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LONG-TERM NITRIC OXIDE (NO) DEFICIENCY INDUCES DETRUSOR OVERACTIVITY BY INCREASING EXTRACELLULAR CA2+ INFLUX THROUGH L-TYPE CA2+ CHANNELS

Hypothesis / aims of study

Nitric oxide (NO) has been recognized as an important neurotransmitter in the lower urinary tract. Previous studies have shown the presence of NO synthases (NOS) in urothelium and detrusor smooth muscle (DSM) in different animal species. Recently, long-term NO blockade has been shown to cause bladder dysfunction and detrusor overactivity in rats [1,2]. However, the mechanisms by which long-term NO deficiency lead to bladder dysfunction still needs further investigation. This study aimed to characterize the bladder dysfunction in mice treated chronically with the NOS inhibitor N^G-nitro-L-arginine methyl ester (L-NAME), focusing our attention to the urodynamic alterations, along with the changes in the contractile responses in isolated DSM. Since NO is reported to reduce extracellular Ca^{2+} influx through L-type voltage operated Ca^{2+} channels (LVOCC) via GMPc/PKG signaling pathway [3], we also investigated the effects of the LVOCC blocker amlodipine in the bladder dysfunction resulting from chronic NO blockade.

Study design, materials and methods

Ten-week old male C57BL6/J mice received the L-NAME (150 mg/kg/day) in drinking water for 3 weeks. DSM strips were prepared and mounted in 10-mL organ baths containing Krebs-Henseleit solution. Concentration-response curves to the muscarinic agonist carbachol (1 nM to 100 µM) were constructed in the absence and in the presence of amlodipine (3 µM). Cystometric study was carried out in anesthetized mice (urethane; 1.8 g/kg) treated or not with amlodipine (25 mg/kg/day), given in the drinking water together with L-NAME.

Results

Carbachol-induced DSM contractions were significantly higher in L-NAME treated group (E_{max}: 4.17 ± 0.61 mN/mg; P<0.001, n=6) compared with control group (1.78 ± 0.17 mN/mg). Pre-incubation with amlodipine prevented the increased carbachol-induced DSM contraction in L-NAME group (E_{max}: 1.05 ± 0.14 mN/mg; P<0.05, n=5). Chronic treatment with amlodipine also normalized the contractile responses to carbachol in the L-NAME-treated mice (E_{max}: 1.70 ± 0.36 mN/mg; P<0.05, n=6). In the cystometric study, control mice showed regular micturition cycles with rare non-void contractions. Mice treated with L-NAME exhibited an irregular micturition pattern accompanied by significant increases in void frequency and non-void contractions (0.72 ± 0.14 and 0.55 ± 0.12 contractions/min, respectively; P<0.05, n=6-7) in comparison with control group (0.14 ± 0.02 and 0.052 ± 0.02 contractions/min, respectively). Bladder capacity, threshold pressure, compliance and amplitude of void contractions were not modified by L-NAME treatment. Chronic treatment with amlodipine nearly restored the increased micturition frequency and non-void contractions in L-NAME group.

Interpretation of results

Chronic treatment with L-NAME results in bladder dysfunction and overactive DSM. Pretreatment with amlodipine (in vitro or chronically) prevented the increases in carbachol-induced DSM contractions in L-NAME-treated mice restoring it to control levels. The cystometric alterations observed in the L-NAME groups were also prevented by chronic treatment with amlodipine. Muscarinic M_3 receptors activation is the physiologically most important pathway to elicit contraction of the urinary bladder, and largely depends on Ca^{2+} entry through LVOCC. On the other hand, NOS inhibition activates LVOCC in afferent and efferent arterioles favoring the smooth muscle contraction [3]. Taken together, our data indicates that chronic NOS inhibition leads to LVOCC activating, causing subsequently an increase in the influx of extracellular Ca^{2+} into cells, and hence the urodynamic alterations.

Concluding message

Our findings show that long-term NO deficiency in C57BL6/J mice induces lower urinary tract dysfunction characterized by detrusor overactivity as a result of increased extracellular Ca^{2+} influx through L-type Ca^{2+} channels.

References

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