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EFFECT OF OXIDATIVE STRESS ON DETRUSOR CONTRACTILITY

Hypothesis / aims of study

Free radicals are produced in vivo even under normal circumstances. In a healthy state the production of radicals and the protection against radicals is balanced. In several pathophysiological conditions (e.g. ischemia-reperfusion) free radical production is enhanced and radicals may overwhelm the defence system, thus disturbing the well-preserved and delicate balance. Such a disbalance between radical formation and protection has been described as oxidative stress [1].

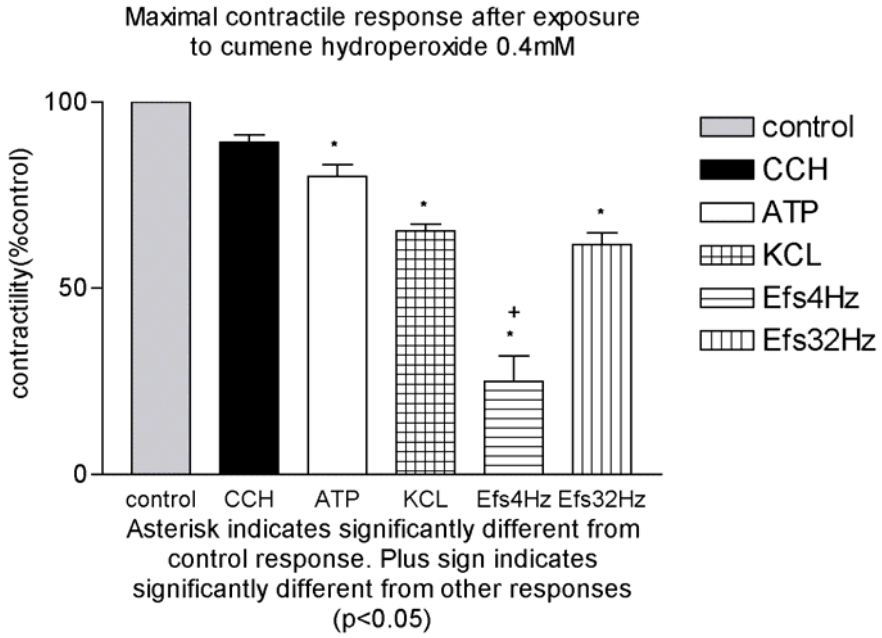
There is increasing evidence that ischemia/reperfusion is a major etiological factor in the progression of bladder dysfunction [2]. The combination of reperfusion and re-oxygenation generates reactive oxygen species that cause membrane injury such as protein oxidation and membrane lipid peroxidation. The consequence is progressive damage to nerve, synaptic and intracellular membranes. The aim of this study was to determine the effect of oxidative stress, induced by addition of cumene hydroperoxide [3], on smooth muscle contractility.

Study design, materials and methods

Pig detrusor strips were mounted in separate 6 ml organ baths containing Krebs-buffer solution which was aerated continuously with 5% CO_2 –95% O_2, at 37^{o}C. Mechanical responses were recorded using an isometric force transducer. Measurements were started after an equilibration period of 60 minutes with an initial tension of 2g. In a preliminary study the effect of tetrodotoxin (1µM) was evaluated on electrical field stimulation in order to determine the neurogenic component. After an initial equilibration time, one group of strips was stimulated with electrical field stimulation at different frequencies (4, 32 Hz) and potassium (60mM). Other strips were subjected to a carbachol concentration response curve (10^{-8}-10^{-5} M) and ATP (1mM) stimulus. Furthermore the detrusor strips were exposed to different concentrations of cumene hydroperoxide (final concentration 0.1, 0.4, 0.8mM) for 30 minutes. At the end of the period of oxidative stress all strips were washed 4 times with fresh buffer and after 20 minutes of recovery, responses to field stimulation (4, 32 Hz), potassium, ATP and carbachol were re-assessed. Differences between mean values were statistically analysed. Student's t-test and one factor ANOVA were used to determine the statistical significance to 0.05 levels.

Results

Electrical field stimulation was almost totally abolished by tetrodotoxin at 4Hz (95%) and partially abolished at 32 Hz (80%). Time controls and exposure to 0.1mM cumene hydroperoxide did not have a significant effect on smooth muscle contractility. The maximal contractile response to EFS (4, 32Hz), potassium, carbachol and ATP after exposure to cumene hydroperoxide (0.4mM) was 25% ($P<0.001$), 62% ($p<0.001$), 65% ($p<0.001$), 89% ($p>0.05$) and 80% ($P<0.01$) of the initial response respectively (figure). The contractile response to electrical field stimulation (4 Hz) was significantly reduced compared to the other responses (figure). Exposure to 0.8mM cumene hydroperoxide resulted in comparable effects as 0.4mM cumene hydroperoxide with an exception for the maximal response to carbachol that decreased significantly after exposure to this higher concentration ($p<0.05$).



Interpretation of results

In this in vitro study we have demonstrated that cumene hydroperoxide significantly reduces bladder smooth muscle contractility. The preliminary study with tetrodotoxin showed that a contraction induced by 32 Hz electrical stimulation was more tetrodotoxin resistant than the contraction caused by 4 Hz electrical stimulation. In the light of this result nerves are shown to be more sensitive to cumene hydroperoxide induced damage. This observation is in accordance with changes due to ischemia/reperfusion by partial outlet obstruction, which are characterized by a greater inhibition of nerve-mediated contractions than those induced by carbachol, ATP and KCL.

Concluding message

This study provides evidence that oxidative stress can induce bladder dysfunction. The involvement of oxidative stress in several pathophysiological bladder conditions needs further exploration in the future.

References

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