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Yamada S[1], Osano A[1], Yokoyama Y[1], Ito Y[1]
1. *University of Shizuoka*

BLADDER ENDOTHELIN-1 RECEPTOR BINDING OF BOSENTAN AND AMBRISENTAN

Hypothesis / aims of study

Endothelin-1 (ET-1) was shown to induce prolonged contractile responses in isolated bladder muscle strips in various species [1]. ET-like immunoreactivity and [^{125}]ET-1 binding sites were identified in detrusor smooth muscles, epithelia, and vascular endothelium [2]. ET-1 is synthesized not only by smooth muscle cells in the urinary bladder, but also by epithelial cells and fibroblasts. Furthermore, the intravenous infusion of ET-1 was shown to cause a decrease in the mean micturition volume of rats in addition to an increase in mean arterial pressure [3]. Selective ET_A receptor antagonists have ameliorating effects on various urinary dysfunctions including benign prostatic hyperplasia. The protective effect of an endothelin converting enzyme inhibitor on rat detrusor function after outlet obstruction has also been demonstrated. These studies clearly indicate the existence of functional receptors for endothelins in the bladder, which could be a promising target for the treatment of lower urinary tract symptoms. Bladder endothelin receptors have been briefly identified. However, bladder ET-1 receptor binding of endothelin receptor antagonists used for the treatment of pulmonary arterial hypertension has not been examined in detail. Based on these pharmacological results, the present study aimed to directly identify ET-1 receptors in the rat bladder by a radioligand binding assay and characterize bladder receptor binding of the clinically used ET-1 receptor antagonists, bosentan and ambrisentan.

Study design, materials and methods

ET-1 receptors in the rat bladder were measured by a radioligand binding technique using a selective radioligand of the endothelin receptor, [^{125}I]ET-1 in the absence and presence of various concentrations (1 nM-100 □M) of ET-1, bosentan, ambrisentan, and CI-2010 *in vitro*. Their receptor binding affinities (IC_{50} values) were estimated. The effects of bosentan and ambrisentan on endothelin receptor binding parameters (apparent dissociation constant: K_d, maximal number of binding sites: B_{max}) were examined to elucidate the mode (competitive or noncompetitive) of antagonism by these agents. Also, endothelin receptor binding of bosentan was measured in the bladder of rats dissected periodically (1, 3, and 6 h) after the oral administration of vehicle or bosentan at two different doses of 100 and 300 mg/kg. The bladder tissue homogenate was incubated with various concentrations of [^{125}I]ET-1 (3-1000 pM) to estimate K_d and B_{max}.

Results

The competitive inhibition of specific [^{125}I]ET-1 binding was measured in the presence of ET-1 and its receptor antagonists. Specific binding of [^{125}I]ET-1 in rat bladder was saturable and of high affinity, which characterized selective labeling of bladder ET-1 receptors. ET-1, bosentan, ambrisentan, and CI-1020 inhibited specific [^{125}I]ET-1 binding in a concentration-dependent manner at nanomolar ranges of IC_{50} (Table 1). Nonlinear least squares regression analysis revealed the presence of high- and low-affinity ET-1 receptor sites for ambrisentan and CI-1020. Bosentan and ambrisentan significantly increased dissociation constant (K_d) for bladder [^{125}I]ET-1 binding without affecting maximal number of binding sites (B_{max}). Oral administration of bosentan at 100 and 300 mg/kg caused a significant (dose-dependent) decrease in B_{max} for bladder [^{125}I]ET-1 binding, without an effect on the K_d (Table 2). The significant decrease at the 100 mg/kg bosentan was seen at 1 and 3 h after the oral administration.

Interpretation of results

[^{125}I]ET-1 labels selectively bladder ET-1 receptors in rats. 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. Also, bosentan and ambrisentan was shown to bind bladder ET-1 receptor in competitive and reversible manner. Oral administration of bosentan caused a dose-dependent decrease in B_{max} for bladder [^{125}I]ET-1 binding, suggesting significant binding of bladder ET-1 receptors *in vivo*. A significant amount of pharmacologically relevant ET-1 receptors may exist in the bladder. These receptors may be implicated in the pathogenesis of lower urinary tract symptoms and may also be promising targets for the development of therapeutic agents.

Concluding message

The major findings of this study are that 1) a significant amount of high affinity receptors for ET-1 exists in the rat bladder and 2) bosenan and ambrisentan may bind to these ET-1 receptors, which suggests some effects on the physiological functions of the bladder.

Table 1. Inhibition of specific [^{125}I]ET-1 binding to the rat bladder by bosentan, ET-1, ambrisentan, and CI-1020. The pIC_{50} values and Hill coefficients were calculated. The pIC_{50} and relative proportions of high- and low-affinity binding sites of [^{125}I]ET-1 for ambrisentan and CI-1020 in the rat bladder were estimated. The values in parentheses represent the relative proportion ratio (%).

Drugs	Selectivity		pIC_{50}	nH
Bosentan	ET_A / ET_B		8.4±0.1	0.9±0.3
ET-1	ET_A / ET_B		8.1±0.1	1.1±0.3
Ambrisentan	ET_A	high	9.4±0.7 (47±10%)	-
		low	7.7±0.5 (53±9%)	-
CI-1020	ET_A	high	10.2±0.2 (58±9%)	-

	low	8.0±0.6 (42±11%)	-

The values represent the mean ± S.E. (n=4-8).

Table 2. Effects of the oral administration of bosentan (100, 300 mg/kg) on the K_d and B_{max} of specific [^{125}I]ET-1 binding in the rat bladder. Rats were orally administered bosentan at doses of 100 and 300 mg/kg, and the bladders were dissected at 1, 3, and 6 h for the [^{125}I]ET-1 binding assay. The values in parentheses represent relative ratio to control value.

Doses	Time (h)	K_d	B_{max}
		pM	*fmol/mg protein*
Control		164±16	255±29 (1.0)
Bosentan			
100 mg/kg	1	142±19	159±17 (0.62)*
	3	153±24	138±24 (0.54)*
	6	174±35	225±22
300 mg/kg	1	189±26	115±9 (0.45)*

Each value represents the mean ± S.E. (n=4-8). Asterisks show a significant difference from control values, *P<0.05.

References
1. 1 Br. J. Pharmacol. 96: 755-757, 1989
2. 2 Eur. J. Pharmacol. 387: 253-263, 2000
3. 3 Pharmacol. 69: 7-11, 2003

Disclosures
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