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Short communication

Role of endothelin ET_A and ET_B receptors in the guinea-pig urinary bladder contraction

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Abstract

The distribution and function of endothelin receptors in the guinea-pig urinary bladder were examined. Specific [^{125}I]endothelin-1 binding sites with both the endothelin ET_A and ET_B receptor subtypes were distributed in the muscle layer. Endothelin-1 elicited a tonic contraction which was inhibited by cyclo(D-Asp-Pro-D-Val-Leu-D-Trp) (BQ123) but not by *N-cis*-2,6-dimethylpiperidinocarbonyl-L-γ-methylleucyl-D-1-methoxycarbonyltryptophanyl-D-norleucine (BQ788) and which was inhibited more strongly by a combination of BQ123 and BQ788. Sarafotoxin S6c elicited a contraction which was abolished by BQ788. The concentration of endothelin-1 in the muscle layer was 707.0 ± 67.5 pg/g wet weight. Thus, endothelin-1 may regulate muscle tone via both subtypes of endothelin receptors in an autocrine manner in the guinea-pig urinary bladder.

Keywords: Endothelin ET_A receptor; Endothelin ET_B receptor; Urinary bladder; Receptor autoradiography

1. Introduction

The endothelins have a variety of biological activities both in cardiovascular and non-cardiovascular tissues, including the urinary tract (Sullivan et al., 2000). The action of ETs is mediated through at least two distinct subtypes of receptors, the endothelin ET_A and the endothelin ET_B receptors. Saenz de Tejada et al. (1992) demonstrated the synthesis of endothelin-1 in epithelia, smooth muscle, and fibroblasts in human and rabbit urinary bladder. Radioligand binding experiments showed the presence of endothelin ET_A and ET_B receptors in the rabbit (Traish et al., 1992) and the rat urinary bladder (Saito et al., 2000). Functional studies indicated that endothelin-1 produced a contraction mediated by the endothelin ET_A receptor but not by the endothelin ET_B receptor in the urinary bladder of these animals (Wada et al., 2000; Donoso et al., 1994). In the case of the guinea-pig urinary bladder, there are conflicting observations about the contractile effect of endothelin, with it causing weak contractile responses (Eglen et al., 1989) or no effect (Wiklund et al., 1989). The functional role of the endothelin ET_B receptor in the urinary bladder is unclear.

The present study was designed to clarify the distribution and the function of the endothelin receptors in the guinea-pig urinary bladder. We found that endothelin-1 produced a clear tonic contraction via both receptor subtypes, the endothelin ET_A and ET_B receptor, and that sarafotoxin S6c also induced a small but significant contraction, thereby indicating the contribution of the endothelin ET_B receptor as well as ET_A receptor to the contraction.

2. Materials and methods

2.1. Tissue preparation

Male guinea-pigs weighing 450–600 g were killed by cervical dislocation. Detrusor strips approximately 10 mm

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long, 2 mm wide were prepared from the dome of the urinary bladder.

2.2. Measurement of endothelin-1 concentration

Detrusor mucosa and muscle were weighed and the concentration of endothelin-1 was determined using an endothelin-1 Enzyme-linked immunosorbent assay kit (Wako, Japan). Results are expressed as pg/g wet weight.

2.3. Receptor autoradiography

The tissue was immediately frozen and 20-μm-thick sections were cut and thaw-mounted onto gelatin-coated slides, and stored overnight under vacuum at 4 °C. Sections were then incubated in vitro with [^{125}I]endothelin-1 (New England Nuclear, USA) according to our method, and the [^{125}I]endothelin-1 binding density in sections was quantified by using a computerized radioluminographic imaging–plate system with [^{125}I]standards ([^{125}I]microscales, Amersham, UK) (Sakurai-Yamashita et al., 1997).

2.4. Measurement of mechanical activity

The preparations without mucosa were placed in 20-ml organ baths containing Krebs-Ringer solution, which was continuously gassed with 95% O_2 and 5% CO_2 and maintained at 36–37 °C and pH 7.4. Tissues were allowed to equilibrate for 1 h under a resting tension of 1 g with bath solution being changed every 15 min. Mechanical responses were recorded by means of an isometric transducer (SD-1T; Nihon Kohden, Japan).

After equilibration, tissues were exposed to 40 mM KCl (replacing by Na^+) for four times at intervals of 30 min. The fourth response was used as a reference for the contraction induced by drugs. The response elicited by endothelin receptor agonists was examined by cumulative addition. To evaluate the effects of an endothelin ET_A receptor antagonist, BQ123, and an ET_B receptor antagonist, BQ788, the preparations were exposed to these drugs 20 min before the addition of agonist.

2.5. Data analysis

All data are presented as means ± S.E. Data were compared by using an analysis of variance (ANOVA) for multiple comparison with the Turkey–Klamer. A p value of 0.05 or less was considered statistically significant.

2.6. Chemicals

Substances used were as follows: endothelin-1 and sarafotoxin S6c were purchased from Peptide Institute, Osaka, Japan. Cyclo(D-Asp-Pro-D-Val-Leu-D-Trp) (BQ123) and *N-cis*-2,6-dimethylpiperidinocarbonyl-L-γ-methyl-leucyl-D-1-methoxycarbonyltryptophanyl-D-norleucine (BQ788) were a gift from Banyu Pharmaceutical Japan. Other chemicals used were of reagent grade.

3. Results

3.1. Concentration of endothelin-1

The concentration of endothelin-1 in the muscle layer and the mucosa of detrusor strips prepared from the dome of the guinea-pig urinary bladder was 707.0 ± 67.5 and 1367.7 ± 95.0 pg/g wet weight, respectively.

3.2. Receptor autoradiography

Fig. 1 shows typical autoradiograms of 10^{-10} M [^{125}I]endothelin-1 binding in the guinea-pig urinary bladder. The binding sites of [^{125}I]endothelin-1 were distributed in the muscle layer (Fig. 1A) and binding was abolished by the addition of nonlabelled endothelin-1 at 10^{-7} M (Fig. 1E). BQ123 at 2×10^{-7} M, a selective endothelin ET_A receptor antagonist, diminished the number of [^{125}I]endothelin-1 binding sites in the muscle layer by about 25% (indicated by an arrow in Fig. 1B), and 2×10^{-7} M of BQ788, the selective endothelin ET_B receptor antagonist, reduced binding in the muscle layer by about 61% (indicated by an arrow in Fig. 1C). Addition

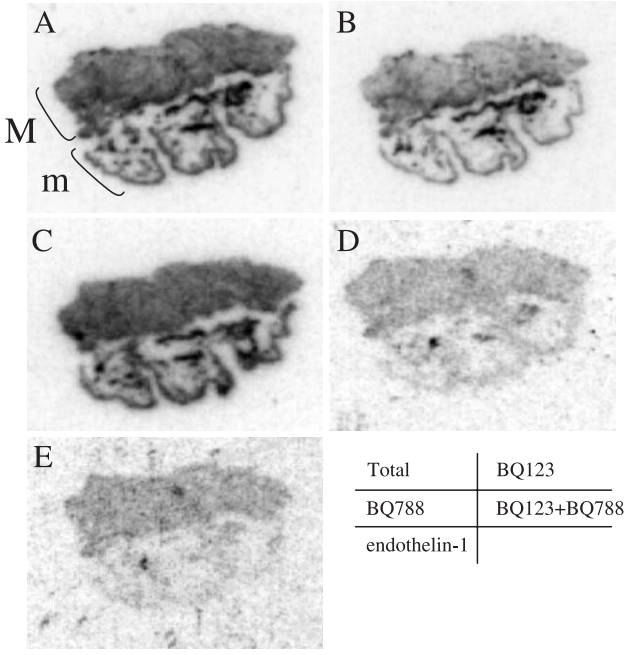

Fig. 1. Representative receptor autoradiograms of [^{125}I]endothelin-1 binding sites in the guinea-pig urinary bladder. Consecutive sections were labelled with 10^{-10} M [^{125}I]endothelin-1 in the absence (A, total binding) or presence of 2×10^{-7} M of BQ123 (B), 2×10^{-7} M of BQ788 (C), and both BQ123 and BQ788 (D) and nonlabelled 10^{-7} M endothelin-1 (E). "M" indicates the muscle layer and "m" indicates the mucosa. The specific binding sites were present in the muscle layer.

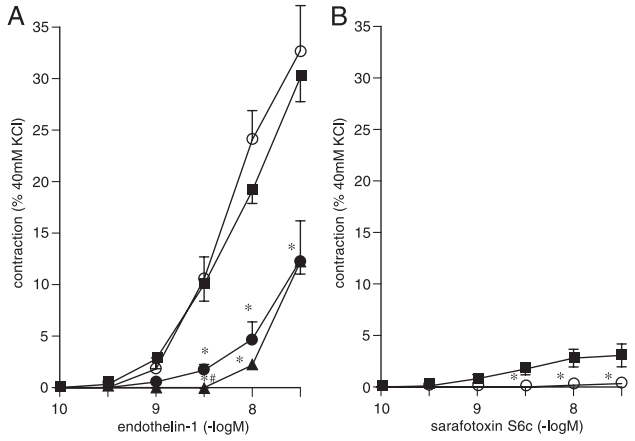

Fig. 2. Concentration–response curves of endothelin-1- and sarafotoxin S6c-induced contractions in the absence and presence of endothelin receptor antagonists. (A) Endothelin-1 was added cumulatively in the absence (■, $n=11$) or presence of 10^{-6} M BQ-123 (●, $n=4$), 10^{-6} M BQ788 (○, $n=4$) and combination of 10^{-6} M BQ123 and 10^{-6} M BQ788 (▲, $n=4$). (B) Sarafotoxin S6c was added cumulatively in the absence (■, $n=5$) and presence of 10^{-6} M BQ788 (○, $n=4$). Each point represents the mean ± S.E. of the contraction induced by endothelin-1 or sarafotoxin S6c relative to the contraction evoked by 40 mM KCl in each preparation. *Significantly different from the value in the absence of antagonist ($p<0.05$). #Significantly different from the value in the presence of BQ123 alone ($p<0.05$).

of both the antagonists almost abolished [^{125}I] endothelin-1 binding (Fig. 1D).

3.3. Effects of endothelin-1 and sarafotoxin S6c on basal tension

Fig. 2A shows the amplitude of the contraction induced by endothelin-1 relative to that induced by 40 mM KCl and the effects of antagonists on the contraction. Cumulative addition of endothelin-1 caused a concentration-dependent tonic contraction. BQ123 at 10^{-6} M significantly inhibited the endothelin-1-induced contraction and caused a rightward shift of the concentration–response curve. BQ788 at 10^{-6} M slightly reduced the contraction induced by endothelin-1. Combined treatment with BQ788 and BQ123 reduced more strongly the contraction induced by a low concentration (3×10^{-9} M) of endothelin-1 than did treatment with BQ123 alone. A selective agonist of the endothelin ET_B receptor, sarafotoxin S6c, produced a small but significant contraction at concentrations higher than 3×10^{-9} M, and the response to sarafotoxin S6c was completely inhibited by BQ788 at 10^{-6} M (Fig. 2B).

4. Discussion

The present study demonstrated that both endothelin ET_A and ET_B receptors are present in the muscle layer of the guinea-pig urinary bladder, and that endothelin-1 produces contraction via both receptors.

Addition of either BQ123 or BQ788 reduced the density of [^{125}I]endothelin-1 binding in the muscle layer, and unlabelled endothelin-1 or a combination of BQ123 and BQ788 abolished this binding. These results indicate the presence of specific endothelin ET_A and ET_B receptors in the muscle layer of the guinea-pig bladder. The presence of endothelin-1 in the muscle layer suggests that endothelin-1 may be involved in the regulation of muscle tension within an autocrine system.

Endothelin-1 caused contraction, and the contraction was significantly reduced by BQ123, but not by BQ788 alone. The different effect of BQ788 on the binding of [^{125}I]endothelin-1 and on the contraction elicited by endothelin-1 may be due to the concentration of endothelin-1 used, 10^{-10} M for binding and higher than 3×10^{-9} M for contraction, which induced a significant contraction. Since the combination of BQ123 and BQ788 reduced the contraction more than BQ123 alone did, the endothelin ET_B receptor may contribute to the contraction induced by endothelin-1. This concept was supported by the finding that the endothelin ET_B receptor agonist, sarafotoxin S6c, produced a small but significant contraction, and that the contraction was abolished by BQ788. Thus, the endothelin ET_B receptor as well as the ET_A receptor may play a role in the regulation of muscle tension in the guinea-pig urinary bladder.

The lack of antagonistic action of BQ788 alone on the contraction induced by endothelin-1 has been reported in various tissues where both subtypes of endothelin receptors are present. In the human bronchi, the contraction induced by endothelin-1 was not inhibited by BQ788 alone, but was inhibited by combined treatment with BQ123 and BQ788, or BQ928, a nonselective antagonist (Fukuroda et al., 1996). Himeno et al. (1998) suggested that heterodimerization of endothelin ET_A and ET_B receptors occurred in the rat pituitary, based on the finding that BQ788 inhibited [^{125}I]endothelin-1 binding only when BQ123 was present.

In the bladder of patients with benign prostatic hypertrophy, the density of endothelin receptors is reduced (Kondo et al., 1995). The expression of the endothelin ET_B receptor is up-regulated in diabetic rabbit urinary bladder (Mumtaz et al., 1999) and the expression of both endothelin ET_A and ET_B receptors is increased in rabbits with partial bladder outlet obstruction (Khan et al., 1999). These results suggest that endothelin receptors in the urinary bladder play a role in the pathophysiology of bladder hypertrophy due to bladder outlet obstruction and diabetic cystopathy.

In conclusion, endothelin-1 and both endothelin ET_A and ET_B receptors exist in the detrusor smooth muscle of the guinea-pig urinary bladder. Endothelin-1 plays a role in the regulation of muscle tension through an autocrine system via not only the endothelin ET_A receptor but also the endothelin ET_B receptor. A pathophysiological role of endothelin should be considered.

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