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SPINAL CORD INJURY INDUCES DEGENERATION OF SYMPATHETIC INNERVATION OF THE MOUSE URINARY BLADDER VIA PRO-APOPTOTIC P75NTR SIGNALLING

Hypothesis / aims of study

The role of increased neurotrophin levels in neurogenic bladder dysfunction has been extensively investigated, however, the role of proneurotrophins has received less attention. Proneurotrophins are released initially from cells that are then proteolytically cleaved to form the mature protein (*e.g.,* nerve growth factor). Mature neurotrophins can then bind to the p75 neurotrophin receptor ($p75^{NTR}$)-TrkA/B complex to promote cell growth and differentiation. Interestingly, proneurotrophins can also bind to $p75^{NTR}$ when it is dimerized with sortilin to initiate apoptotic pathways [1]. Therefore, proneurotrophins may elicit different effects to the mature proteins following nerve injury. Our aim was to determine the expression of the $p75^{NTR}$ and its selective ligand, pro-nerve growth factor (proNGF) in control and spinal cord transected (SCT) mouse bladders.

Study design, materials and methods

SCT surgery: Female C57Bl/6 mice were anesthetized using 1.5-2% isoflurane, a laminectomy performed and the spinal cord completely transected between the T8-T9 vertebrae, packed with haemostatic sponge and the muscle and skin sutured. After surgery, the animals had their bladders expressed twice a day by gentle abdominal compression and given daily prophylactic antibiotics and analgesics. Mice were humanely sacrificed at one, three or seven days after surgery and bladders isolated (N=3 mice per time point).

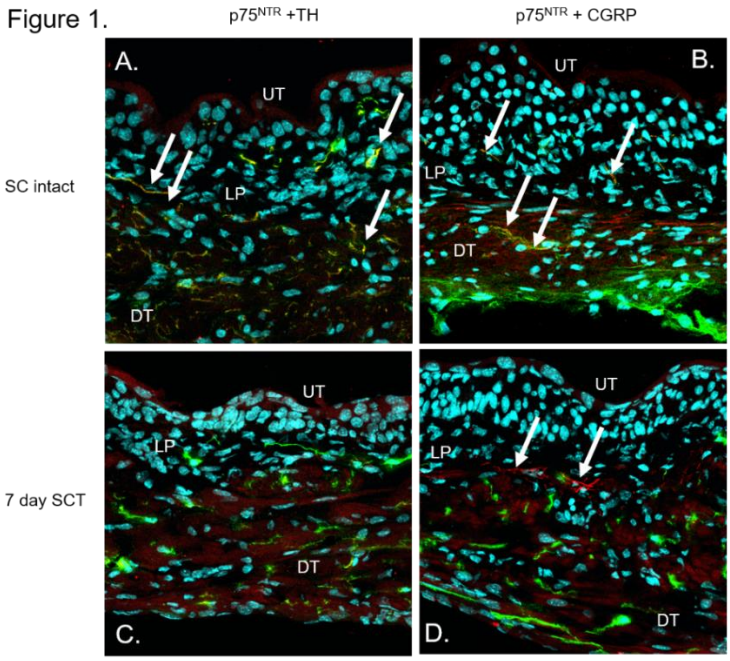

Figure 1. Immunohistochemical labeling of $p75^{NTR}$ and its co-localisation with CGRP and TH-positive nerves in the mouse urinary bladder and the alteration in their respective expression following spinal cord injury.

A: In control mouse bladders $p75^{NTR}$ (green) was highly expressed on TH-positive structures (red) as well as, B: some CGRP-positive nerves (red) throughout the bladder wall (co-localisation is indicated by yellow fluorescence and highlighted with white arrows). C: At seven-days post SCT, TH-positive immunoreactivity (red) was significantly decreased and there was no detectable co-localisation with $p75^{NTR}$ (green). D: Conversely, CGRP-immunoreactivity (red) was increased, particularly in the lamina propria/suburothelium as indicated by the white arrows. Furthermore, there was no co-localisation with $p75^{NTR}$ (green).

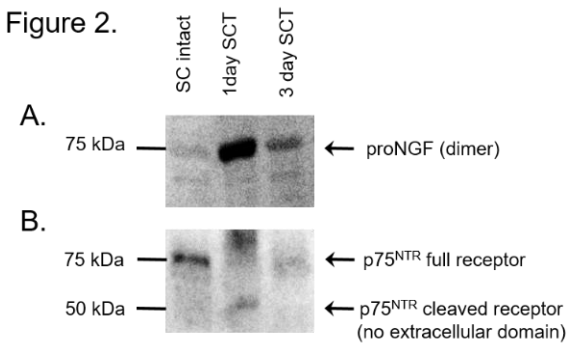

Figure 2. Western blots from control, one- and three-day post SCT mouse bladders for $p75^{NTR}$ and its ligand proNGF. In control mouse bladders, there was minimal expression of proNGF. However, by one day post-SCT there was a significant increase in proNGF levels which began to decrease by three days post injury. This increase in proNGF was accompanied by loss of the full-length $p75^{NTR}$ at one day post-SCT, suggesting that proNGF binding to the $p75^{NTR}$-sortilin complex introduces proteolytic cleavage of the extracellular domain.

Immunohistochemistry and image analysis: Freshly isolated bladders from control, one and seven day post-SCT mice were embedded for cryosectioning. Tissues were examined for immunoreactivity to $p75^{NTR}$ (Neuromics, extracellular domain), the sensory nerve marker calcitonin gene related peptide (CGRP, Sigma), sympathetic nerve marker tyrosine hydroxylase (TH, Abcam), and nuclei were stained with DAPI. Images from labelled tissue sections were obtained using an Olympus FV3000 confocal microscope system.

Western blot: Bladder tissues from control, one and three day post-SCT mice were homogenized in a lysis buffer and run on AnyKD miniProtean gels (Bio-Rad). Expression of $p75^{NTR}$ and proNGF was detected by conventional Western analyses using anti-$p75^{NTR}$ (Abcam, intracellular domain) and anti-proNGF (Thermofisher) antibodies.

Results

Immunohistochemical labelling of control mouse bladder sections demonstrated the expression of $p75^{NTR}$ on nerve-like structures throughout the bladder wall that co-localised with TH and CGRP-positive structures (Figure 1A and B). In bladders from SCT mice (seven days post-injury) there was decreased TH-immunoreactivity (1C) and no apparent co-localisation with $p75^{NTR}$. At this time-point, CGRP-immunoreactivity was increased specifically in the lamina propria (1D) which again did not co-localise with $p75^{NTR}$.

Western blot analysis of control mouse bladders demonstrated that proNGF, the ligand for the $p75^{NTR}$-sortilin complex, was not expressed under normal conditions (Figure 2). However, at one day post-SCT there was a dramatic rise in proNGF levels which was markedly decreased by three days post-SCT. Analysis of $p75^{NTR}$ levels showed that the spike in proNGF at one day post-SCT correlated with the loss of the full-length $p75^{NTR}$, indicating that there was proteolytic cleavage of the extracellular domain initiated by proNGF binding.

Interpretation of results

These data demonstrate that $p75^{NTR}$ is robustly expressed on sensory and sympathetic nerves innervating the mouse urinary bladder. Following spinal cord injury, there is a surge in proNGF levels that can activate the pro-apoptotic $p75^{NTR}$-sortilin complex and this may account for the loss of $p75^{NTR}$/TH and $p75^{NTR}$/CGRP positive nerves seen at seven days post-SCT. However, the increased CGRP labelling following SCT suggests that there is a subset of sensory nerves that are resistant to the apoptotic effects of proNGF, *i.e.,* $p75^{NTR}$-sortilin negative. Therefore, the transient rise in proNGF during the acute stages of spinal cord injury may be the initiator for degeneration of bladder sympathetic nerves.

Concluding message

There are differential roles of pro and mature neurotrophins in neurogenic bladder dysfunction. Selective inhibition of proneurotrophin signalling (*e.g.,* LM11A-31, selective modulator of $p75^{NTR}$ currently in clinical trial for Alzheimer's disease) may be a potential therapy to reduce bladder neural degeneration following spinal cord injury.

References

1. Meeker R and Williams K. Journal of Neuroimmune Pharmacology, 9:615, 2014

Disclosures

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