ABSTRACT

Glaucoma is one of several neurodegenerative diseases of the central nervous system for which a pharmacologic cure is yet to be discovered. In previous studies acetylcholine (ACh) has provided neuroprotection for retinal ganglion cells (RGC) in the mammalian retina under glaucomatous conditions (Wehrwein et al., 2004). More specifically, an α7 nicotinic ACh receptor (nAChR) agonist has demonstrated neuroprotection for RGCs in both *in vitro* and *in vivo* models of glaucoma (Iwamoto et al., 2014). In this study, this α7 nAChR agonist was combined with a positive allosteric modulator (PAM) in dissociated adult porcine retinas to evaluate its effect on isolated RGCs and identify a potential molecular signaling pathway of neuroprotection.

Porcine retinas were dissociated using a two-step panning technique to isolate RGCs. Once isolated, RGCs were cultured under various pharmacologic conditions and incubated for 3 days. Pharmacologic conditions utilized throughout the course of this research included: agonist alone, agonist with low, medium or high dose PAM, and PAM without agonist. In subsequent experiments, enzyme inhibitors were applied thirty minutes prior to pharmacologic intervention to evaluate effects of the drugs in the absence of specific proteins utilized in their hypothesized cellular signaling pathway.

Retinal ganglion cells treated with the α7 nAChR agonist alone demonstrated a 28.0% (± 12.8%) increase in cell survival over untreated control. This agonist in combination with medium or high dose PAM resulted in increased cell survival at 43.0% (± 11.6%) and 52.0% (± 20.9%),

respectfully. However when the PAM was used as monotherapy, cell survival increased by only 3.2% (± 10.4%) over untreated control, supporting its hypothesized allosteric mechanism of action. Enzyme inhibition results suggest that the α7 nAChR agonist utilizes the PI3 to Bcl-2 signaling pathway to produce this neuroprotective effect, and that the PAM works in an allosteric manner through PI3 kinase to produce an enhanced effect. These studies provide support for future research in analyzing the effects of an α7 nAChR agonist and PAM in *in vivo* models of RGC death. Further understanding of these pharmacologic agents could provide important information in the development of new therapeutic options for glaucoma and other neurodegenerative diseases.