

THE LIPID PEROXIDATION PRODUCT 4-HYDROXYNONENAL CONTRIBUTES TO BLADDER SMOOTH MUSCLE DAMAGE.

Hypothesis / aims of study

Ischemia/reperfusion is hypothesised as an etiological factor in the progression of bladder dysfunction (1). Reperfusion and re-oxygenation generates reactive oxygen species that cause injury such as protein oxidation and membrane lipid peroxidation. Bladder outlet obstruction and acute urinary retention are related to an increased peroxidation of lipids (2, 3). Reactive aldehydes that are formed upon lipid peroxidation can contribute to tissue damage mediated by lipid peroxidation. One of the major aldehydes formed during the peroxidation of lipids is 4-hydroxynonenal (HNE). This study was designed to determine which component in the stimulation pathway (cholinergic nerves, membrane bound-tissue receptors and intracellular contractile mechanism) is most sensitive to HNE exposure.

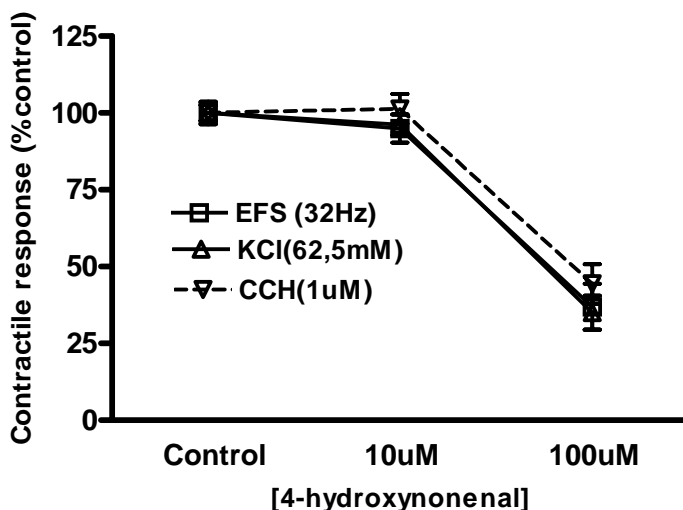
Study design, materials and methods

Experiments were performed on porcine detrusor muscle obtained from the abattoir. Detrusor strips were suspended in a separate 6 ml tissue bath containing Krebs-buffer solution which was aerated continuously with 5% CO₂ -95% O₂, at 37°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. The effects of tetrodotoxin (1µM) and atropine (1µM) were evaluated in combination with electrical field stimulation (EFS) in order to characterize contractile responses. Frequency response curves to EFS (4-32Hz), cumulative concentration response curves for carbachol (10⁻⁸-3.10⁻⁵ M) and potassium responses (65mM) were constructed before and after addition of HNE (10uM and 100uM, exposure:30min). Furthermore the effects of the sulfhydryl inactivator N-ethylmaleimide (NEM: 10uM and 100uM) on mentioned contractile responses were evaluated and compared with the HNE mediated effect. Differences between mean values were statistically analysed using analysis of variance followed by the Newman-Keuls test.

Results

Responses after EFS of the pig bladder strips were for the greater part based on neurogenic stimulation and the release of acetylcholine. When looking at the effect of HNE on different stimulation pathways with a similar degree of initial force development, we found that contractile responses to EFS (32Hz) and carbachol (1uM) were affected in a similar degree (figure). This suggests that HNE did not have an effect on the release of endogenous acetylcholine from postganglionic nerves at 32Hz of electrical stimulation. It is therefore concluded that the cholinergic nerves remain largely unaffected. The pD₂ value of carbachol in strips with HNE treatment did not decrease significantly compared to the time control value. Therefore it seems likely that HNE does not have an effect on postsynaptic muscarinic receptors but damages the membrane function (L-type calcium channels) and/or the contractile apparatus. KCl responses were affected in a similar degree by HNE compared to carbachol responses, which indeed indicates that the L-type calcium channels and or the intracellular contractile mechanisms are mainly affected by HNE (figure). Incubation of bladder strips with NEM had similar effects on pharmacological responses compared to HNE exposure.

Effect of 4-hydroxynonenal on various stimulation pathways with similar force developments.



Interpretation of results

L-type calcium channels and or the intracellular contractile mechanism of the bladder muscle seem to be susceptible to 4-hydroxynonenal mediated damage. The cholinergic nerves and the muscarinic receptors remain relatively unaffected. The effects of 4-hydroxynonenal are most likely mediated by alkylation of sulfhydryl groups.

Concluding message

This study provides evidence that 4-hydroxynonenal, the main reactive aldehyde formed upon lipid peroxidation, contributes to detrusor smooth muscle damage mainly located in the L-type calcium channels and/or the intracellular contractile mechanism.

References

1. Obstructive bladder dysfunction: morphological, biochemical and molecular changes. Eur Urol 2002 (suppl. 1): 14-20.
2. Detrusor mitochondrial lipid peroxidation and superoxide dismutase activity in partial outlet obstruction of rabbits. Neurorol Urodyn 2005; 24(3); 282-287.
3. Bladder dysfunction after acute urinary retention in rats. J Urol 2001; 165 (5); 1745-1747.

FUNDING: NONE

DISCLOSURES: NONE

ANIMAL SUBJECTS: This study did not follow the guidelines for care and use of laboratory animals because Animals (pigs) were obtained from the slaughterhouse. Ethical committee approval is not necessary in Maastricht when animals from slaughterhouse are used for experiments.