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Cristofaro V[1], Chaudhury A[1], Goyal R K[1], Sullivan M P[1]
1. *Boston VA Helathcare System, Harvard Medical School*

PURINERGIC NEUROTRANSMISSION IN THE BLADDER IS REGULATED BY MYOSIN-VA

Hypothesis / aims of study
Smooth muscle neurotransmission is accomplished by the exocytosis of vesicles containing neurotransmitters from nerve varicosities. This process requires the involvement of non-muscle myosin motors, a family of proteins that play a role in translocating secretory vesicles from the cytosol to membrane docking sites where exocytosis occurs. Myosin Va in particular appears to be crucial for neurotransmitter release from enteric varicosities since mice with partial deficiency of myosin-Va exhibit impaired purinergic inhibitory junction potentials in the gut. In the bladder, excitatory neurotransmission is predominantly accomplished by the release of acetylcholine and ATP from nerve varicosities. The purinergic system in particular plays an important role in initiation of neurally-mediated contractile responses in the bladder. However, the mechanisms underpinning vesicle exocytosis and regulation of purinergic neurotransmission in this organ have not been previously investigated. The aim of this study was to examine whether myosin-Va contributes to excitatory neuromuscular transmission in the bladder.

Study design, materials and methods:
Urinary bladders were procured from myosin-Va deficient dilute, brown, nonagouti (DBA) mice. Control tissue was obtained from age matched wild type C57BL/6J (WT) mice. Longitudinal bladder strips from DBA and WT mice were stretched under 0.5 grams of tension in organ baths containing Kreb's solution at 37°C. After 1 hour of equilibration, neurally-mediated contractions were induced by electrical field stimulation (EFS) under baseline conditions, and after administration of purinergic receptor (P2XR) antagonist NF449 or muscarinic receptor antagonist atropine. Post-junctional activation of detrusor smooth muscle was generated by exogenous administration of carbachol (CCh), α-β-methylene ATP (αβmeATP), or KCl. Myosin-Va expression and tissue distribution in the bladder was investigated by confocal microscopy.

Results
Under baseline conditions, the amplitude of EFS-induced contractions was significantly lower in DBA bladders compared to WT bladders at the low-middle frequencies of stimulation. In WT bladders, the administration of purinergic (P2X1) receptor antagonist NF449 significantly decreased EFS-induced contractions at all frequencies compared to baseline response. In contrast, in DBA bladders, NF449 did not affect EFS-induced contractions. Muscarinic receptor antagonist atropine significantly reduced the amplitude of EFS-induced contractions in both WT and DBA compared to respective baseline contractions. The amplitude of the resulting atropine resistant contractions (presumptive purinergic component) was significantly lower in DBA compared with WT bladders. The amplitude of post-junctional contractile responses induced by exogenous administration of either CCh, αβmeATP and KCl were comparable between strains. Myosin Va immunoreactivity was detected in bladder tissue co-localized with the neuronal marker synaptophysin.

Interpretation of results
These findings demonstrate that the purinergic component of nerve-mediated detrusor contractions is impaired in myosin-Va deficient mice. Since post-junctional contractile responses to exogenous ATP were unaltered between strains, our findings suggest that myosin Va may facilitate purinergic neurotransmission in bladder smooth muscle.

Concluding message
Our data are consistent with the hypothesis that myosin-Va plays a major role in the transport of purinergic cargo to membrane varicosities and consequently in driving the contractile response to purinergic neurotransmission in the bladder.

Disclosures
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