
Is PTEN hyperactivity behind poor regeneration in diabetic neuropathy?

Neuropathy is a troublesome complication of diabetes mellitus that commonly affects the sensory and autonomic nervous systems. Impaired nerve conduction and axonopathy lead to pain and/or paraesthesia followed by sensory loss. There is no effective treatment for diabetic neuropathy, in part because its mechanisms are poorly understood. Research has focused on correcting the changes induced by hyperglycaemia, such as oxidative stress, glycation of macromolecules, and mitochondrial dysfunction (Tomlinson and Gardiner, 2008). Neuroprotective strategies that target neurons, Schwann cells, and nerve blood supply have also been suggested (Calcult et al., 2008) but none have been successful (Apfel, 2002; Ropper et al., 2009). However, in this issue of *Brain*, Douglas Zochodne and colleagues present evidence that inhibition of the phosphatase PTEN (phosphatase and tensin homolog) can produce functional improvements in a mouse model of diabetic neuropathy (Singh et al., 2014).

A frequent consequence of diabetic neuropathy, in addition to nerve degeneration, is the failure of axons to regenerate after injury. Recent studies of axon regeneration in the CNS have focused on the phosphatase PTEN. PTEN dephosphorylates the signalling lipid phosphatidylinositol-3,4,5-trisphosphate (PIP3) at position three. PIP3 mediates growth factor receptor signalling (including that of insulin) through activation of PI3-kinase at the plasma membrane. Activation of PTEN thus reduces activity in critical signalling pathways that promote growth and survival downstream of PIP3, such as those mediated by activated Akt/PKB and mTOR, while interfering antagonistic signals (such as GSK3β and FoxO transcription factors). PTEN is a tumour suppressor, but patients with inactivating mutations in PTEN can also present with seizures, learning disability and other neurological symptoms (van Diepen and Eickholt, 2008). Notably, deletion or knockdown of PTEN increases CNS neuron survival and axon regeneration after injury (Park et al., 2010). PTEN is also expressed in dorsal root ganglion (DRG) neurons (Chadbourn et al., 2006) and mediates growth cone collapse and decreased axon elongation, suggesting that PTEN inhibition may also enhance nerve regeneration in the PNS.

In a previous study, Zochodne and colleagues examined whether inhibition of PTEN could reverse regenerative decline in a rat model of sciatic nerve injury (Christie et al., 2010). They showed that PTEN knockdown improved regeneration, and that this effect was additive with the beneficial effect of a preconditioning lesion. Following nerve transection, pharmacological inhibition or knockdown of PTEN by short interfering RNA delivered to the injury site accelerated axon outgrowth in vivo. In the present paper, Zochodne’s group ask whether PTEN might also restrict nerve regeneration in a mouse model of diabetes. The first surprise to emerge from the data is that PTEN messenger RNA and protein expression are substantially increased in sensory neurons in two murine models of diabetes, a type 1 model induced by streptozotocin (STZ) (which depletes insulin by causing rapid pancreatic B cell death), and a type 2 model, the db/db mouse. Sciatic nerve crush injury further increased PTEN messenger RNA in the DRG neurons of diabetic mice compared with injured control mice, although no protein increase above that induced by STZ itself was present 3–6 days after injury.

Singh et al. (2014) then examined functional outcomes after nerve injury in non-diabetic and STZ-treated mice. The latter had confirmed type 1 diabetes and diabetic neuropathy as indicated by elevated blood glucose, reduced weight gain, impaired motor and sensory nerve conduction and loss of sensation to mechanical and noxious stimuli. PTEN knockdown increased motor conduction velocity and compound motor action potentials in the diabetic mice, as well as sensory nerve conduction velocity. These effects were less notable in the injured nerves of control animals. In addition, PTEN knockdown increased both the number and the diameter of myelinated fibres in the tibial nerve of the diabetic mice, increased sensory fibre density in the footpad, and partially restored the response to a noxious mechanical stimulus. As each of these were initially reduced by 50–70% in the diabetic mice compared with control animals, the effects of PTEN short interfering RNA were especially clear in the diabetic mice. Although not all parameters were significantly improved, this partial functional recovery in a model of diabetic neuropathy is a promising start. Whether the beneficial effects persist beyond the 28 days examined by Singh et al. (2014) remains to be seen.

It is less clear whether PTEN elevation in diabetic mice has consequences for downstream signalling pathways. Levels of phospho-S6 kinase, a downstream target of the kinase mTOR, were reduced in diabetic mice, although a role of mTOR in the regeneration of DRG neurons upon PTEN knockdown has previously been excluded *in vitro* (Christie et al., 2010). Nevertheless, as predicted, phosphorylated (inactive) GSK3β was reduced in the diabetic DRG. However, there was no measurable effect on...
phosphorylation of Akt, through which PTEN exerts its main growth-promoting effects. There was also no effect on PTEN phosphatase activity or Forkhead transcription factor FoxO1 localization, although FoxO1 immunoreactivity was detected in nuclei, where it is presumed to be active. Further evidence is thus required for effects via PTEN-dependent downstream pathways.

Singh et al. (2014) extol the virtues of their non-viral method of delivering short interfering RNA by direct application to the nerve injury site (supplemented with injections into the sciatic notch and the plantaris muscle). Whether this method is as effective as delivery through recombinant viruses remains to be tested, especially given the chronic nature of diabetic neuropathy.

However, the big question remaining is whether PTEN activity plays a role in human diabetic neuropathy. The present study shows that thorough investigation into mechanisms of diabetic neuropathy can yield important new lines of research. Such studies should be encouraged, as should the study of diabetic neuropathy in general. To put matters into perspective, between 2010 and 2013, ~7500 papers per annum were listed in PubMed under the topic ‘Alzheimer’s disease’ whereas only ~1100 papers per annum appeared with the subject ‘Diabetic neuropathy’, most being clinical reports. However, according to estimates from the World Health Organization, diabetes affects 347 million people worldwide, of whom 50% are predicted to develop some form of diabetic neuropathy, whereas 35.6 million people were estimated to be living with dementia in 2010. The case for conducting more research into diabetic neuropathy is overwhelming.

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**Consulting the vestibular system is simply a must if you want to optimize gaze shifts**

Even simple activities like reaching for our morning cup of coffee require precisely coordinated movements of multiple parts of the body. Successive attempts at these movements are characterized by ‘repetition without repetition’ (Bernstein, 1967). For this reason, it is thought that the brain does not enforce the details of a specific movement trajectory, but rather uses on-line feedback to optimize acquisition of the movement goal. However, a study in this issue of Brain demonstrates that when we make coordinated movements of the eyes and head to redirect our gaze, we use an optimal strategy that depends on vestibular sensory input: a strategy unavailable to patients with total vestibular loss. These results provide the first evidence that the vestibular system is critical for optimizing voluntary movements (Saglam et al., 2014).

When we make coordinated eye and head movements to redirect our axis of gaze relative to space (gaze = eye-in-head + head-