Neurobiology of Disease 55 (2013) 87–94

Contents lists available at SciVerse ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

TASK-1 channels in oligodendrocytes: A role in ischemia mediated disruption

Virginia Hawkins, Arthur Butt *

Institute of Biomedical and Biomolecular Science, University of Portsmouth, St Michael's Building, White Swan Rd, Portsmouth, PO1 2DT, UK

ARTICLE INFO

Article history:
Received 16 January 2013
Revised 13 March 2013
Accepted 29 March 2013
Available online 6 April 2013

Keywords:
Brain
White matter
Potassium channels
Glia
Oligodendrocyte

ABSTRACT

Oligodendrocytes are the myelinating cells of the CNS and, like neurons, are highly sensitive to ischemic damage. However, the mechanisms underlying cytotoxicity in oligodendrocytes during hypoxic/ischemic episodes are not fully understood. TASK-1 is a K^+ leak channel that mediates hypoxic depolarisation in neurons. The expression and function of TASK-1 in oligodendrocytes had not previously been addressed. In this study, we investigate the expression of TASK-1 in oligodendrocytes and its role in white matter ischemic damage. Expression of TASK-1 in oligodendrocytes was investigated in the mouse brain using immunostaining. TASK-1 channel function was identified by established pharmacological and electrophysiological strategies, using the whole-cell patch clamp technique in cell cultures of oligodendrocytes from the optic nerve, a typical white matter tract. The role of TASK-1 in hypoxia was examined in isolated intact optic nerves subjected to oxygen glucose deprivation (OGD). Oligodendrocytes are strongly immunopositive for TASK-1 throughout the brain. Patch-clamp identified functional TASK-1-like leak currents in oligodendrocytes using two recognised means of inhibiting TASK-1, decreasing extracellular pH to 6.4 and exposure to the TASK-1 selective inhibitor anandamide. Incubation of optic nerves with methanandamide, a non-hydrolysable form of anandamide, significantly protected oligodendrocytes against hypoxic disruption and death in OGD. Our data demonstrate for the first time that oligodendrocytes express functional TASK-1 channels and provide compelling evidence they contribute to oligodendrocyte damage in hypoxia. Since oligodendrocyte damage is a key factor in ischemic episodes, TASK-1 may provide a potential therapeutic target in stroke and white matter disease.

Introduction

Oligodendrocytes are the myelin-forming cells of the central nervous system and are essential for the rapid conduction of neural impulses. Like neurons, oligodendrocytes are highly sensitive to hypoxic/ischemic injury, such as those that occur during stroke, and their damage strongly affects brain function (Back et al., 2007; Dewar et al., 2003). Identification of endogenous factors that mediate oligodendrocyte damage in ischemia, therefore, could contribute to the development of reparative strategies. A key factor in neuronal damage in hypoxic/ischemic episodes is that they cause depolarisation of the resting membrane potential, resulting in a run-down of neuronal activity and ultimately death (Haddad and Jiang, 1997; Lipton, 1999). Oligodendrocytes have a strongly negative resting membrane potential, which is essential for myelination (Bolton and Butt, 2006; Neusch et al., 2001), and is determined by plasmalemmal potassium channels that confer a high selective permeability to potassium ions in oligodendrocyte cell membranes (Bolton and Butt, 2006; Butt and Kalsi, 2006). An emergent family of potassium channels known as two-pore (or tandem-pore) domain potassium channels (K_{2P}) lack voltage dependence and are constitutively open. K_{2P} channels generate the prominent 'leak currents' that set the resting membrane potential and oppose depolarising influences, such as occur during ischemia (Talley et al., 2003). The K_{2P} channel subtype TASK-1 ($K_{2P}3.1$, KCNK3) is sensitive to inhibition by acidic pH and low O_2, and is highly expressed in the brain. TASK-1 channels mediate a standing outward K^+ current in many neurons, notably motor neurons of the cerebellum and pH-sensitive cells of the respiratory centre (Bayliss et al., 2003; Mulkey et al., 2007). TASK-1 channels are inhibited by acute hypoxia and have been shown to mediate neuronal depolarisation and cell death in ischemia (Plant et al., 2002), as well as in response to raised extracellular K^+ (Lauritzen et al., 2003). We hypothesised, therefore, that TASK-1 channels may play a role in the mechanisms setting the resting membrane potential in oligodendrocytes and underlying their susceptibility to ischemia. TASK-1 and other K_{2P} channels and currents have been identified in astroglia (Seifert et al., 2009; Skatchkov et al., 2006; Zhou et al., 2009), but oligodendrocytes had not been studied previously. The aim of the present study was to determine the functional expression of

* Corresponding author. Fax: +44 2392842156.
E-mail address: arthur.butt@port.ac.uk (A. Butt).
Available online on ScienceDirect (www.sciencedirect.com).

0969-9961/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.nbd.2013.03.016

TASK-1 channels in oligodendrocytes and to gain insights into the physiological function of these channels in ischemic conditions in which oligodendrocytes are compromised.

Materials and methods

Animals

Mice aged postnatal day (P)7–15 were used throughout, and killed humanely, by cervical dislocation, in accordance with the UK Animals (Scientific Procedures) Act, 1986. Wild type mice of the C57BL/6 strain were used, or transgenic mouse strains in which the fluorescent reporters DsRed and eGFP are driven by the oligodendroglial genes PLP1 and Sox10, respectively (Azim and Butt, 2011).

Optic nerve explant cultures

Optic nerve explant cultures were prepared as previously described (Greenwood and Butt, 2003). In brief, optic nerves from P7–10 mice were isolated intact in ice cold artificial cerebrospinal fluid (aCSF) comprising of (in mM): NaCl 133; KCl 3; $CaCl_2$ 1.5; NaH_2PO_4 1.2; $MgCl_2$ 1; D-glucose 10; HEPES 8.55, at pH 7.4. Optic nerves were finely chopped with a scalpel to small segments less than 1 mm in length and explants were cultured onto poly-L-lysine/laminin (Invitrogen/Sigma) coated coverslips (1 optic nerve segment per coverslip) in modified Bottenstein and Sato (B&S) medium without thyroid hormones, plus 10 ng/ml recombinant human platelet derived growth factor (PDGF-AA; R&D Systems) and 0.1% gentamicin. After 3–4 days in vitro (DIV) explants were then incubated in B&S with 0.5 mM dibutyryl cyclic adenosine monophosphate (dbcAMP) medium up to 10 DIV to stimulate differentiation.

Immunolabelling

Brain tissue from P15 mice or optic nerve explant cultures were fixed with 4% paraformaldehyde in phosphate buffered saline (PBS, pH7.4); tissue was fixed for either 1 h at room temperature (RT) or overnight at 4 °C, and for 10 min for cultured cells on their coverslips, followed by washes in PBS. For sectioning, brain tissues were placed in cryoprotectant (30% w v^{-1} sucrose in PBS) overnight at 4 °C, then embedded in Cryo-M-Bed (Bright Instruments Company Ltd), before rapidly freezing at −70 °C, sectioning using a cryostat (10 μm; Leica CM3050 S), and transference of sections onto Polysine® coated slides (Thermo-Scientific). Sections and cells were treated the same in subsequent stages. A blocking stage was performed by incubation for 1 h at RT in 5% normal goat serum (NGS) and 0.2% triton-X-100 in PBS. After blocking, samples were incubated overnight at 4 °C with primary antibodies diluted in NGS-PBS: rabbit anti-TASK-1 raised against an intracellular (C-terminal) peptide corresponding to amino acid residues 252–269 of human TASK-1 (Alomone Laboratories), used at 1:200; rat anti-MBP 1:300 (Chemicon); and mouse anti-APC 1:300 (Calbiochem). After washes in PBS, tissues were incubated for 1 h at RT with the appropriate secondary antibodies conjugated with ^{488}Alexafluor or ^{568}Alexafluor (1:500, Molecular Probes). For TASK-1, control experiments were carried out in which tissue was preabsorbed with antigen peptide overnight prior to incubation in the primary antibody; other antibodies have been validated in many studies (Azim and Butt, 2011), and in these cases controls were carried out by omitting primary antibody. Following immunolabelling, coverslips/sections were mounted with Vectasheild® (VectorLabs) and images acquired using a LSM 5 Pascal Axioskop2 confocal microscope (Zeiss), maintaining variables constant between images.

Image analysis

Image analysis was carried out using Volocity 6.1 software (Perkin Elmer). Image acquisition of the different laser lines was performed using multichannel sequential scanning, narrow bandwidths, and minimal laser power and gain to prevent cross-talk between the channels. A pinhole of 1 airy unit or less was used, with an average of 4 scans per image. The number of *z*-sections and resolution were optimised using the Zeiss acquisition software, and in general *z*-sections were <0.75 μm thickness, and approximately 30 *z*-sections were taken for each cell. For confocal photomicrographs, two-dimensional flattened images of the *z*-stacks are presented. For colocalisation analyses, the more accurate technique of Barlow and colleagues was used (Barlow et al., 2010), in which the degree of separation between pixels from the red and green channels was determined in single *z*-sections to provide measurements of signal overlap. First, images were thresholded to separate the positive signal (positive immunolabelling) from background. The threshold was determined by measuring the background intensity value for each channel in negative control sections and setting the threshold as the mean background intensity plus three standard deviations (averaged from a minimum of 6 images). Then, the thresholded Pearson's correlation coefficient (PCC) and Mandersons' overlap coefficients (M1 and M2) were determined as previously described (Barlow et al., 2010), using Volocity 6.1 software. The thresholded PCC determines the statistical strength of the linear relationship between fluorescent intensities from the red and green channels, and the M1 and M2 overlap coefficients provide accurate measurements of the true degree of overlap of red and green. A colocalisation channel was generated from the thresholded PCC to illustrate in three-dimensions the voxels in which the two channels overlap with the same intensity.

Recombinant expression

Optic nerve explant cultures were transiently transfected with mTASK-1pTagFP635, in which mouse TASK-1 cDNA is fused to a N-terminal far-red fluorescent protein TagFP635. This was generated by subcloning mTASK-1 from mTASK-1pEXO into the pTagFP635 mammalian expression vector (Clontech). The construct was verified by sequencing and cells in antibiotic free medium were transfected at 8 DIV using Lipofectamine™ 2000 (Invitrogen), following the manufacturer's guidelines. Cells were fixed 48 h post transfection with 4% paraformaldehyde in PBS, pH7.4 for 10 min with three subsequent washes in PBS prior to mounting with Vectasheild® (VectorLabs) and examined using an LSM 710 Axiovert confocal microscope (Zeiss).

Western blotting

Whole brain and optic nerves (pooled from 3 animals) were removed from P15 mice and tissue was homogenised in buffer containing (in mM): NaCl 12.5; Tris HCl 2, pH 8.0; phenylmethylsulphonylfluoride (PMSF) 0.2, in distilled water with 1x complete mini protease inhibitor cocktail (Roche). Samples were centrifuged at 4 °C, at high speed (14,000 rpm/20,800 rcf) for 4 min and pellets resuspended in PBS. Quantification of protein concentration was carried out using the bicinchoninic acid assay (Sigma) with a standard bovine serum albumin (BSA) concentration curve and UV spectrophotometer absorbance readings at 550 nm. Samples were mixed with Laemmli sample buffer, heated at 95 °C for 10 min with β-mercaptoethanol and 10 μg of protein per lane was loaded for 8% acrylamide sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were then electrophoretically transferred to a polyvinyllidene difluoride membrane (Amersham) which was then incubated in blocking solution (0.5–5% w.v^{-1} dried milk in Tris buffered saline (TBS; 150 mM NaCl, 10 mM Tris pH 7.4) with 0.05% v.v^{-1} Tween 20). Incubation in rabbit anti-TASK-1 antibody at 1:1000 (Alomone Laboratories) was carried out overnight at 4 °C, and following washes, the secondary antibody horseradish peroxidase-conjugated swine anti-rabbit (Dako) was added at 1:10,000 for 1 h at RT; controls were preincubated with the competitive peptide from which the TASK-1 antibody was raised. Extensive washing of the membranes in TBS with 0.05% v.v^{-1}