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Invited review

Purinergic signaling in retinal degeneration and regeneration

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ABSTRACT

Purinergic signaling is centrally involved in mediating the degeneration of the injured and diseased retina, the induction of retinal gliosis, and the protection of the retinal tissue from degeneration. Dysregulated calcium signaling triggered by overactivation of $P2X_7$ receptors is a crucial step in the induction of neuronal and microvascular cell death under pathogenic conditions like ischemia-hypoxia, elevated intraocular pressure, and diabetes, respectively. Overactivation of $P2X_7$ plays also a pathogenic role in inherited and age-related photoreceptor cell death and in the age-related dysfunction and degeneration of the retinal pigment epithelium. Gliosis of micro- and macroglial cells, which is induced and/or modulated by purinergic signaling and associated with an impaired homeostatic support to neurons, and the ATP-mediated propagation of retinal gliosis from a focal injury into the surrounding noninjured tissue are involved in inducing secondary cell death in the retina. On the other hand, alterations in the glial metabolism of extracellular nucleotides, resulting in a decreased level of ATP and an increased level of adenosine, may be neuroprotective in the diseased retina. Purinergic signals stimulate the proliferation of retinal glial cells which contributes to glial scarring which has protective effects on retinal degeneration and adverse effects on retinal regeneration. Pharmacological modulation of purinergic receptors, e.g., inhibition of P2X and activation of adenosine receptors, may have clinical importance for the prevention of photoreceptor, neuronal, and microvascular cell death in diabetic retinopathy, retinitis pigmentosa, age-related macular degeneration, and glaucoma, respectively, for the clearance of retinal edema, and the inhibition of dysregulated cell proliferation in proliferative retinopathies.

This article is part of a Special Issue entitled 'Purines-Neurodegeneration'.

1. Introduction

In the retina, the purines ATP and adenosine act as neuro- and gliotransmitters, and contribute to the bidirectional neuron-glia signaling and to the communication between photoreceptors and the retinal pigment epithelium (RPE) (Newman, 2004a; Mitchell and Reigada, 2008; Housley et al., 2009; Ward et al., 2010; Wurm et al., 2011a). Photoreceptors, neurons, glial cells, the microvasculature, and RPE cells express P1 (adenosine) and P2 (nucleotide) receptors (Housley et al., 2009). Extracellular ATP contributes to the fast excitatory neurotransmission by activating ionotropic, ligand-gated P2X receptors expressed by most classes of retinal neurons (Housley et al., 2009; Ward et al., 2010). ATP has also a neuromodulatory role via activation of metabotropic, G protein-coupled P2Y receptors expressed by neuronal, glial, and RPE cells (Housley et al., 2009; Wurm et al., 2011a). Adenosine is a major inhibitor of the neuronal activity in the retina and suppresses excitatory neurotransmission by various mechanisms such as inhibition of presynaptic voltage-dependent calcium channels resulting in reduced transmitter release (Housley et al., 2009). Purinergic signaling also modulates many of the homeostatic functions of macroglial cells (Müller cells, astrocytes) and the RPE, as well as the activity of microglia (Housley et al., 2009; Wurm et al., 2011a; Sanderson et al., 2014).

In the retinal tissue, purines are tonically released in the dark; the release is increased by neuronal activity (Perez et al., 1986; Neal and Cunningham, 1994). Adenosine is liberated via equilibrative nucleoside transporters from various cells, e.g., retinal ganglion and Müller glial cells, or formed (at least in some species and under pathological conditions) in the extracellular space by enzymatic dephosphorylation of ATP (Newman, 2003, 2004b; Ribelayga and

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Mangel, 2005; Uckermann et al., 2006; Wurm et al., 2008a). ATP is liberated from neurons in a calcium- and/or pannexin-dependent manner (Perez et al., 1986; Santos et al., 1999; Xia et al., 2012), and from non-neuronal cells (glia, RPE) by calcium-independent and -dependent mechanisms (Newman, 2001a,b; Mitchell, 2001; Pearson et al., 2005; Uckermann et al., 2006; Wurm et al., 2010; Brückner et al., 2012; Voigt et al., 2015). Osmotic gradients (Fig. 1H), mechanical or electrical stimulation, and receptor agonists such as ATP, dopamine, thrombin, and glutamate (Fig. 2F) induce a release of ATP from Müller cells (Newman, 2001a,b; 2003; Uckermann et al., 2006; Wurm et al., 2008a, 2010; Krügel et al., 2010), the principal macroglia of the retina (Bringmann et al., 2006).

In addition to the various roles of purinergic receptor signaling in the neuronal information processing and regulation of retinal tissue homeostasis under physiological conditions, purinergic signaling plays crucial roles in the induction of degenerative processes and retinal gliosis under pathophysiological conditions which may contribute to the dysregulation of glial homeostatic functions and the death of photoreceptors, neurons, and vascular cells. Purinergic signaling is activated in the retina under all pathophysiological conditions investigated so far, and purinergic receptor ligands often act as central mediators of signal transduction and effector cascades that induce physiological and degenerative alterations of retinal cells. In this review, we summarize the present knowledge regarding the roles of purinergic signaling in retinal degeneration, induction of retinal gliosis, and protection of the retinal tissue from degeneration.

2. Purinergic regulation of retinal cell death

Purinergic signaling mediated by extracellular ATP and adenosine is involved in inducing of and protection from cell death in the injured and diseased retina. Extracellular ATP is an endogenous danger signal released in large quantities by stressed cells, e.g., during inflammation, oxidative and osmotic stress, nutrient starvation, ischemia–hypoxia, mechanical stimulation, and cell injury (Khakh and North, 2006; Franke et al., 2006; Uckermann et al., 2006; Mitchell and Reigada, 2008; Notomi et al., 2011; Niyadurupola et al., 2013). Elevation of the intraocular pressure is associated with an increased level of extracellular ATP (Zhang et al., 2007; Resta et al., 2007). In addition, high glucose induces raised levels of extracellular ATP, via increasing the exocytotic release of ATP and decreasing its extracellular degradation (Costa et al., 2009; but see Vindeirinho et al., 2013).

Retinal cell degeneration is mediated by multiple death pathways. A critical event is a prolonged cytosolic and mitochondrial calcium overload induced, for example, by overactivation of ionotropic glutamate receptors and voltage-gated calcium channels (Osborne et al., 2004; Bringmann et al., 2005); the excessive calcium influx triggers calpain-dependent apoptosis pathways (Doonan et al., 2005; Azuma and Shearer, 2008; McKernan et al., 2007). Overstimulation of purinergic receptors contributes to the cytotoxic calcium overload. P2X receptors act as direct conduits for the calcium influx (Fig. 1F) and (via membrane depolarization) as indirect activators of voltage-gated calcium channels. Activation of P2Y receptors induces a rapid transient release of calcium from internal stores followed by a more sustained calcium influx (Figs. 1G and 4E).

$P2X_7$ receptors have garnered much attention with regard to their role in inducing retinal cell death. $P2X_7$ receptors are unique in allowing the formation of large plasma membrane pores (Surprenant et al., 1996). $P2X_7$ pores may mediate cytolysis under distinct conditions, and pore formation may induce an inflammasome-dependent cell death pathway (Surprenant et al., 1996; Fowler et al., 2014). $P2X_7$ is expressed by photoreceptors and several classes of retinal neurons including retinal ganglion, amacrine, and horizontal cells (Brändle et al., 1998; Taschenberger et al., 1999; Ishii et al., 2003; Puthussery and Fletcher, 2004; Franke et al., 2005; Puthussery et al., 2006; Niyadurupola et al., 2013). $P2X_7$ is also expressed in pericyte-containing retinal microvessels, RPE cells, microglia, and (in lower vertebrates, chicks, *Macaca*, and humans) Müller glial cells (Fig. 1F) (Morigiwa et al., 2000a,b; Pannicke et al., 2000, 2005a; Kawamura et al., 2003; Innocenti et al., 2004; Sugiyama et al., 2005; Yang et al., 2011; Anccasi et al., 2013; Vitanova and Kupenova, 2014). Upregulation of $P2X_7$ may predispose retinal neurons to damage under pathological conditions, e.g., after elevation of the intraocular pressure (Franke et al., 2005; Sugiyama et al., 2013; Kakurai et al., 2013).

In retinal ganglion cells, short-term stimulation of $P2X_7$ induces a calcium response while prolonged stimulation kill a subpopulation of the cells via activation of voltage-gated calcium channels, sustained increase of the cytosolic free calcium level, and caspase activation (Zhang et al., 2005; Hu et al., 2010). Tissue hypoxia is a pathogenic factor of various retinopathies including glaucoma and diabetic retinopathy (Linsenmeier et al., 1998). Via inducing a sustained increase in the cytosolic free calcium level, activation of $P2X_7$ is involved in mediating the hypoxic death of retinal ganglion and amacrine cells (Sugiyama et al., 2010; Niyadurupola et al., 2013). Activation of $P2X_7$ is also a crucial step in the degeneration of retinal ganglion cells after optic nerve crush (Kakurai et al., 2013). Because the extracellular ATP level increases with elevated intraocular pressure (Zhang et al., 2007; Resta et al., 2007), these responses may exert a deleterious effect on retinal ganglion cells in glaucomatous eyes (Zhang et al., 2007; Resta et al., 2007; Xia et al., 2012). Retinal neurons respond directly to mechanical stimulation with pannexin-mediated ATP release and autostimulation of $P2X_7$ (Xia et al., 2012). Glia-derived ATP may contribute to the degeneration of photoreceptors and neurons under pathological conditions. Retinal glial cells release ATP in response to osmotic-mechanical stimulation (Fig. 1H) (Newman, 2001a,b; 2003; Uckermann et al., 2006); excess ATP may be liberated from glial cells under conditions associated with mechanical perturbations such as retinal detachment and elevated intraocular pressure (Resta et al., 2007; Zhang et al., 2007; Reigada et al., 2008). Glia-derived ATP may also contribute to the propagation of retinal gliosis and degeneration from a local injury into the uninjured retinal tissue (see below).

In addition to inner retinal neurons, photoreceptor cells express $P2X_7$ (Puthussery and Fletcher, 2004). High extracellular ATP induces rapid photoreceptor apoptosis (Puthussery and Fletcher, 2009; Notomi et al., 2011). Inhibition of P2X receptor signaling delays the photoreceptor degeneration in *rd1* mice, a model of human recessive retinitis pigmentosa (Puthussery and Fletcher, 2009). Photoreceptor degeneration is also a hallmark of age-related macular degeneration (AMD). Subretinal hemorrhage, a characteristic of neovascular AMD, is associated with a massive release of ATP from retinal cells induced by extravasated blood; extracellular ATP triggers photoreceptor cell apoptosis by activation of $P2X_7$ (Notomi et al., 2013). Photoreceptor apoptosis is prevented by the $P2X_7$ antagonist brilliant blue G (Notomi et al., 2013), an approved adjuvant in ocular surgery (Rodrigues et al., 2005).

Microvascular cell death is a hallmark of diabetic retinopathy (Mizutani et al., 1996). High extracellular ATP causes cellular death in the pericyte-containing retinal microvasculature by activation of $P2X_7$ resulting in cell depolarization and lethal calcium influx through voltage-gated calcium channels (Sugiyama et al., 2005). Diabetes boosts the vulnerability of retinal microvessels to the lethal effect of $P2X_7$ activation, i.e., the agonist concentration required to trigger lethal transmembrane pore formation decreases markedly soon after the onset of diabetes (Sugiyama et al., 2004;

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