2 (11) and in microglia during in situ recordings from murine brain slices (13). Prominent IR currents have been found in nearly every microglial preparation studied. However, IR channels were not measurable in cultured human microglia isolated from brain tumors (113), and they were only rarely observed in cultured microglia grown with granulocyte/macrophage colony-stimulating factor (GM-CSF) (45).

The properties of IR channels in microglia closely resemble those of IR channels in many other cells. As demonstrated for many classical IR currents (for reviews, see Refs. 71, 108, 119), the open probability of IR channels in cultured microglia increases on hyperpolarization. In most studies on microglia, outward currents through IR channels were not detected at depolarizing potentials. In other cells, small outward IR currents are seen a few tens of millivolts positive to the K\(^+\) equilibrium potential. These outward currents are much smaller than inward currents due to block by intracellular Mg\(^{2+}\) and/or intracellular polyamines (108). IR channels depend on the concentration of external K\(^+\) ([K\(^+\)]\(_o\); Fig. 1). An elevation of [K\(^+\)]\(_o\) shifts the reversal potential of the currents to more positive potentials and increases the chord conductance (36, 83, 87, 111, 112, 134).

IR currents of microglia do not show time-dependent inactivation when measured in K\(^+\) Ringer solution (150 mM [K\(^+\)]\(_o\)) (112) or after replacement of extracellular Na\(^+\) by choline or by N-methyl-D-glucamine (NMG\(^+\)) (36, 112). Thus the time-dependent decay of IR current seen in recordings using normal Ringer solutions (5 mM [K\(^+\)]\(_o\)) is due to a time- and voltage-dependent inhibition by external Na\(^+\), as described in other cells (129). Blockade of IR currents by Na\(^+\) was evident for voltage commands to ~100 mV or to more negative potentials. With increased membrane hyperpolarization, block becomes faster and deeper. In lipopolysaccharide (LPS)-stimulated microglia, replacement of [Na\(^+\)]\(_o\) by choline additionally results in a reduction of IR current amplitudes (112), leading the authors to suggest that some part of IR currents may be due to a Na\(^+\)-activated K\(^+\) conductance. Another possible explanation is that IR currents are reduced due to intracellular acidification (62), since omitting [Na\(^+\)]\(_o\) abolishes Na\(^+\)/H\(^+\) antiport. In macrophage CSF (M-CSF)-grown microglial cells, IR currents are reduced at intracellular pH (pHi) values of <7.0 (36). It is also likely that choline induces a partial current blockade, as has been reported for IR currents in guinea pig myocytes (10). No reduction in IR peak amplitude was observed in murine microglia after substitution of external Na\(^+\) by NMG\(^+\) (36). IR currents of microglia are effectively inhibited by extracellular Ba\(^{2+}\) (36, 83, 109, 112, 121, 134). Ba\(^{2+}\) causes a time- and voltage-dependent blockade (36, 121). Microglial IR currents are also inhibited in a voltage-dependent manner by Cs\(^+\) at micromolar to millimolar concentrations. External Cs\(^+\) caused a deeper block of microglial IR currents at very negative membrane potentials than at potentials near the resting membrane potential (36, 98, 134). After replacement of intracellular K\(^+\) by Cs\(^+\) (150 mM intracellular Cs\(^+\) concentration), an inhibition of IR currents at strongly hyperpolarizing potentials was detected in rat microglia (111, 112). IR currents in microglia are inhibited by tetraethylammonium (TEA\(^+\)) but only at relatively high concentrations compared with other K\(^+\) channels (36, 73, 74, 112). IR currents are also reduced by external application of quinine (112).

In single-channel recordings using symmetric K\(^+\) concentration solutions (150 mM K\(^+\)), a conductance of ~30 pS was determined for microglial IR channels (83, 97, 122, 134). In cell-attached measurements, an additional type of IR channel with a conductance of 43 pS was identified in rat microglia (122). Thus it might be possible that in some preparations whole cell IR currents are generated by at least two (or more) different types of inward K\(^+\) channels, which could explain some heterogeneity of pharmacological data reported in various studies. The extent of expression levels of different IR channels may vary between different microglial preparations or between cells of distinct functional state.

With the use of a corpus callosum slice preparation of juvenile mouse (13), the existence of IR channels in ameboid microglial cells in situ has been demonstrated. Currents activated negative to the K\(^+\) equilibrium potential and were abolished by external Ba\(^{2+}\). They
were weakly sensitive to extracellularly applied TEA⁺ but insensitive to 4-aminopyridine (4-AP) (13). Thus, in mouse tissue slices, IR channels of microglia share nearly identical properties of IR currents described in microglia and macrophages does not reveal significant differences.

The IR channel is also the predominant K⁺ channel in other macrophage preparations (for reviews, see Refs. 30 and 51). Comparison of kinetic and pharmacological properties of IR currents described in microglia and macrophages does not reveal significant differences.

The Kir2.1 channel cloned from mouse macrophage cDNA (89) exhibits characteristics nearly identical to those described in murine microglia. cDNA (89) exhibits characteristics nearly identical to those described in murine microglia. Inward rectifier K⁺ channel (∼ 30 pS) was weakly sensitive to extracellularly applied TEA⁺ but insensitive to 4-AP (IC₅₀ = 23 µM) (13). Thus, in mouse tissue slices, IR channels of microglia share nearly identical properties of IR currents described in microglia and macrophages does not reveal significant differences.

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### Table 1. Ion channels in microglia

<table>
<thead>
<tr>
<th>Channel Type</th>
<th>Species (Preparation)</th>
<th>Pharmacology (IC₅₀ Range)</th>
<th>Regulation/Modulation</th>
<th>References</th>
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</thead>
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<tr>
<td>Inward rectifier K⁺ channel</td>
<td>Rat (culture); mouse (culture)</td>
<td>Ba²⁺ (IC₅₀ = 23 µM); Cs⁺ (µM); TEA⁺ (nM); quinine (mM); Na⁺ (mM)</td>
<td>Downregulation by G protein activation (TNF, GM-CSF, EGF, C5a); PKC activation (PMA, in human); internal acidification; increase in [Ca²⁺]; GM-CSF; LPS; IFN-γ</td>
<td>11, 13, 36, 45, 73, 74, 83, 87, 97, 98, 109, 111, 112, 121, 122, 126, 134, 135, 142</td>
</tr>
<tr>
<td>HERG-like K⁺ channel</td>
<td>Rat (MLS-9 cell line)</td>
<td>E-4031 (µM); Cs⁺ (mM); Ba²⁺ (mM)</td>
<td>Upregulation by adherance; PKC activation (PMA, in rat)</td>
<td>144</td>
</tr>
<tr>
<td>Delayed rectifier K⁺ channel</td>
<td>Rat (culture); mouse (culture)</td>
<td>CTX (IC₅₀ = 1 nM); KTX (IC₅₀ = 1 nM); NTX (IC₅₀ = 1 nM); MTX (nM); 4-AP (IC₅₀ = 0.27 mM); Cd²⁺ (µM); Zn²⁺ (µM); Ba²⁺ (mM); TEA⁺ (IC₅₀ = 10 mM)</td>
<td>Downregulation by increase in [Ca²⁺]; PKC activation (PMA, in rat); intracellular acidification</td>
<td>37–40, 45, 87, 98, 109, 111–113, 121, 134, 135, 142</td>
</tr>
<tr>
<td>Voltage-dependent Ca²⁺-activated K⁺ channel</td>
<td>Bovine (culture); human (culture)</td>
<td>TEA⁺ (µM)</td>
<td>Upregulation by LPS; IFN-γ; GM-CSF; astrocytic factor(s); PKC activation (PMA, in humans)</td>
<td>87, 98</td>
</tr>
<tr>
<td>Voltage-independent Ca²⁺-activated K⁺ channel</td>
<td>Mouse (culture + ACM)</td>
<td>CTX (IC₅₀ = 3 nM); La³⁺ (µM to mM); Ba²⁺ (µM to mM); Cd²⁺ (mM); Zn²⁺ (µM); Ba²⁺ (mM); TEA⁺ (mM)</td>
<td>Downregulation and modulation of kinetics by cyclohexalin D; colchicine; LPS; astrocytic factor(s)</td>
<td>36, 42, 98, 134</td>
</tr>
<tr>
<td>G protein-activated K⁺ channel</td>
<td>Mouse (culture)</td>
<td>4-AP (mM), TEA⁺ (mM)</td>
<td>Modulation of kinetics by Ba²⁺, Ca²⁺, Cd²⁺, Zn²⁺, changes in pHᵢ</td>
<td>36, 42, 98, 134</td>
</tr>
<tr>
<td>H⁺ channel</td>
<td>Mouse (culture); rat (culture)</td>
<td>Zn²⁺ (µM); La³⁺ (µM); Ni²⁺ (µM); Cd²⁺ (µM); Co²⁺ (µM); Ba²⁺ (mM); 4-AP (mM); TEA⁺ (mM)</td>
<td>Downregulation and modulation of kinetics by cyclohexalin D; colchicine; LPS; astrocytic factor(s)</td>
<td>36, 42, 98, 134</td>
</tr>
<tr>
<td>Na⁺ channel</td>
<td>Rat (culture, coculture with astrocytes); human (culture)</td>
<td>TTX (nM)</td>
<td>Upregulation by astrocytic factor(s)</td>
<td>87, 113, 122, 126</td>
</tr>
<tr>
<td>Voltage-activated Ca²⁺ channel</td>
<td>Rat (culture)</td>
<td>TTX (nM)</td>
<td>Uptake by astrocytic factor(s)</td>
<td>87, 113, 122, 126</td>
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<tr>
<td>Ca²⁺-release activated Ca²⁺ channel</td>
<td>Rat (culture)</td>
<td>Ca²⁺ (nM)</td>
<td>Modulation of kinetics by Ba²⁺, Ca²⁺, Cd²⁺, Zn²⁺, changes in pHᵢ</td>
<td>87, 113, 122, 126</td>
</tr>
<tr>
<td>Voltage-dependent Cl⁻ channel</td>
<td>Bovine (culture); human (culture)</td>
<td>Ba²⁺ (mM)</td>
<td>Downregulation and modulation of kinetics by cyclohexalin D; colchicine; LPS; astrocytic factor(s)</td>
<td>36, 42, 98, 134</td>
</tr>
<tr>
<td>Voltage-independent stretch-activated Cl⁻ channel</td>
<td>Rat (culture); mouse (culture, culture + ACM)</td>
<td>DIDS (µM); SITS (µM); NPPB (IC₅₀ = 30 µM); IAA-94 (IC₅₀ = 210 µM); flufenamic acid (IC₅₀ = 80 µM)</td>
<td>Downregulation and modulation of kinetics by cyclohexalin D; colchicine; LPS; astrocytic factor(s)</td>
<td>43, 121, 134</td>
</tr>
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IC₅₀, half-maximal current inhibition; ACM, astrocyte-conditioned medium; CTX, charybotoxin; KTX, kalixotoxin; NTX, naxiotoxin; MTX, margatoxin; TEA⁺, tetraethylammonium; 4-AP, 4-aminopyridine; TTX, tetrodotoxin; NPPB, 5-nitro-2-(3-phenylpropylamino)benzoic acid; IAA-94, 6,7-dichloro-2-cyclopentyl-2,3-dihydro-2-methyl-1-oxo-1H-indol-5-yl(ox)acetic acid; GM-CSF, granulocyte/macrophage colony-stimulating factor; IFN-γ, interferon-γ; LPS, lipopolysaccharide; TNF, tumor necrosis factor; EGF, epidermal growth factor; C5a, complement factor C5a; PKC, protein kinase C; PMA, phorbol myristate acetate; [Ca²⁺], Intracellular free Ca²⁺ concentration; pHᵢ, extracellular pH; HERG, human ether-a-go-go-related gene; GTPγS, guanosine 5′-O-(3-thiotriphosphate).
Inward Rectifier $K^+$ Channels: Regulation

It has been demonstrated that IR channels in microglia are modulated by G proteins, intracellular Ca$^{2+}$ (73, 74), pH$_1$ (36), and protein kinase C (PKC) (135, 142). Activation of G proteins induced by internal perfusion with guanosine 5'-O-(3-thiotriphosphate) (GTP$_{\gamma}$S), by receptor activation with tumor necrosis factor (TNF) or epidermal growth factor (EGF), or by stimulation with complement factor C5a results in a reduction of IR currents in murine microglia (73, 74). A similar IR current inhibition after G protein activation has been described for other macrophage preparations (96, 141). IR currents of murine microglia are also reduced by elevating the concentration of intracellular Ca$^{2+}$ (74) or by acidification of the intracellular milieu (36).

It has been demonstrated that activation of macrophages with LPS or interferon-$\gamma$ (IFN-$\gamma$) induces rapid PKC-dependent phosphorylation of a number of distinct proteins (60, 63). Transformation of microglia from their ameboid into a ramified phenotype is also accompanied by PKC activation (140). Regarding the
effects of phorbol esters on IR currents (135, 142), opposite data have been reported for human and rat microglia. After PKC activation by phorbol 12-myristate 13-acetate (PMA), IR currents of human microglia are inhibited (142), whereas an increase in IR current density has been observed in IFN-γ-stimulated rat microglial cells (135).

Changes in culture conditions or in the microenvironment of the cells can induce dramatic alterations in expression levels and/or in expression patterns of ion channels in microglia. It has been reported that cultured rat microglial cells after 7 days of adherence exhibit larger IR currents than cells measured at the first day of adherence, whereas amplitudes of IR currents are not increased in microglial cells after cultivation for 7 days in suspension (109). A correlation between expression levels of IR channels and cell adherence has also been described in the macrophage cell lines J774.1 (54, 95) and THP-1 (31), in murine peritoneal macrophages (52, 117), and in HL-60 cells (139).

Ion channel patterns differ in microglia, depending on their differentiation pathway (45). Most microglial cells developed with M-CSF exhibit large IR currents, whereas the majority of microglial cells grown with GM-CSF do not express IR channels (45). We found a similar pattern of IR channel expression in bone marrow-derived macrophages that had been developed with either M-CSF or GM-CSF (35). Thus both microglia and macrophages share the ability to regulate their K⁺ channel expression patterns in a similar manner in response to growth factors. Stimulation of rat microglia with LPS causes a decrease in the density of IR currents (134), as has been reported in J774.1 macrophages (95). Furthermore, exposure of murine microglia to IFN-γ results in a reduced percentage of cells expressing IR channels (45). Because LPS and the two cytokines GM-CSF and IFN-γ are known to drive microglial cells in a more activated state that is characterized by an increased secretion of cytokines and the capability to present antigen (46, 48, 55, 58), it is possible that IR channels become downregulated during the process of cellular activation. Intriguingly, this downregulation of IR channels in activated microglia is always accompanied by an upregulation of delayed rectifier channels (see Delayed Rectifier K⁺ Channels: Regulation).

HERG-Like K⁺ Channels: Properties and Expression

Very recently a novel K⁺ conductance has been reported in a cell line derived from rat microglia (144). The K⁺ currents differ markedly from IR and closely resemble the K⁺ channels encoded by the human ether-a-go-go-related gene (HERG) in cardiac muscle and neurons. In high-K⁺-containing solution (160 mM [K⁺]o), HERG-like channels open rapidly on hyperpolarization from 0 mV (Fig. 2B), but, unlike IR channels, they exhibit pronounced inactivation. The pharmacological profile of this current also resembles HERG but not IR: currents are weakly inhibited by external Cs⁺ or Ba²⁺, but the specific HERG channel blocker E-4031 blocks microglial K⁺ channels in the micromolar concentration range. HERG-related K⁺ channels have not been reported previously in any immune cell. The presence of HERG-like K⁺ channels in microglia may reflect their development in the brain environment. Further experiments are required to test whether HERG-like K⁺ channels are also expressed by microglia in situ.

HERG currents would be difficult to detect in a cell studied under “normal” conditions, namely, with physiological solutions (low [K⁺]o) and holding the membrane at a resting-potential-like negative value. Figure 2A illustrates that the channels are inactivated at a holding potential of −80 mV, so that no current is elicited during depolarizing pulses. The only currents seen occur on repolarization following prolonged depolarization. The inward tail currents seen in high [K⁺]o in Fig. 2A would be greatly reduced in amplitude in a “physiological” (low [K⁺]o) solution.

Delayed Rectifier K⁺ Channels: Properties and Expression

The existence of delayed rectifier (DR) channels in microglial cells has been demonstrated in microglial cells cultured from rats (87, 109, 111, 112, 121, 134, 135), mice (37–40, 45), and humans (98, 113, 142). In
microglia, DR currents were seen in a small fraction of unstimulated cells, after adherence, after cellular activation by LPS or various cytokines, during cocultivation with astrocytes, or after exposure to astrocyte-conditioned medium (ACM; see below).

DR currents activate at potentials positive to ~40 mV and increase in amplitude at more depolarizing potentials. The currents exhibit a sigmoidal voltage-dependent activation kinetics and can be fitted to a Hodgkin-Huxley-type \( n \) model (112). Activation and inactivation of DR currents become faster with stronger depolarization up to potentials of ~0 mV, while at more positive potentials time constants of both activation and inactivation are not changed further (37, 112). Inactivation of DR currents can be fitted by a single exponential with time constants of 400–600 ms at a potential of +30 mV in experiments using KCl-containing pipette solutions (37, 112, 134). Values determined for half-maximal steady-state activation (~24 to ~14 mV) and inactivation (~65 to ~34 mV) of microglial DR channels varied somewhat among different preparations (37, 87, 112, 121, 134), which might be due to differences in experimental conditions (e.g., the use of different solutions) or in experimental details (e.g., differences in the time of measurements after break-in or in the age of the cells studied). DR currents in microglia recover slowly from inactivation. In rat microglia, recovery from inactivation was fitted by a sum of two exponentials with time constants of 2 and 16 s (112), whereas in murine microglia the time course of recovery could be fitted with a single time constant of 8 s (37). When the concentration of extracellular K\(^+\) was varied, the reversal potential of the DR currents shifted as expected from the Nernst equation, confirming that microglial DR currents are highly selective for K\(^+\) (37, 112).

Several K\(^+\) channel blockers have been found to be effective blockers of DR channels in microglia. In contrast to observations made for DR channels in neurons (for reviews, see Refs. 64 and 119) or astroglial cells (for review, see Ref. 128) but in agreement with findings in other immune cells (for review, see Ref. 51), DR channels in microglia appear to be much more sensitive to external 4-AP than to external TEA\(^+\) (37, 111, 112). At concentrations of 1–5 mM, 4-AP nearly completely blocks DR channels in rat (111, 112, 134), murine (37), and human (98) microglia. A half-maximal current inhibition at 0.27 mM 4-AP was determined in murine microglia (37). DR currents were only partially blocked by external TEA\(^+\) at millimolar concentrations. During superfusion with 10 mM TEA\(^+\), a current inhibition by ~50% was seen in rat (87, 109, 111, 112) and murine (37) microglia. Inorganic divalent cations, namely, Ba\(^2+\), Ca\(^2+\), or Zn\(^2+\), reduce DR current amplitude and shift the conductance-voltage (g-V) curve to more positive potentials (37). A similar shift in the g-V curve was seen by raising the concentration of extracellular Ca\(^2+\) (112). DR currents in rat and murine microglia are abolished by nanomolar concentrations of several peptide toxins, including charybdotoxin (CTX), kaliotoxin (KTX), margatoxin (MTX), or noxiustoxin (NTX) (37, 39, 121). In murine microglia, currents are half-maximally inhibited by CTX, KTX, or NTX at a concentration of ~1 nM (37, 39). DR currents exhibiting an identical sensitivity to these toxins were also found in THP-1 monocytes (84) and in several T lymphocyte preparations (for review, see Ref. 91). No obvious inhibition of microglial DR currents was observed in the presence of dendrotoxin or mast cell degranulating peptide (37).

Frequency dependence of microglial DR channels was demonstrated by application of repetitive voltage pulses. At 1 Hz stimulation, DR currents decline rapidly during the first pulse, and thereafter reach steady state at ~40% of the amplitudes of the first given pulse (37, 112). The remaining current component shows a lower sensitivity to external TEA\(^+\) (37), as demonstrated under similar experimental conditions for the frequency-independent \( n \) current in T lymphocytes (90), but can be abolished by CTX or NTX (37).

In their kinetics and in their sensitivity to 4-AP, TEA\(^+\), or CTX, whole cell DR currents in microglia are similar to DR currents in peritoneal, alveolar, monocyte-derived, and bone marrow-derived macrophages as well as in the macrophage cell lines J774.1 and THP-1 (35, 54, 84, 105, 107, 117, 143). DR currents expressed in microglia closely resemble cloned Kv1.3 channels (5, 15, 34, 59). With the use of PCR, the presence of Kv1.3 mRNA has been proven in rat microglial cells (109), suggesting that Kv1.3 codes for microglial DR channels.

Delayed Rectifier K\(^+\) Channels: Regulation

DR currents in microglia can be modulated by elevation of intracellular Ca\(^{2+}\) concentration \([\text{Ca}^{2+}]_i\) (112), by PKC activation (135, 142), or by changes in pH\(_i\) and extracellular pH (pHo) (38).

As has been first described for DR currents in T lymphocytes (12), a decrease in DR current amplitude was also observed in microglia in measurements with increased concentration of internal free Ca\(^{2+}\) or after elevation of [Ca\(^{2+}\)] \(_i\) induced by external application of the Ca\(^{2+}\) ionophore A-23187 (112).

In studies on human microglial cells, PKC activation with the phorbol ester PMA led to an enhancement of outward K\(^+\) currents (142), whereas DR currents in rat microglia decreased on treatment with PMA (135). Because experiments have been performed in unstimulated human microglia, whereas rat microglia were activated by LPS or IFN-\(\gamma\), the different changes in IR (see Inward Rectifier K\(^+\) Channels: Regulation) and DR current amplitudes obtained after PKC activation in both microglia preparations might be due to state-dependent differences in expression or activation of PKC isoforms. Controversial effects of PKC activators have also been described for Kv1.3 channels in other cells (2, 4, 21, 116).

Within the central nervous system (CNS), changes in pH\(_i\) have been observed in response to neuronal activity. A prolonged acidification of the extracellular space was detected on electrical stimulation (20) as well as under pathophysiological conditions, such as during...
epileptiform activity or spreading depression (19, 20). In some regions of the CNS, however, an alkalinization was measured after stimulus-evoked neuronal activity (19). Extracellular acidification shifts both steady-state activation and inactivation curves for microglial DR currents in a depolarizing direction, whereas alkalinization results in the opposite effect. Furthermore, DR currents inactivated more slowly on extracellular acidification than on alkalinization (Fig. 3) (38). Variations in pH change current density but do not affect the kinetics of DR currents in microglia. Intracellular acidosis reduces DR currents, whereas alkalosis increases the currents (38). Similar observations have been made for DR channels in human T lymphocytes (32).

The expression level of microglial DR channels can be modulated by the bacterial endotoxin LPS, by cytokines, or by astrocytic factors. Accompanied by a decrease in IR channel density (see Inward Rectifier K⁺ Channels: Regulation), DR channels appear to be upregulated during the process of cellular activation. Large DR currents were observed in microglia activated by LPS (111, 112), IFN-γ (38, 45, 111), or GM-CSF (37, 45), whereas they were only rarely detected in unstimulated microglial cells. A similar upregulation of DR currents after treatment with LPS or by differentiation with GM-CSF has been reported for monocyte- and bone marrow-derived macrophages (35, 106). Surprisingly, DR currents with properties similar to those of activated microglia were identified in ramified microglia developed on an astrocytic monolayer or treated with ACM (39, 40, 122, 126). However, recent evidence suggests that DR channel expression in microglia after exposure to ACM is not strictly correlated with morphological changes of the cells. DR currents are only transiently expressed in ramified microglia and disappear 5 days after treatment with ACM, whereas no changes occur in the ramified phenotype of the cells. Furthermore, DR currents also appear in ameboid microglia, when cells are exposed to low-concentrated ACM (40). The astrocytic factor leading to DR channel expression has not yet been identified. One possible candidate is the growth factor GM-CSF, which is released by astrocytes (94, 115) and known to trigger DR channel expression in murine microglia and macrophages (35, 37, 45).

Ca²⁺-Activated K⁺ Channels: Properties and Expression

Two types of Ca²⁺-activated K⁺ channels have been described in microglia. In bovine (97) and human (98) microglial cells, Ca²⁺-activated K⁺ channels are regulated by both Ca²⁺ and voltage. Voltage-independent Ca²⁺-activated K⁺ channels were described in murine microglial cells (41).

It has been demonstrated in whole cell experiments that the activation of Ca²⁺-activated K⁺ currents in bovine microglia is voltage dependent (97). In single-channel analyses of Ca²⁺-activated K⁺ channels using symmetric Ca²⁺ concentration solutions, a unitary conductance of >200 pS was determined in both bovine and human microglia (97, 98). Moreover, these channels show high sensitivity to external TEA⁺, as reported for a variety of large-conductance Ca²⁺-activated K⁺ channels (BK-type channels) in excitable and other nonexcitable cells (for reviews, see Refs. 85 and 120). In bovine microglia, Ca²⁺-activated K⁺ channels are completely inhibited by extracellularly applied TEA⁺ at a concentration of 2 mM (97). At a potential of 0 mV, a halftime maximal activation at 7 µM Ca²⁺ was determined for Ca²⁺-activated K⁺ channels in human microglia (98). Increasing [Ca²⁺]i shifts the voltage dependence of channel opening to more negative potentials, tending to open more channels at any given membrane potential.

Fig. 3. Variations in extracellular pH (pHₒ) modulate delayed rectifier (DR) K⁺ currents in murine microglia activated with interferon-γ. A-C: DR currents were evoked by voltage steps from Vₒ of −60 mV to potentials between −60 and +80 mV. Voltage pulses were applied in 20-mV increments for a duration of 2,000 ms. Microglial cell was superfused with external solutions of pH 5.8, 6.8, or 7.8. D: monoexponential fit of time-dependent inactivation of DR currents evoked by a voltage pulse from −60 to +80 mV at different pHₒ values. DR currents in microglia inactivate faster with increasing external alkalinization. [From Eder and Heinemann (38) with permission from Elsevier Science.]
Similar large-conductance Ca\(^{2+}\)-activated K\(^+\) channels have been described in human monocyte-derived macrophages, in alveolar macrophages, and in the macrophage cell line THP-1 (31, 49, 53).

Based on their characteristics, Ca\(^{2+}\)-activated K\(^+\) channels in bovine and human microglia resemble Slo channels that were first cloned from Drosophila (1, 3).

A distinct type of Ca\(^{2+}\)-activated K\(^+\) channel has been detected in murine microglia (41). These Ca\(^{2+}\)-activated K\(^+\) currents do not show voltage dependence at test potentials between -120 and +30 mV. In contrast to the BK-type currents of bovine microglia (97), Ca\(^{2+}\)-activated K\(^+\) currents of murine microglia exhibit only a weak sensitivity to extracellular TEA\(^+\) (41). CTX inhibits voltage-independent Ca\(^{2+}\)-activated K\(^+\) currents in murine microglia with a half-maximal current blockade at 3 nM, whereas KTX and apamin fail to block the currents (Fig. 4). Currents are also reduced by several inorganic polyvalent cations, namely, La\(^3+\), Ba\(^2+\), or Cd\(^2+\). Blockade of Ca\(^{2+}\)-activated K\(^+\) currents by Ba\(^2+\) is voltage dependent, with the deepest block seen at strong hyperpolarizing potentials (41).

With respect to their pharmacological profile and their voltage-independent gating, Ca\(^{2+}\)-activated K\(^+\) channels of murine microglia closely resemble small-conductance Ca\(^{2+}\)-activated K\(^+\) channels found in human (31, 50, 75, 78) and murine (61, 117) macrophages. Recently, two channel genes, hSK4 (77) and hIK1 (76), have been described, which might encode for the Ca\(^{2+}\)-activated K\(^+\) channel found in murine microglia.

So far only little is known about regulation of Ca\(^{2+}\)-activated K\(^+\) channels in microglia. In ramified murine microglia, Ca\(^{2+}\)-activated K\(^+\) currents increase in amplitude with time after exposure of microglia to ACM (41). Thus it is likely that the process of microglial ramification is accompanied by an increased level of [Ca\(^{2+}\)], which leads to the activation of Ca\(^{2+}\)-activated K\(^+\) channels.

G Protein-Activated K\(^+\) Channels: Properties and Expression

G protein-activated K\(^+\) currents have been identified in murine microglia (73, 74). These outwardly rectifying K\(^+\) currents were observed after activation of pertussis toxin (PTX)-sensitive G proteins. In the presence of intracellular GTP\(_\gamma\)S, outward K\(^+\) currents appear within a few minutes after establishing the whole cell configuration (74). These currents do not show apparent time-dependent activation or inactivation. Superfusing cells with 4 mM 4-AP reduces current amplitudes by ~40% (74). A similar 4-AP-sensitive outward K\(^+\) current was detected in microglia after extracellular application of ATP or of complement factor C5a, as well as after receptor stimulation with TNF or EGF (73, 74). On inhibition of G proteins by preincubation of microglial cells with PTX, by application of extracellular N-ethylmaleimide, or by intracellular perfusion with guanosine 5'-O-(2-thiodiphosphate), these outward K\(^+\) currents were not observed after receptor stimulation.

Motility of microglia induced by C5a is unaffected in the presence of the K\(^+\) channel blockers 4-AP, TEA\(^+\), or Ba\(^2+\), indicating that activation of G protein-activated K\(^+\) channels is not required for increased motility on C5a receptor stimulation (73).

A PTX-sensitive outwardly rectifying K\(^+\) current that is blocked by 4-AP or quinidine has been observed in the macrophage cell line J 774.1 and in bone marrow-
derived macrophages after G protein activation with GTPγS, Gpp(NH)p, or AlF4− (96) and in J 774.1 cells after stimulation with C5a, ATP, ADP, or interleukin-8 (44). In comparison, G protein-activated K+ currents of microglia exhibit properties nearly identical to those reported for G protein-activated K+ currents in the other macrophage preparations. However, although in both J 774.1 macrophages and microglia G protein-activated K+ currents cannot be induced when [Ca2+]i is buffered with 1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid (BAPTA) at <20 nM, activation of the currents exclusively by elevation of [Ca2+]i as seen in J 774.1 macrophages (44) has not been observed in murine microglia in the absence of receptor stimulation (73).

H+ Channels: Properties and Expression

Voltage-gated H+ currents have been described in murine microglia differentiated with M-CSF (36, 42). The existence of putative H+ currents has also been reported in rat (134) and human (98) microglia. However, since H+ currents were seen only in a small percentage of rat and human microglia, they were not characterized fully in these preparations. H+ currents with properties nearly identical to those in microglia have been described in peritoneal macrophages (81) and in THP-1 macrophages (29), as well as in a variety of other nonexcitable and excitable cells (for review, see Ref. 27).

At a gradient of 1.5 between pH1 (6.0) and pH0 (7.5), slowly activating outward currents were detected in murine microglia at potentials positive to ~40 mV. Activation behavior of H+ currents strongly depends on both pH1 and pH0. A decrease in pH1 shifts the activation threshold of the currents to more negative potentials. Similar shifts in the activation threshold and the g-V curve of H+ currents to more hyperpolarizing potentials, as well as an augmentation of H+ current amplitude, are seen when pH0 is increased. The threshold voltage at which H+ channels first open is shifted by 40 mV/unit change in the pH gradient (ΔpH = pH0 – pH1) in alveolar epithelial cells (18) and seems to behave similarly in microglia. H+ channels open only when there is an outward pH gradient, so that they only carry outward current in the steady state. Measurement of the reversal potential at different pH demonstrates that the H+ currents in microglia are highly selective for H+. Time-dependent activation of H+ currents can be fitted by a single exponential (36, 42).

H+ currents in microglia are inhibited by several inorganic polyvalent cations in the micromolar concentration range (Fig. 5). The following order of blocking activity has been determined: Zn2+ > La3+ > Ni2+ > Cd2+ > Co2+ > Ba2+. Simultaneous with the reduction in current amplitude, a shift in the g-V curve of H+ currents to more positive potentials is seen on application of polyvalent cations. H+ currents in microglia are also inhibited by superfusion with the K+ channel blockers 4-AP or TEA− but to a smaller extent than with inorganic cations (36). In contrast to observations made for H+ currents in epithelial cells and in neutrophils (28), H+ currents in microglia are decreased only slightly by extracellular Na+ (130 mM), suggesting that in microglia Na+/H+ exchange may be less prominent (42).

H+ Channels: Regulation

A possible regulation of H+ channels by PKC stimulation has been suggested for neutrophils (67, 68, 82, 104) and enterocytes (16). In these cells, activation of an electrogenic H+ efflux induced by phorbol esters has been detected using fluorescence imaging measurements of pH1. However, H+ currents in murine microglia were unaffected on extracellular application of the phorbol ester phorbol 12,13-dibutyrate (42).

H+ channels in microglia are modulated by cytoskeletal disruptive agents (42). A decrease in current density and an increase in activation time constant of H+ currents were observed after exposure of microglia to cytochalasin D or colchicine. Modulation of H+ channels due to cytoskeletal reorganization may be a possible explanation for similar changes in current density and activation time constant of H+ currents seen in microglia either after their transformation from an ameboid into a ramified phenotype or after stimulation with LPS (R. Klee and C. Eder, unpublished)
observations), as well as in THP-1 macrophages after their differentiation from monocytes (29).

Arachidonic acid, which is released by activated microglia or other stimulated phagocytes, has been shown to enhance voltage-gated H\(^+\) currents in neutrophils and macrophages (26, 80, 132). It has been suggested that arachidonic acid is the immediate activator of H\(^+\) channels in phagocytes (69, 132). Thus, arachidonic acid may also be important for H\(^+\) channel regulation in microglia in the brain environment.

**Na\(^+\) Channels: Properties and Expression**

Voltage-gated Na\(^+\) channels with properties indistinguishable from fast transient Na\(^+\) channels of neurons (for reviews, see Refs. 17, 22, 64) have been described in rat (87, 122, 126) and human (113) microglia. In whole cell recordings, Na\(^+\) currents activate at depolarizing potentials positive to ~40 mV and reach a current maximum at ~0 mV (87, 113, 122). Currents exhibit rapid time-dependent activation and inactivation. Half-maximal steady-state inactivation at a potential of ~55 mV was determined for Na\(^+\) currents in rat microglia (122). Currents are completely inhibited by superfusion with 2–5 µM tetrodotoxin (TTX) or after substitution of extracellular Na\(^+\) by choline (87, 113).

Microglial Na\(^+\) channels are readily distinguished from "glial" Na\(^+\) channels with respect to their kinetics and sensitivity to TTX. The glial Na\(^+\) channels found in flat, fibroblast-like astrocytes are highly resistant to TTX and exhibit a significantly more negative steady-state inactivation than TTX-sensitive Na\(^+\) channels of neurons and microglia (128).

**Na\(^+\) Channels: Regulation**

In freshly isolated rat microglia, a significantly larger percentage of cells exhibiting Na\(^+\) currents was found within the population of ramified microglia than in the population of ameboid cells (87).

In an intriguing study Siewers and co-workers (122, 126) demonstrated that the expression of Na\(^+\) channels in microglia as well as in monocytes is regulated by astrocytes. Accompanied by the transformation of the cells into a ramified morphology, Na\(^+\) currents were detected in ~80% of both microglia and monocytes after cultivation in direct contact with astrocytes. In contrast, Na\(^+\) currents were not found in ramified spleen macrophages cultured on astrocytes under similar conditions (122). Although fast transient Na\(^+\) currents were obtained in several T cell and B cell lines (51) as well as in murine T lymphocytes (24, 25), to my knowledge they have not been described in any other macrophage preparation.

**Ca\(^{2+}\) Channels: Properties and Expression**

In microglia, two types of Ca\(^{2+}\) channels have been detected, namely, voltage-gated Ca\(^{2+}\) channels (23) and Ca\(^{2+}\)-release activated Ca\(^{2+}\) (CRAC) channels (110). The existence of voltage-gated Ca\(^{2+}\) channels has been reported in one study on rat microglia (23). These microglial Ca\(^{2+}\) currents exhibit some properties of L-type Ca\(^{2+}\) channels described in a variety of excitable cells (for reviews, see Refs. 64, 124, 133). In ~30% of the microglial cells investigated, Ca\(^{2+}\) currents of small amplitude were detected after exposure to the Ca\(^{2+}\) channel opener BAY K 8644. Microglial currents activate at potentials of about −35 mV and show a U-shaped activation curve with a current maximum at about +20 mV.

Voltage-gated Ca\(^{2+}\) channels have not been characterized in other patch-clamp studies of microglia or macrophages. However, an increase in [Ca\(^{2+}\)]\(_i\) during depolarization of the cell membrane induced by elevation of [K\(^+\)]\(_o\) was detected by fluorescence measurements in the macrophage cell line PU5–1.8 (86) and in Kupffer cells (70). The observed increases in [Ca\(^{2+}\)]\(_i\), depended on external Ca\(^{2+}\), were blocked by nifedipine and verapamil (86), and were enhanced by BAY K 8644 (70), suggesting the presence of voltage-gated Ca\(^{2+}\) channels in both macrophage preparations. It is possible that voltage-gated Ca\(^{2+}\) channels are not always expressed in the membranes of microglia and other macrophages. Similar to observations made for microglial K\(^+\), H\(^+\), and Na\(^+\) channels, the expression level of Ca\(^{2+}\) channels may be regulated by microenvironmental factors and may depend on the functional state of the cells. Moreover, Ca\(^{2+}\) currents in microglia undergo rapid rundown (23), which might also explain why voltage-gated Ca\(^{2+}\) channels have not been detected in other whole cell patch-clamp studies of microglia.

In the presence of 10 mM extracellular Ca\(^{2+}\), CRAC currents were induced in rat microglia by perfusing cells with inositol trisphosphate, whereas no currents were detected in Ca\(^{2+}\)-free external solution (110). In the whole cell configuration, CRAC currents develop slowly after a delay of a few seconds. The activation time course was fitted monoexponentially with a time constant of 25 s. CRAC currents in microglia exhibit a strong inward rectification and do not reverse at potentials between −100 and +100 mV.

Evidence for the existence of CRAC channels in microglia has also been demonstrated in Ca\(^{2+}\)-imaging experiments. After depletion of internal Ca\(^{2+}\) stores by thapsigargin, a long-lasting Ca\(^{2+}\) elevation was induced in cultured murine microglia, which was abolished in Ca\(^{2+}\)-free external solution (100).

CRAC channels have been described in various immune cells, including macrophages (47, 93) and lymphocytes (for review, see Ref. 91). However, since CRAC channels in microglia have not been characterized in more detail, at present microglial channels cannot be compared with CRAC channels seen in other cells.

**Cl\(^−\) Channels: Properties and Expression**

The existence of Cl\(^−\) channels has been demonstrated in single-channel measurements or in whole cell recordings of rat (121, 134), bovine (97), human (98), and murine (43) microglia.

Cl\(^−\) channels of large unitary conductance (280–325 pS) were detected in inside-out patches of bovine and human microglial cells (97, 98). It appears that, in microglia, voltage-dependent Cl\(^−\) channels are virtu-
ally silent in whole cell measurements and only become activated after excision of the patch, as has been reported for corresponding Cl− channels in macrophages and other nonexcitable cells (51). All of the properties of the channels in microglia resemble those of voltage-dependent Cl− channels described in murine peritoneal macrophages and in the macrophage cell lines J74.1 and U-937 (79, 117, 123). Cl− currents inactivate during maintained voltage commands, and the time course of inactivation becomes faster with increased depolarization. Activation of the channel appears to be independent of Ca2+. Similar to observations made for Cl− channels in peritoneal macrophages and U-937 cells (79, 123), multiple subconductance states of the Cl− channel were seen in bovine microglia. These subconductance states are reached from either the open or the closed level. Amplitude and kinetics of the Cl− currents are not influenced by lowering pH. Extracellular application of 1 mM Ba2+ induced channel flickering in bovine microglia.

Voltage-independent Cl− channels have been identified in whole cell measurements of rat (121, 134) and murine (43) microglia. In rat microglia, small Cl− currents were seen in a percentage of cells under isosmotic conditions (121, 134). Exposure of microglia to a hyposmotic external solution leading to cell swelling results in a large increase in Cl− current amplitude (121). Cl− currents with properties similar to those of swelling-activated Cl− currents were induced in murine microglia by stretching of the cell membrane (Ref. 43 and unpublished observations). Currents exhibit outward rectification (Fig. 6) and do not show time- or voltage-dependent gating. Lowering the concentration of extracellular Cl− results in a shift of the reversal potential of the current in the depolarizing direction. Substitution of external Cl− by gluconate reveals a relative permeability of 0.34 for gluconate compared with Cl− in rat microglia. Cl− currents undergo rundown, which is prevented by addition of ATP to the pipette solution. DIDS and SITS inhibit Cl− currents in a voltage- and time-dependent manner. Currents are also blocked by 5-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), or flufenamic acid in the micromolar concentration range but are unaffected by extracellular application of Ba2+ or La3+.

With respect to their kinetic and pharmacological properties, both swelling-activated and stretch-activated Cl− currents of microglia closely resemble small-conductance voltage-independent Cl− currents that have been shown to activate either spontaneously or in response to osmotic stress in monocyte-derived macrophages (105), in THP-1 cells (84), and in a number of other immune cells (for review, see Ref. 30).

**FUNCTIONAL ROLE OF ION CHANNELS IN MICROGLIA**

**K+ Channels**

IR channels of microglia seem to be involved in maintaining the membrane potential of the cells. Similar to observations made in macrophages (54), blocking IR channels with Ba2+ results in membrane depolarization in rat microglia (134). Moreover, significantly more positive resting membrane potentials were observed in microglial cells that lacked IR channels than in cells exhibiting large IR currents (45). A similar correlation between the density of IR channels and the value of the resting membrane potential was found in J74.1 macrophages (54, 95).

Several authors have described the properties and regulation of microglial DR currents, but no experimental data are available so far that demonstrate a functional role of DR channels in microglia. However, intensive studies have been performed on other immune cells, suggesting an important role of DR channels in maintaining a large negative membrane potential, in lymphocyte activation and proliferation, as well as in volume regulation (for reviews, see Refs. 30 and 91).

As described in lymphocytes, DR channels in microglia might be involved in repolarizing the membrane potential after depolarizing events. A hyperpolarized membrane potential might be a prerequisite condition for initiating microglial functions, such as phagocytosis, proliferation, motility, cytokine secretion, and oth-

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**Fig. 6. Cl− currents in murine microglia induced by membrane stretch.** A: voltage ramps from −120 to +90 mV were applied for a duration of 400 ms. Currents are shown at 1 and 10 min after application of membrane stretch. For these recordings, a K+ -free intracellular solution was used that contained 4 mM ATP. B: whole cell recordings before (control) and after stretching of the cell membrane. Currents were evoked by voltage pulses from Vr of −10 mV to potentials between −110 and +80 mV for a duration of 400 ms. Stretch-activated Cl− currents do not show time- or voltage-dependent gating.
ers. In immune cells, a negative membrane potential is also important for maintaining a large driving force for Ca$^{2+}$ influx through CRAC channels, which modulate [Ca$^{2+}$], and subsequently regulate gene expression of the cells (for review, see Ref. 91). Thus K$^+$ channels might be involved in regulating the functional state of microglial cells.

Proliferation of microglia appears to be independent of functional DR channels, since the K$^+$ channel blockers CTX or MTX do not inhibit proliferation of rat microglia (121). Moreover, the finding that only a small percentage of proliferating and M-CSF-developed microglia exhibit DR channels (45, 112, 121) also argues against the requirement for DR channels during proliferation of microglia.

It is possible that DR channels are involved in volume regulation in microglia, as reported for lymphocytes (30, 91). Increases in the membrane capacitance as a measure of cell size accompanied by the expression of DR channels have been observed in microglia after exposure to LPS, IFN-γ, or ACM (40, 112, 134). However, blockade of K$^+$ channels by CTX or KTX does not inhibit microglial ramification (40), suggesting a lack of correlation between DR channel expression and cell surface increase in ramified microglia.

G protein activation in microglia inhibits IR channels, whereas both IR and DR channels appear to be inhibited to some extent on elevation of [Ca$^{2+}$]. Thus, under these conditions, either G protein-activated or Ca$^{2+}$-activated K$^+$ channels may play a larger role in maintaining a negative membrane potential. It has been shown that G proteins can be activated in microglia by several external stimuli, such as TNF, EGF, or complement factor C5a (73, 74). [Ca$^{2+}$] is augmented after Ca$^{2+}$ release from internal stores (6, 138) or after Ca$^{2+}$ entry via nonspecific cation channels by ATP receptor stimulation (72), via voltage-gated Ca$^{2+}$ channels (23), or via CRAC channels (110). Activation of voltage-independent Ca$^{2+}$-activated K$^+$ channels is induced by an increase in [Ca$^{2+}$] to 1 μM, a value reached during physiological stimulation. In contrast, as described for large-conductance Ca$^{2+}$-activated K$^+$ channels in human monocyte-derived macrophages (49), BK-type channels of microglia are relatively insensitive to [Ca$^{2+}$]. (98). Thus either large increases in [Ca$^{2+}$], or a moderate [Ca$^{2+}$] elevation accompanied by a large cell depolarization is required for activation of these channels.

**H$^+$ Channels**

Although so far no experimental data have been reported demonstrating the importance of voltage-gated H$^+$ channels in microglia under physiological and pathophysiological conditions, a functional role of microglial H$^+$ channels can be proposed from data obtained in other phagocytes, including macrophages and neutrophils (for reviews, see Refs. 27 and 30). It is suggested that during phagocytosis H$^+$ channels are involved in H$^+$ extrusion. During cellular activation, phagocytes undergo a respiratory burst, in which the NADPH oxidase releases bactericidal superoxide anions into the phagosome and stoichiometrically releases H$^+$ into the cell, tending to produce depolarization and intracellular acidification (for review, see Ref. 66). H$^+$ currents may help maintain both the membrane potential and pH$_i$ during the respiratory burst. By compensating for the efflux of electrons and counteracting internal acidification, H$^+$ efflux through voltage-gated H$^+$ channels may help maintain superoxide production. Blocking H$^+$ channels with Zn$^{2+}$ or Cd$^{2+}$ inhibits the production of superoxide anion in neutrophils (68).

Because in microglia Na$^+$/H$^+$ exchange appears to play only a small role as has been reported for other macrophages (9, 102), microglial H$^+$ channels may also be involved in processes of pH regulation in several other situations. Moreover, outward H$^+$ currents may contribute to the repolarization of the cell membrane in situations that shift the membrane potential above the equilibrium potential for H$^+$ (E$_{H^+}$). This could occur either at physiological pH$_i$ after large membrane depolarization (e.g., after Na$^+$ or Ca$^{2+}$ spikes) or after a moderate depolarization coupled with internal acidification, because then E$_{H^+}$ would be shifted to more negative potentials (e.g., during the respiratory burst or other metabolic processes).

**Na$^+$ Channels**

The role of voltage-gated Na$^+$ channels in microglia is not well understood. It appears that these channels are mainly expressed in ramified microglial cells (87, 122, 126), which are thought to be in an immunologically resting state (88, 101, 131). It can be speculated that the activation of Na$^+$ channels would result in rapid membrane depolarization, which might be necessary to trigger signal cascades for microglial activation and a subsequent immune response.

The observation that an upregulation of Na$^+$ channels occurs in parallel with the process of ramification in microglia on cultivation on astrocytes led us to wonder whether Na$^+$ channels might be involved in regulating morphological changes. However, ramification of microglia was not inhibited in the presence of the specific Na$^+$ channel blocker TTX (unpublished observations).

**Ca$^{2+}$ Channels**

Little is known about the importance of CRAC channels in microglia. Studying the functional role of these channels has been complicated by the absence of a specific channel blocker and because CRAC channels have not been cloned. In lymphocytes the sustained increase in [Ca$^{2+}$] by influx of Ca$^{2+}$ through CRAC channels is essential for proliferation and gene expression (for review, see Ref. 91). Blocking CRAC channels in mast cells inhibits histamine secretion (118). To clarify the functional importance of CRAC channels in microglia, further investigations are required.

Colton and co-workers (23) proposed an involvement of voltage-gated Ca$^{2+}$ channels in microglial superoxide production. This hypothesis is based on the observations that production of superoxide anion is increased...
on depolarization by increasing [K\textsuperscript{+}]\textsubscript{o} is further enhanced by additional treatment of the cells with the Ca\textsuperscript{2+} channel opener BAY K 8644, but is inhibited by nifedipine. An inhibition of the respiratory burst by Ca\textsuperscript{2+} channel blockers has also been reported in alveolar macrophages (103) and peritoneal macrophages (125). During the respiratory burst the membrane of phagocytes is depolarized due to the activity of the NADPH oxidase (66). Because depolarization of the cell membrane limits Ca\textsuperscript{2+} influx through CRAC channels, voltage-gated Ca\textsuperscript{2+} channels may play an important role during processes leading to membrane depolarization but they require an increased level of [Ca\textsuperscript{2+}]\textsubscript{i}. 

**CONCLUSION**

Patch-clamp studies in microglia have revealed a diversity of ion channels. Most of the channels detected have been characterized with respect to their kinetic and pharmacological properties, with the majority of studies focused on microglial K\textsuperscript{+} channels. It can be concluded that microglial ion channels share many properties with the corresponding ion channels in other macrophages.

We are only beginning to learn the functional roles of ion channels in microglia. There is reason to believe that ion channels in microglia maintain the membrane potential and participate in some way in proliferation, ramification, and the respiratory burst. Other functions of microglial ion channels have been proposed from the knowledge about channels in other leukocytes, such as a possible involvement in regulation of the membrane potential, of the internal Ca\textsuperscript{2+} concentration, and of pH\textsubscript{i}. At present it is also not well understood why the expression patterns of the channels change during differentiation and activation of microglia. Further studies will be necessary to determine regulation and functions of the different microglial ion channels. Moreover, to get a better understanding of the behavior of microglia in the brain environment under physiological and pathophysiological conditions, further investigations of microglial ion channels using in situ recording techniques will be required.

I am very grateful to Thomas E. DeCoursey for many helpful discussions and comments on the manuscript.

This work was supported by a fellowship of the Alexander von Humboldt Foundation and by Grant SFB 507/C3 of the Deutsche Forschungsgemeinschaft.


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