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TLR4 INCREASES BLADDER CONTRACTILITY THROUGH MYD88/P38MAPK ACTIVATION IN STREPTOZOTOCIN-INDUCED DIABETIC MICE

Hypothesis / aims of study

Urinary tract infections are responsible for nearly 13 million annual doctor visits in the United States. These infections are most commonly caused by the Gram-negative bacterium *Escherichia coli*, which is responsible for 80-85% of community-acquired urinary tract infections¹. Toll-like receptor 4 (TLR4) is expressed in bladder urothelium, and has been suggested to initiate the expulsion of *E. coli* infected cells from the urinary tract. When activated by lipopolysaccharide (LPS), TLR4 leads to urothelial desquamation and inflammation². Epidemiological studies reported that diabetic patients have greater susceptibility to urinary tract infections than normoglycemic people. Evidence from experimental animal models as well as from humans has indicated that systemic inflammation plays a role in the pathophysiological processes of diabetes that is facilitated by innate immune responses³. Although some evidence suggests a relationship between diabetes and TLR4 activation, the mechanism that underlines these responses in diabetic bladder has not been investigated. The present work aimed to study TLR4 activation and function in isolated bladders from streptozotocin-induced diabetic mice.

Study design, materials and methods

Male C57BL/6 wild type (+/+) and TLR4 knockout (-/-) mice (22-30 g) were injected with streptozotocin (125 mg.kg⁻¹ i.p.). After four weeks, urine spot patterns were registered, and functional and molecular analyses were carried out. For functional experiments, bladders were collected, cut into 2 longitudinal strips and suspended under 5 mN tension in a 10-ml organ bath containing Krebs' solution (pH 7.4, 37°C, 95% O₂ / 5% CO₂). Concentration-response curves to the muscarinic agonist carbachol (0.001-100 µM) and KCl (0.001-1 M) as well as frequency-response curves to electrical-field stimulation (EFS: 10s; 80V; pulse width 1 ms) were constructed in the absence and presence of LPS. Changes in isometric force were recorded (Power Lab v.4 system, AD Instruments, UK). E_{max} and pEC₅₀ values were analyzed by one-way ANOVA followed by a Tukey test. Western blotting to detect MAP kinases p38, ERK1/2, JNK and NFκ-B(p65) were performed in bladders. The expression of TLR4, TLR2, MyD88 and Trif was assessed by RT-PCR.

Results

TLR4^{+/+} and TLR4^{-/-} diabetic mice exhibited increased blood glucose levels and urine spots compared with normoglycemic mice. The ratio between bladder and body weight was increased in diabetic TLR4^{+/+} mice (p<0.05), but unchanged in diabetic TLR4^{-/-} mice. Carbachol (CCh) and KCl produced concentration-dependent bladder strip contractions without genotype differences. Diabetes significantly increased maximal contraction to CCh (CCh: 1.26±0.08 vs. 2.28±0.19 mN/mg, p<0.05) and KCl (KCl: 1.24±0.08 vs. 2.46±0.19 mN/mg, p<0.05) in diabetic strips from TLR4^{+/+} mice, but not in TLR4^{-/-} strips (CCh: 1.26±0.09 vs. 1.27±0.14 mN/mg; KCl: 1.16±0.07 vs. 1.22±0.17 mN/mg). Similarly, contractions to EFS (1-32 Hz) were greater in bladder from diabetic +/+ than non-diabetic +/+ (p<0.05), whereas no significant alterations were observed between diabetic TLR4^{-/-} and non-diabetic TLR4^{-/-}. In separate assays, bladders from non-diabetic mice were incubated with LPS (500 µg/mL) for 60 min, after which functional assays were carried out. LPS caused a 2.3-fold increase in the CCh-induced contractions (p<0.05), which was partially blocked in TLR4^{-/-} mice. Expression of TLR4, TLR2, ERK1/2, JNK, Trif and NFκ-B(p65) were unchanged in diabetic mice, with no genotype differences. Moreover, TLR4 deletion significantly decreased by 70% and 45%, respectively, the MyD88 and p38MAPK activation in diabetic TLR4^{-/-} mice compared with diabetic TLR4^{+/+} (p<0.05).

Interpretation of results

Our data suggest that TLR4 is activated in urinary bladders from diabetic mice. Deletion of this receptor lessened diabetic bladder hypertrophy and normalized bladder contractility. Prior incubation with LPS (TLR4 endogenous ligand) increased CCh-induced contractions. The mechanism that mediates overactive bladder through TLR4 in diabetic mice involves MyD88 activation and p38MAPK phosphorylation.

Concluding message

Inflammation plays an important role in diabetic bladder overactivity. Inhibition of TLR4 decreases the downstream MyD88 and p38MAPK intracellular pathways, thus representing a good pharmacological target to treat urological symptoms associated with diabetes.

References

1. Carton JA, et al. (1992) Eur J Med.1(5):281-7.
2. Song J, et al. (2009) Proc Natl Acad Sci U S A. 106(35):14966-71.
3. Dasu MR, et al. (2012) Clin Sci (Lond). 122(5):203-14.

Disclosures

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