PERSPECTIVE

Interaction between cannabinoid and nucleotide systems as a new mechanism of signaling in retinal cell death

Nervous system emerges from complex signaling interactions where extrinsic (neurotransmitters and trophic factors, among others) and intrinsic factors (transcription factors) interplay in the developing tissue to control gene activity promoting chronic changes in cell genesis, migration, differentiation and death. The retinal microenvironment is regulated by a broad variety of chemicals, including endocannabinoids and nucleotides that modulate embryonic progenitor-neuron-Müller glia signaling in very early developing or pathophysiological conditions. Accumulated evidence demonstrate the presence of a functional cannabinoid system in this tissue, with many retina cell types expressing cannabinoid CB1 and/or CB2 receptors, the two main ligands N-arachidonoylethanolamide (anandamide) and 2-arachidonoylglycerol (2-AG) and enzymes that generate N-acyl phosphatidylethanolamine phospholipase, and diacylglycerol lipase (DAGL) and degrade fatty acid amide hydrolase, monoacylglycerol lipase (MAGL) and cyclooxygenase-2 endocannabinoids (Kokona et al., 2016). As in other areas of the central nervous system, cannabinoids seem to regulate neurotransmission in the retina by inhibiting the release of transmitters such as dopamine, norepinephrine, γ-aminobutyric acid and glutamate. They also play an important role in retinal circuitry and in scotopic vision by modulating Ca^{2+} and K^{+} channels in bipolar cells and photoreceptors. Moreover, under pathological conditions, cannabinoids seem to induce neuroprotection in this tissue (Kokona et al., 2016). Albeit these protective effects, however, recent data are implicating cannabinoid receptors in cell death in the retina, both in the early developing (Freitas et al., 2019) and diseased tissue (Matias et al., 2006; El-Remessy et al., 2011; Chen et al., 2018).

In the developing retina, purinergic signaling is critical to trigger eye and retina development through the activation of distinct P2Y receptor subtypes that regulate neurogenesis and cell migration as well as the activation of P2X7 receptors (P2X7Rs) that mediates neuronal cell death in this tissue (Ventura et al., 2018). Although some reports indicate a possible interaction between endocannabinoid and purinergic systems in other brain regions (Kovacs et al., 2011), evidence demonstrating their interaction during retinal development and the involvement of this interaction in the death of Müller glial progenitors has now emerged (Freitas et al., 2019).

Cannabinoids and nucleotides are strictly related to many types of retinal diseases. In patients with diabetic retinopathy, the most frequent complication of diabetes and one of the leading causes of blindness worldwide, the level of the endocannabinoid anandamide is increased in the cornea, ciliary body, choroid and retina (Matias et al., 2006). In streptozotocin-induced diabetic mice, CB1 receptor deletion or treatment with CB1 receptor antagonist SR141716 prevent retinal cell death by attenuating the retinal oxidative stress mediated by pro-inflammatory mediators. Genetic or pharmacological ablation of CB1 receptor decreases the activity of the pro-apoptotic p38/Jun N-terminal kinase/mitogen-activated protein kinase pathway (El-Remessy et al., 2011). Age-related macular degeneration is a retinal disease associated to excessive inflammatory processes in the retinal pigment epithelium with oxidative stress, mitochondrial dysfunction in the cells, development of new blood vessels, death of photoreceptors and loss of central vision (Kauppinen et al., 2016). In human retinal pigment epithelium cells, activation of CB2 receptor increases the production of pro-inflammatory cytokines and photoreceptor degeneration in an extracellular signal-regulated kinase 1/2-dependent manner, indicating that cannabinoids are able to increase inflammation in the retinal pigment epithelium (Hytti et al., 2017). Accordingly, anandamide levels are increased in the cornea, ciliary body, choroid and retina of age-related macular degeneration patients (Matias et al., 2006) suggesting that endocannabinoids participate in inflammatory retinal diseases.

In a light-induced retinal degeneration model, the CB1 receptor antagonist SR141716A suppresses photoreceptor cell death (Imamura et al., 2017). In mice with photoreceptor degeneration induced by N-methyl-N-nitrosourea, the administration of the CB1 receptor antagonist SR141716A blocks photoreceptor loss with a concomitant decrease in glial reactivity and attenuates N-methyl-N-nitrosourea-induced formation of abnormal vascular complexes (Chen et al., 2018). Together, these data suggest that the endocannabinoid system is involved in some retinal diseases and CB1 receptor antagonists may be potential therapeutic drugs. Although other studies point to cannabinoids as neuroprotective molecules in retinal diseases, such as glaucoma, ischemia, and glutamate-induced neurotoxicity (Kokona et al., 2016), the studies reported above indicate that cannabinoids under certain conditions may participate and increase the retinal degenerative disease.

While a dual neuroprotective-neurotoxic profile is described for cannabinoids, the role of nucleotides in the cell death during retinal development and disease is well established (Ventura et al., 2018). P2X7R is the major nucleotide receptor involved in retinal cell death and its activation by ATP in the developing tissue induces the death of neurons. The trigger and/or progression of some retinal diseases are also associated with nucleotide signaling. In wet age-related macular degeneration, glaucoma and hypoxia, for example, retinal degeneration is triggered by elevated levels of extracellular ATP followed by P2X7 receptors stimulation (**Figure 1**).

The interaction between cannabinoid and P2 nucleotide receptors was reported in previous studies. Synthesis and hydrolysis of endocannabinoids can be modulated by nucleotides in astrocytes, microglia, and neurons. In primary cultures of mouse astrocytes, ATP increases 2-AG synthesis in a P2X7R-dependent manner and both extracellular calcium and DAGL activity are required

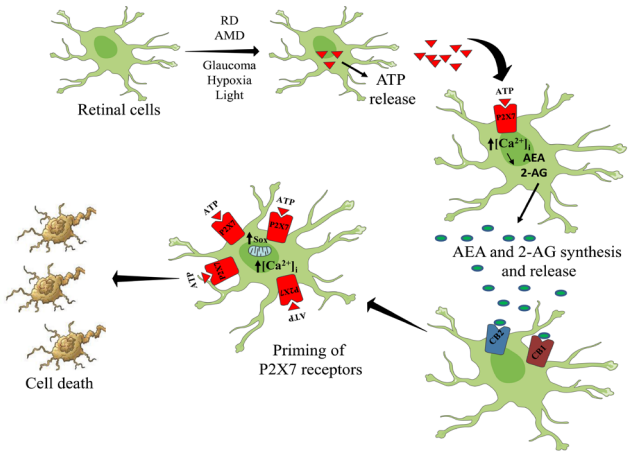

Figure 1 Hypothetical schematic image of the retinal cell death induced by cannabinoids and nucleotides.
Retinal diseases promote ATP release, activating P2X7 receptors and inducing an increase in intracellular calcium (Ventura et al., 2018). The increase in calcium levels activates DAGL at the same time that inhibits MAGL activity, resulting in an increase of endocannabinoid levels at the extracellular medium. Endocannabinoids, in turn, activate CB1 and CB2 receptors and prime P2X7 receptors, triggering calcium entry in the cell. Sustained activation of P2X7 receptors can also promote mitochondrial stress, calcium shifts, fluorescent dye uptake and retinal cell death (Freitas et al., 2019). AEA: Anandamide; RD: retinal diseases; AMD: age-related macular degeneration; 2-AG: 2-arachidonoylglycerol; DAGL: diacylglycerol lipase; MAGL: monoacylglycerol lipase.

(Walter et al., 2004). P2X7Rs are also involved in 2-AG turnover in microglia. Once activated by high concentrations of ATP, P2X7Rs increase the extracellular concentration of 2-AG, an event that is calcium-dependent and inhibits the hydrolysis of 2-AG by MAGL while activating the synthesis of 2-AG by DAGL. This mechanism results in a 2-AG-enriched extracellular environment (Witting et al., 2004). In Purkinje cells, activation of P2XR also leads to calcium influx that triggers endocannabinoid production and CB1 receptor-mediated retrograde synaptic signaling (Kovacs et al., 2011).

Recently, our group identified that cannabinoids induce Müller glial progenitor cell death in embryonic chick retinal cultures (Freitas et al., 2019). Our data also revealed that the agonist WIN55,212-2 (WIN) decreases [^{3}H]-thymidine incorporation in retinal cell cultures, an effect mimicked by inhibitors of the MAGL and fatty acid amide hydrolase enzymes. Accordingly, this agonist also decreases the number of proliferating cell nuclear antigen-positive cells in the cultures. Since the expression of the glial marker 2M6, but not the expression of the neuronal marker beta-tubulin III, decreases in the treated cultures, these findings strongly indicate that cannabinoids can induce the death of developing glial progenitors in culture, reinforcing the idea that cannabinoids may present deleterious effects in the retina.

An important finding from the aforementioned work is the observation that the P2X7R antagonist A438079 blocks WIN55,212-2-induced cell death in the cultures. Moreover, treatment of the cultures with WIN for 24 hours increases the uptake of the fluorescent dye sulforhodamine B by the cells, a response that can also be prevented by A438079. These findings, together with the observation that ATP-mediated increase in intracellular calcium is observed in the cultures after the treatment of the cells with the cannabinoid agonist, strongly suggest that activation of cannabinoid receptors promotes P2X7R-mediated signaling in retinal progenitors in culture (**Figure 1**).

Previous work showed that ATP induces the death newborn neurons in culture via activation of P2X7Rs and blockade of glutamate uptake that accumulate in the extracellular medium and stimulate both N-methyl-D-aspartic acid and non-N-methyl-D-aspartic acid receptors (Ventura et al., 2018). Since activation of P2X7Rs is implicated in the death of retinal cells in several types of retinal injuries, an interesting possibility to explain the deleterious effects of cannabinoids in the retina would be that in certain conditions, cannabinoids modulated the expression/function of P2X7Rs, resulting in increased calcium signaling induced by these nucleotide receptors. To elucidate if the interaction between nucleotide and cannabinoid receptors are increased in retinal pathologies such as glaucoma and diabetic retinopathy could help to clarify which specific conditions favor the pro-survival or pro-apoptotic effects of cannabinoids. Finally, a deeper implication of our work may arise as the role of exogenous and endogenous cannabinoids is further investigated in the context of the human retinal development through pregnancy and early postnatal days. While prenatal exposure to *Cannabis sp*. derivatives have been implicated in negative effects on cognitive development, endogenous cannabinoids are frequently pointed out as crucial to the establishment of migration, fasciculation, differentiation and other time-sensitive events in the nervous system.

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