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Expression and activation of mitogen-activated protein kinases in the optic nerve head in a rat model of ocular hypertension

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ABSTRACT

Background: Glaucoma is a leading cause of irreversible blindness manifesting as an age-related, progressive optic neuropathy with associated retinal ganglion cell (RGC) loss. Mitogen-activated protein kinases (MAPKs: p42/44 MAPK, SAPK/JNK, p38 MAPK) are activated in various retinal disease models and likely contribute to the mechanisms of RGC death. Although MAPKs play roles in the development of retinal pathology, their action in the optic nerve head (ONH), where the initial insult to RGC axons likely resides in glaucoma, remains unexplored.

Methods: An experimental paradigm representing glaucoma was established by induction of chronic ocular hypertension (OHT) via laser-induced coagulation of the trabecular meshwork in Sprague-Dawley rats. MAPKs were subsequently investigated over the following days for expression and activity alterations, using RT-PCR, immunohistochemistry and Western immunoblot.

Results: p42/44 MAPK expression was unaltered after intraocular pressure (IOP) elevation, but there was a significant activation of this enzyme in ONH astrocytes after 6–24 h. Activated SAPK/JNK isoforms were present throughout healthy RGC axons but after IOP elevation or optic nerve crush, they both accumulated at the ONH, likely due to RGC axon transport disruption, and were subject to additional activation. p38 MAPK was expressed by a population of microglia which were significantly more populous following IOP elevation. However it was only significantly activated in microglia after 3 days, and then only in the ONH and optic nerve; in the retina it was solely activated in RGC perikarya.

Conclusions: In conclusion, each of the MAPKs showed a specific spatio-temporal expression and activation pattern in the retina, ONH and optic nerve as a result of IOP elevation. These findings likely reflect the roles of the individual enzymes, and the cells in which they reside, in the developing pathology following IOP elevation. These data have implications for understanding the mechanisms of ocular pathology in diseases such as glaucoma.

1. Introduction

Glaucoma, the leading causes of irreversible blindness worldwide (Quigley and Broman, 2006), is often associated with increased intraocular pressure (IOP). It is characterised as a related group of neurodegenerative diseases with structural damage to the optic nerve resulting in loss of retinal ganglion cells (RGCs) and their axons (Casson et al., 2012). It is believed that RGCs become stressed in glaucoma as a result of altered mechanical and/or vascular influences at the optic nerve head (ONH), the anatomical site where RGC axons converge (Osborne et al., 1999; Flammer et al., 1999; Hernandez, 2000; Burgoyne, 2011). Whatever the initial cause of the stress to RGCs, localised tissue outcomes are thought to include perturbations in metabolic functioning, cessation of axon transport and failure of cellular homeostatic mechanisms (Chidlow et al., 2007). Such events will destabilise intracellular signaling and impact activity of enzymes such as protein kinases, which are physiologically tightly regulated.

The mitogen-activated protein kinases (MAPKs) comprise a group of structurally similar enzymes that phosphorylate a diverse array of target substrates to control many cellular functions including proliferation, differentiation, migration, secretion, apoptosis and inflammation (Cargnello and Roux, 2011; Cowan and Storey, 2003).

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Table 1
Antibodies used in the study.

Name	Cat no./clone#	Host	Company	Dilution IHC	Dilution IF	Dilution WB
Amyloid precursor protein (APP)	22C11#	Mouse	Gift-C. Masters	1:1000	1:250[a]/1:1000	
β-Actin	A5441	Mouse	Sigma-Aldrich			1:10,000
βIII-Tubulin	MAB1637	Mouse	Millipore		1:500[a]	1:1000
Brn 3a	C-20#	Goat	Santa Cruz	1:3000	1:3000	
Iba-1	Ab107159	Goat	Abcam		1:500[a]	
npNFH	SMI32#	Mouse	Sternberger	1:15,000		
OLIG 2	AB9610	Rabbit	Chemicon	1:1000		
p38 MAPK	8690	Rabbit	CST	1:300		1:500
p44/42 MAPK	9102	Rabbit	CST	1:2000	1:1500	1:1000
Phospho-p38 MAPK	4631	Rabbit	CST	1:300	1:300	1:500
Phospho-p44/42 MAPK	4376	Rabbit	CST	1:2000	1:1500	1:1000
Phospho-SAPK/JNK	9251	Rabbit	CST	1:300	1:300	1:250
SAPK/JNK	9258	Rabbit	CST	1:300	1:300	1:250
S100	MAB079-1	Mouse	Millipore		1:1000[a]	
γ-Synuclein	CPTC-SNCG-1	Mouse	DSHB		1:40[a]	
Vimentin	V9#	Mouse	DAKO		1:1000[a]	

CST - cell signaling technologies, IHC - immunohistochemistry, IF - immunofluorescence, WB - Western immunoblot.
[a] Denotes concentration for a 2 step reaction.

Classical activation of MAPKs occurs when they are themselves phosphorylated; this process most often derives from the upstream stimulation of a complex, three-tiered cascade of separate protein kinases (Zeke et al., 2016; Johnson and Lapadat, 2002). Three separate groups of the MAPK family are well defined: extracellular signal regulated kinases (ERK1 and ERK2; p42/44 MAPK) (Roskoski, 2012), stress activated protein kinases/c-Jun N-terminal kinases (SAPK/JNK; JNK-1, JNK-2, JNK-3) (Mehan et al., 2011) and p38 MAPKs (p38α, p38β, p38γ, p38δ) (Cuenda and Rousseau, 2007). In broad terms, SAPK/JNK and p38 MAPK isoforms are primarily stimulated by stress-related effectors or cytokines to cause inflammatory responses, autophagy or apoptosis, while the p42/44 MAPK pathway is stimulated by mitogens or growth factors resulting in cell cycle progression, cell proliferation or differentiation. Because of their widespread expression in the central nervous system (CNS) (Flood et al., 1998), their key roles in cellular functioning, the diversity of signals to which they respond, and the numerous known substrates for their kinase action (Cargnello and Roux, 2011), MAPKs have been implicated in the pathophysiology of many CNS disorders (Grewal et al., 1999; Hetman and Gozdz, 2004; Kim and Choi, 1802). Indeed, roles for this enzyme family have been described in Alzheimer's Disease, Parkinson's Disease and Amyotrophic lateral sclerosis. Additionally, MAPKs have also been shown to have roles in animal models of metabolic stress and ischemia in the CNS (Shackelford and Yeh, 2006; Sugino et al., 2000; Ozawa et al., 1999).

Previous studies have elucidated that members of each of the three major groups of MAPKs are present in the retina. Both activated (by way of phosphorylation) and non-activated p42/44 MAPKs localise to non-neuronal Müller cells, astrocytes and the retinal pigmented epithelium (Zhou et al., 2007; Nakazawa et al., 2002) where they are believed to mediate the effects of endogenous growth factors, such as vascular endothelial growth factor. p38 MAPK and SAPK/JNK, however, are mainly present in their non-activated (non-phosphorylated) forms and are associated with neurons such as RGCs and bipolar cells (Zhou et al., 2007; Nakazawa et al., 2002). MAPKs have been shown to respond to different stressors within the retina. For example, MAPKs are activated in response to *N*-methyl-D-aspartate (Munemasa et al., 2005; Manabe and Lipton, 2003) or glutamate injections (Zhou et al., 2007), retinal ischemia (Roth et al., 2003; Kim et al., 2016) optic nerve transection (Nakazawa et al., 2002; Nitzan et al., 2006; Kikuchi et al., 2000), experimentally-elevated IOP (Dapper et al., 2013), endotoxin-induced uveitis (Takeda and Ichijo, 2002) and experimental diabetes (Ye et al., 2012). Furthermore, Tezel and Wax (Tezel et al., 2003) found increased activation of p42/44 MAPK, as well as both p38 MAPK and SAPK/JNK, in retinal glia and inner retinal neurons, respectively, in human glaucoma patients.

Despite the diversity of studies investigating MAPK in the injured retina there is very little published information regarding the role of this family of enzymes at the ONH, which is believed to represent the likely primary site of injury for RGC axons in glaucoma (Osborne et al., 1999; Chidlow et al., 2011a; Howell et al., 2007). We therefore sought to carry out a detailed spatio-temporal investigation into the potential activation of MAPKs in the ONH of rats subjected to our model of elevated ocular hypertension (OHT). The responses of MAPKs in the retina and optic nerve were also determined, partly to build up a more complete picture of the role of these enzymes in our model and partly to check agreement with previous models of retinal injury.

2. Materials and methods

2.1. Materials

All chemicals were purchased from Sigma-Aldrich (Castle Hill, New South Wales, Australia), unless otherwise stated. A full list of antibodies used in the study is listed in Table 1 with primer sequences shown in Table 2. Antibodies specific to each of p42/44 MAPK, SAPK/JNK and P38 MAPK, or their phosphorylated forms, were reactive with all isoforms associated with that respective MAPK group: anti-p42/44 MAPK recognised p42 MAPK (ERK2) and p44 MAPK (ERK1) and therefore detected proteins of masses 42 and 44 kDa; anti-SAPK/JNK recognised all ten separate isoforms - derived as either 46 or 54 kDa forms from each of five mRNAs (JNK1α, JNK1β, JNK2α, JNK2β, JNK3) - and

Table 2
Primer sequences used for real-time RT-PCR.

mRNA	Primer sequence	Product size (bp)	GenBank accession number
GAPDH	5′-TGCACCACCAACTGCTTAGC-′3 5′-GGCATGGACTGTGGTCATGAG-3′	87	M19533
p44 MAPK	5′-TGGCTTTCTGACCGAGTATGTG-′3 5′-ATTTGGTGTAGCCCTTGGAGTT′-3	80	NM_017347
p42 MAPK	5′-TTGGTCAGGACAAGGGCTCA-′3 5′-CTCGGAACGGCTCAAAGGA-′3	127	NM_053842
SAPK	5′-GCTGCTTTTGATACAGTTCTTGG-′3 5′-AGTTCACGGTAGGCTCTCTTTG-3′	98	NM_001270544, NM_001270556, NM_053829
p38 MAPK	5′-CTGGAAGATGTCGCAGGAGAG-′3 5′-CCTTTTGGCGTGAATGATGG-′3	208	NM_031020