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BENEFICIAL EFFECT OF PRECONDITIONING ON ISCHEMIA-REPERFUSION INJURY IN THE RAT BLADDER IN VIVO

Hypothesis / aims of study

Ischemia and the following reperfusion of the bladder are observed in age-related disorders such as urinary retention, atherosclerosis, vasospasm, embolization, and thrombosis (1). Ischemia-reperfusion injury may cause dysfunction of the urinary bladder, which results in instability and impairment of detrusor contractility during urination (2). Ischemic preconditioning (PC) is defined as brief, non-injurious ischemia-reperfusion (IR) periods that render a tissue more resistant to the harmful effects of a subsequent prolonged period of ischemia through endogenous cellular protective mechanisms. PC may play an important role in developing of bladder dysfunction caused by acute/chronic urinary retention and by ischemia-reperfusion. To our knowledge, however, limited information is available about the effect of PC in the bladder dysfunction. Lorenzi et al. reported the effect of preconditioning in guinea-pig *in vitro* (3). Identified mechanisms of ischemia-reperfusion include altered Ca^{2+} homeostasis, free radical formation, mitochondrial dysfunction, protease activation, altered gene expression, and inflammation. From these points, it is important to perform *in vivo* study to understand effect of PC on the bladder. In order to clarify the effect of PC on ischemia-reperfusion in the bladder, we investigated the role of PC on ischemia-reperfusion injury in the rat bladder *in vivo*.

Study design, materials and methods

Twelve-week-old male SD rats were divided into three groups; sham-operated control (Cont), 30 minutes ischemia-60 minutes reperfusion (IR) and three times of 5 minutes ischemia and then 30 minutes ischemia-60 minutes reperfusion (PC) groups. The bladder functions were estimated by cystometric and functional studies. Contractile response curves to increasing concentrations of carbachol were constructed in the absence and presence of various concentrations of subtype selective muscarinic antagonists, i.e. atropine (non-selective) pirenzepine (M1 selective), methoctramine (M2 selective), and 4-DAMP (M1/M3 selective). We also measured tissue levels of malonaldehyde (MDA) and examined possible histological changes in these rat's bladders.

Results

Preconditioning partially prevented the reduction of bladder dysfunction induced by ischemia-reperfusion. Estimation of the pA2 values for atropine, pirenzepine, methoctramine, and 4-DAMP indicate that the carbachol-induced contractile response in bladder dome is mediated through the M3 receptor subtype in all groups. The MDA concentration in the IR group was significantly larger than that of the control group, and preconditioning significantly reduced MDA production in the bladder. In histological studies, the ischemia-reperfusion with or without preconditioning caused infiltration of leukocytes and rupture of microcirculation in the regions of submucosa and smooth muscle without a corresponding sloughing of mucosal cells.

Table 1. cystometrogram data in the experimental rats

Group	Probability of urination	Pdet (cm H2O)	Bladder capacity (ml)	Residual urine (ml)
Cont	100 %	42.8 ± 2.3	0.70 ± 0.18	0.034 ± 0.010
IR	53.8 %	34.0 ± 2.1*	0.53 ± 0.10	0.233 ± 0.057*
PC	61.5 %	35.4 ± 2.9	0.59 ± 0.14	0.430 ± 0.105*

Data are shown as mean ± S.E.M. of five to nine separated determinations in each group. Pdet means maximum contraction pressure of the detrusor. *:significantly different from the Cont group.

Table 2. Functional studies in the experimental rats

Group	E_{max}/cross-sectional area (g/mm_2)	EC_{50} ($\times 10^{-6}$ M)	KCl/ cross-sectional area (g/mm_2)
Cont	2.25 ± 0.16	4.3 ± 0.5	1.50 ± 0.15
IR	1.61 ± 0.10*	3.1 ± 0.3*	1.04 ± 0.67*
PC	1.87 ± 0.17	2.0 ± 0.2**	1.09 ± 0.90*

Data are shown as mean ±S.E.M. of six to eight separated determinations in each group. E_{max} and ED_{50} values are for carbachol. KCl means contractile force to 100 m mol/l KCL. *: significantly different from the Cont group. **: significantly different from the other groups.

Table 3. MDA concentrations in experimental rat bladders

Group	MDA concentrations (n mol/mg protein)
Cont	3.87 ± 0.16
IR	4.72 ± 0.29*
PC	3.05 ± 0.24**

Data are shown as mean ±S.E.M. of six to eight separated determinations in each group. *: significantly different from the Cont group. **:significantly different from the other groups.

Interpretation of results

Our data indicated that ischemia-reperfusion produced significant damages of bladder function estimated by cystometric and functional studies. Treatment with three times of 5 minutes PC improved this injury. We also

demonstrated that one of these preventive mechanisms was to reduce the production of free radicals produced by ischemia-reperfusion in the bladder. As we suspected that ROS played an important role to prevent ischemia-reperfusion injury in the bladder, we measured the MDA, a marker of lipid peroxidation, concentrations in the experimental bladder. In the present study, the MDA concentrations in the bladder were significantly increased in the IR group. Treatment with PC significantly decreased MDA production by ischemia-reperfusion, and interestingly, the MDA concentration in the PC group was significantly lower than that of the Cont group. These data suggest that at least PC has an effect to reduce ROS production in the ischemia-reperfusion organs. As increases in lipid peroxidation can produce nerve and smooth muscle membrane damage, PC may associate with defensive mechanism that reduce lipid peroxidation. In the functional studies, contractile responses to carbachol and KCl were significantly decreased by ischemia-reperfusion, which was partially prevented by induction of PC. These observed decrease in contractile responses might indicate that ischemia-reperfusion injures or alters the muscarinic receptors on the bladder smooth muscle membrane and their second messenger system. Since we thought a possibility of alterations of these systems, we calculated the pA2 values and their slopes for a series of muscarinic antagonists in order to investigate affinity of receptors. In this study, there were no significant differences of the pA2 values and slopes between any groups in all muscarinic antagonists. These data indicated that alterations of contractile responses of bladder smooth muscles were due to quantitative rather than qualitative changes of muscarinic receptors and their second messenger system. However, roles of opening of surface K_{ATP} channels, regulation of fatty acid metabolism, nitric oxide production, regulation of the mitochondrial permeability transition and opening of K^+ channels in the mitochondrial inner membrane are not clear. In order to understand the precise mechanisms of PC, it is important to investigate these effects on the bladder.

Concluding message

Our data indicate that preconditioning has a beneficial effect on ischemia-reperfusion injury in the rat bladder.

References

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