

CAVEOLAE-MEDIATED REGULATION OF PURINERGIC SIGNALING IN THE BLADDER SUPPRESSES SPONTANEOUS ACTIVITY

Hypothesis / aims of study

Detrusor Overactivity (DO) is characterized by the presence of involuntary bladder contractions during filling. Although the mechanism contributing to these increased contractions remains unclear, particular abnormalities in the morphologic characteristics of smooth muscle (BSM) cells might be responsible for the development of DO.

BSM caveolae, specialized membrane invaginations involved in the regulation of a variety of signalling events, and in the modulation of contractile responses to physiologic stimuli, were found to be reduced in several animal models of DO. In particular, our findings showed that in spontaneously hypertensive rats (SHR), diminished BSM caveolae appear to be responsible for increased spontaneous activity (SA) in bladder tissue and for alterations in the response to purinergic stimuli. Thus, the purpose of this study was to investigate the functional link between the caveolae-mediated regulation of purinergic signalling and the level of SA in the bladder.

Study design, materials and methods

Longitudinal rat bladder strips without mucosa were mounted in organ baths containing Krebs's at 37°C and stretched to 1.5 grams of tension. The amplitude of bladder SA and contractile responses induced by P2X receptor agonist α - β -methylene ATP ($\alpha\beta$ meATP) was measured before and after caveolae were experimentally depleted (which was achieved by methyl- β -cyclodextrin, m β CD). Changes in SA were also determined after P2X receptor inhibition (achieved by NF449, NF279, PPADS, or after $\alpha\beta$ meATP-desensitization) as well as in the presence of ivermectin (IVC, a positive modulator of P2X₄ receptors) or BDBD (a P2X₄ receptor antagonist). Differences in the levels of endogenous ATP released by BSM tissue during these treatments or after KCl stimulation were measured by luciferin/luciferase assay. In addition, levels of basal ATP or induced ATP release (by exposure to hypotonic Krebs's) were determined in isolated BSM cells before and after caveolae depletion. P2X₄ receptor expression in bladder tissue and its molecular interaction with caveolin proteins were determined by western blotting and co-immunoprecipitation respectively.

Results

The experimental depletion of caveolae resulted in inhibition of P2X receptor-mediated contractile responses and augmented SA in BSM tissue. Moreover, exposure to m β CD increased basal and induced release of ATP from BSM cells. The augmentation in SA and in the release of endogenous ATP was similarly induced in BSM tissue by inhibition of P2X₍₁₋₃₎ receptors and by stimulation with IVC. The increase in SA induced by P2X receptor inhibition was prevented by the ATP scavenger apyrase, and also by administration of P2X₄ receptor antagonist BDBD. In addition, in the presence of BDBD, the increase in ATP released from BSM tissue in response to KCl stimulation was prevented. Western blot and immunoprecipitation showed that P2X₄ receptor was expressed in detrusor tissue and co-precipitated with caveolin proteins.

Interpretation of results

The impaired regulation of purinergic signalling and the increase in SA after caveolar depletion are consistent with findings in SHR, an animal model of DO in which caveolae are intrinsically reduced. The molecular interaction between caveolins and P2X₄ receptor is consistent with the localization of this protein complex within caveolar domains and supports the concept that P2X₄-mediated signalling is regulated by caveolin proteins. The augmentation in ATP release induced by caveolae depletion, P2X₁₋₃ receptor inhibition and by P2X₄ activation indicates that the loss of caveolae unmasked an excitatory component, potentially mediated by P2X₄ receptor activation. These findings suggest that SA levels are modulated by the effects of endogenous ATP released from BSM cells and that caveolae may play a role in restraining SA.

Concluding message

The release of endogenous ATP from BSM and its autocrine/paracrine activation of purinergic receptors are suppressed by caveolae. Thus, loss of caveolae-mediated regulation of this process augments ATP release and facilitates purinergic receptor activation, potentially leading to DO.

Disclosures

Funding: Supported by Research Service, Department of Veterans Affairs, Washington, DC, USA. **Clinical Trial:** No
Subjects: ANIMAL **Species:** Rat **Ethics Committee:** VA Boston IACUC