TASK1 modulates inflammation and neurodegeneration in autoimmune inflammation of the central nervous system

Stefan Bittner,1,* Sven G. Meuth,1,* Kerstin Göbel,1 Nico Melzer,1 Alexander M. Herrmann,1 Ole J. Simon,1 Andreas Weishaupt,1 Thomas Budde,2 Douglas A. Bayliss,3 Martin Bendszus4 and Heinz Wiendl1

1 University of Wuerzburg, Department of Neurology, Josef-Schneider-Str. 11, 97080 Wuerzburg, Germany
2 Westfaelische Wilhelms-University Muenster, Institute of Physiology, Robert-Koch Str. 27a, 48149 Muenster, Germany
3 University of Virginia, Department of Pharmacology, Charlottesville, Virginia 22908, USA
4 University of Heidelberg, Department of Neuroradiology, Im Neuenheimer Feld 400, 69120 Heidelberg, Germany

*These authors contributed equally to this work.

Correspondence to: Sven G. Meuth, MD, PhD, University of Wuerzburg, Department of Neurology, Josef-Schneider-Str. 11, 97080 Wuerzburg, Germany. E-mail: meuth_s@klinik.uni-wuerzburg.de

Correspondence may also be addressed to: Heinz Wiendl, E-mail: heinz.wiendl@klinik.uni-wuerzburg.de

We provide evidence that TWIK-related acid-sensitive potassium channel 1 (TASK1), a member of the family of two-pore domain potassium channels relevant for setting the resting membrane potential and balancing neuronal excitability that is expressed on T cells and neurons, is a key modulator of T cell immunity and neurodegeneration in autoimmune central nervous system inflammation. After induction of experimental autoimmune encephalomyelitis, an experimental model mimicking multiple sclerosis, TASK1−/− mice showed a significantly reduced clinical severity and markedly reduced axonal degeneration compared with wild-type controls. T cells from TASK1−/− mice displayed impaired T cell proliferation and cytokine production, while the immune repertoire is otherwise normal. In addition to these effects on systemic T cell responses, TASK1 exhibits an independent neuroprotective effect which was demonstrated using both a model of acutely prepared brain slices cocultured with activated T cells as well as in vitro cultivation experiments with isolated optic nerves. Anandamide, an endogenous cannabinoid and inhibitor of TASK channels, reduced outward currents and inhibited effector functions of T cells (IFN-γ production and proliferation); an effect completely abrogated in TASK1−/− mice. Accordingly, preventive blockade of TASK1 significantly ameliorated experimental autoimmune encephalomyelitis after immunization. Therapeutic application of anandamide significantly reduced disease severity and was capable of lowering progressive loss of brain parenchymal volume as assessed by magnetic resonance imaging. These data support the identification and characterization of TASK1 as potential molecular target for the therapy of inflammatory and degenerative central nervous system disorders.

Keywords: multiple sclerosis; EAE; TASK1; K2P; potassium channels


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Introduction

Potassium channels play a major role in neuronal cell death and immune cell activation. Neuronal apoptosis can be inhibited when K⁺ efflux is prevented (Bortner et al., 1997; Yu et al., 1997; Maeno et al., 2000). The molecular substrates underlying K⁺ efflux in apoptotic volume decrease (AVD), an initial event in apoptosis (Bortner et al., 1997; Burg et al., 2006), depends on the cell type. Probably, K⁺ efflux during apoptosis is mediated by a variety of different potassium channels (Bortner and Cidlowski, 2007). For a number of reasons it is reasonable to assume that tandem pore domain potassium channels (K₂P) play a major role within this context (Patel and Lazdunski, 2004): they are widely expressed in the central nervous system (CNS) and traditionally regarded as background leak channels contributing to setting, maintaining and regulating the resting membrane potential in neurons (Lesage and Lazdunski, 2000; Meuth et al., 2003; Musset et al., 2006; Bean, 2007). TWIK-related acid-sensitive K⁺ channel 1 (TASK1) is a member of this family (for review see Kim, 2005). TASK1 plays a major role in K⁺ dependent cell death and neuronal survival. For example, K⁺ dependent cerebellar granule neuron apoptosis can be induced in neurons lacking the standing outward K current (IKₒ) by transfection with TASK1 or TASK3 (Lauritzen et al., 2003). In addition, we recently described that TASK1 played a critical role in immune cell activation (Meuth et al., 2008). As TASK1 is expressed both on immune cells and neurons, we hypothesized that TASK1 contributes to pathophysiological processes involving pathogenic T cell immunity as well as axonal degeneration.

Multiple sclerosis is a chronic disabling disease with significant implications for patients and society (Sospedra and Martin, 2006; Frohman et al., 2006; Lassmann et al., 2007). Traditional concepts regard multiple sclerosis as an inflammatory demyelinating disorder of putative autoimmune origin, which occurs as a consequence of immune-tolerance breakdown in genetically susceptible hosts. Furthermore, recent data support the importance of primary and secondary neurodegenerative mechanisms (Ferguson et al., 1997; Trapp et al., 1998; Frohman et al. 2006; Lassmann et al., 2007) causing persistent neurological deficits. Axonal damage occurs in early stages of multiple sclerosis and a variety of mechanisms seem to underlie axonal loss (e.g. chronic demyelination, disturbance of axonal ion concentrations, mitochondrial dysfunction, energy failure, alteration of ion exchange mechanisms and accumulation of Ca²⁺) (Trapp et al., 1998; Waxman, 2006; Dutta and Trapp, 2007). Existing therapies in multiple sclerosis have notable effects such as therapy has yet reached clinical standards.

Here we used experimental autoimmune encephalomyelitis (EAE), an animal model of autoimmune CNS inflammation mimicking many relevant aspects of multiple sclerosis (Steinman and Zamvil, 2005; Gold et al., 2006) to investigate the contribution of TASK1 to axonal degeneration and inflammation. Genetic TASK1 deficiency (TASK1⁻/⁻) as well as pharmacological manipulation of TASK channels were investigated in different variants of myelin oligodendrocyte glycoprotein (MOG)-induced EAE in C57BL/6 mice. As pharmacological inhibition of TASK represents a more clinical approach, the endocannabinoid anandamide (arachidonylethanolamine, AEA), a semi-selective inhibitor of TASK channels, was applied under preventive and therapeutic treatment conditions. TASK1 is identified as a modulator of T cell activation and axonal damage in this model of inflammatory CNS degeneration.

Experimental procedures

Induction and assessment of experimental EAE

All animal experiments were approved by local authorities and conducted according to the German law of animal protection. EAE was induced by immunization of 6–8 week old female C57BL/6 mice (Charles River, Sulzfeld, Germany) with varying concentrations of myelin oligodendrocyte glycoprotein (MOG₃₅–₉₉) peptide (Biotrend, Cologne, Germany), depending on the desired disease course (100–200 μg). MOG peptide was added to complete Freund’s adjuvant (CFA) to obtain a 1 mg/ml emulsion and 2 x 50 μl were injected subcutaneously at two different sites of the flank of deeply anaesthetized mice. Pertussis toxin was injected at the day of immunization and 2 days later at a dose of 400 ng (Alexis, San Diego, USA).

One group of mice received 10 mg/kg anandamide (Sigma, Deisenhofen, Germany) from the day of immunization (preventive treatment), while treatment of another group started when animals showed clinical signs of EAE (therapeutic treatment). A control EAE group received the carrier solution alone (without anandamide; 80% NaCl, 10% ethanol, 10% polyethylene glycol; Sigma, Deisenhofen, Germany). In addition, three other groups were administered AM251 (CB1; 3 mg/kg; Tocris, Ellisville, USA), AM630 (CB2; 3 mg/kg; Tocris, Ellisville, USA) or capsazepine (VR1; 10 mg/kg; Sigma, Deisenhofen, Germany) alone (n = 5/group) or in addition to anandamide treatment (n = 10/group). In another set of experiments, EAE was induced following the immunization protocol described above but using 200 μg of MOG peptide in wild-type mice or TASK1⁻/⁻ mice (Mulkey et al., 2007). All mentioned blockers were applied intraperitoneally and the animals were scored and weighed daily. The clinical course of EAE was monitored using the following score system: Grade 0, no abnormality; Grade 1, limp tail; Grade 2, moderate hind limb weakness; Grade 3, complete hind limb paralysis; Grade 4, quadriplegia or premoribund state; Grade 5, death. Animals were scored by two blinded observers.

Abbreviations: AEA = arachidonylethanolamide; APC = antigen-presenting cells; AVD = apoptotic volume decrease; EAE = experimental autoimmune encephalomyelitis; CST = corticospinal tract; PBS = phosphate-buffered saline; PFA = paraformaldehyde
**Proliferation assay —\[^{3}H\]thymidine uptake**

Spleen tissue was homogenized and strained through a 40 µm nylon filter (BD Biosciences, Heidelberg, Germany). The homogenates were rinsed with washing medium Dulbecco’s Modified Eagle’s Medium (DMEM; containing 1% FCS, 1% glutamine, 1% antibiotics) and resuspended in erythrocyte lysis buffer (150 mM NH₄Cl, 10 mM KHCO₃, 0.1 mM EDTA; pH 7.3) for 5 min. Splenocytes from wild-type and knockout mice were cultured for 3 days after isolation and stimulated with CD3/CD28 beads (T cell to bead ratio: 2:1; Dynal Biotech, Hamburg, Germany). Anandamide was added on Day 2 and 1 µCi of \[^{3}H\]thymidine (Amersham, Piscataway, USA) was added for the final 24 h. Radioactivity was measured on a β-scintillation counter (TopCount NXT; PerkinElmer, Rodgau-Jügesheim, Germany). All experiments were performed in triplicate.

**Assessment of cytokine secretion**

Splenocytes from wild-type and TASK1\(^{-/-}\) mice were isolated and 3 x 10⁶ splenocytes in 1 ml medium were plated in DMEM containing 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 25 µg/ml gentamycin, 50 µM mercaptoethanol, 5% foetal calf serum (FCS), 2 mM glutamine and 1% non-essential amino acids (NEAA; Cambrex, Verviers, Belgium). Splenocytes were either left unstimulated or were stimulated with various concentrations of CD3/28 mouse dynabeads in the absence or presence of anandamide (30 µM). The solvent in the final experimental solution did not exceed 1%. Cells were cultured for 2 days at 37°C and 5% CO₂ and supernatants were assessed for IFN-γ protein levels by ELISA (R&D Systems, Wiesbaden, Germany) and for IL2, IFN-γ, IL17, IL4, IL5, IL10, IL6, tumour necrosis factor (TNF)α and Granulocyte-macrophage colony-stimulating factor (GMCSF) by bead-based flow cytometry (Flow Cytomix Mouse Th1/Th2 10plex, Bender MedSystems, Vienna, Austria) according to the manufacturer’s instructions. For MOG recall assay, splenocytes were isolated at disease maximum and 3 x 10⁶ cells per well (duplicates) were re-stimulated with 10 µg/ml MOG peptide (same stock as used for immunization). Cytokine levels were assessed after 2 days by flow cytometry as described above.

**Flow cytometry of CNS cells**

Analysis of CNS cells by flow cytometry was performed as described before (Ortler et al. 2008). In brief, CNS tissue from immunized wild-type and TASK1\(^{-/-}\) at disease maximum was chopped and cells were isolated from the interface of 30–50% Percoll (Amersham) centrifuged for 30 min at 2500 rpm. Mononuclear cells were washed and cell number infiltrates were determined by cell counting and flow cytometry based on the percentage of specific cells (CD4⁺, CD8⁺) from the total cell population acquired.

**Immunophenotyping and flow cytometry**

For analysis of T cell subtype distribution cells were stained for 30 min with appropriate antibodies or isotype controls (all by BD Bioscience): rat anti-mouse CD4-PerCP (No. 553052), rat anti-mouse CD8a-PE-Cy7 (No. 553033), rat anti-mouse CD44-FITC (No. 553133), rat anti-mouse CD62L-APC (No. 553152). Antigen presenting cells from immunized animals were analysed using the following antibodies: rat anti-mouse CD11b-PerCP (No. 350993), hamster anti-mouse CD11c-APC (No. 550261), mouse anti-mouse MCHII-FITC (Serotec, No. MCA1501F), rat anti-mouse CD80-FITC (No. 553691), hamster anti-mouse CD80-FITC (No. 553768), hamster anti-mouse CD40-FITC (No. 553723). In an additional set of experiments, regulatory T lymphocytes were stained by standard intracellular staining protocols using Alexa Fluor 647 rat anti-mouse Foxp3 (No. 560401).

All antibodies were titrated for optimal staining. Probes were measured by flow cytometry (FACS-Calibur, BD Bioscience) and analysed by CellQuest Pro (BD Bioscience).

**Immu-no-histochemistry and -cytochemistry**

Mice were transcardially perfused with phosphate-buffered saline (PBS). Spinal cords were carefully excised from the brainstem to lumbar region, fixed in 4% paraformaldehyde (PFA) in PBS for 2 h and then embedded in Tissue-Tek OCT compound (Miles Laboratories, Elkhart, USA). Ensuring that the same regions were analysed for all mice, 10-µm cross sections of the beginning of the cervical enlargement were cut and incubated with antibodies against phosphorylated neurofilament (SMI31, Sternberger Monoclonals, Lutherville, USA) non-phosphorylated neurofilament (SMI32, Sternberger Monoclonals) in combination or alone, respectively; a method that allows all axons (myelinated and demyelinated) to be quantified (Lo et al., 2003). Sections were sequentially incubated in PBS, horse anti-mouse IgG-biotin (Vector Laboratories, Burlingame, USA), PBS, StreptABCComplex (Dako, Glostrup, Denmark), PBS and diamobenzidin (DAB, Kem-En-Tec Dg, Taastrup, Denmark).

For quantification of axonal density, predefined areas (500 µm²) at specific sites within the dorsal column (DC, cuneate fasciculus) and dorsal corticospinal tract (CST) were examined (Axiohot2, Zeiss, Oberkochen, Germany) with a CCD camera (VisiTron Systems, Tuchheim, Germany). Stained axons were counted utilizing MetaVue Software (Molecular Devices, Downingtown, USA) by a blinded investigator. In one set of experiments we analysed axonal densities in the optic nerve of naive wild-type and TASK1\(^{-/-}\) mice after 24 h ex vivo culturing conditions. Briefly, optic nerves of naive mice were carefully excised and cultured in 24-well plates for 24 h in 95% O₂ and 5% CO₂. CD4⁺ T lymphocytes were isolated from immunized wild-type mice at disease maximum and 1 x 10⁶ cells were added to one optic nerve while the other optic nerve was left without T lymphocytes to ensure high comparability. Optic nerves were then frozen in Tissue Tek and investigated by SMI31/32 staining as described above.
Mice spinal cord cryo sections were selected as described above. Primary antibodies (rat anti-CD11b, 1:100, Serotec, Düsseldorf, Germany; rat anti-CD3, 1:100, Serotec, Düsseldorf, Germany) were added and incubated for 12 h at 4°C. Next, slices were washed three times in PBS and incubated with methanol containing 0.5% H2O2 for 15 min at room temperature. They were subsequently washed with tris-buffered saline (TBS) and biotinylated anti-rat IgG Dako, Hamburg, Germany was added for 60 min at room temperature. Thereafter, avidin/biotin-complex (1:100, Dako, Hamburg, Germany) was performed for 5 min. For detection of demyelination Haemalaun-staining sections were stained by incubation with 1 mg/ml DAB (KemEnTec Diagnostics, Copenhagen, Denmark) for 5 min. Haemalaun-staining (1% haematoxylin) (Sigma-Aldrich, Munich, Germany) in aqua dest. containing 0.2% NaIO3 (Fluka, Sigma-Aldrich), 5% KAISO3 (Merck, Darmstadt, Germany), 5% chloral hydrate (Merck, Darmstadt Germany) and 1% citric acid (Serva, Heidelberg, Germany) was performed for 1 min. For detection of demyelination slides were incubated with Luxol fast blue solution for 12 h at 60°C (0.1%, Sigma-Aldrich, Munich, Germany), washed in 95% ethanol, and then placed in lithium carbonate (0.05%, Sigma-Aldrich, Munich, Germany).

For immunocytochemical stainings, isolated murine CD4+ T cells were placed on coverslips coated with poly-l-lysine (Sigma, Deisenhofen, Germany), fixed with 4% PFA and stained with rabbit anti-TASK1 (Sigma) followed by biotinylated goat anti-rabbit IgG (Vector Laboratories, USA) and streptavidin-FITC (Serotec). Cell nuclei were stained with DAPI (Merck, Darmstadt, Germany) and picture analysis was done as described above.

Preparation of acute brain slices and co-culture experiments with T cells

Preparation of acute brain slices was performed following established procedures (Edwards et al., 1989; Meuth et al., 2003; Na et al., 2009) using naive 6- to 8-week-old C57BL/6 wild-type or TASK1−/− mice, respectively. Mice were anaesthetized and decapitated; the brain was removed and placed in oxygenated artificial cerebrospinal fluid (ACSF) containing (in mM): sucrose, 200; PIPES, 20; KCl, 2.5; NaH2PO4, 1.25; MgSO4, 10; CaCl2, 0.5; dextrose, 10; pH 7.35 for 10 min. Slices of 300 μm were prepared as coronal sections on a vibratome and immediately transferred into separate wells of a 12-well plate filled with artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl, 125; KCl, 2.5; NaH2PO4, 1.25; NaHCO3, 24; MgSO4, 2; CaCl2, 2; dextrose, 10; pH adjusted to 7.35 by bubbling with a mixture of 95% O2 and 5% CO2. Slices were incubated alone or with 5 × 105 CD4+ T cells from MOG-immunized wild-type mice isolated by magnetic-activated cell sorting (MACS) method maximum as described above. After 6 h, slices were harvested and embedded in tissue-Tek optimal cutting temperature (OCT) compound. Immunohistochemical staining was performed on 10 μm coronal sections. Only sections obtained from a depth between 50 and 100 μm from both sides were included in the analysis minimizing the impact of both neuronal apoptosis due to insufficient gaseous diffusion in the inner levels of the 300 μm slices and the cutting procedure at the outer surface as described previously (Edwards et al., 1989). Mean densities of T cells were independent from the slice level after 6 h (data not shown). Slices were post-fixed in 4% PFA for 10 min and incubated in blocking solution (PBS containing 5% bovine serum albumin (BSA), 1% normal goat serum and 0.2% Triton X-100). Slices were then incubated simultaneously with antibodies against neuronal nuclear antigen (NeuN) (1:1000, Chemicon, Billerica, USA) and activated caspase-3 (1:200, Cell Signalling, Boston, USA) overnight at 4°C. Secondary antibodies were Alexa Fluor 488-coupled goat anti-mouse (1:100, BD Pharmingen) or Cy3-coupled goat anti-rabbit (1:300, Dianova). Negative controls were obtained by either omitting the primary or secondary antibody and revealed no detectable signal (data not shown). Pictures were collected and analysed as described above within preselected fields at specific sites (CA2/3 region of the hippocampus and cortex) known to express high levels of MOG antigen (Habib et al., 1998).

Clinical chemistry

At Day 17 (EAE maximum) and Day 50 (EAE remission), sera from three to five animals of different experimental groups were pooled for each group. To obtain control values, the same was done with non-immunized healthy mice. Sera were analysed for GOT, GPT, glucose and urea by spectrophotometric assays (Cobas Integra 800 auto-analysers; Roche, Mannheim, Germany).

Electrophysiological measurements

Splenocytes were isolated as described above, stimulated with CD3/28 mouse dynabeads overnight and CD4+ T cells were isolated using CD4 T-cell isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany). All measurements were conducted in whole-cell configuration of the patch-clamp technique. Individual mouse T lymphocytes were identified by infrared differential interference contrast video-microscopy (Dott and Zieglsbarger, 1990). Whole-cell recording pipettes were fabricated from borosilicate glass (GT150T-10, Clark Electromedical Instruments, Pangbourne, UK; typical resistance was 3–5 MΩ) and filled with an intracellular solution containing (in mM): K-glucuronate, 95; K2-citrate, 20; NaCl, 10; HEPES, 10; MgCl2, 1; CaCl2, 0.5; BAPTA, 3; Mg-ATP, 3; Na-GTP, 0.5. The internal solution was set to a pH of 7.25 and an osmolality of 295 mOsm/kg. Extracellular solution contained (in mM): NaCl, 120; KCl, 2.5; NaH2PO4, 1.25; HEPES, 30; MgSO4, 2; CaCl2, 2; dextrose, 10; pH 7.2 and osmolality was set to 305 mOsm/kg. Membrane currents were recorded using an EPC-10 amplifier (HEKA Elektronic, Lamprecht, Germany). Outward currents were elicited by repeated 500 ms pulses from −80 mV to 40 mV starting from a holding potential of −80 mV, applied at 30 s intervals as described previously (Wulff et al., 2003). Anandamide used for electrophysiological measurements was solved in ethanol. The solvent concentrations in the final recording solution did not exceed 1%. Application of the solvent alone (1%) had no effect on the recorded current. A liquid junction potential of 6 ± 3 mV (n = 5) was measured and taken into account when analysing the data.
Magnetic resonance imaging

MRI measurements were performed immediately after immunization for a baseline image, at Day 16 (gadofluorine M) and at Day 44 on a clinical 1.5T unit (Magnetom Vision; Siemens, Erlangen, Germany). Animals were anaesthetized by inhalation narcosis and were placed in a custom made dual channel surface coil (A063HACG; Rapid Biomedical, Wuerzburg, Germany). The MRI protocol included a T2-w turbo spin echo sequence [repetition time (TR), 2500 ms; echo time (TE), 80 ms, slice thickness, 2 mm] and a high-resolution 3D constructed interference in steady state (CISS) sequence (TR, 16.4 ms; TE, 8.2 ms; slice thickness, 1 mm) in the coronal plane. Ventricular size was assessed on CISS images on a four-step scale from one (normal size) to four (massive ventricular enlargement) (Ip et al., 2006).

Statistical analysis

All results are presented as mean ± SEM. Statistical analysis was performed using a modified student’s t-test (Dixon and Massey, 1969) for normal distribution of data or a Mann–Whitney test for not normally distributed datasets. A Bonferroni-corrected one-way ANOVA was used in case of multiple comparisons using PrismGraph 4.0 software (GraphPad Software, San Diego, CA) or Origin (Microcal). P-values < 0.05 were considered as statistically significant.

Results

Experimental autoimmune CNS inflammation is significantly attenuated in the absence of TASK1

In a first set of experiments we examined the role of TASK1 in MOG35–55 peptide induced EAE. TASK1−/− animals showed a significantly delayed disease onset, reduced disease severity at disease maximum (Day 22; score: 1.1 ± 0.6, P = 0.02, grey line) and a notably ameliorated disease course throughout the whole observation period (50 days) compared with wild-type mice (onset: Day 12; maximum score at Day 17: 3.6 ± 0.19; Fig. 1A, black line versus grey line, Supplementary Table 1). Histological evaluation of mice was done at disease maximum. TASK1−/− mice showed reduced demyelination (n = 5; TASK1−/−: area: 0.2 ± 0.1%; WT: area: 2.1 ± 0.3%; P = 0.01), as well as a reduced inflammatory index (TASK1−/−: 0.6 ± 0.3, WT: 3.5 ± 0.8; P = 0.02), reduced numbers of infiltrating T cells (TASK1−/−: 22 ± 12, WT: 388 ± 166; P < 0.01) and macrophages (TASK1−/−: 66 ± 23, WT: 844 ± 280; P < 0.01) (Supplementary Table 2). Flow cytometry assessment of CNS infiltrating cells at disease maximum also revealed a significantly reduced infiltration of both CD4+ and CD8+ T cells in absolute numbers in TASK1−/− mice while the CD4 to CD8 ratio of the infiltrates was unchanged (Fig. 1B).

In vivo antigen recognition is significantly decreased in TASK1−/− mice

T lymphocytes isolated from wild-type and TASK1−/− mice at disease maximum were re-stimulated with MOG peptide (10 μg/ml, n = 4) and production of different cytokines was measured after 2 days (Fig. 1C). Th1 cytokines (IL2, IFN-γ) were significantly reduced in TASK1−/− mice while no significant changes were observed for Th2 cytokines (IL4, IL5) or IL17. Interestingly, TASK1−/− splenocytes showed a significantly higher production of the suppressive cytokine IL10 upon MOG re-stimulation (Fig. 1C). No differences between TASK1−/− and control animals were found for IL6, TNFα and GMCSF (data not shown).

Absence of TASK1 reduces T cell proliferation and proinflammatory cytokine secretion

We recently demonstrated that TASK1 is expressed on human CD3+ T lymphocytes critically influencing net outward currents and T cell activation (Meuth et al., 2008). Therefore, we analysed TASK1 expression in murine T cells and the effect of TASK1 ablation on immune repertoire and on T cell activation. Isolated murine CD4+ T cells constitutively express TASK1 as assessed by immunocytochemistry (Fig. 2A). A dose-response curve with CD3/28 beads was used to investigate whether naïve wild-type T lymphocytes differ from TASK1−/− T lymphocytes in their general cytokine response upon stimulation (n = 4 each). Indeed, TASK1−/− cells showed a significantly reduced production of Th1 cytokines IL2 (bead to cell ratio 1:1) and IFN-γ (1:4; 1:2; 1:1). No changes were observed for IL17 (Fig. 2B), IL6, TNFα and GMCSF (data not shown) while the cytokine pattern of TASK1−/− mice was not only shifted towards a Th2 phenotype (IL4 and IL5) upon strong stimulation (1:1) but also towards a regulatory phenotype with milder stimuli (IL 10; 1:4; 1:2; 1:1; Fig. 2B). The frequency of regulatory T cells (Tregs; CD4+Foxp3+) per se, however, was unchanged in naïve TASK1−/− mice (n = 4; wt: 2.8 ± 0.3%; TASK1−/−: 2.7 ± 0.1%; Fig. 2C) as well as in immunized mice (data not shown) thus suggesting rather inducible and not naturally occurring Tregs as a potential source of the increased IL10 production in TASK1−/− mice. CD4+ T cells from TASK1−/− mice also showed significantly reduced cell proliferation (thymidine uptake TASK1−/−: 4266 ± 399 cpm, n = 4; WT: 6149 ± 159 cpm, n = 4, P = 0.035; Fig. 2D). Peripheral antigen-presenting cells (APC), identified by the expression of CD11b and CD11c, did not differ in markers of maturation and function (MCH II, CD80, CD86, CD40) between TASK1−/− and WT (Fig. 2E). T cell subset distribution in TASK1−/− was assessed by flow cytometry using CD4, CD8, CD44 and CD62L (Sallusto et al., 2004) as markers (Fig. 2F; Supplementary Table 3). Immunofluorescence analysis showed no significant differences between wild-type and knockout animals. These data indicate that ablation of TASK1 does not affect immune subset distribution and antigen-presenting cells.
phenotype, but critically reduces stimulation-induced T cell proliferation and cytokine secretion.

Neuronal degeneration in EAE is significantly reduced in TASK1<sup>−/−</sup> mice

In a next set of experiments we questioned whether clinical amelioration of EAE in TASK1<sup>−/−</sup> is also correlated with reduced neurodegeneration in experimental CNS inflammation. We quantified numbers of axons in cervical spinal-cord sections by staining for both phosphorylated (SMI31) and non-phosphorylated (SMI32) neurofilament at Day 50 (Fig. 3A). Axon densities in the dorsal CST and dorsal column (DF) were quantified from three to five animals per group (Lo et al., 2003; Craner et al., 2004). As expected, a significant loss of axons was observed in both regions of interest in EAE animals compared with age- and gender-matched controls (Fig. 3A, B and C; Supplementary Table 1). However, TASK1<sup>−/−</sup> mice showed a significantly reduced axonal loss following EAE compared with wild-type controls (CST: 30% reduction, <i>P</i> = 0.01; DF: ~11% reduction, <i>P</i> = 0.02; Fig. 3B and C; Supplementary Table 4). Of note, axonal densities from healthy TASK1<sup>−/−</sup> mice were not different from wild-type controls, excluding an influence of genetic TASK1 deletion on axonal density <i>per se</i> (<i>P</i> = 0.67). To dissect whether TASK1-related neuroprotection is due to silencing of inflammation (indirect) or a direct effect independent from immune cell invasion we cultured
Figure 2 Effects of TASK1 channel knockout on immune cell phenotype and function. (A) Immunocytochemical staining for TASK1 of wild-type CD4⁺ T lymphocyte (middle panel) counterstained with DAPI (left panel) and the merged picture (right panel). (B) IL2, IFN-γ, IL17, IL4, IL5 and IL10 production upon CD3/28 stimulation in naïve T lymphocytes from control animals (black) and TASK1⁻/⁻ mice (grey). CD3/28 beads were applied in a dose-dependent manner to simulate different strength of T cell receptor activation (n = 4 for each value). (C) Splenocytes from naïve wild-type and TASK1⁻/⁻ mice were stained for CD4 and Foxp3 and percentage of CD4⁺Foxp3⁺ cells (naturally occurring Tregs) was assessed by flow cytometry (n = 4). (D) Proliferation of CD3/CD28 bead stimulated naïve CD4 T cells from wild-type (black column) animals and TASK1⁻/⁻ mice (grey column) assessed by thymidine uptake (n = 4). (E) Analysis of CD11c⁺/CD11b⁺ antigen presenting cells by flow cytometry for the surface markers MHCII, CD80, CD86 and CD40 (n = 4). (F) Subset distribution of naïve wild-type and TASK1⁻/⁻ mice lymphocytes was investigated by staining of CD4 and CD8 T cells for CD44 and CD62L. One representative example out of four is shown. See Supplementary Table 3 for detailed analysis. **P<0.05.
Figure 3  TASK1−/− mice are protected against axonal loss during EAE. The number of axons within the spinal cord was measured in naïve wild-type and TASK1−/− mice and compared with spinal cord sections of mice after 50 days of EAE. Neurofilaments were stained with both antibodies against SMI31 and SMI32 and axon density of a defined area (500 µm²) within the corticospinal tract (CST) and dorsal column (DF) was measured. (A) Representative sections of each group are shown (SMI31 + SMI32). (B, C) Quantification of axon counts in the (B) CST and (C) DF from wild-type and TASK1−/− mice before and after 50 days of EAE. (D) SMI32-positive axons in the optic nerve of naïve wild-type (left picture) and TASK1−/− (right picture) mice after 24 h ex vivo culturing and summarizing bar graph representation. (E) Number of SMI32-positive axons in the optic nerve of naïve wild-type and TASK1−/− mice after 24 h ex vivo culturing together with T cells isolated from wild-type EAE mice at disease maximum. **P<0.05. See also Supplementary Table 4.
isolated optic nerves from naïve wild-type or TASK1−/− mice. Damaged axons after 24 h ex vivo culturing were stained by SMI-32 (sensitive marker for injured axons; Fig. 3D). We found significantly reduced numbers of SMI32 positive axons in sections from TASK1−/− mice compared with wild-type controls. In another set of experiments we co-cultured optic nerves from wild-type and TASK1−/− mice with wild-type T lymphocytes isolated from EAE animals at the maximum of disease. Significantly reduced numbers of SMI32 positive axons in knockout mice support a neuroprotective influence of TASK1 deletion under the same inflammatory conditions (wt: 37.8 ± 3.9, n = 4; TASK1−/−: 23.8 ± 3.3, n = 4, P = 0.02; Fig. 3E).

**Acute brain slices indicate a direct neuroprotective impact of TASK1 on neurons**

An acute living brain slice model (Meuth et al., 2003; Na et al., 2009) was chosen to investigate more closely the neuroprotective impact of TASK1 deletion on neurons. 300 µm brain slices of naïve wild-type and knockout mice were incubated for 6 h with CD4+ T lymphocytes isolated from immunized wild-type mice at disease maximum to ensure the same degree of inflammation. Immunohistochemical investigations were conducted thereafter using NeuN as a marker for neurons and activated caspase-3 as a marker of apoptosis. Investigations of the hippocampus (Fig. 4A and C) and the cortex (Fig. 4B and D) revealed a comparable number of NeuN+caspase-3+ cells in wild-type and knockout slices cultured without T cells. Adding MOG-reactive T lymphocytes onto the slices resulted in a significantly higher number of apoptotic neurons in wild-type slices compared with TASK1−/− preparations (hippocampus: 19.3 ± 1.6 cells per mm² versus 14.1 ± 1.2; n = 4; P = 0.04; cortex: 15.6 ± 1.4 versus 11.6 ± 0.9; n = 4; P = 0.05). Taken together these findings indicate a direct impact of TASK1 on neuronal survival and that the absence of TASK1 protects against immune-mediated axonal degeneration in EAE.

**Anandamide, a semi-selective blocker of TASK, attenuates EAE: reversal of established neurological deficits and prevention of progressive CNS tissue loss**

Having established the potential importance of TASK1 in inflammation-induced neurodegeneration, we next asked whether pharmacological blockade of TASK1 exerts beneficial effects *in vivo*. C57BL/6 mice were immunized with high doses of MOG35-55 peptide (200 µg MOG) to induce a strong inflammatory EAE course. The TASK channel inhibitor anandamide (10 mg/kg daily) was applied from the day of disease onset (Day 11). This preventive anandamide treatment led to a significantly reduced peak clinical disease severity (P = 0.01) and an ameliorated disease course over 50 days compared with sham-treated controls (Fig. 5A; summary of clinical data in Supplementary Table 1).

To assess the neurodegenerative EAE component, we reduced the amount of MOG peptide in the immunization protocol to promote a more ‘chronic degenerative’ disease. Immunization with lower MOG peptide doses (100 µg/animal) indeed resulted in a reduced inflammatory disease peak and a more chronic disease course (Fig. 5B; summary of clinical data in Supplementary Table 1). Control animals showed a disease onset at Day 9, followed by a mild and delayed disease peak and a chronic stable disease course over 50 days (Herrero-Herranz et al., 2008). Disease incidence under these immunization conditions was 85% (3 out of 20 animals did not develop a clinical disease). We compared the effect of continuous anandamide treatment (preventive regimen) with the effect of treatment initiated when animals already showed disease symptoms (therapeutic regimen) (Fig. 5B; summary of clinical data in Supplementary Table 1). The preventive treatment regimen induced a delayed disease onset, a notably weaker EAE course and a decreased inflammatory peak. In the therapeutic regimen group, disease onset and inflammatory peak were undistinguishable from the control group indicating that inflammation is not different between both groups. Most importantly, therapeutic application of anandamide led to near complete recovery of clinical symptoms (Fig. 5B; summary of clinical data in Supplementary Table 1).

Aside from its inhibition of TASK channels (Maingret et al., 2001; Meuth et al., 2003), the endocannabinoid anandamide has effects on other receptor and ion channel targets. To rule out that the beneficial therapeutic effects of anandamide are contaminated by non-TASK interferences, we compared anandamide treatment alone with animals receiving anandamide in addition to AM251 (CB1 blocker) (Chen et al., 2006; Hill et al., 2006), AM630 (CB2 blocker) (Werner and Koch, 2003; Moezi et al., 2006) and capsazepine (TRPV1 blocker) (Holt et al., 2005; Jakab et al., 2005). In case this cannabinoid receptor blockade would be of relevance for the observed effects, AM251, AM630 or capsazepine would attenuate the protective effect of anandamide on disease progression. In fact, no significant differences could be observed between anandamide treatment alone and anandamide treatment in the presence of CB1, CB2 or VR1 receptor inhibitors (Fig. 5C; summary of clinical data in Supplementary Table 1). Application of AM251, AM630 or capsazepine alone had no significant effect on EAE course (Supplementary Fig. 1; summary of clinical data in Supplementary Table 1). Of note, application of anandamide was well tolerated by the animals. Severe side effects were absent as revealed by clinical observation as well as by analysis of blood serum samples (Supplementary Table 5) and immunotyping (Supplementary Fig. 2).

**Beneficial effects of anandamide are predominantly mediated by inhibition of TASK1**

To investigate the underlying mechanisms of beneficial anandamide effects in murine EAE we challenged freshly isolated splenocytes from wild-type and TASK1−/− mice. Splenocytes were
stimulated with CD3/CD28 beads in the presence or absence of 30 μM anandamide, a concentration known to inhibit TASK channels (Maingret et al., 2001; Meuth et al., 2003; Veale et al., 2007; Meuth et al., 2008). Anandamide significantly reduced IFN-γ secretion of wild-type splenocytes (wild-type: 6149 ± 159 pg/ml; + anandamide: 4041 ± 231 pg/ml; n = 4, P = 0.03), while it showed no significant effects on IFN-γ release by TASK1−/− cells (TASK1−/−: 4266 ± 400 pg/ml; + anandamide: 3650 ± 319 pg/ml; n = 4, P = 0.45; Fig. 6A).

Anandamide reduced proliferation of wild-type splenocytes as indicated by a reduced thymidine uptake (35 ± 6%, n = 6; P = 0.01). No significant effects were observed in splenocytes from TASK1−/− mice (P = 0.34; Fig. 6B). Next, we used whole-cell patch-clamp techniques to analyse anandamide effects on the

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**Figure 4** Acute brain slices incubated with MOG-reactive T lymphocytes show reduced vulnerability of TASK1−/− neurons. Acute organotypic slices were obtained from naïve wild-type or TASK1−/− mice (n=4) and MACS-purified CD4+ T lymphocytes from wild-type mice at disease maximum of MOG-EAE were added for 6 h. (A) Exemplary immunohistochemical stainings for DAPI (cell nuclei), NeuN (neurons), activated caspase-3 (apoptosis), overlay and enlarged details (from left to right) are shown for wild-type (wt) and knockout (T1) mice in the hippocampus (A) and in the cortex (B). Scale bar is indicated in the lower left and applies for all pictures. (C) Quantitative analysis of caspase-positive neurons per mm² for wild-type (wt) or TASK1−/− brain (T1−/−) alone or with T cells (+TC) in the hippocampus (C) or in the cortex (D). **P < 0.05.
outward current of purified CD4+ mouse T lymphocytes. Voltage protocols stepping the membrane potential from −80 mV to +40 mV (every 30 s) evoked a stable outward current (Fig. 6C). Addition of anandamide (30 μM) reduced the outward current by 32 ± 5% in wild-type mice but showed no effect in TASK1−/− lymphocytes (5 ± 3%, Fig. 6C). Finally, we challenged the specificity of anandamide in vivo. The EAE disease course over 50 days was not significantly altered between TASK1−/− mice in the presence and absence of anandamide (Fig. 6D; preventive protocol). In summary, these results link the anandamide effects mainly to TASK1 channels and rule out major contributions through blockade of known alternative targets.

**Pharmacological blockade of TASK1 protects animals from CNS tissue loss**

Effects of TASK channel modulation on neuronal degeneration were evaluated by magnetic resonance imaging. Ventricular
Figure 6  Effects of anandamide on T lymphocytes from TASK1−/− mice and after application of TASK1−/− mice. (A) Freshly isolated splenocytes from naïve wild type and TASK1−/− mice were stimulated (n=4/group) for 2 days and amounts of interferon γ in supernatants were assessed by ELISA in the presence and absence of anandamide (30 μM). (B) Proliferation was inhibited by anandamide in wild-type cells but not in TASK1−/− cells (in % reduction of proliferation compared to untreated cells). (C) Voltage steps from −80 mV to +40 mV for 500 ms every 30 s (inset 1, right panel) were used to record outward currents in mouse T lymphocytes under control conditions (black lines). Application of 30 μM anandamide leads to a reduction of potassium outward current of wild-type cells while it showed no significant effect on the outward current of TASK1−/− cells (grey lines). Inset 2: Mean bar graph representation of anandamide actions on the potassium outward current in T lymphocytes from wild-type and knockout mice (reduction in%). (D) Application of anandamide in TASK1−/− mice after EAE induction (200 μg MOG) showed no significant effect on disease severity. **P<0.05. See Supplementary Table 1 for detailed analysis.
enlargement was used as marker for degeneration and was quantified in a four-step scale as described earlier (Miller et al., 2002; Lappe-Siefke et al., 2003; Ip et al., 2006) (1: normal ventricular size, 4: massive ventricular enlargement). Mice of each group were scanned for baseline level immediately after immunization and again on Day 44. The atrophy score of the EAE control group increased from 1 (at the beginning) to 3.5±0.5, and the average atrophy progression was 2.5±0.3 (Fig. 7A, B and C). In contrast, anandamide treatment starting from the day of immunization or from the day of disease onset resulted in significantly smaller ventricle sizes and atrophy scores were reduced by 30% (P=0.04; Fig. 7A, B and C). All magnetic resonance images of naïve and anandamide treated mice were also conducted after administration of the novel contrast agent gadofluorine M (Bendszus et al., 2008) to exclude major differences on ventricular enlargement due to different degrees of inflammation or numbers of inflammatory lesions. Only a very low number of inflammatory lesions (wt: four lesions in five animals; wt + anandamide: two lesions in five animals) could be detected in all analysed animals. In summary, this MRI based evaluation of neural degeneration confirms the remarkable CNS-protective effect of anandamide treatment in a longitudinal assessment.

Discussion

We here demonstrate that—based on its unique pharmacological profile and its extensive actions on basic cellular processes—the two-pore domain K⁺-channel (K₂P) TWIK-related acid-sensitive K⁺-channel 1 (TASK1) is a critical modulator of T-cell immunity and neurodegeneration. Based on our results we propose the following hypothetical model concerning the TASK1 channel contribution to the pathogenesis of EAE and multiple sclerosis (Supplementary Fig. 3): TASK1 channels are members of the family of two-pore-domain potassium channels that are commonly regarded as neuronal background channels (Goldstein et al., 2001; Bayliss et al., 2003). By determining the membrane resting potential they are able to influence neuronal excitability and action potential duration. TASK1 channels are among others regulated by hypoxia (Hartness et al., 2001; Lewis et al., 2001) and extracellular acidification (Rajan et al., 2000; Meuth et al., 2003)—both conditions are characteristic for an inflammatory environment as it occurs in EAE brain and spinal cord tissue (Friese et al., 2007). Furthermore, these channels could contribute to an increased loss of potassium ions of neurons which is a known mechanism of apoptosis: loss of potassium is, on one hand, followed by a loss of intracellular water (AVD) and can on the other hand directly activate proapoptotic enzyme cascades (Yu and Choi, 2000). In agreement with this, a recent study demonstrates the involvement of the cation-selective acid-sensing ion channel 1 (ASIC1) in axonal degeneration in EAE (Friese et al., 2007). An elevated concentration of anandamide in EAE brain lesions has been shown previously (Baker et al., 2001) and a neuroprotective effect of anandamide has, for example, been proposed in head trauma (Hansen et al., 2001). Besides this direct impact on CNS neurons and axons, TASK1 channels critically influence T cell effector function (Meuth et al., 2008), thereby providing a dual mechanism of action contributing to T-cell mediated inflammation and neuronal degeneration.
Genetic ablation of TASK1 results in a significantly ameliorated disease course in an animal model of multiple sclerosis, MOG_{35-55}-induced EAE in C57BL/6 mice. TASK1 critically affects T lymphocyte effector function in vivo and in vitro, thereby explaining partially the observed effects in the animal model. While phenotype and subset distribution of T cells and antigen-presenting cells is not altered by the absence of TASK1, T cells from TASK1^{-/-} mice show significantly reduced T cell proliferation and a shift in the cytokine pattern to a more ‘anti-inflammatory’ phenotype (more IL10 production, less IL2 and IFN-γ). No changes in IL17 production were observed. Furthermore, pharmacological blockade by anandamide suppresses T cell proliferation and cytokine secretion. This effect can be clearly explained by predominant action of anandamide on TASK1. While functional consequences of TASK1 channel elimination have been reported with respect to the CNS (Aller et al., 2005; Meuth et al., 2006a, b; Mulkey et al., 2007) and the adrenal gland (Heitzmann et al., 2008), knowledge about the role of TASK1 channels in the immune system is still sparse. Our data show that TASK1 directly modulates T-cell activation by contribution to K+ outward currents (Meuth et al., 2008). Thus, the attenuation of T-effector function can partly explain the beneficial effect of TASK1 neutralization in T cell-dependent inflammatory phases of EAE.

TASK1 is strongly expressed in the CNS (Goldstein et al., 2001, Meuth et al., 2003, Talley et al., 2003). In view of the critical influence of K+ channels for neuronal survival and excitotoxicity, we questioned the impact of TASK1 modulation on neurodegeneration beyond immunomodulation. Analysis of axonal densities in two different regions of the spinal cord (dorsal CST, dorsal column—DF) revealed no differences between TASK1^{-/-} and wild-type mice, therefore excluding any influences of TASK ablation for axonal development and survival. MOG_{35-55} EAE is associated with a significant neurodegenerative component, mirrored by reduction of axons as described earlier (Lo et al., 2003; Craner et al., 2004; Herrero-Herranz et al., 2008). Interestingly, TASK1^{-/-} mice displayed a considerably lower level of axonal loss under these conditions. These data already clearly suggest that TASK1 influences CNS inflammation by a dual mode of action, addressing both T cell activity and neuronal survival. As a note of caution it should be made clearly that the observed experimental design does not allow a clear distinction between the direct CNS-related neuroprotective effects of TASK1 blockade or genetic deficiency and an indirect neuroprotection via attenuation of inflammation. However, axonal densities in optic nerve preparations analysed from wild-type and TASK1^{-/-} mice 24 h after ex vivo culturing without any inflammatory component revealed significantly reduced numbers of SMI-32 positive (injured) axons in TASK1^{-/-} tissue. Co-cultivation of these optic nerves with T lymphocytes from wild-type EAE mice mimicking an inflammatory environment showed similar results with significant lower number of injured axons in optic nerves from TASK1^{-/-} mice. To investigate this finding in further detail we used a model of acutely prepared brain slices from naive wild-type and TASK1^{-/-} mice. We co-cultured the slices with CD4+ T lymphocytes isolated from wild-type EAE animals at disease maximum. This ensures that the inflammatory component of the experimental paradigm is identical in both conditions. Neuronal cell death mediated by inflammation should now only be influenced by the presence or absence of TASK1 in the used brain slices. We analysed neuronal apoptosis (NeuN/caspase-3 positive cells) and obtained a significant reduction in brain slices from TASK1^{-/-} mice compared with controls. This effect cannot be linked to the inflammatory component of the system since infiltrating T cells were isolated from the same wild-type mice strongly arguing in favour of a direct neuroprotective impact of TASK channel inhibition in the chosen model.

While anti-inflammatory treatment options in multiple sclerosis are available, the search for neuroprotective strategies is still disappointing and, so far, no protective agent has reached clinical standards (Kleinschnitz et al., 2007). Therefore, the assumed dual role of TASK1 channels on immune cells and neurons renders them an interesting molecular target, especially for conditions of detrimental autoimmune T cell inflammation in association with neuronal degeneration. To address this aspect and to create a more clinical scenario, we used anandamide as a semi-specific TASK channel modulator in EAE. Treatment with anandamide resulted in a significantly ameliorated disease course in different immunization protocols: anandamide reduced disease severity in a more chronic EAE model evoked by immunization with a reduced MOG_{35-55} peptide concentration. Strikingly, when given after occurrence of clinical symptoms, anandamide led to resolution of clinical deficits, again suggesting a direct neuroprotective impact of TASK1 channel blockade (Herrero-Herranz et al., 2008). This assumption is supported by the beneficial effects of anandamide treatment with respect to CNS tissue loss as assessed by magnetic resonance tomography measuring lateral ventricular sizes at different time points (Ip et al., 2006). Control mice were found to develop severe brain atrophy over 50 days of EAE, whereas mice receiving anandamide from the day of immunization or the day of clinical onset displayed significantly smaller ventricles, while the number of inflammatory lesions as assessed by Gadofluorine M was comparable (Bendzus et al., 2008). This finding clearly supports our data showing less neurodegeneration in TASK1^{-/-} mice under conditions of EAE. Taken together these findings strongly support the assumption of a critical role of TASK1 in modulating direct neuroprotection in the CNS. This hypothesis can find further confirmation in the future using mouse models of mainly inflammatory-independent neurodegeneration. As the neurodegenerative process in EAE is rather acute and mostly driven by T cell infiltration it is still hard to dissociate effects of TASK1 on immune cells and on neurons in vivo despite our experimental results arguing for a dual mechanism of TASK channels. Targeting the role of TASK1 in mouse models of Parkinson’s, Alzheimer’s or motor neuron disease would provide further insight into the role of TASK1 channels in the CNS.

More than 15 years after the description of the first endocannabinoid (Devane et al., 1992) many aspects of its biochemistry and pharmacology are poorly understood. The sites of physiological action of anandamide in particular seem to be much more complex than first anticipated after its discovery. To further specify the beneficial effects of anandamide treatment in EAE, we co-applied AM251 (CB1 blocker) (Chen et al., 2006; Hill et al., 2006), AM630 (CB2 blocker) (Werner and Koch, 2003; Moezi et al., 2006) and capsazepine (TRPV1 blocker) (Holt et al., 2005;
Jakab et al., 2005) and showed that protective effects of anandamide in the EAE model were fully retained, ruling out a major role for cannabinoid CB receptors or TRPV1. Of notice, application of these blockers alone had no significant effect on EAE course in wild-type mice. Moreover, very recently it could be shown that CB2 receptor knockout mice showed an exacerbated clinical score compared with wild-type mice, and this occurred in concert with extended axonal loss, T cell infiltration and microglial activation (Palazuelos et al., 2008). In addition, we established directly a role for TASK1 in anandamide action by using TASK1−/− mice. While anandamide reduced IFN-γ levels and proliferation in CD3/CD28 stimulated T cells from wild-type animals, no effect was observed in T lymphocytes from TASK1−/− mice. Patch-clamp recordings of murine CD4+ lymphocytes displayed an anandamide sensitive standing outward current while application of the endocannabinoid showed no effect on the outward current of TASK1−/− lymphocytes. Finally, in vivo treatment of TASK1−/− EAE mice with anandamide had no additional effect. Taken together, these data congruently indicate that the beneficial effects of anandamide are predominantly mediated via TASK1 channel modulation in vitro and in vivo. However, since (i) TASK1 channels can form heterodimers with TASK3 channels (Czirjak and Enyedi, 2002); and (ii) a TASK3 channel region that is critical for its blockade by methanandamide was identified recently (Veale et al., 2007) we cannot formally rule out TASK3 channel-mediated contributions to the observed effects. Summarizing the data establishes a role of TASK1 channels in inflammation and neurodegeneration in an animal model of multiple sclerosis and suggests this channel as promising molecular target structure for the treatment of the disease.

Supplementary material

Supplementary material is available at Brain online.

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References


