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"The role of purines in photoreceptor death during retinal degeneration"

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A/Prof Erica Fletcher, The University of Melbourne

The aim of this project was to examine whether certain substances released from dying photoreceptors could contribute to the death of neighbouring cells. The purine, ATP, is commonly known for its role as an energy molecule. However, when it acts outside cells, it regulates communication between neurons, via receptors called P2X receptors. Our theory was that following neuronal death, large amounts of ATP could be released from dying photoreceptors, which in turn could lead to excessive activation of neighbouring cells and their death.

This project had three aims. First, we examined the mechanism by which ATP induces photoreceptor death. We found that when ATP levels are high, photoreceptor death occurs within 8 hours, via mechanisms involving elevated intracellular calcium and programmed cell death. This work has allowed us to develop a large animal model of retinal degeneration that is currently being used for preclinical testing of the Australian retinal implant produced by Bionic Vision Australia.

Secondly, we examined the effect of inhibition or loss of P2X receptors on retinal structure during degeneration. We have tested a number of compounds known to block the action of P2X receptors. To date, these compounds all reduce photoreceptor death by around 30% in the rd1 mouse model of retinal degeneration. We created and analysed retinal structure and function of P2X7/rd1 double knockout mice and also P2X7null mice. We found that photoreceptor death was slowed in the double knockout also by around 30%, consistent with our pharmacological experiments. We also examined the mechanism by which blockade of P2X receptors reduces photoreceptor death. We expected these experiments to show that photoreceptors die more slowly because they are less activated by the large amounts of ATP. However, to our surprise, we also found that the inflammatory response that normally develops during degeneration was substantially reduced. Thus, the positive effects of drugs that block P2X receptors on photoreceptors could act as anti-inflammatory agents.

The third aim, was to establish that anomalies in purine regulation contribute to retinal degeneration. We anticipated that if excessive amounts of ATP contribute to cell death, then one should see evidence that P2X receptors, or the enzymes that degraded ATP are altered prior to or during the active phase of photoreceptor death. We quantified the expression of a range of P2X receptors and also enzymes that degrade eATP in control and rd1 retina. We found that P2X7, P2X4 and NTPDase 1 expression were elevated during the active phase of rod death suggesting that dysregulation of purines occurs during the active phase of degeneration.

In summary, this project has established a possible role for purines in exacerbating photoreceptor death. Moreover, this project has implicated inflammation as a contributor to photoreceptor death. Further work is needed, however, to optimise the use of this class of drug for those with retinal degenerations.