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Title
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Permalink
https://escholarship.org/uc/item/7h56z82r

Journal
Channels, 11(4)

ISSN
1933-6950

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Publication Date
2017-07-04

DOI
10.1080/19336950.2017.1300738

Peer reviewed

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Title:

Potassium channel expression and function in microglia: Plasticity and possible species variations

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Key words:

Microglia, K^+ channel, $K_V1.3$, $K_{Ca}3.1$, $K_{ir}2.1$

Abstract

Potassium channels play important roles in microglia functions and thus constitute potential targets for the treatment of neurodegenerative diseases like Alzheimer's, Parkinson’s and stroke. However, uncertainty still prevails as to which potassium channels are expressed and at what levels in different species, how the expression pattern changes upon activation with M1 or M2 polarizing stimuli compared to more complex exposure paradigms, and - most importantly - how these findings relate to the *in vivo* situation. In this mini-review we discuss the functional potassium channel expression pattern in cultured neonatal mouse microglia in the light of data obtained previously from animal disease models and immunohistochemical studies and compare it with a recent study of adult human microglia isolated from epilepsy patients. Overall, microglial potassium channel expression is very plastic and possibly shows species differences and therefore should be studied carefully in each disease setting and respective animal models.

Introduction

Microglia are the resident immune cells of the central nervous system and have many properties in common with peripheral macrophages, including expression of the cytochemical marker Iba-1 (Fig. 1). In the unperturbed brain these cells exist in a “not activated” or “surveillant” state where they scout neurons and tissues for invading pathogens and damage via a ramified network of fine cellular processes equipped with receptors for chemical “danger signals”, such as Toll-like receptors for bacteria and purinergic receptors for ATP and other phosphorylated nucleotides released from damaged or over-activated neurons (Fig. 2). Upon stimulation, microglia can attain various activated states characterized by both morphological and functional changes, such as rounding up and retraction of processes, directed migration along stimuli gradients, increased proliferation and phagocytosis, as well as polarization to phenotypes with distinct patterns of cytokine and chemokine production.[1] A correctly orchestrated chain-of-events in a microglia response thus leads to identification and destruction of pathogens, removal of cellular and tissue debris, and eventually initiates healing processes involving other cells in the damaged area.

The action of microglia can also in some instances become detrimental and promote neuronal death via excessive secretion of noxious substances such as nitrous oxide (NO) and superoxide (O_2^-) or excessive synaptic pruning. For example, in Alzheimer’s disease (AD) activation of microglia by soluble amyloid-β oligomers is widely viewed as an early and central event in the pathogenesis.[2-4] While microglial phagocytosis of amyloid-β aggregates is impaired in AD, oligomeric amyloid-β stimulates microglia to produce inflammatory cytokines, disrupt synaptic plasticity[5] and

to kill neurons in organotypic hippocampal slices.[6] In ischemic stroke and traumatic brain injury inflammation induced in the wake of neuronal death causes secondary damage and significantly expands the primary insult,[7,8] while in multiple sclerosis[9,10] and some degenerative eye diseases activated microglia contribute to the demyelination of axons or phagocytosis of photoreceptors.[11]

A popular concept claims that, similar to macrophages,[12] microglia polarized by stimulation with bacterial cell wall constituents, like lipopolysacccarides (LPS) and/or the cytokine interferon-γ (IFN-γ) to the "classical" pro-inflammatory M1-phenotype are predominantly neurotoxic based on their production of IL-1β, TNF-α, IL-12, IL-6, and NO, whereas microglia stimulated by IL-4 to the M2 phenotype are thought to suppress inflammation and promote tissue repair by secreting anti-inflammatory mediators and neurotrophic factors (Fig. 2).[13,14] It is becoming clear, however, that the presence of distinct M1/M2 subpopulations is an oversimplification,[15] and it has been proposed to abandon this concept in favour of a stimulus based terminology (e.g. M(LPS) or M(IL-4)) and assume no fixed, stable phenotype *in vivo*.[16] Despite this consideration the M1/M2 concept is helpful for hypothesis generation based on *in vitro* experiments, since it represents well-defined stimuli conditions eliciting opposite extremes in a spectrum of activated microglia phenotypes, which can be compared to more complex stimulation paradigms or to the conditions prevailing *in vivo*. From a therapeutic perspective the ultimate goal is of course to understand how to strengthen restorative properties and to avoid microglia-mediated neurotoxicity in patients.

Potassium channels in microglia

Potassium (K^+) channels are important in microglia since their activation can induce membrane hyperpolarizations, which are essential for driving Ca^{2+} influx trough inward rectifying Ca^{2+}-Release-Activated-Ca^{2+}-channels (CRAC),[17,18] ATP-activated P2X receptors[19] and other Ca^{2+}-permeable cation channels (Fig. 2).[1] K^+ channels facilitate refilling of intracellular Ca^{2+} stores following ATP or UTP activation of metabotropic P2Y-receptors,[20] thus maintaining high Ca^{2+} and timing of intracellular signalling events important for microglia activation and proliferation. K^+ channels can also participate in microglia volume regulation (working together with Cl^- channels in setting up local osmotic gradients) and thereby in important cellular functions such as shape changes, phagocytosis, and migration towards chemotaxic stimuli.[1]

Various specific inhibitors of $K_{Ca}3.1$ and $K_V1.3$ channels have proven efficient in rodent models for neurodegenerative diseases and conditions such as stroke,[21,22] multiple sclerosis,[23] retinal degeneration,[24] and radiation induced brain damage[25]. K^+ channels are therefore considered a highly relevant target class for pharmacological intervention in microglia-derived pathologies. Based primarily on classical molecular techniques it has been reported that isolated rodent microglia can express a plethora of voltage-gated ($K_V1.3$, $K_V1.1$, $K_V1.5$, $K_V3.1$), Ca^{2+} activated ($K_{Ca}3.1$, $K_{Ca}2.3$, $K_{Ca}1.1$), and inward rectifying K^+ channels ($K_{ir}2.1$).[1] In addition, large gene array studies have identified $K_V4.1$ and $K_{2P}13.1$ as microglia signature genes in mice with experimental autoimmune encephalomyelitis and human microglia.[26]

Surprisingly, however, reports are quite inconsistent concerning which K^+ channel types can be identified functionally by electrophysiological and pharmacological

approaches. This may be hypothesized to relate to a combination of experimental variables such as species differences, different isolation procedures and culturing methods, quantification methods or age and sex of animals. However, systematic investigations of the relative importance of these factors for channel expression and function in rodent microglia are uncommon in the literature, and so is the translational aspect comparing data from neonatal and adult microglia with human data. This is obviously problematic for attempts to use isolated microglia for drug screening and predicting which specific K^+ channel targets to pursue in drug discovery programs.

We have recently investigated some aspects of K^+ channel expression in isolated microglia, to delineate this variability and better understand the expression of microglia K^+ channels and their function under different conditions: 1) Nguyen et al.[27] for the first time performed a systematic investigation of differential K^+ channel expression (assessed by electrophysiology, qPCR, and immunocytochemistry) and functional importance (release of cytokines) in neonatal cultured mouse and foetal human microglia activated under “classically” pro-inflammatory (LPS, IFN-γ) vs. “alternatively” (IL-4) activating conditions. 2) In parallel, Blomster et al.[28] investigated the functional expression of K^+ channels - with specific focus on quantifying the $K_{Ca}3.1$ component by electrophysiology and pharmacology - in unstimulated vs. LPS and IL-4 polarized cultured human microglia from adult epilepsy patients who received therapeutic surgery in order to control their seizures.

$K_V1.3$, $K_{ir}2.1$, and $K_{Ca}3.1$ are the main K^+ channels expressed in mouse neonatal microglia

Unstimulated cultured mouse microglia express very low total K^+ currents typically consisting of one to three distinct biophysical components: An inwardly rectifying current dominating at potentials negative to E_K, a linear current most easily discernible in an intermediate potential range (-60 to -40 mV), and finally a classical voltage-dependent current activating positive to -40 mV and showing use-dependent inactivation upon repeated stimulation (Fig. 3). These biophysically defined components are also clearly pharmacologically distinguishable since Ba^{2+} selectively abolishes the inward rectifier, and the KCa3.1 inhibitor TRAM-34[29] and the Kv1.3 inhibitors PAP-1[30] and ShK-186[31] eliminate the linear and voltage-dependent components, respectively. In support of this biophysical and pharmacological evidence $K_V1.3$, $K_{ir}2.1$, and $K_{Ca}3.1$ are also clearly identified by qPCR and immunohistochemistry, whereas other candidates (see above) are expressed at much lower levels or could not be detected.

Stimulation of neonatal mouse microglia with ATP or differentiation into M1/M2 phenotypes with LPS/IFN-γ or IL-4 respectively, differentially modulates expression of these K^+ channels as measured by electrophysiology in a highly specific way (Fig. 3): LPS, the classical M1 "invading bacteria-stimuli", selectively upregulates $K_V1.3$, but causes no increase in either $K_{ir}2.1$ or $K_{Ca}3.1$ currents (actually a transient decrease in mRNA levels detected with qPCR). However, the exact opposite pattern is seen for stimulation with ATP, which leaves $K_V1.3$ expression unaltered, while it strongly increases the expression of both $K_{ir}2.1$ and $K_{Ca}3.1$. Activation with the pro-inflammatory cytokine IFN-γ essentially mimics the ATP effect in that $K_{ir}2.1$ and $K_{Ca}3.1$ (but not $K_V1.3$) are up-regulated. Noteworthily, combining IFN-γ with LPS completely abolishes the IFN-γ effect on $K_{ir}2.1$ and $K_{Ca}3.1$, whereas the combination has the same stimulating effect

on $K_V1.3$ as LPS alone. Finally, activation of microglia with the receptor-bypassing pro-inflammatory stimuli PMA+ionomycin closely mimics the pattern induced by ATP and IFN-γ, in other words: a phenotype dominated by $K_{ir}2.1$ and $K_{Ca}3.1$. In contrast to the various pro-inflammatory stimuli, the M2 cytokine IL-4 yields yet another distinct profile with specific up-regulation of $K_{ir}2.1$, leaving $K_{Ca}3.1$ and $K_V1.3$ at their pre-stimulation levels.

These results provide an excellent reference for discussion of *in vivo* data as well as comparisons to other species, but first we will briefly address the functional importance of microglia K^+ channels.

The importance of specific microglia K^+ channels for expression, synthesis and secretion of mediators

One aspect of K^+ channel subtypes in microglia is their expression as determined by electrophysiological experiments, another is the functional impact of specifically inhibiting these channels on pro- and anti-inflammatory responses from differentially activated microglia cells. To elucidate this question, we used selective $K_{Ca}3.1$ and $K_V1.3$ inhibitors, at concentrations previously determined sufficient to ascertain full inhibition of the target channel, while staying well below values causing non-selective actions on other channels (an aspect often neglected when using pharmacological tools) or having non-specific cytotoxic effects. Thus, the specific effect of $K_{ir}2.1$ blockade could unfortunately not be determined due to the high cytotoxicity of the available pharmacological tools in our *in vitro* system.

LPS stimulation of mouse neonatal microglia causes the expected M1 response pattern characterized by very strong increases in both mRNA and protein secretion of the pro-inflammatory cytokines IL-1β, IL-6 and TNF-α as well as up-regulation of iNOS and a corresponding increase in NO release.[27] As expected from the strongly increased expression of $K_V1.3$ by LPS, both the small molecule inhibitor PAP-1 and the peptide inhibitor ShK-186 significantly reduced both expression and release of these mediators. More surprisingly, considering that LPS polarization did not significantly increase $K_{Ca}3.1$ expression above basal, undifferentiated levels, TRAM-34 had similar or even stronger effects on IL-1β than the $K_V1.3$ inhibitors, which may well reflect a critical role of the relatively few $K_{Ca}3.1$ channels present under these conditions. It is worth noting that the effect sizes of both K^+ channel inhibitors are at par with the standard microglia activation blocker minocycline (which acts by a very different mechanism), but also that none of the inhibitors (including minocycline) causes a complete block of cytokine and NO production. Whether the blockers possibly might show additive or synergistic effects was not investigated in this study.

IL-4 stimulation selectively up-regulated the cancer promoting enzyme Arginase 1, the membrane saccharide receptors CD206 and YM1, as well as the insulin-like growth factor, IGF1. In contrast to the overall similar inhibiting effects of $K_{Ca}3.1$ and $K_V1.3$ blockers on secretion of pro-inflammatory microglia, the effect on these M2 markers were much more variable with only TRAM-34 showing a significant inhibition of CD206 and YM1.[27]

Comparisons between K^+ channel expression in cultured neonatal microglia and acutely isolated adult microglia from normal and inflamed mouse brains

It is of course often questioned how findings made with cultured neonatal microglia translate to microglia in the adult brain. While *in vitro* studies typically show very clear distinctions between a LPS-induced M1-like state and an IL-4 induced M2-like state,[32] studies in human inflammatory and neurodegenerative diseases often report heterogeneous microglia phenotypes co-expressing M1 and M2 markers.[33,34] In a previous publication we therefore acutely isolated CD11b$^+$ microglia/macrophages from the brains of mice subjected to either ischemic stroke or intraventricular LPS injection using magnetic beads and immediately afterwards studied their K^+ channel expression by whole-cell patch clamp.[22] Microglia acutely isolated from non-infarcted, normal brains exhibited very small K^+ currents, which on average were even smaller than what was observed in unstimulated cultured neonatal microglia but clearly consisted of combinations of $K_V1.3$, $K_{Ca}3.1$ and $K_{ir}2.1$. In contrast, microglia isolated from the infarcted brain area eight days after an ischemic stroke showed greatly increased functional K^+ channel expression. Interestingly, while some cells predominantly expressed $K_{ir}2.1$ currents similar to IL-4 stimulated neonatal microglia, other cells exhibited large Kv1.3 currents similar to LPS stimulated neonatal microglia. However, many acutely isolated cells from the infarcted area had no “clean” phenotype but were found to possess K^+ currents consisting of various combinations of $K_V1.3$, $K_{Ca}3.1$ and $K_{ir}2.1$.[22] In contrast to these findings from an ischemic stroke model, where microglia activation likely occurs in response to a combination of released ATP, neuronal debris, inflammatory cytokines and ischemia/reperfusion injury, microglia isolated from LPS

injected mouse brains typically expressed either large $K_V1.3$ currents or a combination of $K_V1.3$ and $K_{Ca}3.1$ but virtually no K_{ir} currents. The increase in $K_{Ca}3.1$ expression, which was not observed following LPS stimulation *in vitro*, in our opinion is probably a response to neuronal injury following the LPS injection.

Comparison to functional K^+ channel expression in isolated prenatal and adult human microglia

Using commercially available human microglia derived from human foetuses, we demonstrated prominent expression of $K_V1.3$ currents and a minor K_{ir} component irrespective of LPS or IL-4 stimulation ($K_{Ca}3.1$ was not quantified due to the need of using F^- based pipette solutions for successful patch-clamping of these cells).[27]

In contrast, functional K^+ channel expression in adult neocortical microglia from epilepsy patients varies significantly from the pattern observed with mouse microglia - both cultured neonatal cells and acutely isolated ones from adults - as well as the prenatally derived human microglia.[28] Isolated adult human microglia have much larger cell capacitances (20-25 pF compared to 4-5 pF for both neonatal and adult mouse as well as prenatal human microglia) and much lower input resistance reflecting a 5-6 times larger cell size as well as a significantly higher K^+ channel conductance at negative membrane potentials. At first glance, the summed K^+ channel whole-cell ramp current mimics quite well the neonatal phenotype with distinguishable inward rectifying, linear, and voltage-dependent components. Quantitatively, however, the linear current typically dominates in the isolated adult microglia, often obscuring the other components. The linear conductance is activated by low concentrations of the potent

$K_{Ca}3.1/K_{Ca}2.x$ activator NS309 and fully inhibited by the $K_{Ca}3.1$ blocker NS6180,[35] hence represents a high functional expression of $K_{Ca}3.1$. Quantification based on the "difference current" revealed a minimal estimate of an average number of 585 channels per cell. Based on the small residual current left after complete NS6180 inhibition, and in line with the neonatal and adult mouse data, $K_{Ca}2$ channels did not contribute significantly to the Ca^{2+} activated and voltage-insensitive current. Importantly, the high expression level of $K_{Ca}3.1$ also translates to a dominating effect on the membrane potential (Fig. 4), since activation/block of the channel immediately switched the membrane potential between -20 and -75 mV, thus predicting $K_{Ca}3.1$ channel expression as a powerful mechanism for amplification of conductive Ca^{2+}-influxes via changes in membrane potential.

As judged from the few microglia cells expressing no $K_{Ca}3.1$ or from cells with NS6180-blocked $K_{Ca}3.1$, the inward rectifying K^+ current (K_{ir}) seems biophysically undistinguishable from its mouse counterpart, but formal identification as $hK_{ir}2.1$ has not yet been achieved. In contrast, the dominating voltage-dependent outward component activates at much more positive potentials (at 0 mV or higher) than described above for mouse microglia (~ 0 vs. -40 mV), exhibits much larger relative "gating noise" (indicative of a high single channel conductance), does not appreciably inactivate, and is modulated positively by the BK channel activator NS1619[36] and negatively by the BK inhibiting tremorgenic alkaloid paxilline.[37] We therefore conclude that this current is attributable to expression of the big conductance Ca^{2+} and voltage-activated $K_{Ca}1.1$ channel. The BK channel has previously been reported from human and bovine microglia in culture[38,39] as well as human macrophages,[40] but is usually not found in

rodent microglia under any stimulation condition (but can occasionally be seen as a small residual current in microglia from adult $K_V1.3$ KO mice; Heike Wulff, unpublished data). The highly surprising finding from this study was, however, that neither $K_V1.3$ nor any other "classic" K_V current could be detected. Based on the mouse experiments it is no surprise that M2 polarization with IL-4 did not upregulate $K_V1.3$, but one might have expected an effect of M1 polarization with LPS (which significantly upregulated $K_V1.3$ in the mouse microglia, see Fig. 3).

These results make us conclude that cultured human adult microglia from epilepsy patients predominantly exists in a state where $K_{Ca}3.1$ is very strongly expressed and where K_{ir} and $K_{Ca}1.1$ provide the main - if not the only - accompanying K^+ conductances. It is worth noting that despite these cells being able to respond "normally" to LPS-stimulation *in vitro* through increased secretion of pro-inflammatory cytokines, neither LPS nor IL-4 caused a significant change in functional expression of any of the K^+ channels probably reflecting that adult microglia cells are less plastic.

Future directions towards an integrated view of K^+ channel expression and function in microglia

As seen from the preceding chapters microglia are remarkably plastic in their expression of K^+ channels as summarized for neonatal microglia in Fig. 5 (left): M1 polarizing stimuli (LPS, IFN-γ) selectively up-regulate $K_V1.3$, M2 polarizing stimuli (IL-4) up-regulate $K_{ir}2.1$, whereas IFN-γ and ATP induce a $K_{Ca}3.1/K_{ir}2.1$ profile. Microglia in brains in animal models of stroke, Alzheimer's or Parkinson's disease cannot be expected to fall exactly into the well-defined *in vitro* phenotypes as demonstrated by

other combinations of $K_{ir}2.1$, $K_{Ca}3.1$, $K_V1.3$ in microglia acutely isolated from adult stroke mice.[22] This is easily rationalized from their individual and probably complex *in vivo* "stimulation history". However, the K^+ channel "players" are the same as in the *in vitro* experiments. The situation is somewhat different in human microglia from epilepsy patients (Fig. 5, right): The $K_{Ca}3.1$ current is by far the most prominent, no matter if cells are stimulated with LPS or IL-4 or not.[28] Kir channels are also expressed, but the noteworthy finding is the lack of $K_V1.3$ expression and presence of $K_{Ca}1.1$ instead. What is particularly surprising about the missing functional $K_V1.3$ expression, is that several immunohistochemistry studies have previously shown strong $K_V1.3$ specific staining on activated microglia in human ischemic infarcts,[22] microglia surrounding amyloid-plaques in Alzheimer's disease,[41] as well as on cells with a glia morphology in active multiple sclerosis lesions.[42] Combined with our finding that cultured prenatal human microglia clearly express $K_V1.3$ and that microglia exhibit such a pronounced and stimuli-dependent expression plasticity, a reasonable assumption is that the "adult epilepsy microglia phenotype" carries a unique set of K^+ channels dictated by their specific aging and/or epilepsy/medication history. High expression of $K_{Ca}3.1$ and Kir combined with low $K_V1.3$ may, for example, be the result of a "stimulation milieu" dominated by an augmented purinergic drive, likely to be the case in epilepsy because of increased synaptic release of ATP/UTP or secretion from astrocytes.[43] In contrast, we have no idea what may drive $K_{Ca}1.1$ expression and suspect it could relate to a species difference.

To settle the question of plasticity vs. species differences in the expression of microglia K^+ channels in health and disease, we recommend the following approaches:

1) Differentiation of cultured microglia cells: There is a huge need for extending the *in vitro* polarization studies of cultured microglia using multiple well-defined stimuli and combinations thereof to other species. Even taxonomically closely related species or different strains of the same species are worth investigating in detail as clearly demonstrated by papers describing M2(IL-4) mediated upregulation of $K_{Ca}3.1$ in cultured neonatal rat microglia[44] and expression of $K_{Ca}2.3$;[45] both findings that we have not been able to corroborate using mouse and human microglia. Additionally, microglia senescence during aging or in prolonged *in vitro* culture can alter their responsiveness to stimuli.[46] Thus, particular attention should be paid to the age of the animals as well as to the length of time microglia are kept in culture for differentiation studies to avoid unintentional skewing of microglia activation and the resulting K^+ channel expression. 2) Acutely isolated microglia cells and slices from animal models: Functional K^+ channel expression in microglia should be studied more thoroughly in both mouse and rat models of diseases accompanied by neuroinflammation such as ischemic stroke, Alzheimer's disease, Parkinson's disease, traumatic brain injury, and epilepsy. Compared to isolated cells acute slices obviously offer the advantage that microglia can be studied in their natural 3D-environment. So far the only electrophysiological data available in literature is our characterisation of K_V, K_{Ca} and K_{ir} channel expression in acutely isolated microglia from mice subjected to middle cerebral artery occlusion (MCAO),[22] two studies from the year 2000 reporting small K_{ir} currents under normal conditions and increased K_V channel expression following facial nerve axotomy or 48-hours after MCAO in acute rat brain slices,[47,48] and a study showing increased $K_V1.3$ expression in microglia in hippocampal slices prepared from mice 48 hours after kainate

induced status epilepticus.[49] Similarly, it would be very interesting to study K^+ channel expression in acute microglia isolated from electrically kindled rats or from genetic epilepsy models such as Frings audiogenic seizure (AGS)-susceptible mice.[50] 3) Acute and cultured microglia from human diseased tissue: Despite the technical and practical difficulties involved there is a need for confirming our current results from cultured cells from epilepsy patients[28] by comparing to the functional K^+ channel expression profile in acutely isolated microglia. Using one of the currently available electrophysiological HTS platforms for "instant parallel profiling" of acutely isolated microglia as fast as possible after surgery is a realistic way forward for such studies. Furthermore, the currently investigated microglia are all isolated from the supposedly "healthy" neocortical tissue, which unavoidably had to be removed as part of the amygdahippocampectomy (the position of the drug-resistant epileptic focal point). Therefore, it would also be highly interesting to characterize the currently obtained data with the K^+ channel expression in microglia from the much more seriously affected limbic structures. We also need to investigate if inhibition of specific K^+ channels has the same impact on human microglia functions (secretion, migration etc.) as in mice microglia. Finally, we believe that the recent success in obtaining microglia-like cells from human pluripotent stem cells[51] should greatly aid this translational work and even make it possible to investigate changes in channel expression in a broader disease specific context.

Taken together, such studies would help to inform decisions about whether to target microglial K^+ channels to suppress detrimental microglia functions in neurological diseases. So far there is only limited *in vivo* target validation available. Pharmacological $K_{Ca}3.1$ inhibition has been demonstrated to reduce infarct area and neurological deficit

in mouse and rat models of ischemic stroke,[21,22] reverse tactile allodynia in rats with peripheral nerve injury,[52] and reduce glioma associated microglia activation in a mouse model.[53] Based on this data two groups have already proposed to repurpose the $K_{Ca}3.1$ blocker senicapoc for the treatment of Alzheimer's disease,[6,54] but so far, no data validating the therapeutic hypothesis has been published. $K_V1.3$, which based on the *in vitro* data showing $K_V1.3$ expression in "M1-like" microglia,[27] might seem an even more attractive target but, as discussed above, many questions about the conditions of $K_V1.3$ expression in human microglia remain and only one *in vivo* study has shown that $K_V1.3$ inhibition can reduce microglia mediated radiation induced brain damage.[25]

Funding details

This work was supported by the National Institute of Neurological Disease and Stroke under Award NS098328 and by the European Union's Seventh Framework Programme (FP7/2007-2013) under Grant HEALTH-F2-2011-278850 (INMiND) from the Strategic Research Council (COGNITO).

Disclosure statement

Linda Blomster and Palle Christophersen are full time employees of Saniona A/S.

Abbreviations and acronyms:

Iba-1: Ionized Ca^{2+} binding adaptor molecule; NS309: 6,7-dichloro-1H-indole-2,3-dione 3-oxime; NS6180: 4-[[3-(trifluoromethyl)phenyl]methyl]-2H-1,4-benzothiazin-3(4H)-one; PAP-1: 5-(4-phenoxybutoxy)psoralen; PMA: phorbol-12-myristate-13-acetate; ShK-186: Stichodactyla toxin-186; TRAM-34: 1-[(2-chlorophenyl)diphenylmethyl]-1H-pyrazole.

Figure legends

Figure 1

“Surveillant” microglia in normal adult mouse brain (right) and activated microglia in the border of an ischemic infarct (left) stained for the cytochemical marker Iba-1. The ischemic infarct was induced by middle cerebral artery occlusion as described in ref. 22.

Figure 2

Cartoon showing the regulation of Ca^{2+} signalling in microglia by a selected number of ion channels and receptors. (Abbreviations: K_V, voltage-gated K^+ channel; K_{ir}, inward-rectifier K^+ channel; K_{Ca}, calcium-activated K^+ channel; IP_3, ionositol-triphosphate; Orai1, calcium release-activated calcium channel protein 1; P2R, purinergic receptor; SERCA, sarco/endoplasmic reticulum Ca-ATPase; STIM, stromal interaction molecule; TRP, transient receptor potential channel). Activation of K^+ channels hyperpolarizes the microglia membrane and thus facilitates Ca^{2+}-influx through Ca^{2+} and cation channels.

Figure 3

Scatterplots of the functional $K_V1.3$, $K_{Ca}3.1$ and $K_{ir}2.1$ expression levels measured by whole-cell patch-clamp in unstimulated neonatal mouse microglia and in microglia 40-48 h after stimulation with LPS, LPS plus IFN-γ, IL-4, IFN-γ, ATP or PMA plus ionomycin. Redrawn from Nguyen et al.[27]

Figure 4

Effects of sequential activation and inhibition of $K_{Ca}3.1$ in a cultured human microglia cell isolated from an epilepsy patient. The upper panel shows the effect on membrane potential and the lower depicts the corresponding effect on the K^+ currents. The activator is the positive modulator NS309 and the inhibitor the highly selective NS6180. Redrawn from Blomster et al.[28]

Figure 5

Cartoon summarizing the observed functional expression profiles of various K^+ channels in cultured microglia isolated from prenatal mouse and adult human epilepsy patients, respectively. The cultured microglia were activated by classical M1(LPS, IFN-γ) and alternative M2(IL-4) stimulation paradigms, compared to stimulation with the damage or danger signal, ATP.

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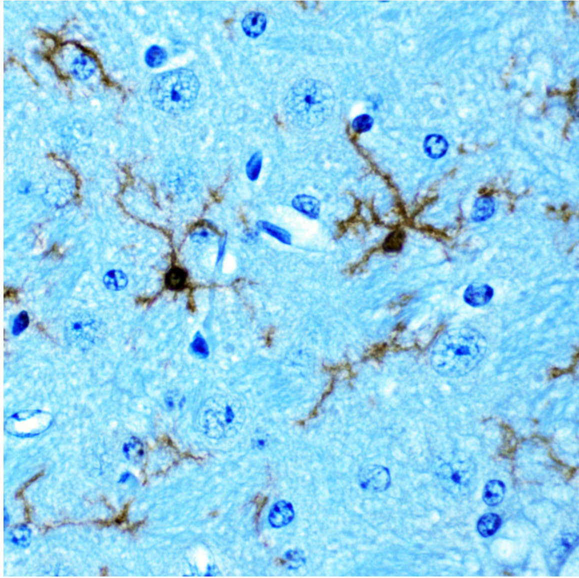

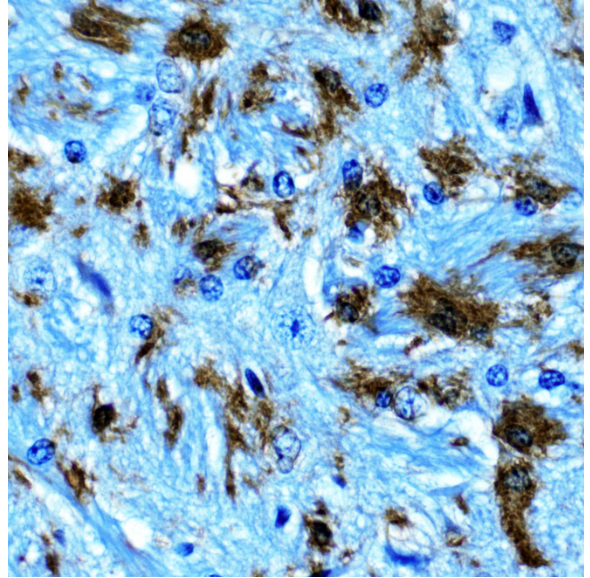

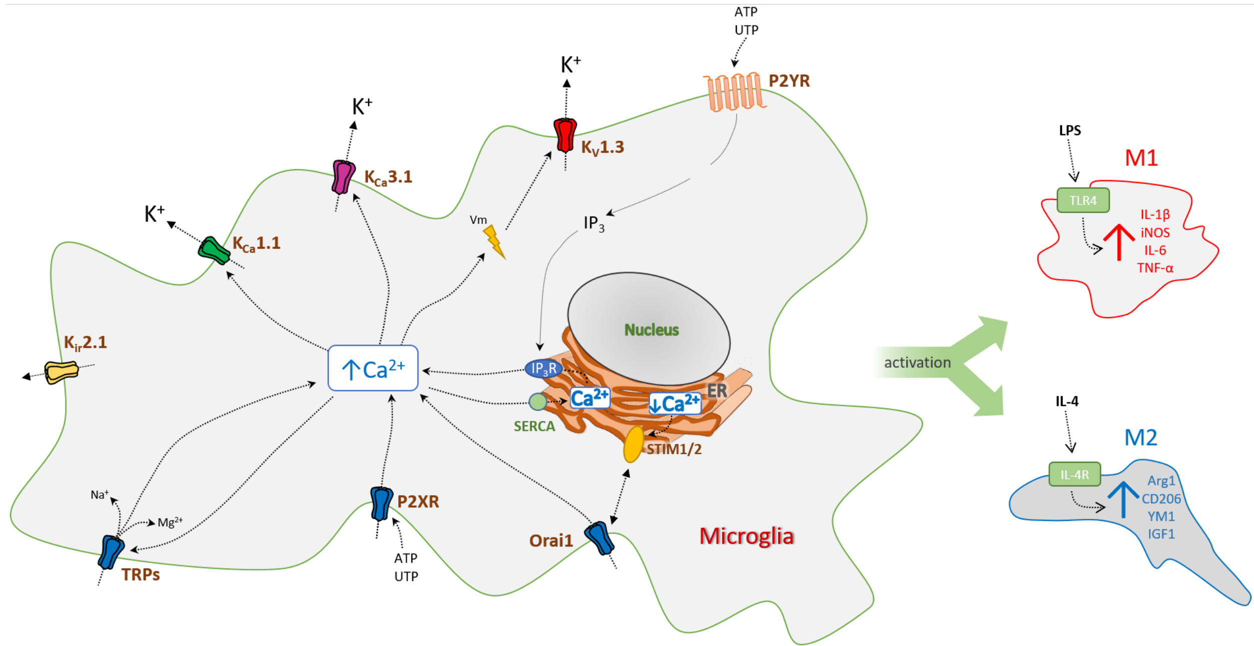

ATP
UTP
P2YR
K+
Kv1.3
K+
Kca3.1
K+
Kca1.1
Vm
IP3
Nucleus
Kir2.1
↑Ca2+
IP3R
Ca2+
↓Ca2+
ER
SERCA
STIM1/2
Na+
Mg2+
P2XR
TRPs
ATP
UTP
Orai1
Microglia
activation
LPS
M1
TLR4
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M2
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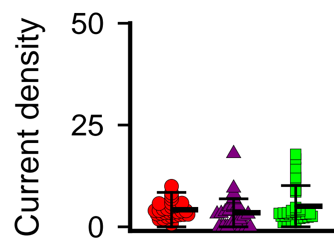
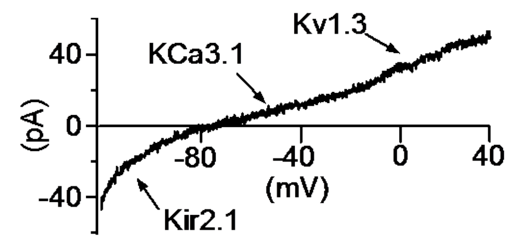
Arg1
CD206
YM1
IGF1

Unstim.





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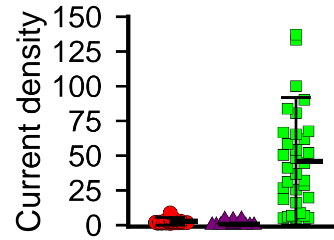
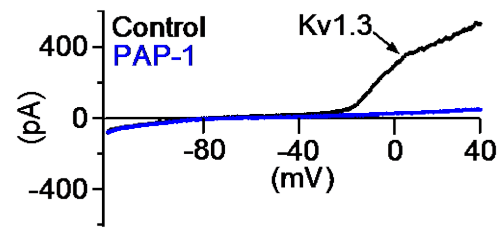
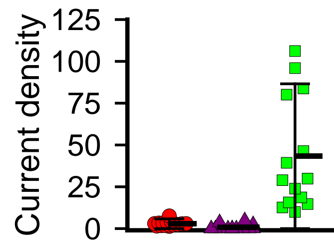
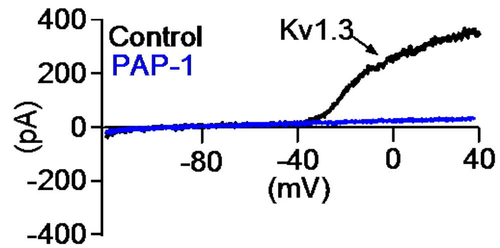


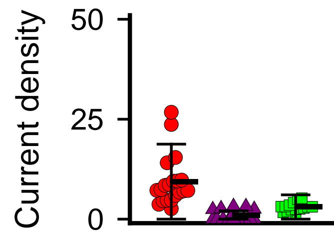


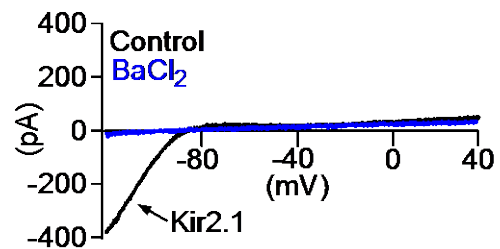
LPS+IFN-γ





IL-4





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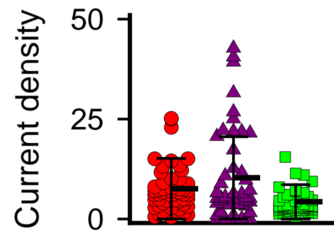

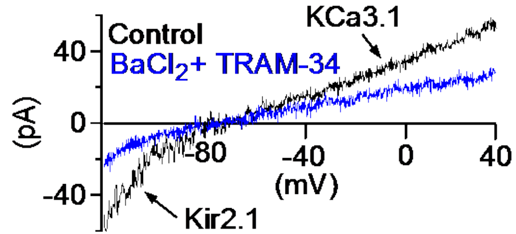

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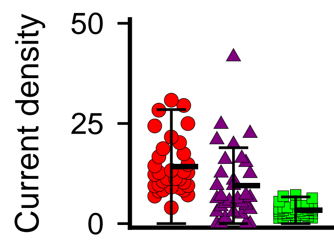
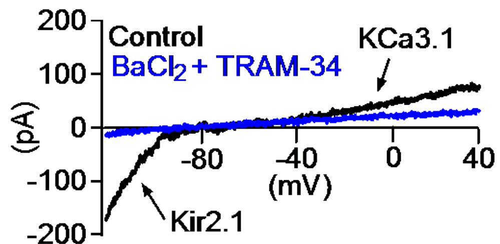
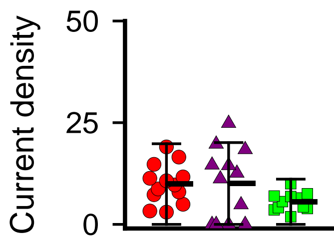
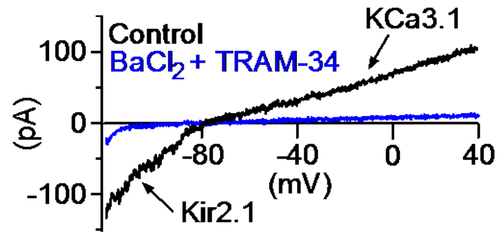


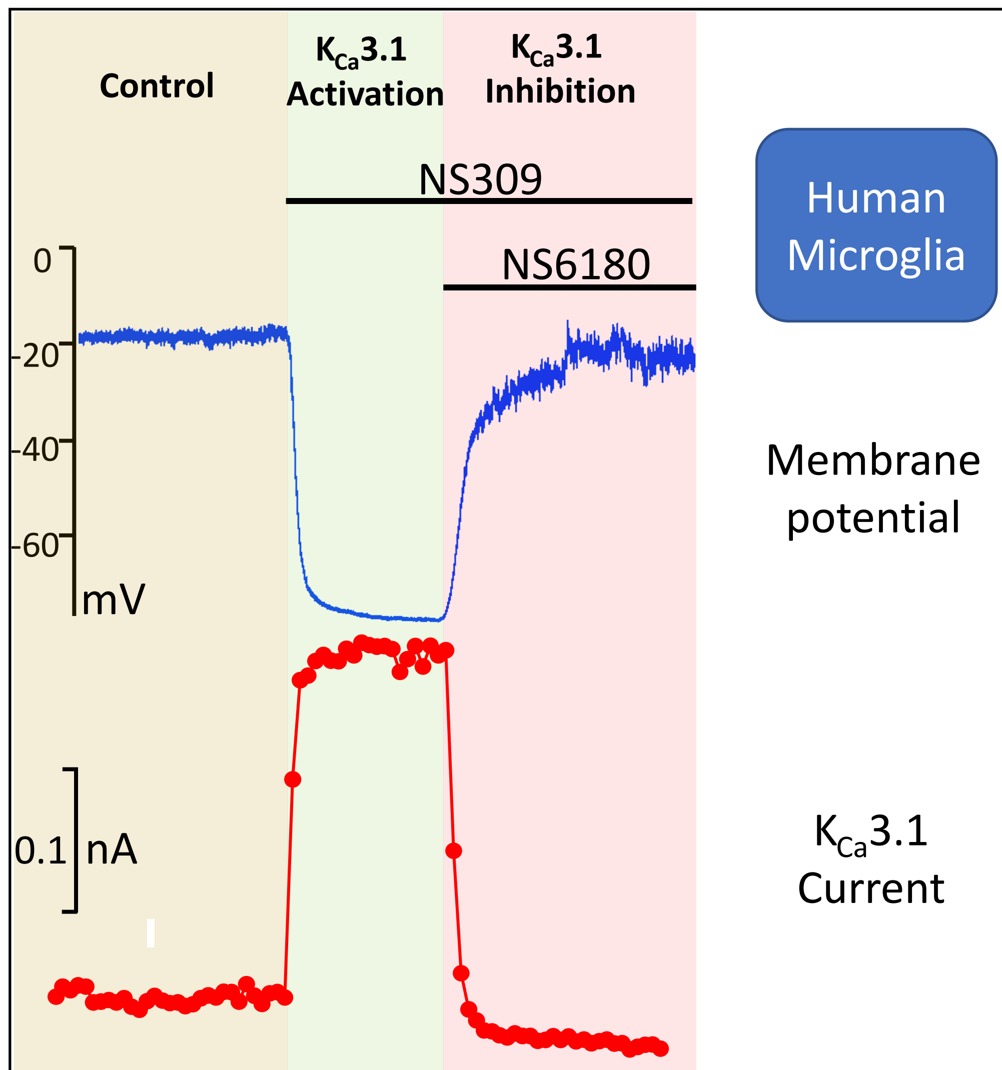


PMA+Iono

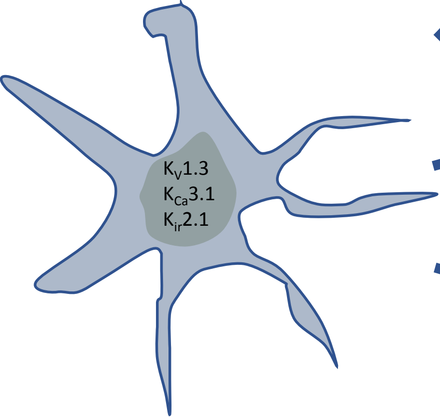




Kir2.1 KCa3.1 Kv1.3


Control
$K_{Ca}3.1$ Activation
$K_{Ca}3.1$ Inhibition
NS309
NS6180
Human Microglia
0
-20
-40
-60
mV
Membrane potential
0.1
nA
$K_{Ca}3.1$ Current

MOUSE
Neonatal
HUMAN
Adult, epilepsy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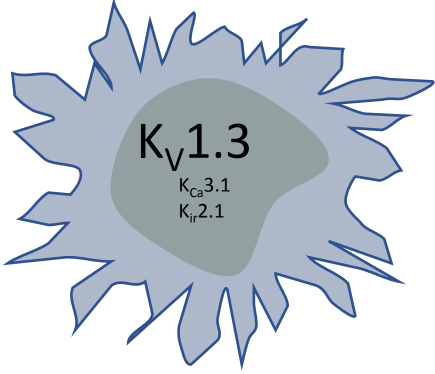
$K_{Ca}3.1$
$K_{ir}2.1$
LPS
LPS+IFN-γ
IFN-γ
ATP
IL-4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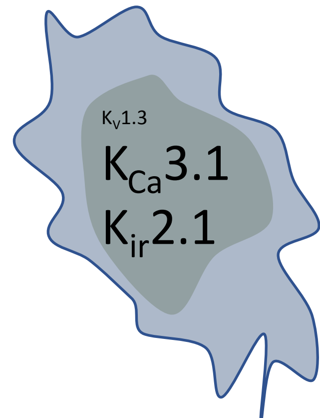
M1
TNF-α
IL-1β
IL-6
NO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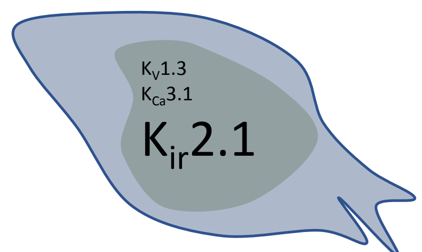
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$K_{Ca}3.1$
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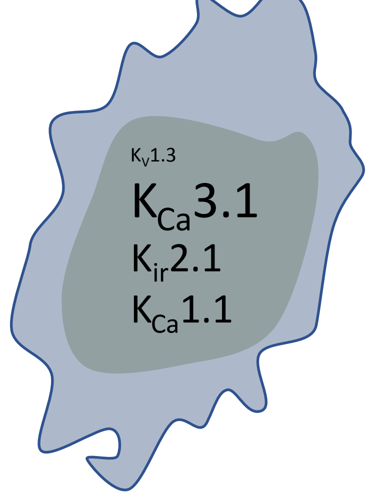
M2
Arg-1
IGF-1
YM-1
CD206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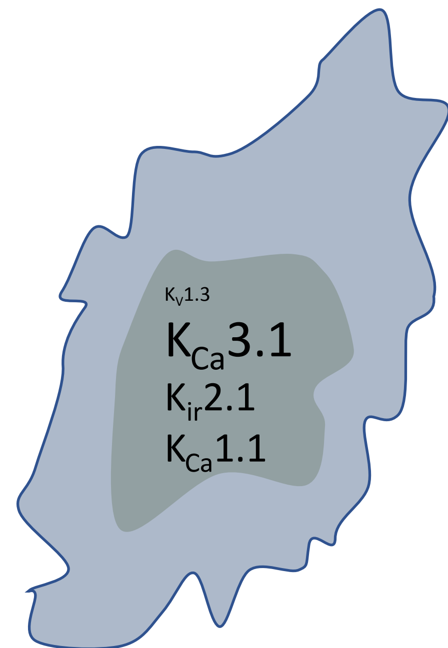
$K_V1.3$
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$K_V1.3$
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LPS
IL-4
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