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Birder L. [1], de Groat W. [1], Nakamura Y. [1], Kiss S. [1], Barrick S. [1], Nealen M. [2], Kanai A. [1], Ruiz G. [1], Apodaca G. [1], Burke N. [1], Caterina M. [2]
1. University of Pittsburgh School of Medicine; Department of Medicine, 2. Johns Hopkins University

INVOLVEMENT OF VR1 IN BOTH CHEMOSENSITIVE AND MECHANOSENSITIVE MECHANISMS IN THE URINARY BLADDER.

Aims of Study

In the urinary bladder, capsaicin-sensitive nerves have been implicated in the regulation of reflex voiding. However, the importance of capsaicin receptors to bladder function has remainded unclear. Thus, to directly address the importance of VR1 in urinary bladder function, we examined in detail the distribution of VR1 protein in the bladder reflex arc and also evaluated bladder function in mice lacking the VR1 capsaicin receptor (VR1 null).

Methods

For continuous cystometry, we inserted a polyethylene tube connected to a pressure transducer through the dome of the bladder. Continuous cystometrograms were performed by constant infusion (0.04 ml/min) of either physiological saline, acetic acid (0.1%) or infusion of drug (capsaicin) solutions into the bladder to elicit repetitive voiding. In some animals, two hours after recordings were complete, animals were sacrificed via intracardiac perfusion (Krebs, 4% paraformaldehyde) and tissues were removed for immunocytochemistry.

For immunocytochemistry, sections of urinary bladder whole mounts were processed for vanilloid receptor type 1, VR1, and alternate sections of lower lumbo-sacral spinal cord were immuno-stained for either VR1 or the Fos protein using specific antisera. In addition, stained sections were imaged with a Leica DMIRB confocal microscope using a 100x objective. Z-series taken every 0.5 microns were processed for 3-dimentional reconstruction.

For ultrastructure (scanning and transmission electron micrographs), tissue was fixed (cacodylate buffer) and the samples were osmicated (1.5% OsO_4) and then stained overnight in aqueous uranyl acetate. Cells were sectioned with a diamond knife (Diatome; Fort Washington, PA) and mounted on butvarcoated nickel grids, contrasted (uranyl acetate and lead citrate), and viewed in a JEOL (Japan) 100CX electron microscope.

Following enzymatic digestion (dispase/trypsin) of urinary bladder mucosa, urothelial cells were plated (50-150,000 cells/ml) onto collagen-coated dishes. ATP levels were quantitated using the luciferin-luciferase assay following superfusion of oxygenated (hypo-osmotic) solution to either excised urinary bladders or urothelial cells.

Results

VR1-immunoreactivity (IR) is located throughout the micturition pathway with staining in nerve fibers passing through the major pelvic ganglion, submucosal and mucosal nerve fibers in the bladder wall. In the urinary bladder, nerves exhibiting VR1-IR are localized in close proximity to VR1-positive epithelial cells in the basal layer of the urothelium. Both basal and superficial epithelial cells as well as the bladder smooth muscle stain for VR1-IR and exhibit VR1-mRNA expression. In addition, VR1 nerve fibers are present within the superficial spinal cord dorsal horn as well as in nerve fibers close to identified preganglionic cell bodies.

We used mice lacking VR1 to evaluate bladder responses. We first evaluated two stimuli, capsaicin and protons, both of which can activate VR1. Nociceptive stimuli (acetic acid) increased both the frequency of voluntary voiding contractions (as indicated by the inter-contraction interval, ICI) and Fos-immunoreactivity (a measure of afferent activity) in spinal cord neurons to similar extent in both wild type and null mice. However, while intravesical capsaicin decreased the micturition frequency in wild type mice, VR1 null mice exhibit a deficient urodynamic response to capsaicin.

VR1 null mice are also deficient in response to distension induced voiding. VR1 null mice exhibit increased bladder capacity (45%) and an increased frequency (90%) of non-voiding contractions (intravesical pressure waves whose amplitudes were greater than 15 cm H_20 which did not result in obvious voiding). In contrast to wild type, VR1 null mice also exhibit a decreased Fos-IR induced by saline infusion.

In either excised bladder or cultured urothelial cells, mechanical (stretch or hypo-osmotic) stimuli evoked the release of ATP. Compared to wild type mice, this release was significantly decreased in both excised bladders and urothelial cells isolated from VR1 null mice. The absence of VR1 had no significant effect on phenylephrine (used as control) evoked ATP release.

Conclusions

These results demonstrate that VR1 is expressed throughout the afferent limb of the micturition reflex pathway. In addition, the location of VR1-positive bladder nerves in close proximity to VR1-positive epithelial cells suggests there may be a chemical communication between the urothelium and adjacent nerves. Our results also reveal that while VR1 is essential for capsaicin-evoked bladder responses, acid detection in the bladder appears to result largely from VR1-independent mechanisms.

The deficits in both voiding reflexes and distension evoked ATP release from both excised bladder and urothelial cells observed in VR1 null mice suggest that VR1 may be involved in both mechanosensitive as well as chemosensitive afferent mechanisms in the urinary bladder.