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INCREASED UPREGULATION OF BRADYKININ RESPONSE IN SMOOTH MUSCLE FROM URINARY BLADDER WITH INFRA-VESICAL OUTLET OBSTRUCTION

Aims of Study: Bradykinin has a contractile action on detrusor smooth muscle. The physiological role of bradykinin (BK) in urinary bladder function is however largely unknown. Two BK receptors have been found: B_1 and B_2. Under normal conditions the B_2 receptor seems to be the predominant in mediating the contractile response in urinary bladder.In vascular muscle the B_1-dependent contractile response is low or absent under normal conditions, but can be upregulated by prolonged incubation in vitro. Interleukin-1 has been proposed as a possible mediator of this increased response. It has recently been shown that several growth factors can activate oncgenes in cultured smooth muscle cells and increase the expression of BK-receptors.

The aim of the present study was to characterize the time-dependent upregulation of the contractile response to BK agonists in normal human detrusor muscle.We also compared the bradykinin response in control and hypertrophic rat bladder muscle in order to evaluate whether there was an increased expression of BK receptors in growing detrusor smooth muscle cells.

Methods: Detrusor muscle was obtained from patients undergoing cystectomy for cancer, control rats, and rats with bladder hypertrophy secondary to 10 days partial infravesical outlet obstruction. The preparations were mounted in organ baths and repeatedly contracted by high-K^+ solution. Cumulative dose-response relations (expressed relative to the high K^+ response) were determined for BK and [des-arg^9]-BK, (a B_1 selective agonist) at onset of the experiment, and at 4 hours.The influence on the BK response by the following drugs was tested: Indomethacin (inhibits cyclo-oxygenase), cycloheximide (inhibits protein synthesis), enalaprilate (inhibits BK degradation), L-NAME (inhibits NO synthase), tetrodotoxin (TTX, blocks nerve activity).

Results: BK and [des-arg^9]-BK induced dose-dependent (1 nM to 1μM) contractions in freshly isolated preparations from both human and normal rat detrusor muscle. Infravesical obstruction induced at 5-fold increase in rat bladder weight. In freshly isolated strips the BK-induced contractions were of similar amplitudes in control and hypertrophic muscle. After the 4 hour period the contractile responses to BK and [des-arg^9]-BK had increased slightly but significantly in the control human and rat bladder but had increased 6-fold in the hypertrophic muscle. The upregulated reponse was in all tissues abolished by indomethacin and decreased to 50% by cycloheximide. Enalaprilate, L-NAME, and TTX did not affect the upregulated response.

Conclusions: The present investigation shows that the contractile response to BK in vitro is upregulated in the hypertrophic smooth muscle of the rat

urinary bladder to a higher extent than in control rat and human detrusor muscle. The upregulated response was of the B_1-receptor type and seems to include an increase in both the generation of prostanoids along the cyclo-oxygenase pathway, and in a *de novo* syntesis of receptors or specific proteins involved in the activation pathway of the BK receptor.

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THE EFFECT OF NITRIC OXIDE IN ISCHEMIA-REPERFUSION RAT BLADDER

Aims of Study: Since there is an increasing evidence suggesting that nitric oxide (NO) plays important roles in ischemia-reperfusion injury in the bladder,[1,2] we evaluated the effect of NO inhibitors on ischemia-reperfusion injury in the rat utilizing muscle bath and continuous cystometory studies.

Methods: Rat abdominal aorta was clamped with a small clip to induce ischemia-reperfusion (I-R) injury in the rat bladder dome as previously reported.[1] Since our preliminary experiments revealed that 30 minutes reperfusion caused more severe damage than 5, 10 or 20 minutes reperfusion, rats in the I-R and I-R+NA, I-R+NM groups were exposed to 30 minutes and 7 days reperfusion.[1] In the I-R+NA, I-R+NM groups, L-NAME (30 mg/kg) and L-NMMA (30 mg/kg) were injected i.p. 30 minutes prior to the ischemia, respectively, since our previous report indicated that this dose was effective in causing a significant reduction in the histological damages induced by ischemia-reperfusion in the rat urinary bladder.[1,2] Furthermore, detrusor pressure during voiding (Pdet) and capacity of the bladder were evaluated with continuous cystometry (C.CMG, infusion speed 12.6 ml/hr) in control, 30 minutes ischemia and 30 minutes ischemia-30 minutes reperfusion rats with or without the treatment of L-NAME (30 mg/kg).[3]

Results: Contractile responses to carbachol of the bladder strips, C.CMG and bladder capacity are shown in the Table 1 and 2. The contractile responses of the rat bladder dome under 30 minutes ischemia differed slightly but not significantly from those of controls. Reperfusion (30 min) gave significant reduction in contractile response to carbachol in the rat bladder (40.5% of control group). The treatment with L-NAME (30 mg/kg) significantly prevented the injury of reperfusion (59.4% of control group). Seven days after the induction of ischemia-reperfusion, the contractile response to carbachol was significantly improved compared to 30 minuets reperfusion group. Treatment with 30 mg/kg of L-NAME and L-NMMA significantly increased the contractile response to carbachol compared to the I-R group without L-NAME or L-NMMA measured seven days after ischemia-reperfusion induction. In the C.CMG studies, 30 minutes ischemia significantly decreased the Pdet, and subsequent reperfusion slightly recovered the Pdet in the rat. The Pdet from 30 minutes ischemia-30 minutes reperfusion rats receiving treatment with L-NAME (30 mg/kg) returned to basel revel and, was significantly recovered compared to that from the 30 minutes ischemia rats.

Conclusion: 1) Ischemia induced by clamping of the rat abdominal aorta caused reduction in contractile responses to carbachol of the bladder dome, and subsequent reperfusion caused additional damage to smooth muscle judged by functional study; 2) In contrast to these findings, *in vivo* study Pdet in 30 minutes ischemia rats was