544

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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, ensogenous 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 sepcies [1]. ET-like immunoreactivity was identified in detrusor smooth muscles, epithelium and vascular endothelimum [2]. Selective ET_A receptor antagonists has 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}I]ET-1 as a selective radioligand of the receptor.

Study design, materials and methods

Endothelin-1 receptors in the rat bladder homogenates were measured by the radioligand binding assay using [^{125}I]ET-1, and binding parameters of apparent dissociation constant (K_d) and maximal number of binding sites (B_{max}) for [^{125}I]ET-1 were estimated by nonlinear regression analysis using Graph Pad Prism. The competitive inhibitory effects of specific [^{125}I]ET-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}I]ET-1 binding was estimated. Specific [^{125}I]ET-1 binding was also comparatively measured in the cerebral cortex, lung, heart and kidney of rats

Results

Specific binding of [^{125}I]ET-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}I]ET-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}I]ET-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}I]ET-1 binding (Fig. 1), with IC_{50} values of 5.10 and 8.70 nM, respectively.

Interpretation of result

[^{125}I]ET-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}I]ET-1 binding in the bladder, cerebral cortex, lung, heart, and kidney of rats.

Tissues	K_d (pM)	B_{max} (fmol/mg protein)
Bladder	110 ± 32	272 ± 36
Cerebral cortex	78.4 ± 7.8	172 ± 8
Lung	1607 ± 936	3988 ± 1851
Heart	98.4 ± 14.8	132 ± 9
Kidney	814 ± 236	256 ± 50

Each value represents the mean ± SEM for three to four experiments.

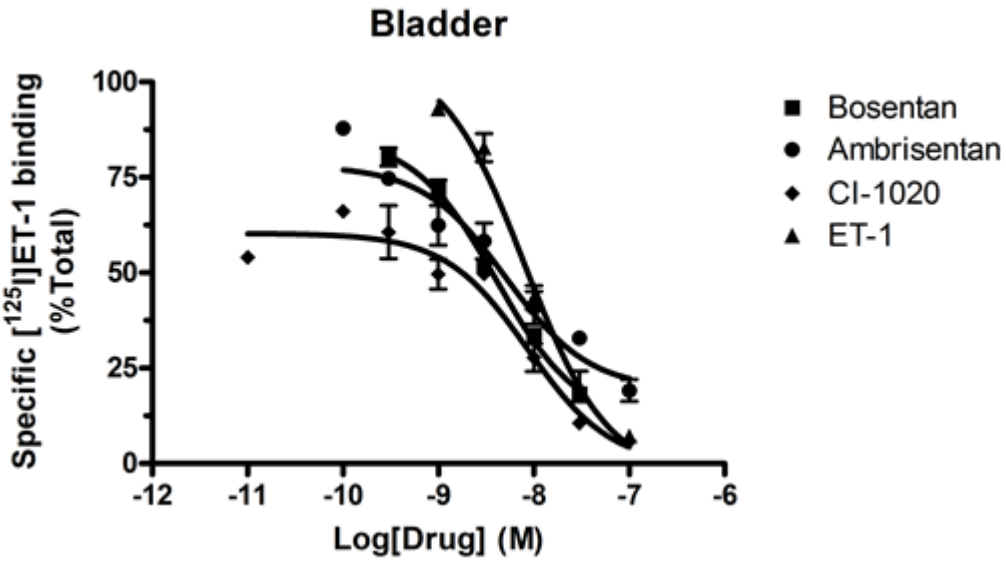

Fig. 1. Competitive inhibition by bosentan, ambrisentan, CI-1020 and ET-1 of specific [^{125}I]ET-1 binding in the bladder of rats. Each value represents the mean ± SEM for two to three experiments.

References

1. BJU Int 84: 714-719, 1999
2. J Urol 148: 1290-1298, 1992
3. Eur J Pharmacol 580: 394-400, 2008

Disclosures

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