

● PERSPECTIVES

Role of P2X₇ receptors in neuronal death in the retina

The P2X₇ receptor is a member of the family of purinoceptors, which are ligand-gated membrane ion channels activated by extracellular adenosine 5'-triphosphate. A unique feature of the P2X₇ receptor is that its activation can result in the formation of large plasma membrane pores that allow the flux of ions as well as hydrophilic molecules of up to 900 Da. Recent studies indicate that P2X₇-mediated signaling can trigger apoptotic cell death after ischemia and during the course of certain neurodegenerative disorders. Expression of the P2X₇ receptor has been demonstrated in most cell types in the retina and is related to retinal neurotransmission, and may modulate both photoreceptor and rod bipolar cell responses. In addition to its physiological roles, receptor stimulation has been reported to be involved in neuronal death in the retina. Stimulation of P2X₇ receptors can kill retinal ganglion cells (RGCs) by a mechanism dependent on increased intracellular Ca²⁺. This mechanism might play a role in ischemia-induced neuronal damage and optic nerve injury. Given that extracellular ATP levels and P2X₇ receptor expression in the retina increase with elevated intraocular pressure, stimulation of P2X₇ receptors may exert a deleterious effect on RGCs in glaucomatous eyes. P2X₇ receptor activation may also be associated with the up-regulation of inflammatory cytokine expression, e.g., interleukin-1 β and tumor necrosis factor- α . Based on its reported effects, the P2X₇ receptor is a potential therapeutic target of pharmacological strategies designed to prevent neuronal death in ocular diseases including glaucoma.

What P2X₇ receptors are?

Extracellular adenosine 5'-triphosphate (ATP) is an excitatory transmitter in both the peripheral and central nervous systems. P2X receptors are a family of ligand-gated membrane ion channels activated by extracellular ATP. P2X receptors consist of seven isoforms designated P2X₁ to P2X₇ (North, 2002; Kaczmarek-Hájek et al., 2012) and are widely distributed in most types of cells of nearly every origin. These receptors have many functions such as synaptic transmission in the peripheral and central nervous systems, contraction of smooth muscle, platelet aggregation, macrophage activation, cell death and immunomodulation (Burnstock et al., 2010, 2011).

In contrast to other ligand-gated channels in the purinoceptor family, the P2X₇ receptor possesses unique features that are likely to be of both physiological and pathophysiological significance. Most

importantly, not only does the initial activation of these receptors result in the opening of a non-selective plasma membrane channel, but in many types of cells, sustained activation causes the formation of trans-membrane pores that are permeable to hydrophilic molecules of up to 900 Da (Valera et al., 1994; Falzoni et al., 1995).

Indicative of the P2X₇ receptor having a role in cell pathology, this receptor has been found to be highly up-regulated in neurons and glial cells located in the ischemic cerebral cortex (Franke et al., 2004). P2X₇-mediated signaling is also implicated in neurodegenerative diseases, such as Parkinson's disease, Alzheimer's disease and multiple sclerosis (Romagnoli et al., 2008).

P2X₇ receptors in the retina

Expression of the P2X₇ receptor has been demonstrated in most cell types in the retina; these include neurons such as the retinal ganglion cells (RGCs) (Brändle et al., 1998; Ishii et al., 2003; Wheeler-Schilling et al., 2001), as well as glia (Morigiwa et al., 2000; Pannicke et al., 2000) and vascular cells (Kawamura et al., 2003). In the adult rat retina, immunolabeling for the P2X₇ receptor is detected in a number of cells in the inner nuclear layer and ganglion cell layer, suggestive of amacrine cells and RGCs (Brändle et al., 1998). This research group later confirmed that P2X₇ receptors are expressed in identified RGCs using reverse transcription polymerase chain reaction (Wheeler-Schilling et al., 2001). These receptors were also found in presynaptic processes of rod bipolar cells, as well as other conventional synapses, suggesting that purines play a role in neurotransmission within the retina, and may modulate both photoreceptor and rod bipolar cell responses (Puthussery and Fletcher, 2004). Moreover, based on experiments using the P2X₇ receptor knockout mouse, it was suggested that these receptors provide excitatory input to photoreceptor terminals or to inhibitory cells that regulate both the rod and cone pathway response (Vessey and Fletcher, 2012). Another group suggested that activation of this receptor may affect uptake of neurotransmitters from the extracellular space by Müller cells in the retina (Pannicke et al., 2000).

P2X₇ receptors and neuronal death in the retina

In addition to the putative physiological roles of P2X₇ receptors, stimulation of these receptors has been reported to be involved in neuronal death in the retina. It was reported that ATP induces the death of developing avian retinal neurons in culture *via* activation of P2X₇ receptors (Ancasí et al., 2013). The potential neuroprotective effect of a P2X₇ receptor antagonist on photoreceptor cell death was reported using primary retinal cell cultures (Notomi et al., 2011). This antagonist has also been reported to inhibit apoptosis in cultured human retinal pigment epithelium (Yang et al., 2011). Moreover, stimulation of P2X₇ receptors can kill RGCs *in*

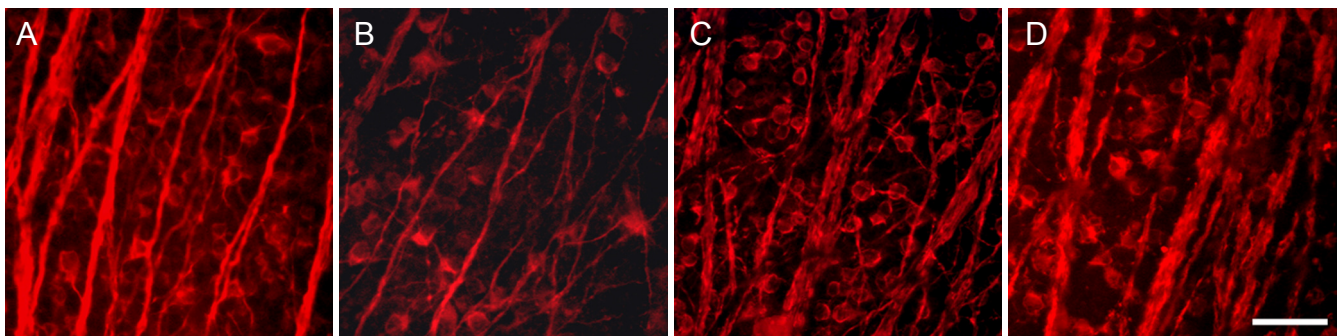


Figure 1 Representative photomicrographs of TUJ1-positive cells on day 7 after optic nerve crush (ONC) injury, with and without P2X₇ antagonists (OxATP and BBG) in corresponding regions (1 mm from the optic nerve head).

(A) Normal eye; (B) an eye with ONC + phosphate buffered saline; (C) an eye with ONC + 30 $\mu\text{mol/L}$ OxATP; (D) an eye with ONC + 0.3 $\mu\text{mol/L}$ BBG. Scale bar: 50 μm . TUJ1: Neuron-specific β -tubulin; OxATP: oxidized adenosine triphosphate; BBG: brilliant blue G. From Kakurai et al. (2013).

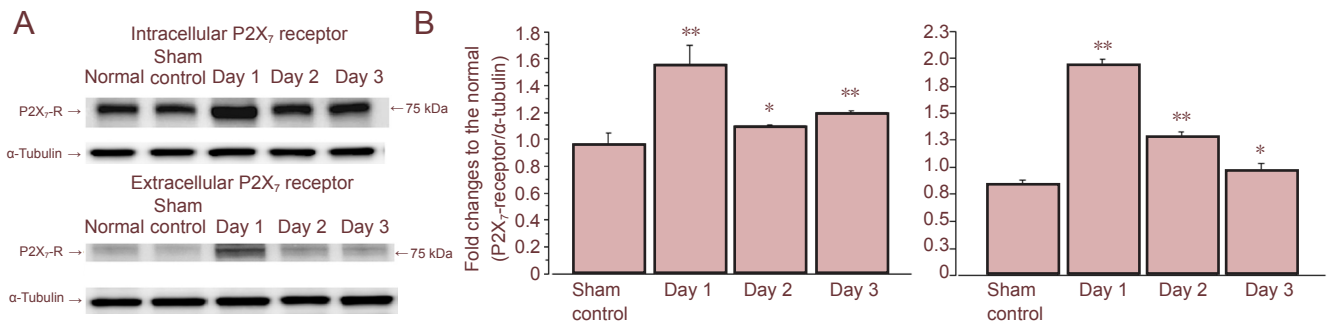


Figure 2 Western blot analysis of P2X₇ receptor (P2X₇-R) protein levels in whole retina after intraocular pressure (IOP) elevation. (A) Representative immunoreactive bands of intracellular and extracellular P2X₇ receptors in normal, sham control, and treated retinas on days 1, 2, and 3 after IOP elevation. (B) Densitometric quantification of immunoreactive bands of intracellular (left) and extracellular (right) P2X₇ receptor in sham control and treated retinas on days 1, 2, and 3 compared to normal retina. Data are expressed as mean ± SD (*n* = 3). The asterisks indicate significant differences from the sham control eyes (unpaired *t*-test, **P* < 0.05, ***P* < 0.01). From Sugiyama et al. (2013).

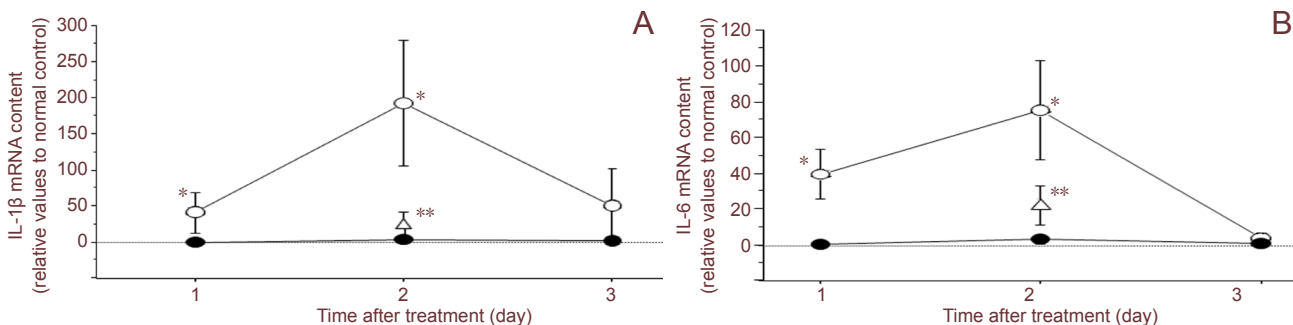


Figure 3 Effects of intraocular pressure (IOP) elevation on the expression of interleukin (IL)-1β and IL-6 mRNA in the retina. (A) Alterations in IL-1β mRNA content in the retina after IOP elevation (open circles) or after sham treatment (closed circles). (B) Alterations in IL-6 mRNA content in the retina after IOP elevation (open circles) or sham treatment (closed circles). Open triangles represent samples from eyes treated with 10 μmol/L O_xATP just after IOP elevation. Data are expressed as mean ± SEM (*n* = 4–5). The asterisks indicate significant differences from the sham control eyes (*) or from the eyes after IOP elevation (**; Mann-Whitney U-test, *P* < 0.05). From Sugiyama et al. (2013).

vitro and *in vivo* through a mechanism that is likely dependent on a rise in intracellular Ca²⁺ (Zhang et al., 2005; Hu et al., 2010). It was also suggested that the balance between extracellular ATP and its protective metabolite adenosine can influence RGC survival in the eye (Hu et al., 2010). Another study demonstrated that early up-regulation of neuronal P2X₇ receptors may cause injury to retinal neurons which may contribute to retinal damage (Franke et al., 2005). Furthermore, data from our laboratory indicate that the activation of P2X₇ receptors is involved in hypoxia-induced death of retinal neurons (Sugiyama et al., 2010). Using human organotypic retinal cultures, it was demonstrated that stimulation of P2X₇ receptors can mediate RGC death and that this mechanism plays a role in ischemia-induced neurodegeneration (Niyadurupola et al., 2013). Moreover, it was also reported that ischemic damage was attenuated by P2X₇ receptor antagonists in isolated optic nerves as well as in cultured oligodendrocytes (Domercq et al., 2010). Consistent with this, our recent study revealed that P2X₇ antagonists prevent loss of RGCs after optic nerve crush (Figure 1), and that this protective effect is possibly mediated through suppression of P2X₇ receptor over-expression in the retina (Kakurai et al., 2013). In addition, multiple combinations of three Ca²⁺ channel inhibitors including a P2X₇ receptor antagonist have been indicated in the preservation of visual function in a rat model of partial optic nerve transection (Savigni et al., 2011).

P2X₇ receptors and glaucoma

A group of researchers have demonstrated that ATP is released in response to an acute rise in ocular pressure both *in vitro* (Reigada et al., 2008), and *in vivo* (Zhang et al., 2007) and have recently

shown that ATP release accompanies chronic elevation in pressure (Li et al., 2011). It has also been suggested that mechanical strain triggers ATP release directly from retinal ganglion cells and that this released ATP autostimulates P2X₇ receptors (Xia et al., 2012). Recently, our group has also demonstrated that acute elevation of intraocular pressure can induce the up-regulation of P2X₇ receptor expression (Figure 2) (Sugiyama et al., 2013). Given that extracellular ATP levels and P2X₇ receptor expression in the retina increase with elevated intraocular pressure, and stimulation of P2X₇ receptors in RGCs can be lethal, this autocrine response may exert a deleterious effect on RGCs in glaucomatous eyes.

There are a number of reports linking P2X₇ receptor activation in the retina with the expression of inflammatory cytokines (Skaper et al., 2010; Weisman et al., 2012). For example, P2X₇ agonists enhance the release of interleukin (IL)-1β and tumor necrosis factor (TNF)-α from hypoxia-activated retinal microglia (Morigiwa et al., 2000). In addition, our data suggest that the up-regulation of TNF-α, IL-1β, and IL-6 may be involved in RGC death that occurs when P2X₇ receptors are activated after an increase in intraocular pressure (Figure 3) (Sugiyama et al., 2013).

In conclusion, a variety of recent experimental studies are providing evidence that the P2X₇ purinoceptor is a potential therapeutic target of pharmacological strategies designed to diminish or prevent neuronal death in ocular diseases including glaucoma.

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