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NERVE GROWTH FACTOR SIGNALLING IN BLADDER IS MODULATED BY CAVEOLIN

Hypothesis / aims of study

Nerve Growth Factor (NGF), an important neurotrophin (NT) involved in inflammatory responses and in maintenance of sensory and sympathetic innervation, has been shown to be elevated in urine of patients with overactive bladder in proportion to the severity of overactivity [1]. Accordingly, the levels of NGF are up-regulated in the bladder of animal models with overactivity, and the blockade of NT specific receptors has a beneficial effect in reducing bladder overactivity [2]. Although these data suggest a role for NGF in influencing bladder function, the exact mechanisms by which neurotrophin signalling is regulated in the bladder have not been studied. Previous studies in other cell types indicate that several components of the TRK-A signalling cascade are localized in caveolae [3]. These cell membrane invaginations sequester and regulate a variety of signalling molecules, and thus may facilitate, organize and integrate cellular responses to extracellular stimuli. The purpose of this study was to investigate if caveolae are involved in the regulation of NT signaling in the bladder and whether caveolin protein expression is potentially affected by NGF.

Study design, materials and methods

Bladders were excised from male adult Sprague Dawley rats, cut in longitudinal strips and placed in Kreb's solution at 37°C. Bladder tissue was exposed to NGF (1μM) for different periods of time (from 30 minutes to 6 hours) or left untreated for control. In different experiments tissue was pre-incubated with methyl-β-cyclodextrin (15mM, 30 minutes), an agent that depletes caveolae from the cell membrane, and subsequently exposed to NGF stimulation. After incubation, total protein was extracted from bladder tissue for western blotting experiments. In parallel, bladder tissue samples were embebbed in cryoprotective compound and frozen for immunofluorescence analysis. For co-immunoprecipitation, TRK-A immunocomplex was precipitated from bladder lysate using specific TRK-A antibody coupled to magnetic beads; the samples obtained were subsequently immunoblotted with caveolin-1 (Cav-1) antibody in order to investigate the molecular interaction between Cav-1 and TRK-A. Western blotting was carried out to examine the potential effects of caveolar depletion (achieved by methyl-β-cyclodextrin) on the NGF-induced phosphorylation of ERK1/2 in bladder tissue before and after short term stimulation with NGF (1μM, 30 minutes). The effect of long term NGF stimulation (up to 6 hours) on caveolin protein expression was also investigated by immunofluorescence and western blotting techniques.

Results

Cav-1 was found to co-precipitate with NGF receptor TRK-A in bladder tissue, and its association with TRK-A was time dependently increased upon NGF stimulation. The incubation of bladder tissue with NGF for 30 minutes induced an increase in the phosphorylation of ERK1/2 compared with unstimulated tissue. The experimental depletion of caveolae resulted in a decrease of the NGF-induced phosphorylation of ERK1/2 compared with tissue in which the caveolar integrity was preserved. Long term stimulation of bladder tissue with NGF increased Cav-1, Cav-2 and Cav-3 expression in a time dependent manner.

Interpretation of results

The molecular interaction between Cav-1 and TRK-A receptor as well as the inhibition of NGF-induced ERK1/2 phosphorylation after caveolar depletion suggested that caveolae play a role in modulating NT signaling. These results, taken together with the NGF-induced up-regulation of caveolin protein expression, indicate a complex reciprocal regulation between NGF and caveolar elements.

Concluding message

Alterations in the regulation of the caveolae-NGF interaction may potentially contribute to the development of bladder dysfunction associated with increased NGF levels in the bladder.

References

1. Kim JC, Park EY, et al: Changes of urinary nerve growth factor and prostaglandins in male patients with overactive bladder symptom. Int J Urol. 2005;12(10):875-80.
2. Pinto R, Frias B, et al: Sequestration of brain derived nerve factor by intravenous delivery of TrkB-Ig2 reduces bladder overactivity and noxious input in animals with chronic cystitis. Neuroscience. 2010; 166 (3):907-16.
3. Peiró S, et al: PC12 Cells Have Caveolae That Contain TrkA. Caveolae-disrupting drugs inhibit nerve growth factor-induced, but not epidermal growth factor-induced, MAPK phosphorylation. J Biol Chem. 2000; 275:37846-52.

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