66

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Title: NOS INHIBITORS PREVENT APOPTOSIS INDUCED BY ISCHEMIA - REPERFUSION IN THE RAT BLADDER

Aims of Study:

Ischemia and then reperfusion of the bladder are observed in age-related disorders such as urinary retention, atherosclerosis, vasospasms, embolization, and thrombosis [1-2]. The mechanism of ischemia-reperfusion injury is complicated but is thought to be at least in part related to the free radical and arginine-nitric oxide (NO) system. We have demonstrated that peroxynitrite, reacting with NO and superoxide radicals, plays an important role as a cell/tissue-damaging agent during ischemia-reperfusion in the rat bladder. Recently, after overdistention, catheterization induces reperfusion injury in the bladder and reactive oxygen species are one of the main contributing factors in this injury [3-4]. In the present study, we evaluated the reversibility and preventive effects of N^G-nitro-L-arginine methylester (L-NAME), an NO synthase (NOS) inhibitor, and investigated apoptotic cells in ischemia-reperfusion injury in the rat urinary bladder.

Methods:

All animal experiments were performed in accordance with the guidelines set by the Tottori University Committee for Animal Experimentation. The rat abdominal aorta was clamped to induce ischemiareperfusion (I-R) injury in the rat bladder dome with or without L-NAME (30 mg/kg, i.p. 30 minutes prior to the ischemia) according to our previous reports [1-2]. Rats were exposed to 30 minutes of ischemia only and subsequent 30 minutes, 3 days and 7 days of reperfusion. Muscle bath studies to carbachol and 100 mM KCI were performed to confirm the bladder function in order to investigate the bladder function. TUNEL and H&E staining were performed in the experimental rat bladder in order to detect apoptosis in the bladder.

Result:

Data of our experiments are shown in the Tables. 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 and KCI in the rat bladder. The treatment with L-NAME significantly prevented the injury of reperfusion. Three and seven days after the induction of ischemia-reperfusion, the contractile responses were improved compared to 30 minutes reperfusion group. Treatment with L-NAME significantly increased the contractile responses compared to the l-R group without LNAME in each duration. Apoptosis was induced by ischemia-reperfusion, and the peak in TUNEL-positive cells was observed 3 days after the insult. The induction of apoptosis was prevented by treatment with LNAME.

Conclusion:

1) the ischemia Induced by clamping of tile rat abdominal aorta causes reduction in contractile responses of the bladder dome to carbachol, and subsequent reperfusion causes additional damage to smooth muscle as judged by a functional study;
2) the peak ratio of apoptosis in the bladder can be observed 3 days after the insult of 30 minutes of ischemia, an effect that can be significantly prevented by treatment with L-NAME;
3) ischemia-reperfusion injury can be prevented by treatment with NO inhibitors.

TABLE 1. FUNCTIONAL DATA OF ISCHEMIA-REPERFUSION RAT BLADDER

	Emax, gm/mm^2	ED_{50}, µm	KCl (100mM), gm/mm^2
Control	11.1 ±1.3	1.9±0.3	8.2±1.1
I	8.8 ± 0.7#	2.4 ± 0.3	1. ± 0.8#
I-R (30 min)	4.5 ± 0.6*	4.8 ± 0.9*	3.9 ±0.6*
+ L-NAME (30 mg/kg)	6.6 ± 0.4*	4.5 ± 0.9*	4.9 ± 0.3*
I-R (3 days)	7.0 ± 0.6*#	3.8 ± 0.9*	5.9 ± 0.6*#
+ L-NAME (30 mg/kg)	7.6 ± 0.4*#	3.5 ± 0.8*	6.9 ± 0.2*#
I-R (1 Week)	7.9 ± 0.7*#	0.9 ± 0.1#	6.8 ± 0.5*#
+ L-NAME (30 mg/ka)	9.0 ±0.8#+	1.3 ± 0.2#+	7.5 ± 0.3#+

*) significantly different from control group. #) significantly different from I-R (30 min) group. +) significantly different from I-R (30 min) + L-NAME group.

TABLE 2. TUNEL POSITIVE CELLS IN ISCHEMIA-REPERFUSION RAT BLADDER

	Ratio of TUNEL Dositive cells (%)
Control	0.08 ± 0 02
30 min. ischemia	
+ 30 min. reperfusion	0.61 ±0.15*
+ 3 days reperfusion	13.88 ±i 1.88*#
+ 7 days reperfusion	6.16 i± 1.17*#
30 min. ischemia (+ L-NAME 30mg/kg)	
+ 30 min. reperfusion	0.52 ± O.14*
+ 3 days reperfusion	6.79 ± 1.01*
+ 7 days reperfusion	3.53 ± 0.47*

* significantly different from control and each group in the same medical treatment. # significantly different from comparable values in L-NAME treated animals.

References

1. Saito M, Wada K, Kamisaki Y. Miyagawa I. 1998. Effect of ischemia-reperfusion on contractile function of rat urinary bladder: Possible role of nitric oxide. Life Sol 62: PL149-156.

2. Saito M, Miyagawa I. 1999. Direct detection of nitric oxide on rat urinary bladder during ischemiareperfusion. J Urol 162: 1490-1495.

3. Lin A T-L, Chen K-K, Yang CH, Chang LS. 2000. Mannitol facilitates rabbit urinary bladder recovery from overdistension injury. Urology 56: 702-707.

4. Saito M, Miyagawa I. 2001. Bladder dysfunction after acute urinary retention in the rat. J Urol In press.

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