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HYPERGLYCEMIA ENHANCES BLADDER SMOOTH MUSCLE EXCITABILITY THROUGH A CAVEOLAE DEPENDENT FACILITATION OF RHO-ROCK SIGNALING

Hypothesis / aims of study

Bladder dysfunction is a common urologic complication in patients affected by diabetes. Diabetic bladder dysfunction is characterized by an early compensatory phase in which bladder smooth muscle (BSM) is hyperactive, followed by a late stage in which the bladder contracts less efficiently. While it appears that a loss in neuromuscular transmission might be responsible for the late decompensated stage of diabetic bladder, the mechanism leading to BSM hyperactivity in the early phase remains unclear. Our previous findings in an animal model of type 2 diabetes showed that the increased BSM contractility in the early stage of diabetes was correlated with upregulation of Rho signaling and increased myosin light chain (MLC) phosphorylation. In other smooth muscle systems, Rho–associated signaling is regulated by caveolae [1], specific membrane microdomains enriched in cholesterol that play a pivotal role in regulating tissue contractility. The purpose of this study was to investigate whether caveolae are involved in Rho-dependent changes in contractility induced by hyperglycemia.

Study design, materials and methods

Longitudinal BSM strips from male C57 (10-12 weeks old) mice were mounted in organ bath at 37°C and stretched under 0.5 grams for isometric tension studies. Hyperglycemia was induced by exposing BSM tissue to a high glucose (23mM) Kreb's solution. Separate tissue was exposed to normal Kreb's (11.5mM glucose) supplemented with 11.5mM mannitol to control for the hyperosmotic conditions of Krebs with high glucose. Bladder contractions were induced by administration of carbachol (CCh, 1μM) under euglycemic conditions, and repeated at intervals of 1, 2, 4 and 8 hours under either hyperglycemic or control conditions. The effect of hyperglycemia on the contractile response to CCh stimulation was investigated in the presence of a Rho kinase inhibitor (Y27632), using a dose that does not affect the response to CCh under normal glucose conditions (1μM). The response to CCh under hyperglycemic conditions was also determined after depletion of caveolae, achieved by incubation with methyl-β-cyclodextrin (mβCD, 10mM, 1 hour). For molecular studies, hyperglycemia-induced changes in myosin light chain phosphorylation (pMLC) were analyzed by western blot. The molecular interaction between Rho associated protein kinase 2 (ROCK-2) and caveolin proteins in mouse BSM tissue were investigated by immunoprecipitation experiments. High glucose-induced changes in caveolin protein expression in BSM tissues from db/db mice, an animal model of diabetes, was compared with non-hyperglycemic control mice.

Results

The contractile response to CCh after 2, 4 and 8 hours of hyperglycemia was significantly higher than CCh responses recorded under euglycemic conditions at the same time points. The amplitude of CCh responses in tissue exposed to Kreb's supplemented with mannitol were not increased over the same time period, indicating that the augmented CCh responses under hyperglycemic conditions were not due to osmotic changes. In contrast, the high glucose-induced increase in CCh responses was prevented by the ROCK inhibitor Y27632, as well as by caveolae depletion using mβCD. Phosphorylation of MLC in response to CCh stimulation was significantly higher in BSM tissue exposed to high glucose compared to pMLC levels detected under euglycemic conditions. Cav-3, but not Cav-1 expression was upregulated in BSM tissue from hyperglycemic, db/db mice compared to non-diabetic bladders. ROCK-2 co-precipitated with Cav-3 in mouse bladder tissue.

Interpretation of results

The augmented responses to CCh stimulation in the presence of high glucose indicates that hyperglycemia contributes to the hypercontractile phenotype characteristic of the early stage of diabetic bladders. The increased MLC phosphorylation induced by hyperglycemia, together with the prevention of hyperglycemia-induced hypercontractility by a ROCK inhibitor, suggest that hyperglycemia alters Rho/ROCK signaling. The molecular interaction between ROCK-2 and Cav-3 along with the inhibitory effect of caveolae depletion on high glucose-induced BSM hyperreactivity suggest that caveolae facilitate the activation of Rho-ROCK signaling in the bladder during hyperglycemia.

Concluding message

The upregulation of Cav-3 in bladder tissue from hyperglycemic/diabetic mice may enhance Rho-ROCK signaling and potentially contribute to the development of bladder hyperreactivity in these animals. Our findings suggest a potential link between hyperglycemia-induced BSM hyperreactivity and the caveolae-mediated facilitation of Rho-ROCK signaling.

References

1. Physiol Res. 63:179-87, 2014

Disclosures

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