

ACTIVATION OF CALCIUM-SENSING RECEPTOR MODULATES BLADDER SMOOTH MUSCLE CONTRACTION

Hypothesis / aims of study

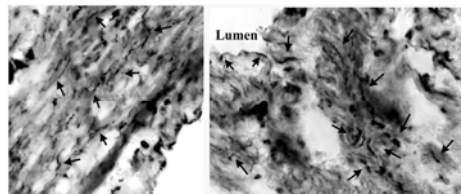
Many fundamental aspects regarding the regulation of bladder contractility are not completely understood. For example, the role of calcium, organic compounds and other polyvalent cations as extracellular signalling molecules has not been critically investigated. In addition to the established role of calcium in determining intracellular signalling events, calcium ions can function as autocrine/paracrine messengers by activating extracellular targets such as the Calcium-sensing Receptor (CaR) [1]. This G-protein coupled receptor is abundantly expressed in tissues that are involved in maintaining systemic calcium homeostasis, including the parathyroid, kidney, bone and GI tract. However, the distribution of this receptor in organs that do not explicitly regulate calcium (brain, lung, skin, eye and prostate) suggests that the CaR may be involved in other essential physiologic processes. In the bladder, pharmacomechanical coupling, excitation-contraction coupling and neurotransmitter release are calcium dependent processes that may however induce local changes in extracellular calcium. Ensuing fluctuations in the concentration of calcium or other extracellular CaR activators may potentially impact bladder smooth muscle function. Therefore, CaR may provide a mechanism for sensing extracellular calcium or other polyvalent cations and for regulating contractile responses to these local changes. Spermine is an endogenous polyamine associated with growth and protein synthesis in the bladder. In other tissues, spermine has been shown to effectively activate CaR [1]. The goal of this study was to determine the distribution of CaR and investigate the potential role of CaR in bladder function by determining the contractile response to spermine.

Study design, materials and methods

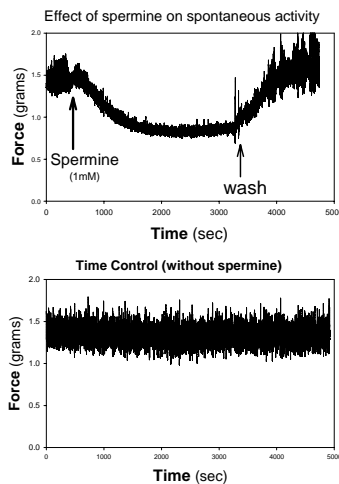
Western blotting was performed to detect the presence of CaR protein in rat bladder. Total bladder lysate was separated by gel electrophoresis and transferred to a nitrocellulose membrane. After blocking, the membrane was incubated with CaR antibody followed by an alkaline phosphatase conjugated secondary antibody and processed for chemiluminescence detection. To determine the distribution of the CaR in the rat bladder and pelvic plexus, immunohistochemistry was performed using frozen tissues sections (10 μm) fixed in acetone or formalin. Sections were incubated overnight at 4°C with primary antibody (CaR, 5 $\mu\text{g}/\text{ml}$). After washing, sections were incubated with a biotinylated secondary antibody, followed by a peroxidase-avidin complex and visualized by DAB staining. To exclude non-specific staining, parallel sections were processed as above but specific primary antibody was omitted. Rat parathyroid gland was used as a positive control. In vitro studies were performed to determine the contractile responses to activation of the CaR. The urinary bladder was removed from male rats and placed in Krebs's solution. Longitudinal strips of bladder tissue were suspended in a temperature-controlled organ bath (37°C) between platinum electrodes, placed under 1.5 grams of force and equilibrated for 45 minutes. Tissue was exposed to increasing concentrations (100 μM -3mM) or a single dose (1mM) of spermine. The amplitude and frequency of spontaneous activity, as well as the contractile response to electrical field stimulation (EFS, 2-64 Hz), were determined before and after each dose of spermine. In addition, the contractile responses to carbachol (1 μM) and KCl were measured before and after exposure to spermine.

Results

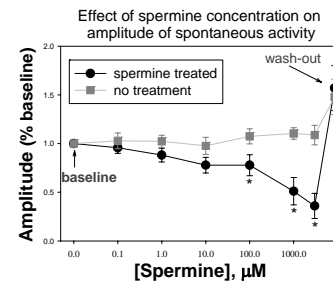
Western blot analysis confirmed the expression of CaSR protein rat bladder. Immunoreactivity was observed in a punctate distribution projecting through smooth muscle layers of the bladder, suggesting localization of CaSR to nerve fibers in bladder tissue (arrows). Positive immunoreactivity was also detected in the urothelium (right panel). In the pelvic plexus, immunoreactivity was detected in neurons and fibers innervating blood vessels. Immunostaining



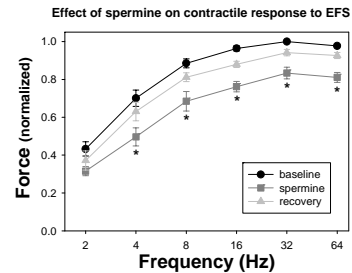
was not detected in sections incubated without primary antibody.



Spontaneous activity was significantly decreased following administration of spermine (left panels). With increasing doses of spermine (10 μ M to 3mM), a significant reduction in both frequency and amplitude of spontaneous activity was demonstrated. This inhibitory effect was completely reversed after washout with Krebs. Spermine



administration significantly attenuated the contractile response to EFS at all frequencies. Contractile responses to carbachol and



KCl were not affected by exposure to spermine, thus supporting the assumption that spermine did not adversely affect smooth muscle contractile elements.

Interpretation of results

Our findings indicate that CaR protein is expressed throughout the bladder and is localized in nerve fibers. This distribution is similar to the reported presence of CaR protein and mRNA in neurons in numerous regions of the brain [2], as well as the myenteric plexus of the small and large intestine [3]. Thus, our findings suggest that the CaR provides a novel mechanism for regulating nerve activity in the bladder. Our *in vitro* studies demonstrated that activation of CaR by spermine attenuated spontaneous activity and reduced the contractile response to EFS. These functional effects are consistent with the hypothesis that CaR may couple local changes in extracellular calcium, whether spontaneous or depolarization induced, with contractile response.

Concluding message

Bladder function may be modulated by activation of the CaR. In addition to physiologic levels of extracellular calcium, polyamines such as spermine can serve as agonists for the CaR. Since spermine is present in bladder tissue, endogenous spermine may activate the CaR not only under physiologic conditions, but in pathologic conditions that are associated with alterations in polyamines, including bladder hypertrophy, denervation, bladder cancer and interstitial cystitis. Thus, the CaR may present a reasonable target for pharmacologic intervention in the management of bladder dysfunction.

1. Nat Rev Mol Cell Biol. 4:530-538, 2003.
2. Eur J Neurosci. 12:872-884, 2000.
3. Am J Physiol. 274:G122-G130, 1998.

FUNDING: Department of Veterans Affairs