CHARACTERIZATION OF ENDOTHELIN-1 RECEPTORS IN THE RAT BLADDER BY RADIOLIGAND BINDING ASSAY

Hypothesis / aims of study
Endothelin (ET)-1 is a 21 amino acid, endogenous vasoactive peptide that binds to two receptor subtypes, namely ET_A and ET_B receptor. ET-1 induces prolonged contractile responses in isolated bladder muscle strips in various species [1]. ET-like immunoreactivity was identified in detrusor smooth muscles, epithelium and vascular endothelium [2]. Selective ET_A receptor antagonists have ameliorating effects on various urinary dysfunctions including benign prostatic hyperplasia [3]. Based on these pharmacological results, the current study aimed to identify directly and characterize ET-1 receptors in the bladder by radioligand binding assay using [125]IET-1 as a selective radioligand of the receptor.

Study design, material and methods
Endothelin-1 receptors in the rat bladder homogenates were measured by the radioligand binding assay using [125]IET-1, and binding parameters of apparent dissociation constant (K_d) and maximal number of binding sites (B_max) for [125]IET-1 were estimated by nonlinear regression analysis using Graph Pad Prism. The competitive inhibitory effects of specific [125]IET-1 binding in the rat bladder were measured in the presence of various concentrations of ET-1 and its receptor antagonists (bosentan, ambrisentan and CI-1020). The IC_50 representing the molar concentration of agents necessary to displace 50% of specific [125]IET-1 binding was estimated. Specific [125]IET-1 binding was also comparatively measured in the cerebral cortex, lung, heart and kidney of rats.

Results
Specific binding of [125]IET-1 in the rat bladder homogenates was saturable and of high affinity (K_d=110 pM, B_max=272 fmol/mg protein), which characterized a selective labeling of bladder ET-1 receptors. High affinity of specific [125]IET-1 binding was also detected in the cerebral cortex, lung, heart and kidney of rats, and K_d and B_max values showed some difference among tissues (Table 1). ET-1 and bosentan (a mixed ET_A and ET_B receptor antagonist) at the concentrations of 0.3-100 nM inhibited specific [125]IET-1 binding in the rat bladder in a concentration-dependent manner (Fig. 1), and their IC_50 values were 5.75 and 8.41 nM, respectively. Similarly, ambrisentan (ET_A-selective antagonist) and CI-1020 (ET_A-selective antagonist) inhibited competitively the bladder [125]IET-1 binding (Fig. 1), with IC_50 values of 5.10 and 8.70 nM, respectively.

Interpretation of result
[125]IET-1 labels selectively ET-1 receptors in rat tissues such as the bladder. Selective antagonists of ET-1 receptors at pharmacological doses may bind to these ET-1 receptors, thereby suggesting some effects on the physiological functions of bladder.

Concluding message
It is concluded that there exists a significant amount of pharmacologically relevant ET-1 receptors in the rat bladder. Selective antagonists of ET-1 receptors may cause pharmacological effects on the bladder functions. To our knowledge, this study provides the first evidence for the direct identification of pharmacologically relevant ET-1 receptors in the bladder.

Table 1. K_d and B_max for specific [125]IET-1 binding in the bladder, cerebral cortex, lung, heart, and kidney of rats.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>K_d (pM)</th>
<th>B_max (fmol/mg protein)</th>
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<tbody>
<tr>
<td>Bladder</td>
<td>110 ± 32</td>
<td>272 ± 36</td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>78.4 ± 7.8</td>
<td>172 ± 8</td>
</tr>
<tr>
<td>Lung</td>
<td>1607 ± 936</td>
<td>3988 ± 1851</td>
</tr>
<tr>
<td>Heart</td>
<td>98.4 ± 14.8</td>
<td>132 ± 9</td>
</tr>
<tr>
<td>Kidney</td>
<td>814 ± 236</td>
<td>256 ± 50</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SEM for three to four experiments.
Fig. 1. Competitive inhibition by bosentan, ambrisentan, CI-1020 and ET-1 of specific $[^{125}\text{I}]$ET-1 binding in the bladder of rats. Each value represents the mean ± SEM for two to three experiments.

References
1. BJU Int 84: 714-719, 1999

Disclosures
Funding: None Clinical Trial: No Subjects: ANIMAL Species: Rat Ethics Committee: the guidelines for the care and use of laboratory animals of the University of Shizuoka.