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Title: EXTRACELLULAR CA2+ MEDIATES STRETCH-INDUCED ACTIVATION OF C-JUN NH2 TERMINAL KINASE IN BLADDER SMOOTH MUSCLE CELLS

Aims of study

Bladder outlet obstruction is known to cause the structural alterations in the bladder wall, including smooth muscle hypertrophy, hyperplasia and collagen proliferation. Although the mechanisms responsible for these pathologic changes are still obscure, an excessive mechanical overload may be involved in the pathogenesis. In cultured bladder smooth muscle cells, mechanical stress was also shown to induce the release of various growth factors, such as HB-EGF and IGF-1. However, the molecular mechanisms that underlie these physiological responses are largely unknown. The mitogen-activated protein kinases (MAPKs) constitute a family of serine/threonine kinases that mediate the transduction of external stimuli into intracellular signals. These include extracellular signal-regulated kinase (ERK), c-Jun NH_2 terminal kinase (JNK) and P38. The present study, focusing on the primary effects of mechanical stretch, investigates how mechanical stress may be sensed and whether the signal transduction pathway are involved in MAPKs in rat bladder smooth muscle cells (BSMCs).

Materials and methods

The primary cultures of rat BSMCs were plated on six-well silicone Elastomer-bottomed culture plates that had been coated with collagen type I. BSMCs were subjected to mechanical stretch using a controlled vacuum unit (Flexercell Strain Unit: FX-3000). Using this unit, cells were subjected to sustained stretch with 5 - 25 % elongation by applying a vacuum at 15 - 20 kPa. The activities of MAPKs were measured by *in gel* kinase assay and *in vitro* kinase assay methods using radioisotope.

Results

Among three subsets of MAPK family members, activation of JNK was most relevant: the activity was elevated from 5 min after stretch and peaked at 10 min with 11-fold stimulation. Activation of P38 was small as compared with that of JNK, and ERK was not activated at all. JNK activation by mechanical stretch was totally dependent on extracellular Ca^{2+}, and inhibited by Gd^{3+}, a blocker of stretch-activated ion channels. As JNK was activated by ionomycin, suggesting an important role of extracellular Ca^{2+} on stretch-induced activation of JNK. Nifedipine and velapamil, inhibitors for voltage-dependent Ca^{2+} channel had no effect on this JNK activation.

Conclusion

These results suggest that in BSMCs, mechanical stretch opens Gd^{3+} sensitive Ca^{2+} channel, and increased intracellular Ca^{2+} activates JNK, which may stimulate transcriptional factors leading to smooth muscle hypertrophy.