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Systemic and renal effects of an ET_A receptor subtype-specific antagonist in healthy subjects

[1,2,3]Leopold Schmetterer, [1]Susanne Dallinger, [1]Barbara Bobr, [1]Nicole Selenko, [1]Hans-Georg Eichler & [1]Michael Wolzt

[1]Department of Clinical Pharmacology and [2]Institute of Medical Physics, Währinger Gürtel 18-20, A-1090, Vienna, Austria

1 Endothelins (ETs) might play a pathophysiological role in a variety of vascular diseases. The aim of the present study was to characterize the effects of BQ-123, a specific ET_A receptor antagonist on systemic and renal haemodynamics in healthy subjects. This was done at baseline and during infusion of exogenous ET-1.

2 The study was performed in a balanced, randomized, placebo-controlled, double blind 4 way cross-over design in 10 healthy male subjects. Subjects received co-infusions of ET-1 (2.5 ng kg^{-1} min^{-1} for 120 min) or placebo and BQ-123 (15 μg min^{-1} for 60 min and subsequently 60 μg min^{-1} for 60 min) or placebo. Renal plasma flow (RPF) and glomerular filtration rate (GFR) were assessed by the *para*-aminohippurate (PAH) and the inulin plasma clearance method, respectively.

3 BQ-123 alone had no renal or systemic haemodynamic effect. ET-1 significantly reduced RPF (-24%, $P<0.001$) and GFR (-12%, $P=0.034$). These effects were abolished by co-infusion of either dose of BQ-123 (RPF: $P=0.0012$; GFR: $P=0.020$).

4 BQ-123 reversed the renal haemodynamic effects induced by exogenous ET-1 *in vivo*. This indicates that vasoconstriction in the kidney provoked by ET-1 is predominantly mediated by the ET_A receptor subtype.

Keywords: Endothelins; renal plasma flow; glomerular filtration rate; renal vascular disease; endothelin$_A$ receptors

Introduction

Endothelins (ETs) are a family of amino acid peptides, which have been shown to be potent vasoactive agents. Three members of the endothelin family have been identified so far: ET-1, ET-2 and ET-3 (Inoue *et al*., 1989). Two subtypes of receptors with different sensitivities to the three isoforms exist. The ET_A receptor, which is expressed on vascular smooth muscle, is characterized by its very high affinity to ET-1 and plays an important role in the pronounced vasoconstrictor effects of ET-1 (Arai *et al*., 1990). Two subtypes of ET_B receptor have been identified. The ET_{B1} receptor, which is present on endothelial cells has equal affinity for each endothelin isoform and mediates vasorelaxation through release of nitric oxide. The ET_{B2} receptor subtype has a high affinity for ET-3 and mediates direct vasoconstriction (Sokolovsky *et al*., 1992).

Endothelins are assumed to play a pathophysiological role in a variety of vascular diseases including hypertension, vasospasm, ischaemia, congestive heart failure, shock, atherosclerosis, diabetic retinopathy and nephropathy, acute and chronic renal failure, hepatorenal syndrome and stroke (Rubanyi & Polokoff, 1994). In man, bosentan, a nonpeptide, mixed ET_A and ET_B receptor antagonist, has been studied by several investigators. In healthy subjects intravenous infusion of bosentan reverses ET-1 induced reductions in skin blood flow (Weber *et al*., 1996). In patients with severe chronic heart failure, bosentan reduces mean arterial pressure, pulmonary artery pressure and right atrial pressure and increases cardiac index (Kiowski *et al*., 1995). However, specific ET_A receptor antagonists, such as BQ-123 may be more attractive in the treatment of vascular disease in man, as the vasoconstrictive action of ET-1 is mainly attributed to its action on the ET_A receptor subtype.

In man the effect of BQ-123 has already been investigated in the forearm (Haynes & Webb, 1994; Haynes, 1995). Brachial artery infusion of BQ-123 produced forearm vasodilatation, which indicates that endogenous production of ET-1 contributes to the basal vascular tone in man. Additionally, BQ-123 prevented the vasoconstriction to locally administered ET-1. Intravenous infusion of BQ-123 increased renal plasma flow and glomerular filtration rate in patients with hepatorenal syndrome, without affecting systemic blood pressure, heart rate or cardiac output (Soper *et al*., 1996). However, the haemodynamic actions of systemically administered BQ-123 have not yet been characterized in healthy subjects and little is known about the importance of ET receptor subtypes in the regulation of blood flow in man. We hypothesized that intravenous infusion of BQ-123 may decrease systemic blood pressure and increase renal plasma flow in healthy subjects by blocking the effect of basal ET-1 on vascular tone. In addition, we hypothesized that BQ-123 may prevent the systemic and renal haemodynamic effects of exogenous ET-1. To test these hypotheses we administered BQ-123 in the absence and presence of exogenous ET-1 to healthy subjects.

Methods

Subjects

After approval from the Ethics Committee of Vienna University School of Medicine was obtained, 10 healthy male subjects were studied (age range: 20–31 years, mean $\pm$ s.d.: 24.1 $\pm$ 3.0 years). The nature of the study was explained and all subjects gave written consent to participate.

Study design

The study was performed in a balanced, randomized, placebo-controlled, double blind 4 way cross-over design. After a 20 min resting period in a sitting position an infusion of *para*-

[3]Author for correspondence at: Department of Clinical Pharmacology, Währinger Gürtel 18-20, A-1090 Vienna, Austria

aminohippurate (PAH) and inulin was started. Fifty minutes after the start, baseline readings of renal haemodynamic parameters were obtained. Thereafter, the subjects received BQ-123 or placebo with co-infusion of ET-1 or placebo for 120 min. BQ-123 (Clinalfa AG, Läufelfingen, Switzerland) was infused intravenously at a dose of 15 μg min^{-1} for 60 min and subsequently at a dose of 60 μg min^{-1} for the next 60 min. Concomitantly, ET-1 (Clinalfa AG) was also infused intravenously in a dose of 2.5 ng kg^{-1} min^{-1} for 120 min. Physiological saline solution was infused as placebo. Every 30 min blood samples were drawn to determine PAH and inulin plasma concentration. This was continued until 240 min after the start of drug infusion.

Methods

Systemic haemodynamics Systolic and diastolic blood pressures were measured on the upper arm by an automated oscillometric device. Pulse rate was automatically recorded from a finger pulse-oxymetric device (HP-

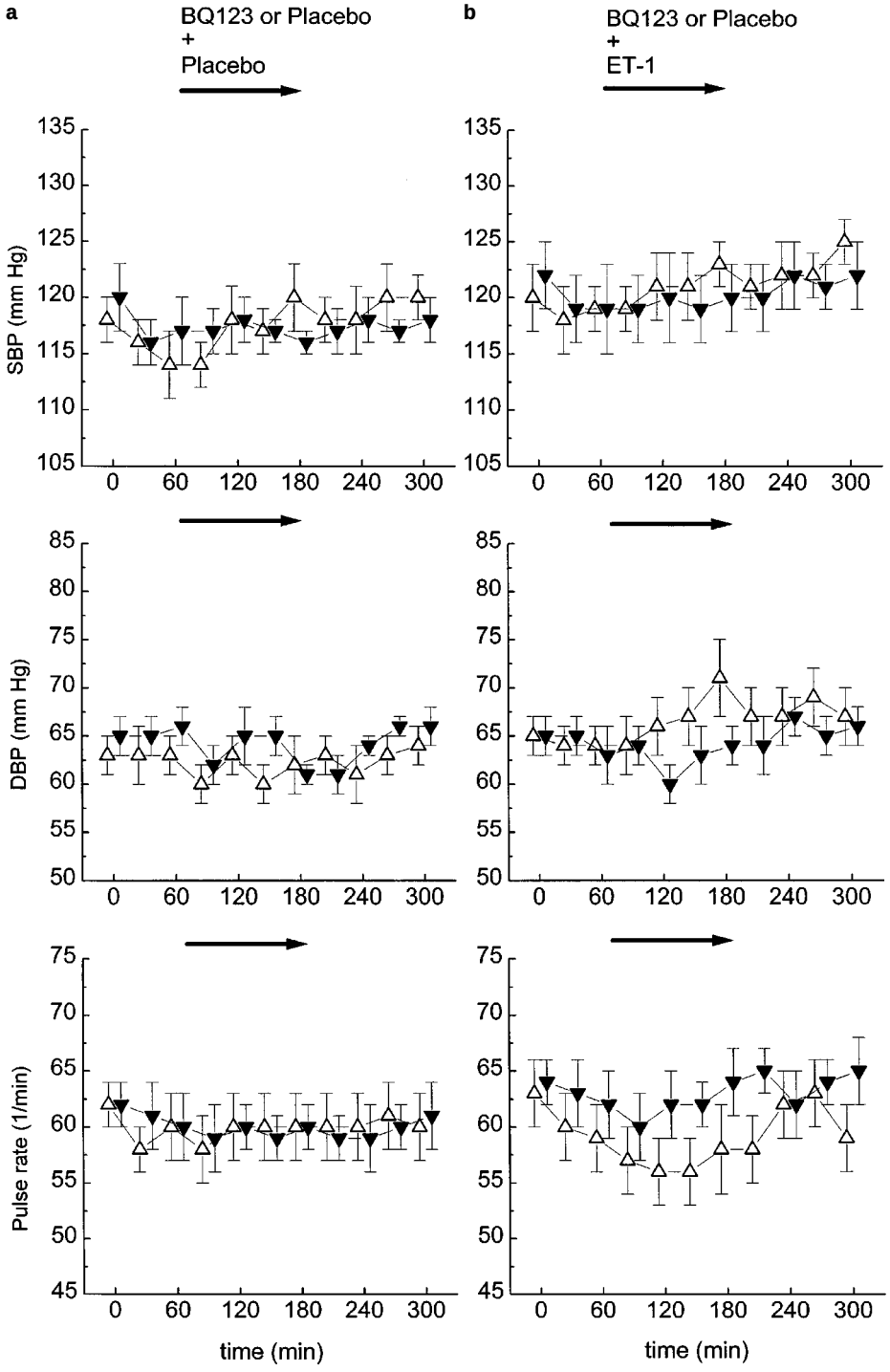

Figure 1 Systemic haemodynamic effects of BQ-123 (solid triangles, 15 μg min^{-1} for 60 min and subsequently 60 μg min^{-1} for 60 min) or placebo (open triangles, $n=10$). Effects were studied in the absence (a) or presence (b) of exogenous ET-1. Data are presented as means and vertical lines show s.e.mean. The arrow indicates the infusion period.

CMS patient monitor, Hewlett Packard, Palo Alto, CA, U.S.A.).

Renal plasma flow and glomerular filtration rate Renal plasma flow (RPF) and glomerular filtration rate were assessed by the PAH and the inulin plasma clearance method, respectively. PAH and inulin plasma concentrations were measured at baseline and at the scheduled time points during drug administration by photometric analysis (Schnurr *et al*., 1980).

Data analysis

Data are presented as mean ± s.e.mean. Haemodynamic effects of ET-1 were assessed with ANOVA *versus* placebo. The effect of BQ-123 alone (*versus* placebo) and BQ 123 in the presence of exogenous ET-1 (*versus* ET-1 alone) was also tested with ANOVA. *Post hoc* analysis was done with Wilcoxon signed rank test using Bonferroni's correction for multiple comparisons. A P value of <0.05 was considered significant.

Results

There were no significant differences between the baseline values on the four study days. BQ-123 did not affect systemic haemodynamics (Figure 1). ET-1 did not cause any significant changes in systolic, diastolic blood pressures or pulse rate.

BQ-123 alone did not alter renal haemodynamic parameters (Figure 2). ET-1 caused a significant reduction in RPF (-24%, $P<0.001$) and GFR (-12%, $P=0.034$). When BQ-123 was co-administered with ET-1 the effect of exogenous ET-1 on RPF and GFR was abolished. This antagonistic effect was significant *versus* ET-1 alone for RPF ($P=0.0012$) and GFR ($P=0.020$). Co-administration of BQ-123 and ET-1 did

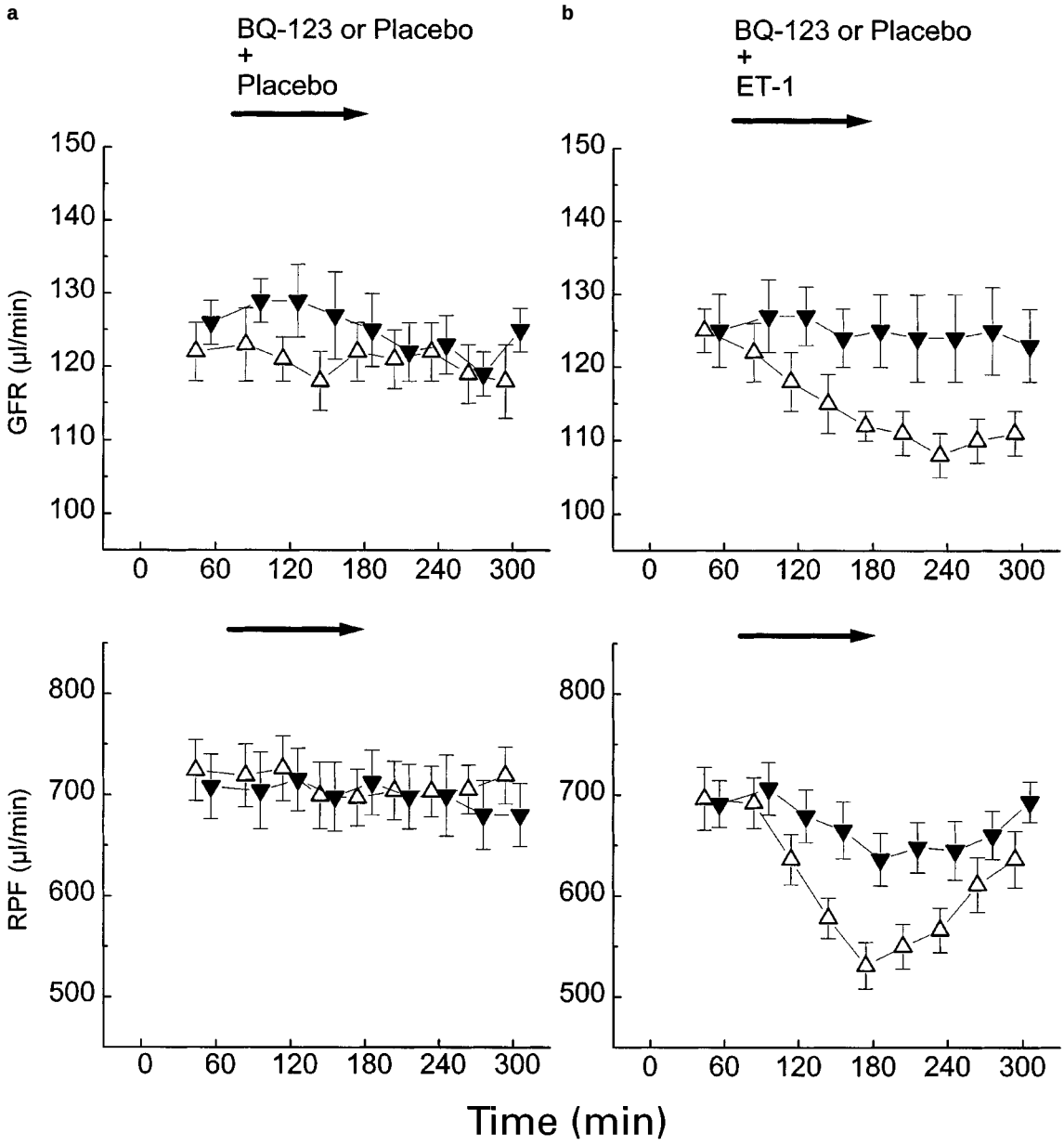

Figure 2 Renal haemodynamic effects of BQ-123 (solid triangles, 15 μg min^{-1} for 60 min and subsequently 60 μg min^{-1} for 60 min) or placebo (open triangles, $n=10$). Effects were studied in the absence (a) or presence (b) of exogenous ET-1. Data are presented as means and vertical lines represent s.e.mean. The arrow indicates the infusion period.

not exert a significant effect on RPF (−8%; NS *versus* baseline) or GFR (−1%; NS *versus* baseline).

Discussion and conclusions

In the present study an intravenous infusion of ET-1 at a dose of 2.5 ng kg^{-1} min^{-1} caused a significant reduction in RPF and GFR. This is in keeping with results from previous studies (Gasic *et al*., 1992; Rabelink *et al*., 1994; Schwartz Sorensen *et al*., 1994) and indicates that the renal vasculature is particularly sensitive to changes in local ET-concentration. By contrast, the effect of exogenous ET-1 on systemic haemodynamics was small, which also has been observed previously with comparable doses (Vierhapper *et al*., 1990; Gasic *et al*., 1992; Rabelink *et al*., 1994; Schwartz Sorensen *et al*., 1994; Schmetterer *et al*., 1997). However, the central finding of this study is that both doses of BQ-123 fully reversed the renal haemodynamic effects of ET-1 (Figure 2).

To our knowledge this is the first human study to demonstrate that ET-1 induced vasoconstriction in the kidney can be reversed by co-administration of a specific ET$_A$ receptor antagonist. We did not measure ET-1 plasma levels, but the plasma concentrations achieved by infusion of the dose selected for our study is likely to be in the range between 8.5 and 10 pmol l^{-1} (Vierhapper *et al*., 1990; Schwartz Sorensen *et al*., 1994). This is well above the physiological ET-1 plasma concentrations in healthy subjects, but in the same range as those observed in patients with hepatorenal syndrome and renal failure (Tomita *et al*., 1989; Moore *et al*., 1992). Hence the selected doses of BQ-123 may also be adequate to abolish the renal vasoconstrictor action provoked by pathologically increased ET-1 production. However, the haemodynamic effects of BQ-123 observed in the present study may differ from those in the above mentioned disease states, where counter-regulatory mechanisms co-exist. Our results are in keeping with the findings of Soper *et al*. (1996), who treated three patients with hepatorenal syndrome with short term infusions of BQ-123 at the same doses. In all these patients PAH and inulin clearance strongly increased. However, it is not yet clear whether circulating levels of ET-1 observed in hepatorenal syndrome and renal failure are directly causually linked to the disease or rather reflect circulatory imbalance.

Interestingly, specific blockade of the ET$_A$ receptor almost completely reversed the renal haemodynamic effects of exogenous ET-1 in our study. Hence, the vasoconstriction caused by ET-1 in the kidney seems to be mediated almost exclusively by the ET$_A$ receptor subtype. An effect of BQ-123 on ET$_B$ receptors in the present study is unlikely, as this compound has a more than 9000 fold selectivity for the ET$_A$ receptor over the ET$_B$ receptor *in vitro* (Haynes, 1995). However, in the human forearm both ET$_A$ and ET$_B$ receptors are likely to be involved in the vasoconstrictor response to ET-1 (Haynes, 1995). On the other hand our results are compatible with the findings of Allcock & Warner (1995) who found that BQ-123 attenuated the effect of ET-1 on blood pressure and peripheral resistance in the rat. In contrast, BQ788, a selective ET$_B$ receptor antagonist potentated the effects of ET-1 and PD145065, a nonselective ET receptor antagonist, had almost no effect on ET-1 induced haemodynamic changes.

In our experiments BQ-123 alone exerted no renal or systemic haemodynamic effects. This was unexpected, as previous studies have suggested that endogenous generation of ET-1 contributes to the maintenance of vascular tone, at least in the forearm resistance bed of normal subjects (Haynes & Webb, 1994; Haynes, 1995; Berrazueta *et al*., 1997). Our results do not support the hypothesis that this is also the case in the kidney. In addition, the lack of effect of BQ-123 on systemic haemodynamic parameters indicates that ET-1 does not play a major role in basal blood pressure regulation in healthy subjects. The difference in results obtained in the forearm studies and our experiments may well be caused by regional differences in ET receptor subtype densities. In addition, it is possible that we have missed a change in peripheral vascular resistance in relying solely on blood pressure and pulse rates as an index, because reflex changes may mask any effect.

Recently it has been speculated that the vasodilatation induced by BQ-123 in the human forearm is not solely caused by displacement of ET-1 from its receptor, but also by another unknown effect on vascular tone (Berrazueta *et al*., 1997). This hypothesis is based on the time course of the effects of BQ-123 in the human forearm (Berrazueta *et al*., 1997). Our experimental design with consecutive infusion of increasing doses of BQ-123 does not allow for characterization of the time course of the renal and systemic haemodynamic effects of BQ-123. However, as BQ-123 alone did not exert any significant effects, a haemodynamic action not connected to the ET-system is unlikely.

It is not apparent from the results of this study, which dose of BQ-123 is necessary to prevent ET-1-induced renal vasoconstriction. Due to the long-lasting pharmacodynamic effect of ET-1 in the kidney, our study was not designed to answer this question. Based on the results obtained during co-infusion of the lower dose of BQ-123 and exogenous ET-1 it may be speculated that 15 μg min^{-1} BQ-123 is probably appropriate to prevent ET-1-induced vasoconstriction *in vivo* and this dose seems favourable for future human studies.

In conclusion, we have shown that BQ-123 abolishes the renal haemodynamic effects induced by exogenous ET-1 *in vivo*. This indicates that vasoconstriction in the kidney provoked by ET-1 is predominantly mediated by the ET$_1$ receptor subtype. In addition, our results suggest a potential for a new therapy with ET$_A$ receptor subtype antagonists in renal diseases associated with increased ET-1 plasma levels.

ET, endothelin; GFR, glomerular filtration rate; RPF, renal plasma flow

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