Contractile Effects of Endothelins on Isolated Human Ureter

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Summary

The aim of our study was to investigate mechanism of action of endothelins 1, 2 and 3 on spontaneous activity, tone and intraluminal pressure of human ureter. Both longitudinal tension and intraluminal pressure were recorded from the isolated segments of proximal human ureter. Endothelins 1, 2 and 3 (5.35×10^{-11} M - 5.05×10^{-8} M) produced concentration-dependent tonic contraction and sustained increase in intraluminal pressure of isolated preparations of human ureter. Endothelins 1 and 3 produced also concentration-dependent inhibition of spontaneous, phasic contractions of the isolated preparations. Selective antagonist of ET_A receptors BQ123 and selective antagonist of ET_B receptors BQ788 produced significant inhibition of endothelin-1-induced tonic contraction (pA_2=8.80 and 6.55, respectively) and increase in intraluminal pressure (pA_2=8.68 and 7.02, respectively), while they did not affect endothelin-1-induced inhibition of spontaneous activity. Endothelin 1 produces increase in tone and intraluminal pressure of isolated human ureter acting on both ET_A and ET_B receptors, the first one being functionally more important. Only endothelins 1 and 3 inhibit spontaneous, phasic activity of human ureter, but this effect was not blocked by selective antagonists of ET_A and ET_B receptors.

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Introduction

The three 21-amino-acid signaling peptides: endothelin-1 (ET-1), endothelin-2 (ET-2), and endothelin-3 (ET-3), produce contraction of smooth muscles through activation of two G protein-coupled receptor sub-types (ET-A and ET-B) (Brunner *et al.* 2006, Davenport and Maguire 2006, Polikepahad *et al.* 2006). ET_A receptors have higher affinities for ET-1 and ET-2 than ET-3, while ET_B receptors have the same affinities for ET-1, ET-2 and ET-3 (Huang 2005). Although endothelins primarily contract smooth muscles in blood vessels (Brunner *et al.* 2006), there are significant effects of these peptides on extravascular smooth muscles. Endothelins contract smooth muscles in esophagus, stomach, ileum, colon, gallbladder, common bile duct (Huang 2005), airways (Henry 1999), myometrium (Cameron *et al.* 1995) and Fallopian tubes (Jankovic *et al.* 2009).

Up to nowadays, it was shown in rabbit, human and canine urinary tract that ET-1 produces significant contractile responses of smooth muscle in bladder dome, trigone, and urethra (Langenstroer *et al.* 1997, Okamoto-Koizumi *et al.* 1999, Wada *et al.* 2000), and immunohistochemistry studies in dogs located ET-1 to epithelial elements of these organs (Langenstroer *et al.* 1997). ET_A receptors were found in rabbit and rat ureter by binding studies (Latifpour *et al.* 1995, Nakamura *et al.* 1997), and there are proofs that these receptors are upregulated in diabetes (Saito *et al.* 1999) and after ligation of ureter (Feldman *et al.* 2000). Although in a study on isolated human ureter contractile effect of

endothelin-1 was demonstrated as part of the experimental protocol (Iselin *et al.* 1998), effects of other two endothelins were not investigated, and receptors for endothelins in human ureter were not characterized, leaving us without clear picture about role of endothelins in regulation of human ureter motility.

The aim of our study was to investigate effects of endothelins 1, 2 and 3 on spontaneous activity, tone and intraluminal pressure of isolated segment of human ureter, and to analyze receptors through which the most potent of them exerts its action.

Materials and Methods

Patients

Segments of ureter were taken from 17 patients (7 male and 10 female) during nephrectomy. All patients underwent surgery because of renal cell carcinoma in stage T1N0M0. The mean age of the patients was 61.75 ± 10.12 years, with the range from 42 to 69 years. The study was approved by Ethics Committee of Clinical Center "Kragujevac", and the patient signed the informed consent forms.

All patients underwent surgery from 2004 to 2007 in the Urology and Nephrology Clinic of Clinical Centre "Kragujevac" in Kragujevac, Serbia. The operations were performed under general anesthesia.

Isolated preparations

The segment of ureter (6 cm long, starting 3 cm distal from pyeloureteral junction) taken from a patient in the operating room was transferred to the laboratory within the next 15 minutes, and there mounted in an isolated organ bath, according to modified method of Magnus (Magnus 1904). Distal end of the ureteral segment was tied at the bath bottom circumferentially, permitting entrance of a catheter in the lumen of the preparation; the catheter was used for measurement of intraluminal pressure. Proximal end of the ureteral segment was ligated, and hanged for isometric transducer.

The bath and the transducer

The isolated preparations were mounted in 75 ml isolated organ bath, filled with Krebs solution (in mM/l: NaCl 113.0, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ x $7H_2O$ 1.2, $NaHCO_3$ 25.0, KH_2PO_4 1.2 and glucose 11.6). The bath solution was maintained at 37 °C and aerated with 95 % O_2 and 5 % CO_2. The longitudinal tension and the intraluminal pressure of the isolated preparations were continuously recorded with the isometric transducer (Palmer Bio Science, Los Angeles, CA, USA) and the pressure transducer (Majk Electronic, Mladenovac, Serbia), and registered on personal computer using Majk Electronic interface and software (Majk Electronic, Mladenovac, Serbia). The isolated preparations were given a passive load of 10 mN, and intraluminal pressure was set at 0.4 kPa; it was then allowed to equilibrate for 1 h before an experiment started.

The agonists

The effects of experimental substances on area under the contraction curve were measured. The AUC for tonic contractions was measured from the horizontal line which extrapolated the basal tone line (recorded before an agonist was added to the isolated organ bath) to the right, up to the line recorded after the agonist was added to the isolated organ bath, which connects the dips of phasic contractions. The AUCs of phasic contractions recorded after each agonist dose were summarized, and divided with time (in minutes) elapsed until addition of the next dose of the agonist.

The experimental substances were added to the isolated organ bath cumulatively, without washing between the subsequent doses, from serosal side of the isolated preparations. The interval between two adjacent doses was always 5-6 minutes. After cumulating all doses of an agonist, the bath was washed three times, and the isolated preparation was allowed to rest for further 30 minutes. In separate experiments, the antagonists were added to the bath 20 minutes before the agonists. The effect of each experimental substance was observed on at least four isolated preparations, taken from different individuals.

Chemicals

In this study the following substances were used: endothelin 1 human (Sigma-Aldrich, St. Louis, USA), endothelin 2 human (Sigma-Aldrich, St. Louis, USA), endothelin 3 human (Sigma-Aldrich, St. Louis, USA), selective antagonist of ET_B receptors (Feldman *et al.* 2000) *cis*-2,6-dimethylpiperidinocarbonyl-*g*-methylleucyl -D-Trp(1-CO_2CH_3)-D-Nle-OH (BQ788) (Sigma-Aldrich, St. Louis, USA), and selective antagonist of ET_A receptors (Feldman *et al.* 2000) cyclo(D-Asp-L-Pro-D-Val-L-Leu-D-Trp-) (BQ123) (Sigma-Aldrich, St. Louis, USA) (Davenport 2002).

For the purpose of the study, endothelin 1, endothelin 2, endothelin 3 and BQ123 were dissolved in

distilled water, while BQ788 was dissolved in methanol.

Statistics

The concentration-response relationship was determined by linear regression on logarithmically transformed data calculated according to the method of least squares. The range of values used for the linear regression was from 15 % to 85 % of the maximal response, in the more linear part of the curve. The concentration of an agonist eliciting 50 % of the maximum response (EC_{50}) and its confidence limits (1.96 x standard error) were determined graphically for each concentration-response curve by linear interpolation (Bowman and Rand 1980, Kenakin 1984).

The results are expressed as means ± 95 % confidence intervals. Statistical differences between the EC_{50} of the control curve and EC_{50} in the presence of an antagonist were determined by Student's t-test for paired observations. A value of $p < 0.05$ was considered to be statistically significant. The extent of an antagonist effect was expressed by pA_2 value (-log molar concentration of antagonist reducing the agonist response by a factor of two), which was estimated from the Schild equation: ($EC_{50}e/EC_{50}c$) - 1 = $[B]/K_b$, where $EC_{50}e$ is EC_{50} for the agonist in the presence of the antagonist, $EC_{50}c$ is EC_{50} for the agonist in the absence of the antagonist, $[B]$ is concentration of the antagonist and K_b is dissociation constant of the antagonist, which is further mathematically transformed to pA2 (pA2 = -log K_b) (Jankovic *et al.* 1999).

Results

Spontaneous activity

The isolated preparations of human ureter exhibited spontaneous activity which was simultaneously recorded as increase in longitudinal tension and in intraluminal pressure. The frequency of the spontaneous activity was 1.22±0.57 per minute, with range from 0.55 to 2.68 per minute (n=12). The area under the curve of spontaneous increase in longitudinal tension was 213.33±173.64 mN/min (range from 3.60 to 499.80 mN/min; n=12). The area under the curve of spontaneous increase in intraluminal pressure was 9.69±12.02 kPa/min (range from 0.31 to 37.12 kPa/min; n=12).

The effects of endothelins on longitudinal tension of the isolated ureter

Endothelin-1 (from 5.35×10^{-11} M to 5.05×10^{-8} M)

produced concentration-dependent tonic contraction of the isolated ureter (EC_{50}=1.07±1.89x10^{-9} M; r=0.68; $p < 0.01$) (Figs 1A and 2A), and concentration-dependent inhibition of its spontaneous rhythmic contractions (EC_{50}=3.90±1.59x10^{-9} M; r=0.80; $p < 0.05$) (Fig. 1A). The maximal extent of endothelin-1 - induced tonic contraction was on average 994.46±470.48 mN, and maximal extent of endothelin-1 – induced inhibition of spontaneous rhythmic contractions was 99.4±1.8 %.

Endothelin-2 (from 5.0×10^{-11} M to 4.94×10^{-8} M) produced concentration-dependent tonic contraction of the isolated ureter (EC_{50}=4.99±1.84x10^{-9} M; r=0.88; $p < 0.001$) (Figs 1A and 2B). However, endothelin-2 (from 5.0×10^{-11} M to 4.94×10^{-8} M) did not influence spontaneous rhythmic contractions of the isolated ureter (F=0.83; r=0.16; $p > 0.05$) (Fig. 1B). The maximal extent of endothelin-2 - induced tonic contraction was on average 360.12±333.85 mN.

Endothelin-3 (from 5.0×10^{-11} M to 4.76×10^{-8} M) produced concentration-dependent tonic contraction of the isolated ureter (EC_{50}=3.90±12.34x10^{-9} M; r=0.65; $p < 0.05$) (Figs 1A and 2C), and concentration-dependent inhibition of its spontaneous rhythmic contractions (EC_{50}=3.05±0.31x10^{-8} M; r=0.67; $p < 0.01$) (Fig. 1C). The maximal extent of endothelin-3 - induced tonic contraction was on average 482.51±276.62 mN, and maximal extent of endothelin-3 – induced inhibition of spontaneous rhythmic contractions was 76.7±26.9 %.

The effects of endothelins on intraluminal pressure of the isolated ureter

Endothelin-1 (from 5.35×10^{-11} M to 5.05×10^{-8} M) produced concentration-dependent sustained increase in intraluminal pressure of the isolated ureter (EC_{50}=2.03±1.41x10^{-9} M; r=0.83; $p < 0.001$) (Fig. 1A), and concentration-dependent inhibition of its spontaneous rhythmic increases in intraluminal pressure (EC_{50}=5.50±1.65x10^{-9} M; r=0.80; $p < 0.01$) (Fig. 1A). The maximal extent of endothelin-1 - induced sustained increase in intraluminal pressure was on average 66.79±51.91 kPa, and maximal extent of endothelin-1-induced inhibition of spontaneous rhythmic increases in intraluminal pressure was 99.9±0.1 %.

Endothelin-3 (from 5.0×10^{-11} M to 4.76×10^{-8} M) produced concentration-dependent sustained increase in intraluminal pressure of the isolated ureter (EC_{50}=1.17±2.81x10^{-9} M; r=0.62; $p < 0.01$) (Fig. 1C), and concentration-dependent inhibition of spontaneous rhythmic increases in intraluminal pressure

($EC_{50}=3.74\pm3.19 \times 10^{-9}$ M; r=0.62; p<0.05) (Fig. 1C). The maximal extent of endothelin-3 - induced tonic contraction was on average 36.57±28.14 kPa, and maximal extent of endothelin-3 - induced inhibition of spontaneous rhythmic contractions was 96.7±6.7 %.
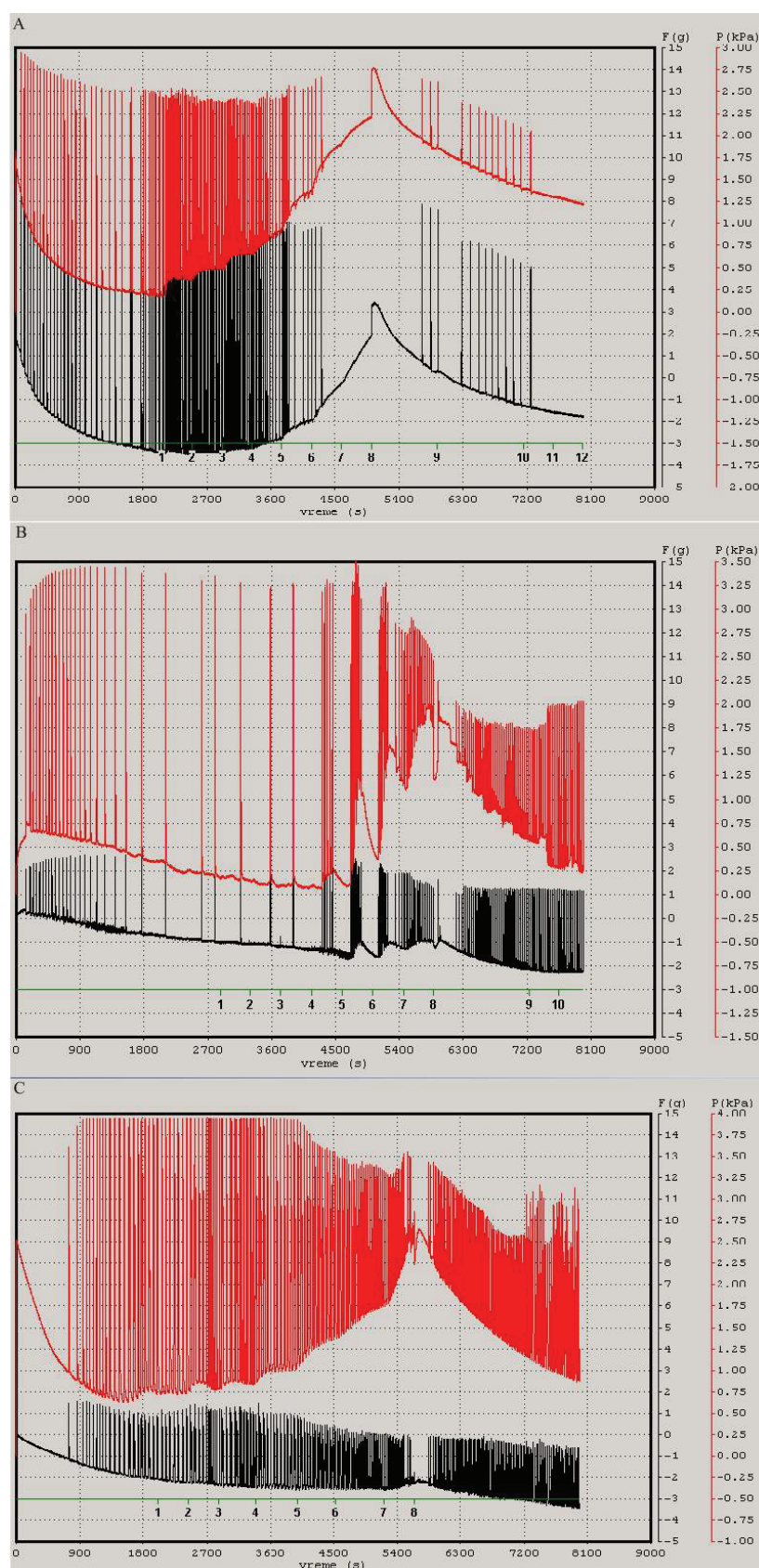

Fig. 1. Original tracings of effects of endothelin-1 **(A)**, endothelin-2 **(B)** and endothelin-3 **(C)** on isolated human ureter tone, intraluminal pressure and spontaneous activity. F(g) = force (grams); P(kPa) = intraluminal pressure (kilopascals) vreme (s) = time (seconds). Tracings in black − force of longitudinal contraction. Tracings in red − intraluminal pressure. **A** - Numbers from 1 to 7 indicate addition of subsequent doses of endothelin-1. Number 8 indicates the bath washing. Number 9 indicate addition of BQ123, and numbers 10, 11 and 12 addition of subsequent doses of endothelin-1. Note: continuation of this experiment with further doses of endothelin-1 in the presence of the antagonist BQ123 are recorded at other tracing. **B** - Numbers from 1 to 7 indicate addition of subsequent doses of endothelin-2. Number 8 indicates the bath washing. Numbers 9 and 10 indicate addition of two doses of endothelin-1. Note: continuation of this experiment with further doses of endothelin-1 is recorded at other tracing. **C** - Numbers from 1 to 7 indicate addition of subsequent doses of endothelin-3. Number 8 indicates the bath washing.
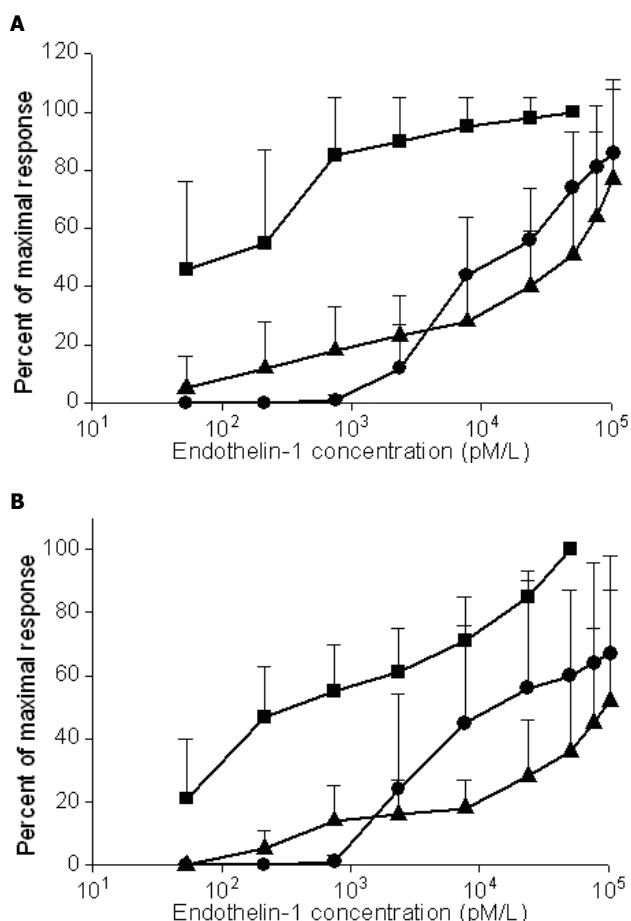
A

B

Fig. 2. Influence of selective antagonist of ET_A receptors BQ123 and selective antagonist of ET_B receptors BQ788 on endothelin-1 effects on longitudinal tone **(A)** and intraluminal pressure **(B)** of isolated segment of human ureter. Endothelin-1 (■), endothelin-1 + BQ788 (1.0 x 10^{-6} M) (●) and endothelin-1 + BQ123 (1.09 x 10^{-6} M) (▲). Each point is mean response of five to eight isolated preparations. Error bars: standard deviations around mean responses. One half of the error bars was omitted for the sake of clarity.

Influence of selective antagonist of ET_A receptors BQ123 and selective antagonist of ET_B receptors BQ788 on endothelin-1 effects on isolated human ureter

BQ123 (1.09x10^{-6} M) produced significant inhibition of endothelin-1 effect on tone (T-test=11.23; df=75; p<0.001; pA_2=8.80) (Fig. 2A) and of endothelin-1 effect on increase of intraluminal pressure (T-test=11.28; df=75; p<0.001; pA_2=8.68) (Fig. 2B). However, BQ123 (1.09x10^{-6} M) did not influence inhibitory effects of endothelin-1 on spontaneous rhythmic contractions (T-test=1.55; df=59; p>0.05) and on spontaneous rhythmic increases in intraluminal pressure (T-test=0.31; df=74; p>0.05) of isolated human ureter.

BQ788 (1.0x10^{-6} M) produced significant inhibition of endothelin-1 effect on tone (T-test=3.35; df=58; p<0.01; pA_2=6.55) (Fig. 2A) and of endothelin-1

effect on increase of intraluminal pressure (T-test=3.77; df=58; p<0.01; pA_2=7.02) (Fig. 2B). However, BQ788 (1.0x10^{-6} M) did not influence inhibitory effects of endothelin-1 on spontaneous rhythmic contractions (T-test=1.22; df=58; p>0.05) and on spontaneous rhythmic increases in intraluminal pressure (T-test=0.79; df=58; p>0.05) of isolated human ureter.

Methanol (62.5x10^{-3} M) did not influence effect of endothelin-1 on longitudinal tension (T-test=0.58; p>0.05) and on intraluminal pressure (T-test=0.34; p>0.05) of the isolated ureteral segment.

Discussion

Human ureter has smooth muscle layer with characteristics of a syncitium (Engelmann 1869, Bozler 1942), which easily transfers changes in membrane potentials from cell to cell. Spontaneously active cells in the renal pelvis generate electrical impulses, which are then conducted to ureteral smooth muscle cells. These impulses initiate contraction of the smooth muscle cells and formation of peristaltic waves which travel down the ureter and push urine to the bladder. Ureteral peristalsis is basically a myogenic process, which is then modulated by numerous nervous, endocrine and paracrine factors (Uehara and Burnstock 1970, Santicioli and Maggi 1998).

Endothelins are produced by different types of cells, including endothelial and epithelial cells, fibroblasts, macrophages, neurons (endothelin-1), intestinal epithelial cells (endothelin-2), and neurons and renal tubular cells (endothelin-3) (Pinet 2004). Endothelin-1 is involved in regulation of acid-base balance; its synthesis increases in renal tissue during metabolic acidosis, and it elevates expression of sodium/hydrogen (Na+/H+) transporter at luminal membrane of renal tubular cells (Laghmani *et al.* 2001, Peng *et al.* 2001), acting through ET_B receptors. However, precise expression of endothelins and their receptors in ureter remains mostly unresolved; previous studies have confirmed existence of endothelin-1 immunoreactivity at canine and rabbit ureter, but without more detailed insight into its location and possible function (Latifpour *et al.* 1995, Langenstroer *et al.* 1997, Nakamura *et al.* 1997, Saito *et al.* 1999). The only study on human ureters in patients with renal cell carcinoma has shown that endothelin-1 caused contraction of isolated ureters, but it remained unclear whether this contraction was mediated by ET_A, ET_B or non-ET_A/

non-ET_B receptors; the effects of both endothelin-2 and endothelin-3 were not elucidated (Takeda *et al.* 1994).

Our results show strong, concentration-dependent contractile action of endothelin-1, endothelin-2 and endothelin-3 on human ureter; the contraction is tonic and accompanied by sustained increase in intraluminal pressure, suggesting possible role of endothelins in development of renal-ureteral colic. With exception of endothelin-2, endothelins also inhibit spontaneous, phasic contractions of ureter, which normally make basis of ureteral peristalsis. This further strengthens the assumption about possible role of endothelins in renal-ureteral colic, since after initial hyperperistalsis, peristaltic waves disappear, and pain is constant, due to sustained increase of intraluminal pressure proximal to the site of ureteral obstruction by stone or other solid material (Travaglini *et al.* 2004).

In our study selective antagonist of ET_A receptors BQ123 and selective antagonist of ET_B receptors BQ788 produced significant inhibition of endothelin-1-induced tonic contraction and increase in intraluminal pressure, suggesting that both ET_A and ET_B receptors are involved in action of endothelins on ureteral smooth muscles. However, while pA_2 value of BQ123 in the ureteral tissue was in the same range as pA_2 values for the same antagonist observed in smooth muscles of Fallopian tubes (Jankovic *et al.* 2009, 2010), pA_2 value of BQ788 in ureter was much lower than the values obtained in that tissue (the magnitude of difference was more

than 2). Such results suggest functional predominance of ET_A receptors, although ET_B receptors are still important, and participate in the tissue response on endothelins. Endothelin-3 has higher affinity for ET_B than for ET_A receptors, and we have observed somewhat higher sensitivity of isolated human ureter to endothelin-3 than to other two endothelins (Fig. 1). Therefore, in order to completely abolish the effects of endothelin-1 on human ureter, dual blockade of ET_A and ET_B receptors is needed. Further research using different methods (binding studies, immunohistochemical studies and receptor expression studies) is necessary to characterize ET_A and ET_B receptors in more detail, and give complete insight in their physiological roles.

In conclusion, endothelins 1, 2 and 3 produce increase in tone and intraluminal pressure of isolated human ureter acting on both ET_A and ET_B receptors, the first one being functionally more important. Only endothelins 1 and 3 inhibit spontaneous, phasic activity of human ureter, but this effect is not mediated through conventional ET_A and ET_B receptors.

Conflict of Interest
There is no conflict of interest.

Acknowledgements

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