

## **Axonal Regeneration The Key to Therapeutic Advancement?**

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Neurons of the central nervous system lack the ability to regenerate an axon after injury, due to an inhibitory glial environment. However, an acute injury to the optic nerve (e.g. optic nerve crush) transforms retinal ganglion cells (RGCs) into a regenerative state in vitro, as shown in different retinal culture models. We have examined the effect of elevated intraocular pressure (representing a chronic injury) on axonal regeneration of RGCs in buphthalmos. A rat mutant with hereditary buphthalmos was used to (1) determine whether the extent of RGC loss corresponds to the severity and duration of elevated intraocular pressure (IOP), (2) examine whether RGCs exposed to an elevated IOP are able to regenerate their axons in a retina culture model, and (3) analyze the proteome of the regenerating retina in order to identify putative regeneration-associated proteins. Retrograde labeling of RGCs revealed a decrease in their numbers in the retinas of buphthalmic eyes that increased with age. Quantification of axons growing out of retinal explants taken at different stages of the disease demonstrated that buphthalmic RGCs possess a remarkable potential to regrow axons. As expected, immunohistochemistry and immunoblotting revealed that elevated IOP was associated with upregulation of certain known proteins, such as growth-associated protein 43, glial fibrillary acidic protein, and endothelin-1. In addition, two-dimensional polyacrylamide gel electrophoresis and mass spectrometry revealed several spots corresponding to proteins that were specifically regulated when buphthalmic RGCs were permitted to regrow their axons. Out of the proteins identified, heat-shock protein (HSP)-60 was constantly expressed during axonal growth at all stages of the disease. Antibodies against HSP-60 reduced axonal growth, indicating the involvement of this protein in regenerative axonal growth. These data show that glaucomatous retinal neurons can grow their axons, and that HSP-60 supports neuritogenesis. This model may help to elucidate the fundamental mechanisms of optic neuropathies at stages preceding death caused by chronic injury, and aid in the development of neuroprotective strategies.