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Properties of Voltage-gated Potassium Currents of Microglia Differentiated with Granulocyte/Macrophage Colony-stimulating Factor

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Abstract. Voltage-gated whole-cell currents were recorded from cultured microglial cells which had been developed in the presence of the macrophage/microglial growth factor granulocyte/macrophage colony-stimulating factor. Outward K^+ currents (I_K) were most prominent in these cells. I_K could be activated at potentials more positive than –40 mV. Half-maximal activation of I_K was achieved at –13.8 mV and half-maximal inactivation of I_K was determined at –33.8 mV. The recovery of I_K from inactivation was described by a time constant of 7.9 sec. For a tenfold change in extracellular K^+ concentration the reversal potential of I_K shifted by 54 mV.

Extracellularly applied 10 mM tetraethylammonium chloride reduced I_K by about 50%, while 5 mM 4-aminopyridine almost completely abolished I_K. Several divalent cations (Ba^{2+}, Cd^{2+}, Co^{2+}, Zn^{2+}) reduced current amplitudes and shifted the activation curve of I_K to more positive values. Charybdotoxin (IC_{50} = 1.14 nM) and noxiustoxin (IC_{50} = 0.89 nM) blocked I_K in a concentration-dependent manner, whereas dendrotoxin and mast cell degranulating peptide had no effect on the current amplitudes.

The outward K^+ currents showed a frequency dependence when depolarizing pulses were applied at a frequency of 1 Hz. A frequency-independent outward current (I_K') characterized by the same activation behavior as I_K was detected. I_K' was blocked completely by 10 nM charybdotoxin or by 10 nM noxiustoxin. In contrast to its effect on I_K, 10 mM tetraethylammonium chloride did not reduce I_K'.

Key words: Microglia — Granulocyte/macrophage colony-stimulating factor — Whole-cell recording — Outward K^+ currents — Frequency-independent K^+ current — Peptide toxins

Introduction

In the mouse, granulocyte/macrophage colony-stimulating factor (GM-CSF) is a 23 kD glycoprotein cytokine identified to promote the development of various bone marrow-derived cell populations, i.e., granulocytes, macrophages (Metcalf, 1989) or dendritic cells (Inaba et al., 1992). Besides its effects on growth, differentiation and immunofunctional activity (Metcalf, 1989; Hamilton, 1993), GM-CSF also acts as a survival factor by preventing hematopoietic cells from apoptosis (Williams et al., 1990). In the central nervous system an expression of both GM-CSF and its specific receptor has been detected in association with microglia activation (Raivich et al., 1991; Hunter et al., 1992). These findings are consistent with in vitro data which demonstrate that cultured microglia respond to GM-CSF by proliferation (Frei et al., 1987; Giulian & Ingeman, 1988) and initiation of the antigen-directed immune cascade (Fischer et al., 1993).

Considering the potent modulatory effects of GM-CSF on microglial activity, its influence on electrophysiological properties remained unnoticed. Recent studies on voltage-gated K^+ currents of GM-CSF-treated microglial cells reveal an unusual K^+ channel profile: In contrast to cultured resting cells which exhibit inward

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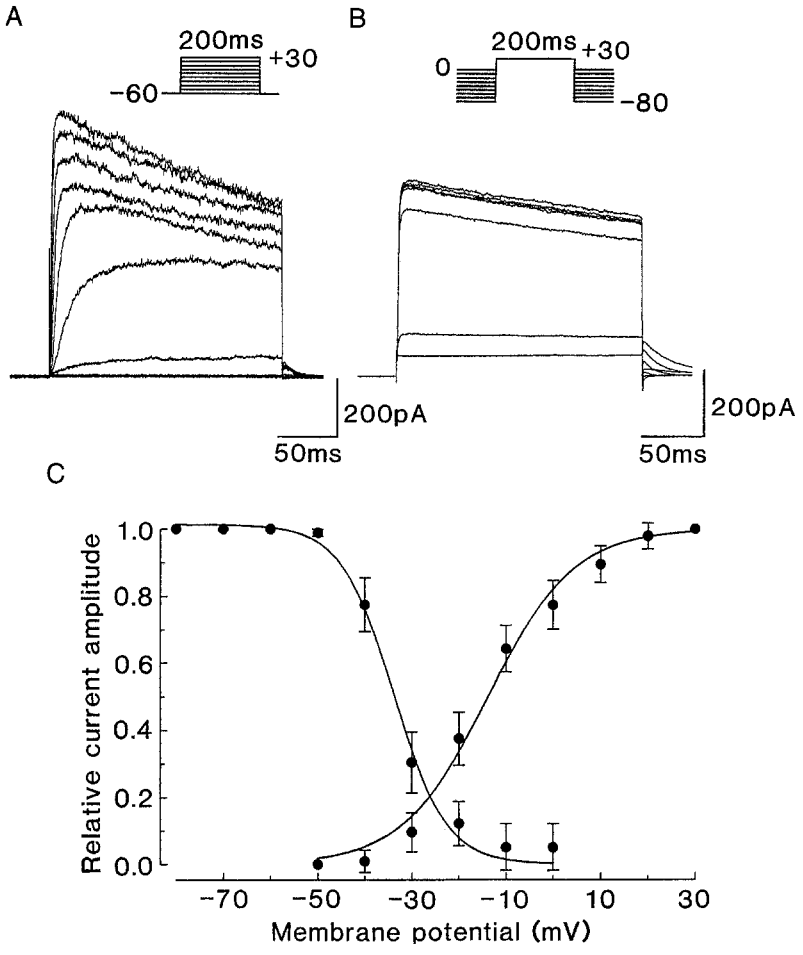

Fig. 1. Activation and steady-state inactivation behaviour of I_K. (*A*) Outward K^+ currents were evoked by 200 msec depolarizing voltage commands applied every 8 sec in 10 mV steps from −60 to +30 mV. Cells were held at −60 mV. (*B*) Varying the holding potential between −80 and 0 mV microglial cells were pulsed for 200 msec to a test potential of +30 mV. (*C*) Steady-state inactivation and activation curves of normalized peak currents using the voltage protocols shown in *A* and *B*.

rectifying K^+ currents (Kettenmann et al., 1990; Fischer et al., 1994; Eder et al., 1995), exposure to GM-CSF results in the sole expression of outward K^+ currents (I_K) by most recorded cells (Fischer et al., 1994). The expression of I_K has been detected in isolated microglial cells for a duration of more than one week, but it was already seen in microglia during their development in GM-CSF-supplemented primary cell cultures. In comparison, upon stimulation with lipopolysaccharide (LPS) (Nörenberg et al., 1992, 1994) activated microglia simultaneously show both inward and outward currents. Therefore, the detection of only I_K on microglia may provide evidence for their distinct functional state which is generated during cultivation with GM-CSF.

In the present study detailed analyses of the kinetic and pharmacological properties of the voltage-gated potassium currents on GM-CSF-grown microglia have been performed.

Materials and Methods

Cell Culture

Microglia were obtained from brain cell cultures of newborn (B10xC3H/HeJ)F_1 mice which originated from a breeding stock supplied by the Zentralinstitut für Versuchstierforschung (Hannover, Germany). Mixed brain cell cultures were prepared with minor modifications of the standard method (Frei et al., 1987) and were enriched for microglia by administration of GM-CSF. In detail: brain cortices had been enzymatically dissociated (1 hr at 37°C with 1.2 U/ml dispase and 0.2 U/ml collagenase, both from Boehringer, Mannheim, Germany) and a single-cell suspension was achieved by repeated triturations. Cells were seeded into tissue culture flasks at a density of 2–4 × 10^6/5 ml in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal calf serum and 50 ng/ml recombinant murine GM-CSF (F. Seiler, Behringwerke Marburg, Germany). After at least 10 days of incubation microglial cells were harvested by shaking the cultures (1 hr, 300 rpm) to detach weakly adherent cells from the astrocytic monolayer. As previously tested by FACS analysis the cells recovered represent a nearly homogenous microglia population and stain positive for CD11b and F4/80 markers but fail to express astrocytic GFAP cytoskeleton marker (Fischer et al., 1993). Isolated microglia were propagated up to 8 days on glass coverslips in 48-well Costar plates (5 × 10^4/1 ml) for patch clamp experiments while maintaining the GM-CSF-supplemented medium.

Electrophysiological Recordings

Membrane currents were measured using the whole-cell configuration of the patch clamp technique (Hamill et al., 1981). An EPC-9 patch clamp amplifier (HEKA, Lambrecht/Pfalz, Germany) was interfaced to an Atari computer for pulse application and data recording. Series

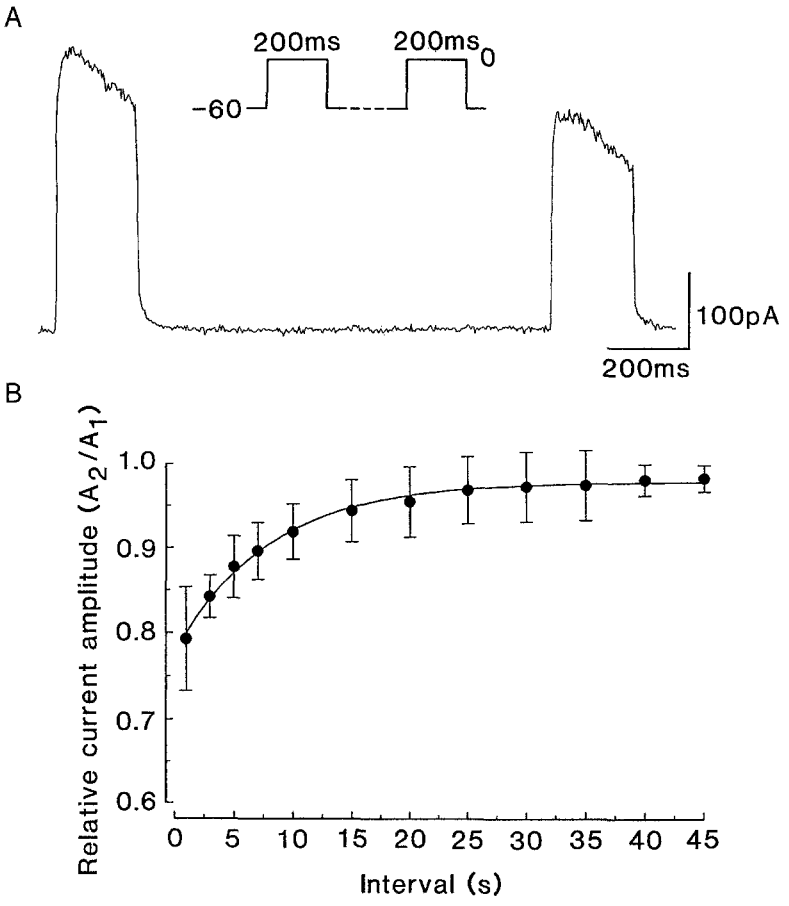

Fig. 2. Recovery of I_K from inactivation. Cells were depolarized from the holding potential (–60 mV) for 200 msec to a potential of 0 mV to inactivate I_K before they were stimulated again to a 200 msec pulse to 0 mV. The interpulse interval was varied between 1 and 45 sec. (*A*) Recordings of I_K applying 1 sec between the two test pulses. (*B*) Time course of recovery of I_K from inactivation. The ratio of the peak current during the second pulse to that during the first pulse is plotted as a function of the interpulse interval.

resistance compensation was routinely used to reduce the effective series resistance by about 70%. Patch electrodes of 3–4 MOhms were fabricated on a two-stage puller (Narishige PP-83, Tokyo, Japan) from borosilicate glass (outer diameter 1.5 mm and inner diameter 1 mm; Hilgenberg, Malsfeld, Germany). The electrodes were filled with the following solution (in mM): KCl, 120; $CaCl_2$, 1; $MgCl_2$, 2; HEPES, 10; EGTA, 11; D-glucose, 20. This solution was adjusted to pH 7.3 with KOH. The extracellular solution contained (in mM): NaCl, 120; KCl, 5.4; $CaCl_2$ 2; $MgCl_2$, 1; HEPES, 10; D-glucose, 25. The pH of the extracellular solutions was adjusted to 7.4. Using these solutions voltage-gated currents could be measured for a duration of more than 1 hr, while they did not show any kind of rundown. All recordings were done at room temperature (20–23°C). Voltage-gated currents were filtered at 3 kHz and stored on computer disk for subsequent analyses. Analyses were performed on Atari computers with the Review program (Instrutech, Mineola, NY). Current recordings were not subtracted for leak currents which were very small compared with the voltage-gated currents. Membrane capacitances of the cells analyzed in this study varied between 10 and 30 pF. Data are presented as mean values ± SD of the number of experiments indicated.

Pharmacological Studies

For drug application a 4-barrel or a 6-barrel microperfusion pipette was positioned at a distance of about 30–50 μm from the recorded cell to permit a rapid exchange of solutions outside the cell, which was achieved in less than one second. The flow rate was adjusted by hydrostatic pressure.

Tetraethylammonium chloride (TEA) (Sigma, Germany), 4-aminopyridine (4-AP) (Sigma, Germany), $BaCl_2$, $CdCl_2$ or $ZnCl_2$ were added to the extracellular superfusing solution. In some experiments a Ca^{2+}-free external solution was used or the extracellular KCl was varied between 2 and 50 mM by equimolar substitution of NaCl. The following peptide toxins were tested: charybdotoxin (CTX) (Latoxan, Rosans, France), dendrotoxin (DTX) (F. Dreyer, Institute of Pharmacology, Gießen, Germany), mast cell degranulating peptide (MCDP) (Latoxan, Rosans, France), noxiustoxin (NTX) (L. Possani, Institute of Biotechnology, Cuernavaca, Mexico). Peptide toxins were dissolved in 0.1% bovine serum albumin containing solutions.

Results

Activation Behavior of Outward K^+ Currents

Outward K^+ currents (I_K) were measured when applying 200 msec voltage commands in 10 mV increments from the holding potential (–60 mV) to +30 mV. An example of superimposed current traces is illustrated in Fig. 1*A*. Potassium currents were seen first at pulses to –30 mV and became larger in amplitude at more depolarizing voltage commands. After plotting normalized peak current amplitudes *vs.* membrane potential, the activation curve of I_K could be described by a Boltzmann equation.

In 11 cells studied with this protocol a half-maximal activation voltage of −13.8 mV was estimated (Fig. 1*C*). The outward current time to peak decreased when stepping to more positive potentials. 2000 msec voltage pulses were used to determine the inactivation behaviour of I_K. The decay of the outward K^+ currents could be fitted by one exponent with a time constant of 568 ± 23 msec (n = 8) when pulsed to +30 mV. The decay time course of I_K did not markedly change at voltage pulses to potentials more positive than 0 mV.

Steady-state Inactivation of I_K

The steady-state inactivation of I_K was studied by varying the holding potential between −80 and 0 mV in 10 mV increments. Several minutes after establishing the actual holding potential cells were pulsed to a potential of +30 mV for 200 msec. At holding potentials above −50 mV I_K decreased in amplitude. The example of current recordings in Fig. 1*B* shows that no outward K^+ current could be activated when cells were held at potentials positive to −20 mV. Peak amplitudes of the evoked currents were measured, normalized and then plotted as a function of the holding potential (Fig. 1*C*). Using a Boltzmann function a half-maximal inactivation of −33.8 mV (n = 9) was determined.

Recovery of I_K from Inactivation

The time course of recovery from inactivation was measured using a two-pulse voltage protocol (Fig. 2). From the holding potential of −60 mV, a 200 msec voltage pulse to a potential of 0 mV was applied. Cells were then returned to the holding potential for intervals between 1 and 45 sec before again pulsing to a potential of 0 mV (200 msec). To ensure complete recovery of the currents between each paired pulse application there was an interval of 60 sec. The peak current at the second step (A_2) was expressed as a proportion of the peak current elicited by the first step (A_1) and plotted as a function of the interpulse interval (n = 8) (Fig. 2*B*). With increasing interpulse interval durations the peak current of the second pulse became larger indicating the removal of inactivation. The time course of recovery could be fitted monoexponentially with a time constant of 7.9 sec (n = 8).

Potassium Dependence of I_K

To examine the K^+ selectivity of the outward currents, reversal potentials were estimated by measuring tail currents evoked by the application of 200 msec voltage pulses to potentials between −10 and −110 mV in 5 mV steps following a depolarizing pulse to 0 mV (200 msec).

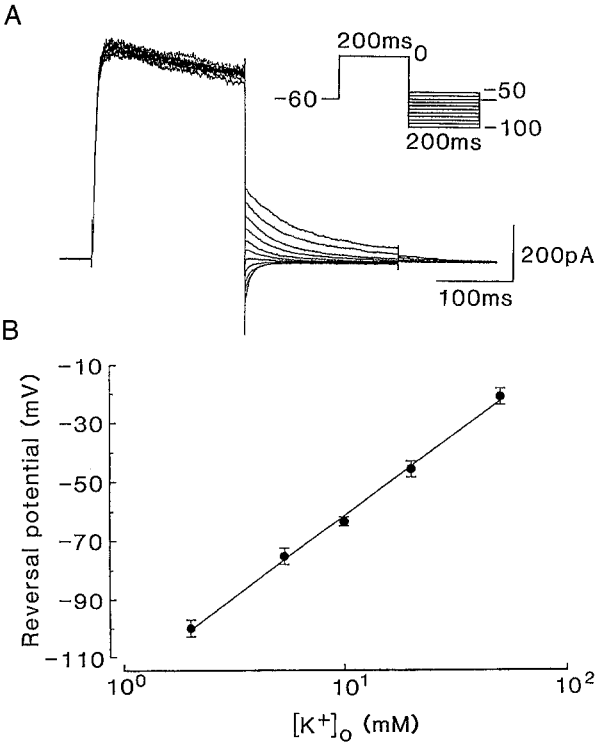

Fig. 3. Potassium dependence of I_K. (*A*) Following a test pulse to 0 mV (200 msec) the cell was pulsed to potentials between −50 and −100 mV. The external K^+ concentration was set to 5.4 mM. (*B*) Reversal potentials of I_K are plotted as a function of log $[K^+]_o$.

Using this protocol the reversal potentials for I_K were obtained in the presence of external potassium concentrations varying between 2 and 50 mM. As shown in Fig. 3 a tenfold increase in the extracellular potassium concentration shifted the reversal potential by 54 mV which is in good agreement with the value of 58 mV predicted by the Nernst equation.

Pharmacology of I_K

The pharmacological properties of I_K were investigated using the following depolarizing voltage protocol: 200 msec voltage commands were applied from the holding potential of −60 mV in 10 mV increments from −60 to +30 mV. Peak amplitudes of the whole-cell outward currents were measured when extracellularly superfusing cells with control solution or with solutions containing K^+ channel blockers.

Even at high concentrations of TEA, an effective delayed rectifying channel blocker in many tissue preparations (Cook & Quast, 1989; Hille, 1992), no complete block of I_K was demonstrable. At a concentration of 10 mM TEA I_K was reduced by 48.4 ± 1.2% (n = 4) whereas at 20 mM a reduction of 55.8 ± 3.2% (n = 5) was determined (Fig. 4). In contrast to TEA, 4-AP appears to be

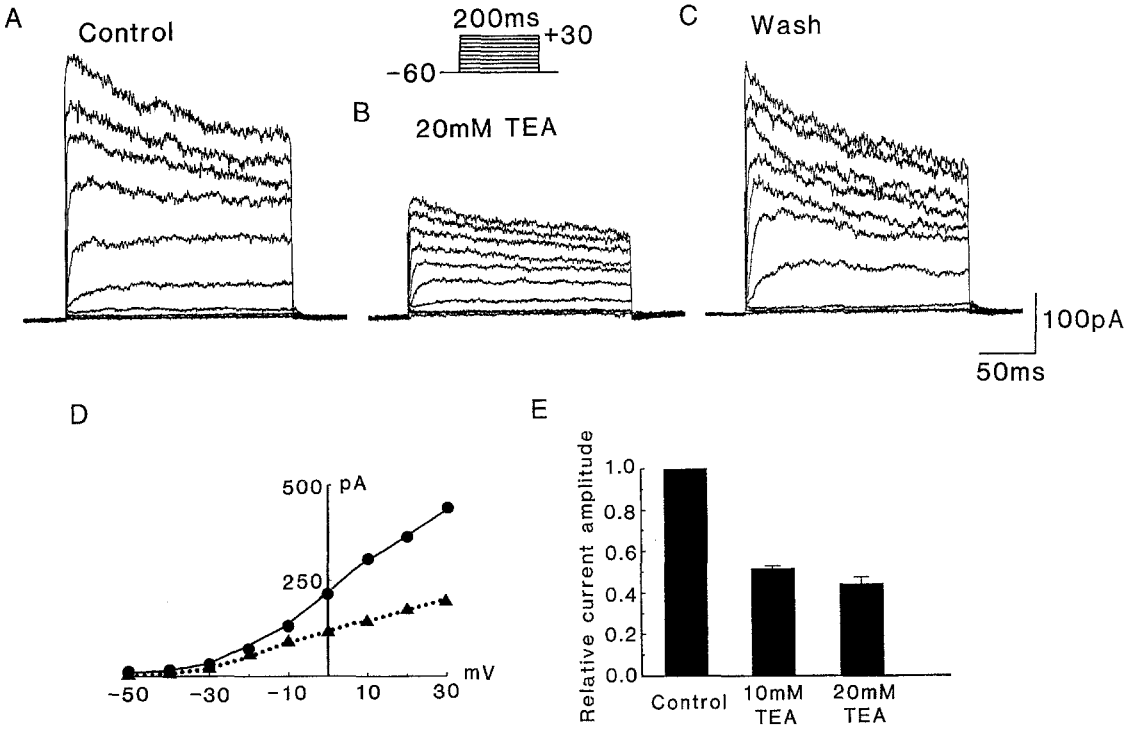

Fig. 4. Effect of extracellularly applied TEA on I_K. Using the pulse protocol as illustrated in the inset currents were recorded before (*A*), during (*B*) and after (*C*) superfusion with 20 mM TEA. (*D*) Corresponding *IV*-curves of peak currents in the presence of control solution (solid line) and in the presence of 20 mM TEA (broken line). (*E*) Reduction of peak current amplitudes after application of 10 and 20 mM TEA obtained at +30 mV.

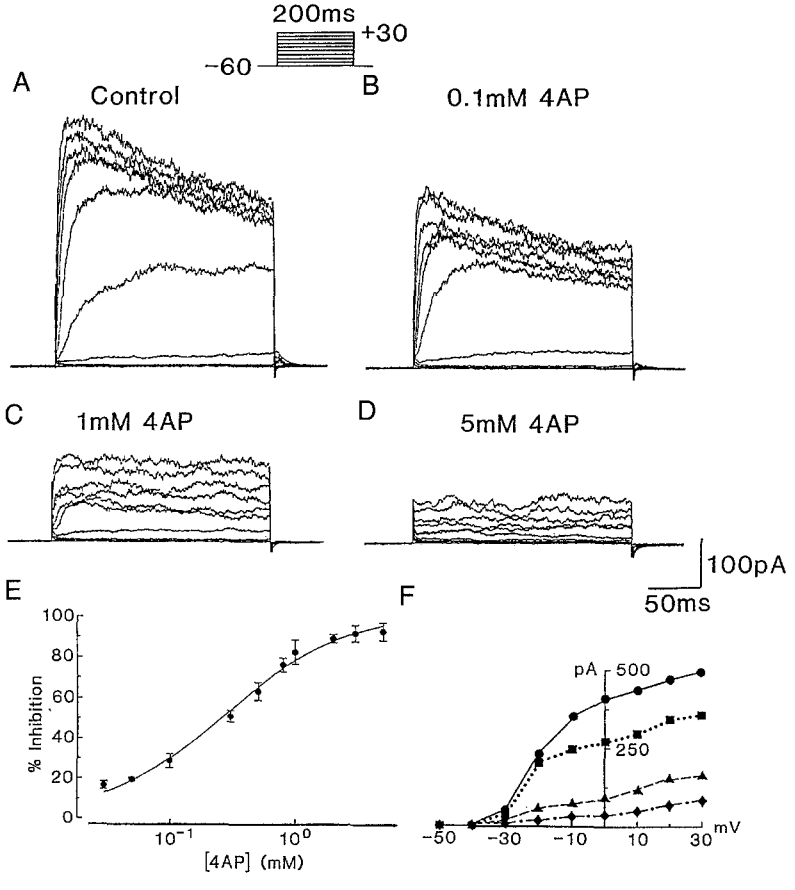

Fig. 5. Effect of 4-AP on I_K. Outward K^+ currents were evoked by the voltage protocol shown in the inset. Cells were superfused with control solution (*A*), 0.1 mM (*B*), 1 mM (*C*), and 5 mM (*D*) 4-AP (*E*) Concentration-response-curve for 4-AP. Peak current amplitudes were measured at 200 msec pulses to +30 mV (*F*) *IV*-relationship for the peak currents in control solution (continuous line) and in the presence of 0.1 mM 4-AP (dotted line), 1 mM 4-AP (dashed line) and 5 mM 4-AP (dot-dash line).

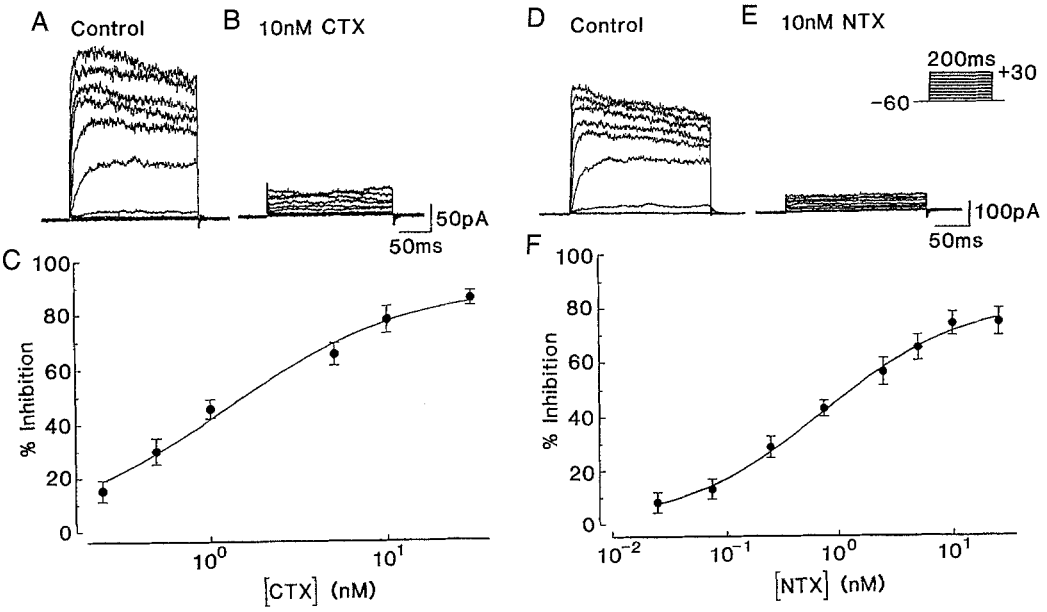

Fig. 6. Effect of peptide toxins on I_K. Current recordings in control medium (*A*, *D*) and in the presence of 10 nM CTX (*B*) and 10 nM NTX (*E*). The pulse protocol shown in the inset was used to activate I_K. Concentration-response-curves for CTX (*C*) and NTX (*F*) were investigated analysing peak current amplitudes after application of a 200 msec voltage pulse to +30 mV.

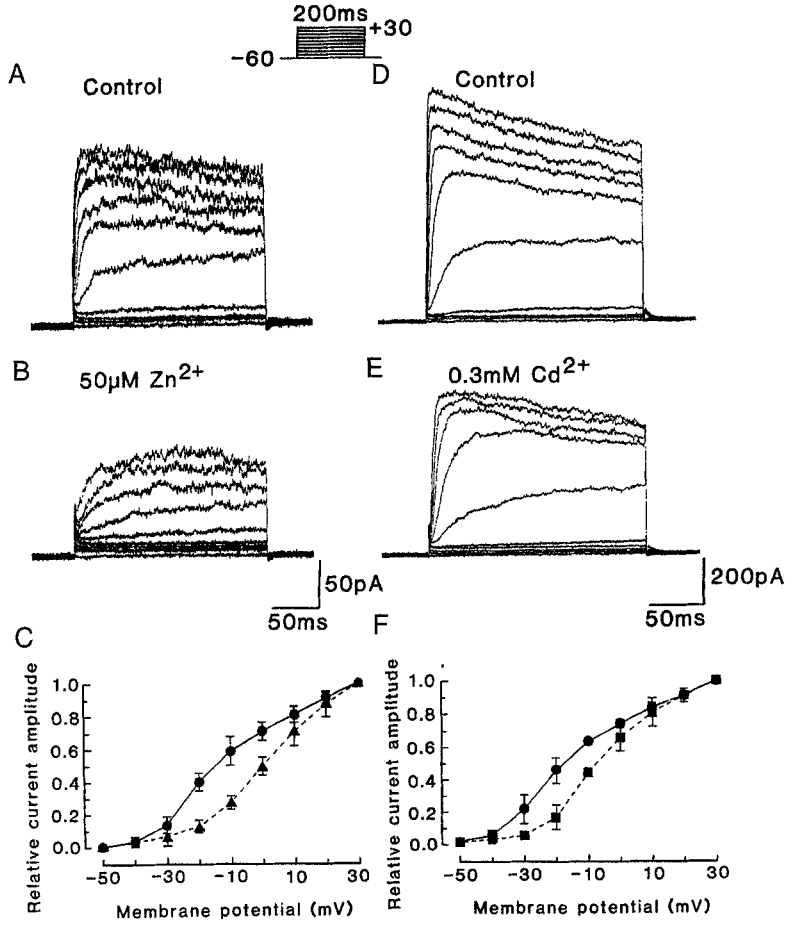

Fig. 7. Effects of divalent cations on I_K. Recordings of I_K elicited with the voltage protocol illustrated in the inset applying control solutions (*A*, *D*) and in the presence of 50 μM Zn^{2+} (*B*) or 0.3 mM Cd^{2+} (*E*). (*C*), (*F*) Shift of the normalized activation curves to more positive values during superfusion with 50 μM Zn^{2+} or 0.3 mM Cd^{2+} (dotted lines). Control solutions (continuous lines).

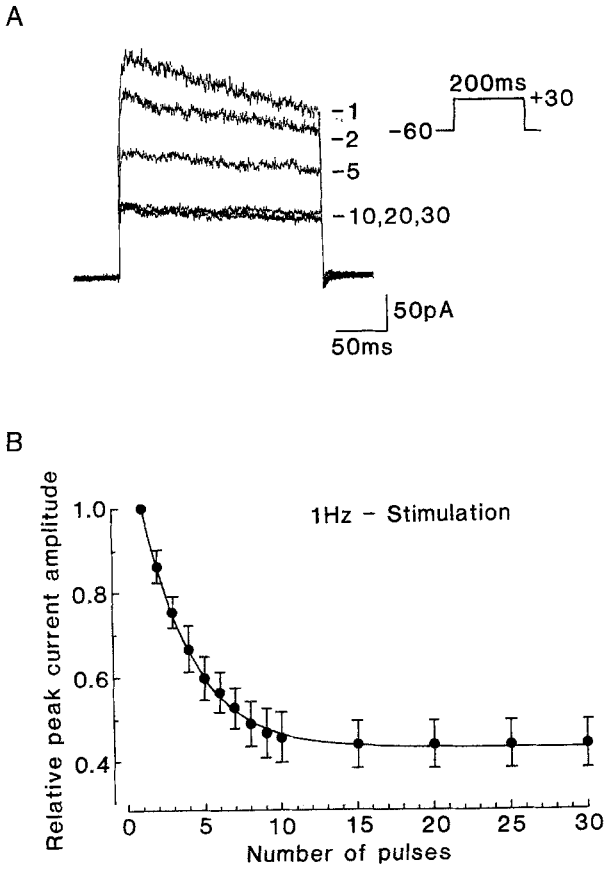

Fig. 8. Frequency dependence of K^+ outward currents. Cells were stimulated by repetitive pulses from the holding potential (–60) to +30 mV (200 msec) applied at a frequency of 1 Hz. (*A*) Example of current recordings. The numbers of the given pulses are indicated. (*B*) Normalized peak current amplitudes are plotted as a function of the number of applied pulses.

a more potent blocker of outward K^+ currents in activated microglial cells. An example of current recordings before and during application of 0.1, 1 and 5 mM 4-AP is illustrated in Fig. 5*A–D, F*. An IC_{50} value of 0.27 mM 4-AP was determined from effects of 4-AP on peak current amplitudes at a test potential of +30 mV (200 msec) (Fig. 5*E*).

Several peptide toxins are known to show a high specificity for different types of K^+ channels (for review, *see* Dreyer, 1990; Garcia et al., 1991). DTX and MCDP applied in concentrations of up to 500 nM did not markedly reduce I_K. In contrast, the scorpion toxins CTX or NTX completely blocked I_K at concentrations of 10 nM suggesting a high specificity for outward K^+ currents in GM-CSF-activated microglia (Fig. 6). To estimate the concentration-response-relation for block of I_K by CTX or NTX a voltage pulse to +30 mV was applied for 200 msec. The peak amplitudes of the evoked currents were measured when superfusing cells with control solution or solution containing either CTX or NTX at different concentrations. For each tested concentration the percentage inhibition of I_K was determined from 8 microglial cells. Using the Hill equation IC_{50} values of 1.14 nM CTX and 0.89 nM NTX were calculated (Fig. 6).

The influence of divalent cations on I_K was tested by applying extracellularly Zn^{2+}, Cd^{2+} or Ba^{2+}. As illustrated in Fig. 7 the superfusion with divalent cations resulted in different effects on I_K. Outward K^+ currents were reduced in the presence of divalent cations (Fig. 7*A–B, D–E*). Moreover, the steady-state activation curve for I_K was shifted in a depolarizing direction after the application of divalent cations (Fig. 7*C, F*). 50 μM Zn^{2+} shifted the activation curve for I_K by 15.4 mV (n = 6), 0.3 mM Cd^{2+} by 11.3 mV (n = 7) and 10 mM Ba^{2+} by 10.8 mV (n = 5). Additionally, divalent cations modulated the kinetic properties of I_K. In the presence of the tested divalent cations I_K activated more slowly than under control conditions (Fig. 7). When omitting Ca^{2+} from the extracellular solution no influence on amplitude or duration of the outward K^+ currents by divalent cations was observed (*not shown*).

Frequency Dependence of Outward K^+ Currents

To study the frequency dependence of outward K^+ currents in GM-CSF-activated microglial cells repetitive voltage commands to +30 mV (200 msec) were applied at a frequency of 1 Hz. As demonstrated in Fig. 8*A* the amplitude of I_K initially decreased rapidly and thereafter reached a steady-state at 43.7% of the normalized amplitude of the first given pulse. The time course of decay at 1 Hz stimulation was fitted monoexponentially with a time constant of 3.28 sec (n = 9) (Fig. 8*B*).

The remaining outward current (I_K') which did not show any frequency dependence was characterized by the same activation behaviour as described for I_K: An example of I_K' and its corresponding I-V curve is illustrated in Fig. 9*B, C*. To record I_K' 200 msec voltage pulses were applied from –60 to +30 mV in 10 mV increments at a frequency of 1 Hz following 50 repeated pulses to +30 mV (200 msec) applied at the same frequency. The activation threshold of I_K' was determined at –40 mV and a half maximal activation voltage for I_K' of –14.4 mV (n = 6) was calculated (Fig. 9).

Two different types of frequency-independent currents have been described in lymphocytes, the TEA-insensitive n′-type and the CTX-resistant l-type (Lewis & Cahalan, 1988). To test the possibility of equal characteristics of I_K' with the pharmacological properties of one of these lymphocytic K^+ currents the effects of CTX, NTX and TEA on I_K' were studied. When superfusing cells with extracellular solutions containing 10 nM CTX or 10 nM NTX I_K' could be abolished as shown in Fig. 10*A, B*. In contrast to these peptide toxins, TEA did not influence I_K' when applied in concentrations between 1 and 10 mM. A slight reduction of I_K' of 13.2 ± 2.6% (n = 4) only was obtained at a concentration of 20 mM TEA

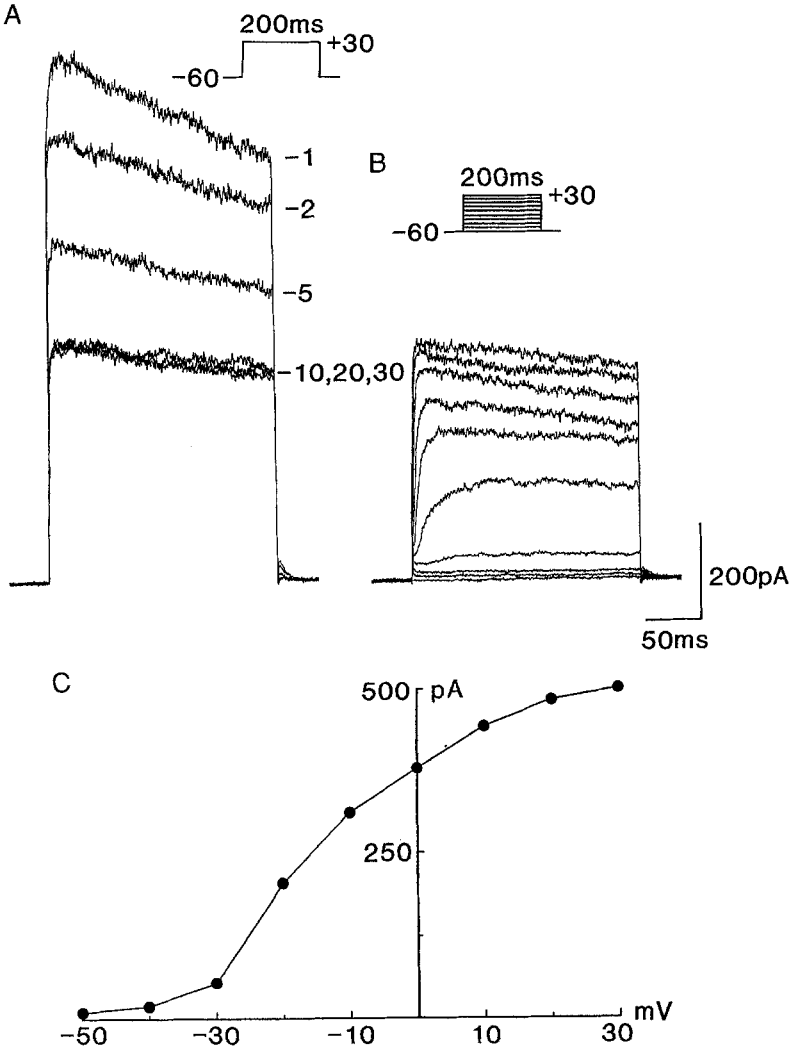

Fig. 9. Activation behaviour of I_K'. Following 50 repetitive pulses to +30 mV applied at a frequency of 1 Hz (*A*) the cell was pulsed at the same frequency in 10 mV steps from –60 to +30 mV for 200 msec (*B*). (*C*) Activation curve of I_K' corresponding to *B*.

(Fig. 10*C*). Divalent cations modulated I_K' in a similar way as obtained for I_K. In their presence I_K' activated more slowly than under control conditions. Superfusing cells with 50 μM Zn^{2+} or 0.3 mM Cd^{2+} resulted in a reduction of the amplitude and in a depolarizing shift of the activation curve for I_K' by 15.2 mV (n = 4) and 12.1 mV (n = 4), respectively.

Discussion

In microglial cells matured in the presence of GM-CSF, two types of voltage-gated outward K^+ currents were detected, distinguished by their behavior during repetitive pulse application: a frequency-dependent K^+ current (I_K) and a frequency-independent K^+ current (I_K').

This correlates with the appearance of an outward K^+ current on cultured microglia as a result of stimulation with the bacterial endotoxin LPS as described by Nörenberg et al. (1992, 1994). Both LPS-stimulated and GM-CSF-cultured microglial cells express a frequency-dependent outward K^+ current with rather similar kinetic properties: Their threshold of activation was reached at about –40 mV. The currents displayed a moderate inactivation and recovered slowly from inactivation.

Outward K^+ currents in GM-CSF-treated microglial cells could be blocked by several K^+ channel blockers such as TEA, 4-AP, CTX and NTX. Interestingly, in contrast to most delayed rectifying currents (Cook & Quast, 1989; Hille, 1992), I_K appear to be much more sensitive to externally applied 4-AP than to extracellular TEA. This corresponds with the current properties described for LPS-stimulated microglia (Nörenberg et al., 1994). In contrast, the I_K analyzed in the present paper displays a high sensitivity to the scorpion toxin CTX which distinguishes it from LPS-treated cells where CTX reduced outward K^+ currents with a 100 times lower potency (Nörenberg et al., 1994). It should be noted, however, that the toxins used in both studies were purified from different sources.

As has been reported for outward currents in LPS-treated microglia (Nörenberg et al., 1994) extracellular

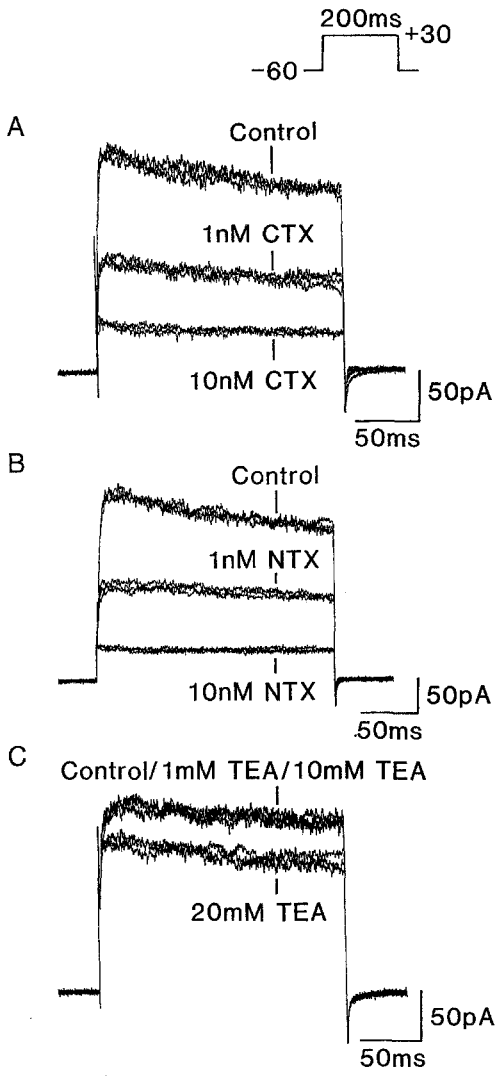

Fig. 10. Pharmacology of I_K'. Isolation of I_K' was achieved after application of repetitive pulses from −60 to +30 mV at a frequency of 1 Hz. I_K' were recorded during application of 1 and 10 nM CTX (*A*), of 1 and 10 nM NTX (*B*) and of 1, 10 and 20 mM TEA (*C*).

application of Cd^{2+} resulted in a decrease of the amplitude of I_K in GM-CSF-grown cells. Additionally, we found a modulatory effect on the activation behaviour of I_K in the presence of several other divalent cations which appeared as a positive shift of the voltage dependence and as a slower time-dependent activation of the current. Similar observations have been made at neuronal transient A currents (Mayer & Sugiyama, 1988), neuronal delayed rectifying currents (Spires & Begenisich, 1992) and lymphocytic n-type currents (DeCoursey et al., 1985).

In summary, with respect to their kinetics and pharmacology, the frequency-dependent outward K^+ current in GM-CSF-cultured microglial cells closely resembles outward K^+ currents in LPS-treated microglia as well as in macrophages (Ypey & Clapham, 1984; Nelson et al., 1990; Gallin, 1991), the non-neuronal tissue microglia counterparts. Moreover, I_K of the cytokine-treated microglial cells are also similar to the n-type current described in lymphocyte preparations (Sands et al., 1989; Choquet & Korn, 1992; Chandy et al., 1993). When comparing the characteristics of I_K with those of channels which had already been cloned, I_K shows identical properties with Kv 1.3 channels, the gene of which encodes the n-type voltage-gated K^+ channels of T lymphocytes. These channels had been cloned from T lymphocyte cDNA of mice (Grissmer et al., 1990), rat (Douglass et al., 1990) and human (Attali et al., 1992; Cai et al., 1992).

When GM-CSF-grown microglial cells were pulsed repeatedly at a frequency of 1 Hz, a frequency-independent outward K^+ current (I_K') was separately detected. Its voltage dependence of activation and its sensitivity to CTX and NTX did not differ from those of I_K, however, in addition to the lack of the frequency dependence, I_K' could be distinguished from I_K by its lower sensitivity to extracellularly applied TEA. Based on these characteristics I_K' of GM-CSF-grown microglial cells strongly resembles the n′-type currents of T lymphocytes (Lewis & Cahalan, 1988). Lymphocytic n′-type currents also could not be blocked by 10 mM TEA but they were effectively reduced following application of CTX at low concentrations. However, a frequency-independent current similar to the CTX-resistant lymphocytic l-type K^+ current (Lewis & Cahalan, 1988) was not detected in GM-CSF-activated microglial cells.

In contrast to *in situ* measurements of microglial cells in corpus callosum slice preparations (Brockhaus et al., 1993), outward K^+ currents have been observed only in cultured microglia, in response to stimulation with LPS (Nörenberg et al., 1992, 1994) or with cytokines (Fischer et al., 1994). This coincides with the immunofunctional activation of the cells. However, the existence of outward K^+ currents on microglia *in situ* can not be excluded. Pathological alterations within the CNS are associated with a local production of cytokines (Hunter et al., 1992) combined with histochemical evidence of microglia activation. Accordingly, an upregulated release of GM-CSF and of GM-CSF-receptor expression has been observed following neural injury (Raivich et al., 1991).

The physiological role of outward K^+ currents in microglia and macrophages is not well understood at present. Several attempts have been made to study the function of these currents in T lymphocytes using a pharmacological approach. It has been shown that blocking n-type K^+ channels induced by charybdotoxin, noxiustoxin, 4-AP or TEA caused an inhibition of T cell activation, proliferation and production and secretion of interleukin 2 (Chandy et al., 1993). Since a blockade of n-type channels resulted in a membrane depolarization, it was concluded that these channels may participate in the regulation of the resting membrane potential of lympho-

cytes. Moreover, it has been reported by Deutsch and Chen (1993) that voltage-gated outward K^+ channels play a role in volume regulation in T lymphocytes: The T lymphocyte line CTLL-2 which is normally unable to regulate volume regained this function after the transfection of cells with Kv 1.3. It would be interesting to see whether similar functional effects could be detected in microglial cells.

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