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ROLE OF EXTRACELLULAR CA2+ INFLUX THROUGH L-TYPE CA2+ CHANNELS (LVOCC) TO BLADDER DYSFUNCTION AND OVERACTIVE DETRUSOR SMOOTH MUSCLE IN OBESE MICE

Hypothesis / aims of study

Obesity and metabolic syndrome are common risk factors for the lower urinary tract symptoms (LUTS), including overactive bladder and stress or urge incontinence. There are, however, few experimental studies exploring the physiopathology of the bladder dysfunction resulting from metabolic syndrome. The present study was designed to investigate the *in vivo* (cystometry) and the *in vitro* functional alterations of detrusor smooth muscle (DSM) in high-fat fed mice. Recently, extracellular Ca^{2+} influx through L-type voltage operated Ca^{2+} channels (LVOCC) was shown to play a major role in bladder dysfunction and overactive detrusor in diabetic mice [1]. Therefore, the contribution of Ca^{2+} sensitization and of Ca^{2+} influx through L-VOCC to the bladder alterations in the high-fat fed mice was also investigated.

Study design, materials and methods

Four-week old male C57BL6/J mice were fed for 10 weeks with either a standard chow or a high-fat diet that induces obesity [2]. Insulin tolerance tests (ITT), oral glucose tolerance test (OGTT) and lipid profile analysis were measured. Detrusor smooth muscle (DSM) strips with intact urothelium were mounted in 10-mL organ baths containing Krebs-Henseleit solution. Concentration-response curves to the muscarinic agonist carbachol (1 nM to 30 µM) were constructed in DSM in the presence of the LVOCC blockers nifedipine or amlodipine. Contractile responses to α,β-methylene ATP (purinergic agonist), KCl and $CaCl_2$, as well as to electrical-field stimulation (EFS; 1-32 Hz) were carried out. Relaxant responses to the Rho-kinase inhibitor Y27632 in DSM were performed. Cystometric study was carried out in anesthetized mice (urethane; 1.8 g/kg) treated or not for three weeks with amlodipine. Messenger RNA expression of muscarinic M_3, M_2 and P2X1 receptors, as well as of L-VOCC was determined in DSM strips by real-time RT-PCR.

Results

High-fat fed mice exhibited significant increases in body weight, epididymal fat mass, total cholesterol and LDL levels, as well as insulin resistance and impaired glucose tolerance. Carbachol-induced contractions were significantly greater ($P<0.001$) in obese group compared with control group (E_{max}: 5.02 ± 0.89 and 1.83 ± 0.36 mN/mg, respectively; n=6-7). Contractile responses to KCl and extracellular $CaCl_2$ were also higher in obese mice (E_{max}: 2.98 ± 0.52 and 2.67 ± 0.39 mN/mg, respectively) compared with control group (E_{max}: 1.97 ± 0.28 and 1.66 ± 0.22 mN/mg, respectively; n=5-7). EFS produced frequency-dependent DSM contractions, which were higher in obese group at all frequencies employed. The relaxant responses to Y27632 did not significantly differ between obese and control groups. Prior DSM incubation with nifedipine (3 nM) or amlodipine (3 µM) restored the enhanced carbachol-induced DSM contractions in obese group to control levels (E_{max}: 2.06 ± 0.80 and 1.26 ± 0.24 mN/mg, respectively; $P<0.05$, n=5-7). In the cystometric study, control mice exhibited regular micturition cycles with rare non-void contractions, whilst obese mice exhibited an irregular micturition pattern and a significant increase in void frequency and non-void contractions. Three-week oral treatment with amlodipine (25mg/Kg/day) normalized the micturition frequency and non-void contractions in obese mice. Levels of mRNA expression for M_3 receptor and L-VOCC were significantly higher in DSM of obese mice, while M2 and $P2X_1$ remained unaltered.

Interpretation of results

Obese mice showed some features of metabolic syndrome such as dislypidemia, central obesity, impaired glucose tolerance and insulin resistance. Receptor-dependent and -independent DSM contractions were higher in obese mice, evidencing an overactive DSM in these animals. The cystometry revealed an increase in void frequency and non-void contractions in obese mice. Nifedipine and/or amlodipine treatments restored the overactive DSM in vitro and normalized the bladder dysfunction (cystometry) in obese mice, strongly suggesting that such alterations are the result of increased extracellular Ca^{2+} influx through L-VOCC. Extracellular Ca^{2+} influx through LVOCC coupled to muscarinic M_3 receptors has been shown to play a major role in bladder dysfunction and overactive detrusor in diabetic mice [1]. Since a common link between diabetes and obesity is the insulin resistance, it is likely therefore that bladder dysfunction in obese mice reflect a defective insulin action.

Concluding message

The results presented herein shows that high-fat diet induced obesity in mice displays bladder dysfunction and an overactive DSM as a result of increases in extracellular Ca^{2+} influx through LVOCC, which be may linked to enhanced expression and activity of muscarinic M_3 receptors and LVOCC.

References

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Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**The present work was approved by the Ethical Principles in Animal Research adopted by Brazilian College for Animal Experimentation (COBEA)**