PERSPECTIVE

RhoA/ROCK activation in major pelvic ganglion mediates caspase-3 dependent nitrergic neuronal apoptosis following cavernous nerve injury

Severe erectile dysfunction (ED) remains a significant side effect among men following surgical intervention for treatment of clinically localized prostate cancer, despite development of nerve-sparing techniques (Weyne et al., 2015a; Sopko and Burnett, 2016). The type of axonal injury due to prostatectomy is a classic peripheral neuropathy with an acute inflammatory phase that leads to Wallerian degeneration of the canvernous nerve (CN), which can be followed by a regenerative phase characterized by enhanced neurotrophic factor activation and reduction in neuroinflammation (Weyne et al., 2015b). The current treatment for post-RP ED is type 5 phosphodiesterase inhibitor (PDE5I) (Weyne et al., 2015a). Though, PDE5I are known only to affect the function corporal smooth muscle cells and do not act as a neurotrophic agent. Mounting evidence suggests that axonal regeneration of the CN and successful functional reinnervation of the end-organ penis with intact neuronal nitric oxide (NO) release can, at least in part, recover lost erectile function (Albersen et al., 2010).

The study by Hannan et al. (2016) looked at the role of the RhoA/Rho-associated protein kinase (ROCK) pathway in CN regulation following CN injury (CNI) in rats. The animal model, a bilateral CNI model, that was used in this study mimics the partial nerve damage similar to what occurs due to nerve-sparing radical prostatectomy and has been validated for the study of post-prostatectomy ED mechanisms (Albersen et al., 2012). This model causes a time-dependent decline in the neurogenic erectile response to CN stimulation.

There is evidence to support the idea that the activation of the RhoA/ROCK pathway hampers nerve regeneration (Cheng et al., 2008; Auer et al., 2012; Sopko et al., 2014) and inhibits neurogenesis after injury in peripheral nerves (Hiraga et al., 2006). The study found that RhoA and ROCK gene and protein expression increase in the major pelvic ganglion (MPG) following CNI as did caspase-3 mediated apoptosis. The upregulation of the RhoA/ROCK pathway led to direct activation of caspase-3 causing selective apoptosis of nitrergic neurons. Apoptosis in the MPG was measured by TUNEL positive cells and caspase-3 gene expression, which directly correlated with increases in gene expression and activation of RhoA and ROCK in the MPG. This expression and activity of RhoA and ROCK isoforms in the MPG peaked 14 days following CNI, which supports the idea that a temporal increase in RhoA and ROCK contributes to loss of autonomic ganglion cells of the MPG. RhoA and ROCK expression was localized to both the ganglion cell bodies as well as the nerve fibers. This provides evidence that RhoA/ROCK may affect CN function. The activation of RhoA/ROCK that occurs in the MPG after CNI represents a newly identified part of the signaling pathway that regulates neurite outgrowth after injury. This finding is consistent with results from other studies (Gratzke et al., 2012; Hannan et al., 2013).

Furthermore, the study showed that ROCK inhibitor (ROCK-1), also known as faudasil or Y-27632 partially suppressed up regulation of RhoA/ROCK pathway, apoptosis in the MPG and activation of caspase-3 in the MPG. The study found that ROCK-1 significantly suppressed caspase-3 dependent apoptosis 14 days post-CNI. Inhibition of ROCK also improved nitrergic signaling and suppressed detrimental effects of axonal degeneration to the penis that occur after CNI.

The study showed how inhibition of ROCK could improve nitrergic degeneration in the MPG, encourage neuritogenesis of the MPG neurites *ex vivo* and preserve autonomic innervation to the penis. The proposed mechanism of action is related to the inhibition of caspase-3 dependent apoptosis of ganglia in the MPG. It was found that TUNEL-positive apoptotic cells increased in the MPG 7 and 14 days following CNI. Additionally, there was an associated increase of both caspase-1 and caspase-3 gene expression between 48 hours and 21 days following CNI. Activated caspase-3 protein expression was found to be most elevated 14 days following CNI. One interesting finding was that there was a positive temporal correlation in gene expression of RhoA and ROCK to caspase-3 in the MPG. The finding suggests the increase in apoptosis in the MPG following CNI is partly mediated by the RhoA/ROCK signaling axis, which has not previously been identified.

Additionally, while CNI caused symptoms consistent with ED including a reduction in intracavernous pressure and impairment of non-adrenergic non-cholinergic-mediated relaxation in the penis, inhibition of ROCK was found to improve the intracavernous pressure and non-cholinergic-mediated relaxation in the penis after CNI.

The neurotransmitter NO and the three nitric oxide synthase (NOS) isoforms play important roles in facilitating penile erection (Sopko et al., 2014). The constitutively active NOS isoform neuronal NOS (nNOS) is expressed in the somas of the neurons of the MPG, which innervate the smooth muscle cells of the erectile tissues. The study demonstrated a significant decrease in nNOS positive ganglion and gene expression after bilateral CNI

in the MPG. The study found that CNI reduces nitrergic (nNOS) gene expression, uncouples nNOS protein causing a decrease in nNOS enzymatic activity, and reduces axonal staining of nNOS in the MPG 14 days after CNI. The nNOS uncoupling was found to contribute to the decreased expression and activity of nNOS in the MPG 14 days following bilateral CNI. When animals were treated with ROCK-I, nNOS levels and neurogenic-mediated erectile response was preserved indicating that autonomic innervation to the corpora cavernosa was conserved. Furthermore, the study showed that the use of ROCK-I also improved the uncoupling of nNOS and increased nNOS gene expression and activity in the MPG. ROCK-I did this by barring a decrease in the dimer to monomer ratio of nNOS protein to prevent the impairment of NOS activity caused by CNI. The prevention of uncoupling of nNOS preserved neurogenic mediated *in vivo* and *ex vivo* nitrergic relaxation of the corpora cavernosa. Thus, it can be said that RhoA and ROCK have inhibitory functions on the nitrergic axons of the MPG.

The results of this study provide evidence that the activation of the RhoA/ROCK pathway is implicated in the mediation of capase-3 dependent nitrergic neuron apoptosis in the MPG following CNI. The evidence suggests that inhibition of the RhoA/ROCK pathway could improve post-prostatectomy ED.

Looking forward, in addition to furthering our knowledge in the field of ED, investigation on RhoA/ROCK signaling in axonal degeneration following crush injury to the CN could hold important insights to the field of peripheral axonal regeneration. This study showed activated RhoA was important for the neuroregulation of penile erection and evidenced the therapeutic effects of inhibitors of ROCK. It revealed the mechanisms by which inhibitors of ROCK could benefit peripheral nerve regeneration such as inhibition of caspase-3 dependent apoptosis and prevention of nitrergic neuronal loss in the MPG following peripheral CN injury. RhoA/ROCK pathway inhibitors could be an important therapeutic target moving forward.

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