Data are shown as mean ± S.E.M. of six to eight separated determinations in each group. E_max and E_D50 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

<table>
<thead>
<tr>
<th>Group</th>
<th>MDA concentrations (n mol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cont</td>
<td>3.87 ± 0.16</td>
</tr>
<tr>
<td>IR</td>
<td>4.72 ± 0.29*</td>
</tr>
<tr>
<td>PC</td>
<td>3.05 ± 0.24**</td>
</tr>
</tbody>
</table>

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 injury 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 KATP 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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ANIMAL SUBJECTS: This study followed the guidelines for care and use of laboratory animals and was approved by Tottori University Committee for Animal Experimentation #06S-13