Current Topics

Ion Channels as Therapeutic Targets for the Immune, Inflammatory, and Metabolic Disorders

Review

Physiological and Pathophysiological Roles of Transient Receptor Potential Channels in Microglia-Related CNS Inflammatory Diseases

Hisashi Shirakawa* and Shuji Kaneko

Department of Molecular Pharmacology, Graduate School of Pharmaceutical Sciences, Kyoto University; 46–29 Yoshida-shimoadachi-cho, Sakyo-ku, Kyoto 606–8501, Japan.

Received April 30, 2018

Central nervous system (CNS) inflammation is a potential therapeutic target for neurodegenerative diseases. In recent years, a number of studies have focused on the links between neurodegenerative diseases and CNS glial cells, especially microglia. Microglia are the main resident immune cells in the CNS and represent approximately 10–15% of all CNS cells. Microglia play an important role in maintaining brain homeostasis at rest by surveying the environment, and engulfing apoptotic cells and debris in the healthy brain. However, under certain pathological conditions, microglia can generate neurotoxic factors, such as pro-inflammatory cytokines and molecules like nitric oxide (NO), which lead to CNS inflammatory diseases. In this review, we discuss the evidence that regulation of microglial ion channels may modulate CNS inflammation and subsequent tissue damage in neurological disorders. In particular, we discuss the role of transient receptor potential (TRP) channels in microglia in both acute and chronic inflammatory conditions, and describe the physiological and pathophysiological roles of TRP channels in CNS inflammatory pathways. Additionally, we describe the benefits of stimulation/inhibition of TRP channels in animal models of microglia-related CNS inflammatory diseases.

Key words microglia; cytokine; transient receptor potential (TRP) channel; central nervous system (CNS) inflammation; Ca^{2+} signaling; reactive oxygen species

1. INTRODUCTION

The brain has long been regarded as an immune-privileged organ, where inflammation can only occur after the breakdown of the blood-brain barrier and subsequent infiltration by peripheral immune cells.^{1,2)} However, immunocompetent cells residing in the central nervous system (CNS) express pattern recognition receptors (PRRs), such as toll-like receptors (TLRs) and nucleotide binding oligomerization domain (NOD)-like receptors, which mediate innate immunity.^{3,4)} PRRs recognize microbial molecular motifs, known as pathogen-associated molecular patterns (PAMPs), which are not generally found in normal brain tissue but which typically accumulate in infected tissues. Recent studies clearly indicate that, in the absence of CNS infection, endogenous PRR ligands-so-called damage associated molecular patterns (DAMPs), also known as alarmins-are released in damaged CNS tissue, resulting in activation of the innate immune system within the CNS.^{3,4)} These pro-inflammatory responses in the immunocompetent cells in the brain contribute to the progression of neurodegenerative diseases by sustained release of pro-inflammatory mediators, which leads to CNS inflammation.^{5,6)} Although the molecular mechanisms remain to be elucidated, the inflammatory response during a wide variety of chronic CNS diseases is likely controlled by microglia, the main type of immunocompetent cells residing in the CNS.^{7,8)} In various chronic disease states, microglia are primed by prior pathological changes in the CNS to respond more vigorously to subsequent inflammatory stimulation, leading to more deleterious consequences.^{7,8)} Conversely, microglia also engulf and degrade apoptotic cells in the injured brain, resulting in neuroprotection, tissue repair, and regeneration.^{2,9)} Microglia switch to an activated phenotype in response to multiple factors, including PAMPs and DAMPs^{2,9)}; therefore, the mechanisms underlying microglial regulation of neuronal damage and dysfunction are the subject of much research.

Microglia express a wide variety of ion channels that are involved in their cellular inflammatory responses.¹⁰⁾ Some of these channels are of particular interest because of their potential as targets for treatment of neurological diseases.^{10,11} In this review, we focus on transient receptor potential (TRP) channels, which are widely expressed in many different tissues and cell types. The TRP family currently comprises 28 mammalian cation channels, subdivided into six subfamilies according to their sequence homology: TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPA (ankyrin), TRPML (mucolipin) and TRPP (polycystic). Most TRP channels are nonselective Ca²⁺-permeable cation channels that serve as cellular sensors for mechanical, thermal, and chemical stimuli.^{12,13)} TRP channels are involved in cellular processes such as cytokine production, proliferation, and migration, all of which are important cellular activities in microglia.^{13,14)} In this review, we will discuss the preclinical evidence for a significant role of TRP channels in microglial activation, particularly in neurological diseases. Mechanisms by which microglia may affect neuronal activity will be highlighted.

2. PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL ROLES OF TRPC CHANNELS IN MICROGLIA

The TRPC subfamily comprises seven members, namely TRPC1-C7, all of which are Ca2+-permeable nonselective cation channels that are strongly expressed in neurons and glia in the brain.¹⁵⁾ TRPC members are classified into two groups on the basis of their sequence homology and functional similarity: the TRPC1/TRPC4/TRPC5 and TRPC3/TRPC6/ TRPC7 groups.¹⁶⁾ TRPC3, TRPC6, and TRPC7 form the diacylglycerol (DAG)-sensitive group and are activated by DAG after stimulation of phospholipase C (PLC)-coupled receptors such as G_{a/11}-type G-protein-coupled receptors or receptor tyrosine kinases. In microglia, there are only a few studies on the functional expression of TRPC channels. Quantitative comparisons of mRNA expression have shown that TRPC1, TRPC3, and TRPC6 are highly expressed in cultured microglia.¹⁷⁾ In line with this finding, double-label immunofluorescence studies show that TRPC3 and TRPC6 are weakly localized in microglia in vivo.^{18,19} Recently, Liu et al. reported that amyloid β -protein (A β) upregulates TRPC6 via nuclear factorkappaB (NF- κ B) and promotes the production of cyclooxygenase-2 (COX-2) in BV-2 microglia, which could be involved in microglial activation-induced hippocampus neuronal damage.²⁰⁾ Moreover, Mizoguchi et al. reported that brain-derived neurotrophic factor (BDNF) induces sustained elevation of the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) through the upregulation of surface TRPC3 channels.²¹⁾ They also found that TRPC3 channels is important for the BDNF-induced suppression of nitric oxide (NO) production in activated microglia, which might be important for the regulation of inflammatory responses and involved in the treatment of neuropsychiatryic disorders including depression. Further investigation would be expected to reveal the physiological and pathophysiological roles of TRPC channels in microglia.

In additional to their physiological role, TRPC channels also play a pathophysiological role in microglia-related inflammatory diseases in the CNS. In this context, Munakata et al. demonstrated that, in the collagenase/autologous blood infusion mouse model of intracerebral hemorrhage (ICH) that is a subtype of hemorrhagic stroke with high morbidity and mortality, Pyr3, a selective TRPC3 inhibitor, can reduce the perihematomal accumulation of microglia and astrocytes and attenuate neurological deficits, neuronal injury, and brain edema, clearly suggesting that TRPC3 contributes to the outcomes after ICH.²²⁾ Since thrombin, a critical blood-derived factors that invades the brain tissue in ICH, has been shown to activate TRPC3 via protease-activated receptor-1 (PAR-1, a G-protein-coupled receptor that activated by thrombin proteolytically) in 1321N1 human astrocytoma cells²³⁾ and cultured rat primary astrocytes in vitro,²⁴⁾ thrombin in the hematoma may induce TRPC3-mediated astrocyte activation via PAR-1 and result in neuroinflammation and neurodegeneration after ICH. However, PAR-1 is important for ICH-induced microglia activation,²⁵⁾ implying TRPC3 in microglia might be a new therapeutic target for the prevention of secondary brain injury and neurological deficits after ICH.

3. PHYSIOLOGICAL ROLES OF TRPM CHANNELS IN GLIA

The TRPM subfamily comprises eight members, namely TRPM1–M8, some of which are sensitive to oxidative stress.²⁶⁾ TRPM2 was the first TRP channel to be identified as redoxsensitive.²⁷⁾ It is strongly expressed in the brain and immune cells and its role in the CNS has been studied extensively. TRPM2 forms a Ca²⁺-permeable cation channel that is activated by oxidative stress mediated by reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) , through the production of nicotinamide adenine dinucleotide and its metabolites, such as ADP-ribose (ADPR) and cyclic ADPR.^{27,28)} Studies indicate that TRPM2 mediates H_2O_2 -induced Ca^{2+} influx, which modulates physiological and pathological cellular functions. TRPM2 is expressed in both neurons and glia, and oxidativestress-induced TRPM2 activation is implicated in neuronal diseases. Several studies have focused on the physiological and pathophysiological roles of TRPM2 in microglia because of the myeloid-monocytic lineage of microglia. TRPM2 functions as a Ca²⁺-permeable channel in mouse monocytes.²⁹⁾ Kraft et al. reported that TRPM2 is functionally expressed at high levels in cultured rat microglia.³⁰⁾ Using cultured microglia derived from wild-type and TRPM2 knockout mice, Haraguchi et al. showed that TRPM2 is involved in NO production.³¹⁾ Subsequently, Miyake et al. examined the intracellular signaling mechanisms underlying these phenomena and demonstrated that combined application of lipopolysaccharide (LPS) and interferon- γ can stimulate TRPM2-mediated extracellular Ca²⁺ influx in cultured microglia.³²⁾ They also showed that activation of TRPM2 results in proline-rich tyrosine kinase 2 (Pyk2)-mediated activation of p38 mitogen-activated protein kinase and c-Jun N-terminal kinase (JNK) signaling, leading to the increased NO production in microglia. The sulfonylurea receptor 1-TRPM4 channel regulates NO synthase 2 transcription in TLR4-activated microglia.33) TRPM7 is functionally expressed in cultured rat microglia,³⁴⁾ and is essential for the enhanced ability of microglia to migrate and invade during anti-inflammatory states.35)

4. PATHOPHYSIOLOGICAL ROLES OF TRPM CHANNELS IN MICROGLIA

4.1. Microglial/Macrophagic TRPM2 Channels in Neuropathic Pain TRPM2 is thought to be involved in a range of pathological pain states, including neuropathic pain.^{31,36,37)} Evidence suggests that peripheral and spinal neuroinflammation mediated by the interaction between nociceptive neurons and immune/glial cells plays a pivotal role in neuropathic pain.³⁸⁾ After peripheral nerve injury, pro-nociceptive inflammatory mediators, such as pro-inflammatory cytokines, chemokines, and excess ROS produced by peripheral tissues and the spinal cord, can lead to peripheral and central sensitization of nociceptive neurons. In this context, Haraguchi et al., demonstrate that TRPM2 expressed in macrophages and microglia plays a critical role in neuropathic pain. TRPM2 is responsible for chemokine (C-X-C motif) ligand 2 (CXCL2) and NO production in macrophages and microglia, which aggravate peripheral and spinal pro-nociceptive inflammatory responses, respectively, in inflammatory and neuropathic pain models.^{31,32)} Furthermore, TRPM2 may play a role in the

infiltration of peripheral immune cells into the spinal cord after peripheral nerve injury.³⁶⁾ Consequently, TRPM2 is involved in a wide range of pathological pain states induced by peripheral and spinal neuroinflammation—such as inflammatory pain, osteoarthritic pain, neuropathic pain induced by peripheral nerve injury, chemotherapy-induced peripheral neuropathy, and painful diabetic neuropathy—rather than in physiological nociceptive pain.³⁷⁾

4.2. TRPM2 Channels in Cerebrovascular Disease TRPM2 is thought to be involved in stroke, especially in ischemic cerebral infarction, which is the leading cause of death and permanent disability in adults worldwide. TRPM2 is thus a potential therapeutic target for stroke. The injury mechanisms following ischemic stroke are multifaceted. Oxidative stress induced by cerebral ischemia-reperfusion injury is considered to be the main event leading to neuronal death. Several lines of evidence indicate that TRPM2 mediates ROS-induced neuronal death. TRPM2 acts as a redox-sensitive Ca²⁺-permeable channel and thus has a pivotal role in H₂O₂-induced neuronal death in primary cultured neurons.³⁹⁾ TRPM2 is activated by intracellular ADPR that is overproduced in response to oxidative stress and ROS, such as H₂O₂.²⁷⁾ In line with these findings, several studies indicate that TRPM2 mediates ischemic brain damage. For example, Alim et al. used wild-type and TRPM2 knockout mice to show that the genetic ablation of TRPM2 causes a shift in the expression ratio of the GluN2A/GluN2B subunits of the N-methyl-D-aspartate receptor, which may selectively upregulate survival pathways and result in neuroprotection from cerebral-ischemia-induced neuronal cell death in vivo.⁴⁰ Shimizu et al. reported an extended therapeutic window for treatment with a novel peptide inhibitor of TRPM2 channels after focal cerebral ischemia, demonstrating that TRPM2 is a promising candidate for treatment of acute cerebral ischemic infarction.⁴¹⁾ However, recent studies have focused extensively on the pathophysiological role of TRPM2 in microglia/macrophages. Application of lysophosphatidylcholine induces intracellular Ca²⁺ influx and increases phosphorylation of p38 mitogen-activated protein kinase (MAPK) via TRPM2, which in turn activates microglia.⁴²⁾ In addition, TRPM2-mediated Ca²⁺ influx induces the production of pro-inflammatory cytokines/chemokines in monocytes.²⁹⁾ TRPM2 is also involved in chemokine CXCL2 and NO production in cultured macrophages and microglia.^{31,32)} In line

with these findings, TRPM2 regulates the migratory capacity of neutrophils and macrophages in response to ischemic brain injury, thereby secondarily perpetuating brain injury after the ischemic event.⁴³⁾ Taken together, these results suggest that TRPM2 in microglia/macrophages could mainly contribute to the development of cerebral ischemic injury.

4.3. Microglial TRPM2 Channels in Cognitive Impairment in CNS Diseases Recently, TRPM2 channels in resident microglia were shown to play a critical role in the pathogenesis of chronic cerebral hypoperfusion through the aggravation of inflammatory responses.⁴⁴⁾ Chronic cerebral hypoperfusion manifests in a wide range of CNS diseases, including neurodegenerative disorders and mental disorders that are accompanied by cognitive impairment; however, the underlying mechanisms remain unclear. In this study, chronic cerebral hypoperfusion can be modeled in mice by bilateral common carotid artery stenosis (BCAS), which results in a decline in cognitive function and excessive release of pro-inflammatory cytokines such as tumor necrosis factor α (TNF α) and interleukin 6 (IL6), all of which were suppressed in TRPM2 knockout mice. TRPM2 knockout mice also had significantly decreased accumulation of microglia in the corpus callosum, although there was no difference in the number of glial fibrillary acidic protein (GFAP)-positive cells between genotypes. Moreover, BCAS mice treated daily with the anti-inflammatory antibiotic minocycline showed significant improvements in their cognitive function.⁴⁴⁾ The schematic in Fig. 1 summarizes the role of TRPM2 in pathology caused by chronic cerebral hypoperfusion. Taken together, these findings suggest a new hypothesis, in which TRPM2-mediated activation of microglia specifically contributes to the pathology through the aggravation of inflammatory responses.

In this context, Ostapchenko *et al.* clearly demonstrate that genetic elimination of TRPM2 normalizes deficits in synaptic markers in aged mice, improves age-dependent spatial memory deficits and reduces microglial activation of hippocampus in mice designed to model Alzheimer's disease.⁴⁵⁾ More recently, Alawieyah Syed Mortadza *et al.* report that exposure to $10-300 \text{ nm} \text{ A}\beta42$ induces concentration-dependent microglial activation and generation of TNF α that are ablated by genetically deleting of TRPM2 or pharmacologically inhibiting TRPM2, revealing a critical role of TRPM2 in A $\beta42$ -induced microglial activation.⁴⁶⁾ These reports shed light on



Fig. 1. Schematic of the Pathophysiological Role of TRPM2 during Chronic Cerebral Hypoperfusion

ROS are produced locally to the chronic cerebral hypoperfusion, resulting in the specific activation of microglial TRPM2 channels. Excessive release of pro-inflammatory cytokines such as $TNF\alpha$, $IL1\beta$, and IL6, presumably from microglia, can induce CNS inflammation and white matter injury, ultimately leading to cognitive impairment (see ref. 44).

our understanding of the mechanisms of chronic cerebral hypoperfusion-related inflammation, and suggest a novel therapeutic target for the treatment of cognitive impairment in CNS diseases.

5. PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL ROLES OF TRPV CHANNELS IN MICROGLIA

The TRPV subfamily is expressed in glial cells, especially astrocytes and microglia. Microglia express several TRPV isoforms, particularly TRPV1, TRPV2, and TRPV4. Activation of TRPV1 triggers Ca²⁺ signaling-dependent cell death in microglia⁴⁷⁾ and contributes to reduced nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase-mediated production of ROS in microglia.⁴⁸⁾ Miyake *et al.* demonstrated that activation of TRPV1 triggers an increase in intramitochondrial Ca²⁺ concentration and following depolarization of mitochondria, which results in mitochondrial ROS production, MAPK activation, and enhancement of chemotactic activity in microglia⁴⁹⁾ (see Fig. 2). Marrone *et al.* clearly demonstrated that stimulation of microglial TRPV1 controls cortical micro-



Fig. 2. Schematic of the Physiological Role of TRPV1 in Microglial Migration

TRPV1 is functionally expressed in mitochondria and its activation by capsaicin or endovanilloids triggers an increase in intramitochondrial Ca²⁺ concentration, depolarization of mitochondria, resultant mtROS production, JNK/p38 activation and enhancement of chemotactic activity in microglia (see ref. 49).



Fig. 3. Schematic of the Role of TRPV4 in Microglial Activation

TRPV4 is functionally expressed in mitochondria and opening of TRPV4 channels, not by blocking of TRPV4, induces membrane depolarization mainly by increasing Na⁺ influx. The LPS-induced morphological changes and increases in TNF α release, galectin-3 expression and K⁺ current amplitude are all attenuated by TRPV4-mediated depolarization of microglia, leading to the attenuation of Ca²⁺ influx and activation of microglia are finally suppressed (see ref. 54). glia activation *per se* and indirectly enhances glutamatergic transmission in neurons by promoting extracellular microglial microvesicles shedding.⁵⁰⁾ In this context, in an experimental model of Parkinson's disease, the TRPV1 selective agonist capsaicin inhibits microglia-mediated ROS production, leading to the resultant blockade of the death of dopaminergic neurons.⁵¹⁾ Moreover, TRPV1 partially regulates cytokine production induced by elevated hydrostatic pressure in retinal microglia.⁵²⁾ Further investigations are warranted to clarify the pathophysiological roles of microglial TRPV1 in CNS diseases.

With respect to TRPV2 and TRPV4, activation of TRPV2 (and also TRPV1) by cannabidiol results in enhanced microglial phagocytosis.⁵³⁾ In addition, Konno *et al.* demonstrates that stimulation of TRPV4 in cultured rat microglia suppresses LPS-induced TNF α release and galectin-3 upregulation, and also suppresses augmentation of voltage-dependent K⁺ currents, suggesting that depolarization in response to opening of the TRPV4 channel attenuates the driving force for extracellular Ca²⁺ and suppresses microglial activation⁵⁴) (see Fig. 3).

6. CONCLUSION

Recent studies emphasize the significance of TRP channels and microglial activation in CNS inflammation and the pathologies of various neurological diseases. In this review, we summarized the evidence for the potential molecular mechanisms underlying stimulation or inhibition of TRP channels, and the pathways leading from TRP activation to the modulation of microglia and resultant CNS inflammation. Notably, microglial TRPV1 and TRPM2 play essential roles in various pathological CNS conditions, such as Parkinson's disease, neuropathic pain, Alzheimer's disease and chronic cerebral hypoperfusion, and, as such, are promising therapeutic targets for these diseases. Of note, suppression of microglial activation and subsequent downstream signaling can be achieved not only through the loss or inhibition of TRP channel function, but also through TRP stimulation (for example, stimulation of microglial TRPV1 and TRPV4). This suggests that the different types of TRP channel affect microglial activation independently. Although the precise mechanisms underlying TRP channel function remain to be verified in vivo, our hope is that TRP channel activators and/or inhibitors will be developed as therapeutic tools to treat neurological diseases in which CNS inflammation is a factor.

Acknowledgments This work was supported by Ministry of Education, Culture, Sports, Science and Technology (MEXT)/JSPS KAKENHI Grant Numbers 17K19486 (to H.S.), 24390016 (to S.K.), and also supported by the Takeda Science Foundation and the Novartis Foundation (to H.S.).

Conflict of Interest The authors declare no conflict of interest.

REFERENCES

- Engelhardt B, Vajkoczy P, Weller RO. The movers and shapers in immune privilege of the CNS. *Nat. Immunol.*, 18, 123–131 (2017).
- Li Q, Barres BA. Microglia and macrophages in brain homeostasis and disease. *Nat. Rev. Immunol.*, 18, 225–242 (2018).

- Kigerl KA, de Rivero Vaccari JP, Dietrich WD, Popovich PG, Keane RW. Pattern recognition receptors and central nervous system repair. *Exp. Neurol.*, 258, 5–16 (2014).
- Gadani SP, Walsh JT, Lukens JR, Kipnis J. Dealing with danger in the CNS: the response of the immune system to injury. *Neuron*, 87, 47–62 (2015).
- Glass CK, Saijo K, Winner B, Marchetto MC, Gage FH. Mechanisms underlying inflammation in neurodegeneration. *Cell*, 140, 918–934 (2010).
- Becher B, Spath S, Goverman J. Cytokine networks in neuroinflammation. *Nat. Rev. Immunol.*, 17, 49–59 (2017).
- Block ML, Zecca L, Hong JS. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nat. Rev. Neurosci.*, 8, 57–69 (2007).
- Salter MW, Stevens B. Microglia emerge as central players in brain disease. *Nat. Med.*, 23, 1018–1027 (2017).
- Lan X, Han X, Li Q, Yang QW, Wang J. Modulators of microglial activation and polarization after intracerebral haemorrhage. *Nat. Rev. Neurol.*, 13, 420–433 (2017).
- Kettenmann H, Hanisch UK, Noda M, Verkhratsky A. Physiology of microglia. *Physiol. Rev.*, 91, 461–553 (2011).
- Madry C, Attwell D. Receptors, ion channels, and signaling mechanisms underlying microglial dynamics. J. Biol. Chem., 290, 12443–12450 (2015).
- 12) Wu LJ, Sweet TB, Clapham DE. International union of basic and clinical pharmacology. LXXVI. Current progress in the mammalian TRP ion channel family. *Pharmacol. Rev.*, **62**, 381–404 (2010).
- 13) Sawamura S, Shirakawa H, Nakagawa T, Mori Y, Kaneko S. TRP channels in the brain: what are they there for? *Neurobiology of TRP Channels*. (Emir TLR ed.) 2nd edition, CRC Press/Taylor & Francis, Boca Raton, FL, Chapter 16 (2017).
- Echeverry S, Rodriguez MJ, Torres YP. Transient receptor potential channels in microglia: roles in physiology and disease. *Neurotox. Res.*, 30, 467–478 (2016).
- Vennekens R, Menigoz A, Nilius B. TRPs in the brain. *Rev. Physi*ol. Biochem. Pharmacol., 163, 27–64 (2012).
- 16) Vazquez G, Wedel BJ, Aziz O, Trebak M, Putney JW Jr. The mammalian TRPC cation channels. *Biochim. Biophys. Acta*, **1742**, 21–36 (2004).
- 17) Ohana L, Newell EW, Stanley EF, Schlichter LC. The Ca²⁺ releaseactivated Ca²⁺ current (I(CRAC)) mediates store-operated Ca²⁺ entry in rat microglia. *Channels*, **3**, 129–139 (2009).
- 18) Fusco FR, Martorana A, Giampà C, De March Z, Vacca F, Tozzi A, Longone P, Piccirilli S, Paolucci S, Sancesario G, Mercuri NB, Bernardi G. Cellular localization of TRPC3 channel in rat brain: preferential distribution to oligodendrocytes. *Neurosci. Lett.*, 365, 137–142 (2004).
- 19) Lee KP, Choi S, Hong JH, Ahuja M, Graham S, Ma R, So I, Shin DM, Muallem S, Yuan JP. Molecular determinants mediating gating of transient receptor potential canonical (TRPC) channels by stromal interaction molecule 1 (STIM1). *J. Biol. Chem.*, 289, 6372–6382 (2014).
- 20) Liu N, Zhuang Y, Zhou Z, Zhao J, Chen Q, Zheng J. NF-κB dependent up-regulation of TRPC6 by Aβ in BV-2 microglia cells increases COX-2 expression and contributes to hippocampus neuron damage. *Neurosci. Lett.*, **651**, 1–8 (2017).
- 21) Mizoguchi Y, Kato TA, Seki Y, Ohgidani M, Sagata N, Horikawa H, Yamauchi Y, Sato-Kasai M, Hayakawa K, Inoue R, Kanba S, Monji A. Brain-derived neurotrophic factor (BDNF) induces sustained intracellular Ca²⁺ elevation through the up-regulation of surface transient receptor potential 3 (TRPC3) channels in rodent microglia. *J. Biol. Chem.*, **289**, 18549–18555 (2014).
- 22) Munakata M, Shirakawa H, Nagayasu K, Miyanohara J, Miyake T, Nakagawa T, Katsuki H, Kaneko S. Transient receptor potential canonical 3 inhibitor Pyr3 improves outcomes and attenuates astrogliosis after intracerebral hemorrhage in mice. *Stroke*, 44, 1981–1987

(2013).

- 23) Nakao K, Shirakawa H, Sugishita A, Matsutani I, Niidome T, Nakagawa T, Kaneko S. Ca²⁺ mobilization mediated by transient receptor potential canonical 3 is associated with thrombin-induced morphological changes in 1321N1 human astrocytoma cells. *J. Neurosci. Res.*, **86**, 2722–2732 (2008).
- 24) Shirakawa H, Sakimoto S, Nakao K, Sugishita A, Konno M, Iida S, Kusano A, Hashimoto E, Nakagawa T, Kaneko S. Transient receptor potential canonical 3 (TRPC3) mediates thrombin-induced astrocyte activation and upregulates its own expression in cortical astrocytes. *J. Neurosci.*, **30**, 13116–13129 (2010).
- 25) Wan S, Cheng Y, Jin H, Guo D, Hua Y, Keep RF, Xi G. Microglia activation and polarization after intracerebral hemorrhage in mice: the role of protease-activated receptor-1. *Transl. Stroke Res.*, 7, 478–487 (2016).
- 26) Simon F, Varela D, Cabello-Verrugio C. Oxidative stress-modulated TRPM ion channels in cell dysfunction and pathological conditions in humans. *Cell. Signal.*, 25, 1614–1624 (2013).
- 27) Hara Y, Wakamori M, Ishii M, Maeno E, Nishida M, Yoshida T, Yamada H, Shimizu S, Mori E, Kudoh J, Shimizu N, Kurose H, Okada Y, Imoto K, Mori Y. LTRPC2 Ca²⁺-permeable channel activated by changes in redox status confers susceptibility to cell death. *Mol. Cell*, 9, 163–173 (2002).
- 28) Perraud AL, Fleig A, Dunn CA, Bagley LA, Launay P, Schmitz C, Stokes AJ, Zhu Q, Bessman MJ, Penner R, Kinet JP, Scharenberg AM. ADP-ribose gating of the calcium-permeable LTRPC2 channel revealed by Nudix motif homology. *Nature*, **411**, 595–599 (2001).
- 29) Yamamoto S, Shimizu S, Kiyonaka S, Takahashi N, Wajima T, Hara Y, Negoro T, Hiroi T, Kiuchi Y, Okada T, Kaneko S, Lange I, Fleig A, Penner R, Nishi M, Takeshima H, Mori Y. TRPM2mediated Ca²⁺influx induces chemokine production in monocytes that aggravates inflammatory neutrophil infiltration. *Nat. Med.*, 14, 738–747 (2008).
- 30) Kraft R, Grimm C, Grosse K, Hoffmann A, Sauerbruch S, Kettenmann H, Schultz G, Harteneck C. Hydrogen peroxide and ADPribose induce TRPM2-mediated calcium influx and cation currents in microglia. Am. J. Physiol. Cell Physiol., 286, C129–C137 (2004).
- 31) Haraguchi K, Kawamoto A, Isami K, Maeda S, Kusano A, Asakura K, Shirakawa H, Mori Y, Nakagawa T, Kaneko S. TRPM2 contributes to inflammatory and neuropathic pain through the aggravation of pronociceptive inflammatory responses in mice. *J. Neurosci.*, 32, 3931–3941 (2012).
- 32) Miyake T, Shirakawa H, Kusano A, Sakimoto S, Konno M, Nakagawa T, Mori Y, Kaneko S. TRPM2 contributes to LPS/IFNyinduced production of nitric oxide via the p38/JNK pathway in microglia. Biochem. Biophys. Res. Commun., 444, 212–217 (2014).
- 33) Kurland DB, Gerzanich V, Karimy JK, Woo SK, Vennekens R, Freichel M, Nilius B, Bryan J, Simard JM. The Surl-Trpm4 channel regulates NOS2 transcription in TLR4-activated microglia. J. Neuroinflammation, 13, 130 (2016).
- 34) Jiang X, Newell EW, Schlichter LC. Regulation of a TRPM7-like current in rat brain microglia. J. Biol. Chem., 278, 42867–42876 (2003).
- 35) Siddiqui T, Lively S, Ferreira R, Wong R, Schlichter LC. Expression and contributions of TRPM7 and KCa2.3/SK3 channels to the increased migration and invasion of microglia in anti-inflammatory activation states. *PLOS ONE*, **9**, e106087 (2014).
- 36) Isami K, Haraguchi K, So K, Asakura K, Shirakawa H, Mori Y, Nakagawa T, Kaneko S. Involvement of TRPM2 in peripheral nerve injury-induced infiltration of peripheral immune cells into the spinal cord in mouse neuropathic pain model. *PLOS ONE*, 8, e66410 (2013).
- 37) So K, Haraguchi K, Asakura K, Isami K, Sakimoto S, Shirakawa H, Mori Y, Nakagawa T, Kaneko S. Involvement of TRPM2 in a wide range of inflammatory and neuropathic pain mouse models. *J. Pharmacol. Sci.*, **127**, 237–243 (2015).

- 38) Ren K, Dubner R. Interactions between the immune and nervous systems in pain. *Nat. Med.*, 16, 1267–1276 (2010).
- 39) Kaneko S, Kawakami S, Hara Y, Wakamori M, Itoh E, Minami T, Takada Y, Kume T, Katsuki H, Mori Y, Akaike A. A critical role of TRPM2 in neuronal cell death by hydrogen peroxide. *J. Pharmacol. Sci.*, **101**, 66–76 (2006).
- 40) Alim I, Teves L, Li R, Mori Y, Tymianski M. Modulation of NMDAR subunit expression by TRPM2 channels regulates neuronal vulnerability to ischemic cell death. J. Neurosci., 33, 17264– 17277 (2013).
- 41) Shimizu T, Dietz RM, Cruz-Torres I, Strnad F, Garske AK, Moreno M, Venna VR, Quillinan N, Herson PS. Extended therapeutic window of a novel peptide inhibitor of TRPM2 channels following focal cerebral ischemia. *Exp. Neurol.*, **283** (Pt A), 151–156 (2016).
- 42) Jeong H, Kim YH, Lee Y, Jung SJ, Oh SB. TRPM2 contributes to LPC-induced intracellular Ca²⁺ influx and microglial activation. *Biochem. Biophys. Res. Commun.*, 485, 301–306 (2017).
- 43) Gelderblom M, Melzer N, Schattling B, Göb E, Hicking G, Arunachalam P, Bittner S, Ufer F, Herrmann AM, Bernreuther C, Glatzel M, Gerloff C, Kleinschnitz C, Meuth SG, Friese MA, Magnus T. Transient receptor potential melastatin subfamily member 2 cation channel regulates detrimental immune cell invasion in ischemic stroke. *Stroke*, **45**, 3395–3402 (2014).
- 44) Miyanohara J, Kakae M, Nagayasu K, Nakagawa T, Mori Y, Arai K, Shirakawa H, Kaneko S. TRPM2 channel aggravates CNS inflammation and cognitive impairment *via* activation of microglia in chronic cerebral hypoperfusion. *J. Neurosci.*, **38**, 3520–3533 (2018).
- 45) Ostapchenko VG, Chen M, Guzman MS, Xie YF, Lavine N, Fan J, Beraldo FH, Martyn AC, Belrose JC, Mori Y, MacDonald JF, Prado VF, Prado MA, Jackson MF. The transient receptor potential melastatin 2 (TRPM2) channel contributes to β-amyloid oligomerrelated neurotoxicity and memory impairment. J. Neurosci., 35, 15157–15169 (2015).
- 46) Alawieyah Syed Mortadza S, Sim JA, Neubrand VE, Jiang LH. A critical role of TRPM2 channel in $A\beta_{42}$ -induced microglial activa-

tion and generation of tumor necrosis factor-*α*. *Glia*, **66**, 562–575 (2018).

- 47) Kim SR, Kim SU, Oh U, Jin BK. Transient receptor potential vanilloid subtype 1 mediates microglial cell death *in vivo* and *in vitro via* Ca²⁺-mediated mitochondrial damage and cytochrome c release. J. Immunol., 177, 4322–4329 (2006).
- Schilling T, Eder C. Stimulus-dependent requirement of ion channels for microglial NADPH oxidase-mediated production of reactive oxygen species. J. Neuroimmunol., 225, 190–194 (2010).
- 49) Miyake T, Shirakawa H, Nakagawa T, Kaneko S. Activation of mitochondrial transient receptor potential vanilloid 1 channel contributes to microglial migration. *Glia*, 63, 1870–1882 (2015).
- 50) Marrone MC, Morabito A, Giustizieri M, Chiurchiù V, Leuti A, Mattioli M, Marinelli S, Riganti L, Lombardi M, Murana E, Totaro A, Piomelli D, Ragozzino D, Oddi S, Maccarrone M, Verderio C, Marinelli S. TRPV1 channels are critical brain inflammation detectors and neuropathic pain biomarkers in mice. *Nat. Commun.*, 8, 15292 (2017).
- Sappington RM, Calkins DJ. Contribution of TRPV1 to microgliaderived IL-6 and NFkappaB translocation with elevated hydrostatic pressure. *Invest. Ophthalmol. Vis. Sci.*, 49, 3004–3017 (2008).
- 52) Chung YC, Baek JY, Kim SR, Ko HW, Bok E, Shin WH, Won SY, Jin BK. Capsaicin prevents degeneration of dopamine neurons by inhibiting glial activation and oxidative stress in the MPTP model of Parkinson's disease. *Exp. Mol. Med.*, **49**, e298 (2017).
- 53) Hassan S, Eldeeb K, Millns PJ, Bennett AJ, Alexander SP, Kendall DA. Cannabidiol enhances microglial phagocytosis via transient receptor potential (TRP) channel activation. Br. J. Pharmacol., 171, 2426–2439 (2014).
- 54) Konno M, Shirakawa H, Iida S, Sakimoto S, Matsutani I, Miyake T, Kageyama K, Nakagawa T, Shibasaki K, Kaneko S. Stimulation of transient receptor potential vanilloid 4 channel suppresses abnormal activation of microglia induced by lipopolysaccharide. *Glia*, 60, 761–770 (2012).