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UROTHELIAL RELEASE OF NERVE GROWTH FACTOR (NGF): MODULATION BY PROTEIN KINASE C AND MITOGEN-ACTIVATED PROTEIN KINASE PATHWAYS

Hypothesis / aims of study

Neurotrophins, including nerve growth factor (NGF) and brain-derived nerve factor (BDNF), have been implicated in the regulation of bladder sensory function, as well as bladder overactivity. Classically, detrusor smooth muscle has been viewed as a prominent site of NGF production in the bladder based on the rich innervation of this cell type. Recently however, urothelium has been suggested to play an active role in the modulation of afferent activity in the bladder by releasing several paracrine modulators including ATP and nitric oxide. Although several studies have demonstrated the presence of NGF immunoreactivity in the urothelium of experimental animals, a possible release of neurotrophins from the human urothelium has not yet been studied. Therefore, we have explored NGF presence in human urothelium and investigated the mechanisms of its release from a human urothelial cell line.

Study design, materials and methods

The human immortalized urothelial cell line UROtsa was grown to 80-90% confluence and starved for 16 hours in serum-free medium before the experiments. Urothelial origin of the cells was confirmed by specific immunostaining with a cytokeratin 17 antibody. The release of NGF and BDNF from the cells was quantified by ELISA following the sampling of the conditioned medium at various time-points and pre-incubation with inhibitors of signal transduction pathways. NGF mRNA expression in cell lysates was studied by real-time PCR, whereas NGF protein expression and mitogen-activated protein (MAP) kinase ERK1/2 phosphorylation were examined by immunoblotting. To verify the relevance of the findings in UROtsa cell line, immunohistochemical staining for NGF was performed in human bladder biopsies obtained from patients with neurogenic detrusor overactivity and control subjects. Immunohistochemistry was performed using a Leica Microsystems Bond Max automated immunostainer. The validation of the staining was performed by omitting the primary antibody. Quantitative data are shown as means ± SEM and the experimental groups were compared using one-way ANOVA.

Results

In UROtsa cells, the expression of NGF was detected both at mRNA and protein levels. Additionally, UROtsa constitutively released NGF (11.5 ± 1.4 pg NGF/mg protein/h), but not BDNF to the medium. Pre-incubation with the protein kinase C (PKC) inhibitor Gö6976 (1 µmol/l), down-regulation of PKC by treatment with phorbol 12-myristate 13-acetate (PMA, 100 nmol/l) and the inhibitor of mitogen-activated kinase kinase (MEK1/2) U0126 (1 µmol/l) significantly reduced the constitutive 24-hours NGF release to 70 ± 6 %, 71 ± 3 %, 34 ± 4 % of control, respectively (all $p<0.05$), whereas the cyclic AMP elevating agent forskolin (10 µM) was without a significant effect.

In addition, PKC-dependent phosphorylation of MEK1/2 target ERK1/2 MAP kinase by bradykinin (100 nmol/l) was associated with enhanced (121 ± 5%, $p<0.05$, *Figure 1*) NGF release and increased NGF mRNA expression (273 ± 53%, $p<0.05$) in UROtsa. Both bradykinin-induced effects were prevented by pre-incubation with Gö6976 and U0126, suggesting the involvement of PKC-MAP kinase pathway.

Finally, the presence and localisation of NGF in native human urothelium was confirmed with specific positive urothelial immunostaining of biopsies from healthy controls and patients with neurogenic detrusor overactivity (*Figure 2*).

Interpretation of results

The current results indicate that human urothelial cells in culture express and spontaneously release NGF following the activation of PKC-MAP kinase pathway. Importantly, activity of this pathway is modulated by inflammatory mediators binding to G-protein coupled receptors, such as bradykinin, resulting in enhanced NGF expression and release. Such modulation could represent an important mechanism leading to excessive NGF production in bladder pathologies. Finally, NGF seems to be localized in urothelium of human bladder biopsies as well, confirming the relevance of the cellular model.

Concluding message

Urothelium could represent an important cellular source of NGF in healthy, as well as in inflamed and overactive bladder. Targeting the urothelial receptors or signalling pathways modulating NGF release may provide a novel strategy in the treatment of urinary bladder overactivity.

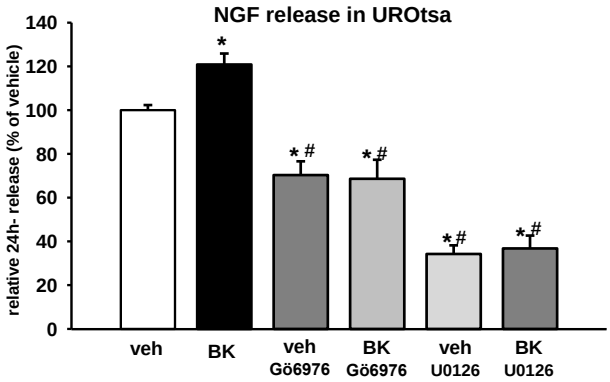

Figure1. 24 hours-release of NGF from UROtsa cells into medium (vehicle) and the effect of 100 nM bradykinin (BK), 1 µmol/l PKC inhibitor Gö6976 and 1 µmol/l MEK1/2 inhibitor U0126, * $p<0.05$ *vs* vehicle, # $p<0.05$ *vs* bradykinin.

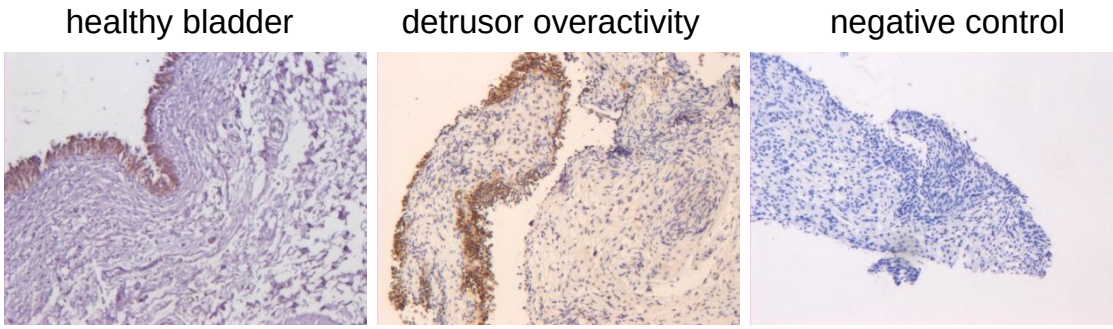

Figure 2. Positive NGF immunostaining found in urothelium of healthy human bladder (left panel), material from a patient with neurogenic detrusor overactivity (middle panel), but absent in negative control (right panel).

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Is this a clinical trial?	**No**
What were the subjects in the study?	**HUMAN**
Was this study approved by an ethics committee?	**Yes**
Specify Name of Ethics Committee	**NHS research ethics committee**
Was the Declaration of Helsinki followed?	**Yes**
Was informed consent obtained from the patients?	**Yes**