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Systemic ETA receptor antagonism with BQ-123 blocks ET-1 induced forearm vasoconstriction and decreases peripheral vascular resistance in healthy men

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1 The effect on systemic haemodynamics of BQ-123, a selective endothelin A (ETA) receptor antagonist, was investigated in healthy men by giving, on separate occasions, ascending intravenous doses of 100, 300, 1000 and 3000 nmol min^{-1} BQ-123, each for 15 min, in a randomized, placebo-controlled, double-blind study. The response of forearm blood flow to brachial artery infusion of endothelin-1 (ET-1; 5 pmol min^{-1} for 90 min) was also studied using bilateral forearm plethysmography, after systemic pre-treatment, on separate occasions, with one of two doses of BQ-123 (300 and 1000 nmol min^{-1} for 15 min) or placebo.

2 Systemic BQ-123 dose-dependently decreased systemic vascular resistance ($P<0.01$ for all doses vs placebo) and mean arterial pressure ($P<0.05$ for 300 nmol min^{-1} and $P<0.01$ for 1000 and 3000 nmol min^{-1}) during the 60 min following infusion. There were concurrent increases in heart rate and cardiac index. BQ-123, when infused systemically for 15 min, appeared to reach a maximum effect at 1000 nmol min^{-1}.

3 Intra-brachial ET-1 infusion, after pre-treatment with placebo, caused a slow onset progressive forearm vasoconstriction without systemic effects. This vasoconstriction was attenuated by pre-treatment with BQ-123 at 300 nmol min^{-1} and abolished by BQ-123 at 1000 nmol min^{-1} ($P<0.01$ vs placebo).

4 These effects occurred at concentrations of BQ-123 in the plasma (510 ± 64 nmol l^{-1}) that were ETA receptor selective, and were not accompanied by an increase in plasma ET-1 that would have indicated ETB receptor blockade.

5 We conclude that ETA-mediated vascular tone contributes to the maintenance of basal systemic vascular resistance and blood pressure in healthy men.
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Abbreviations: ET, endothelin; FBF, forearm blood flow; IC_{50}, half-maximal inhibitory concentration

Introduction

Endothelin-1, which was first identified by Yanagisawa *et al.* (1988), is a well characterized, potent and sustained vasoconstrictor and pressor agent involved in the endothelium-mediated regulation of vascular tone (Haynes & Webb, 1998). Two ET receptor subtypes have been identified at a molecular level and characterized pharmacologically in blood vessels. ETA receptors (Arai *et al.*, 1990) have higher affinity for ET-1 than ET-3, are found on vascular smooth muscle cells, and mediate vasoconstriction. ETB receptors have equal affinity for ET-1 and ET-3 (Sakurai *et al.*, 1990) and are found on vascular endothelial cells, where they mediate endothelium dependent vasodilatation (De Nucci *et al.*, 1988; Tsukahara *et al.*, 1994). ETB receptors are also present on vascular smooth muscle cells, where they may contribute to vasoconstriction (Clozel *et al.*, 1992; Reizebos *et al.*, 1994; Seo *et al.*, 1994; Tschudi & Luscher, 1994).

Local studies in human forearm resistance vessels using phosphoramidon, an endothelin converting enzyme inhibitor, and BQ-123, a selective ETA receptor antagonist, first demonstrated the importance of ET-1 in maintaining basal resistance vessel tone, in large part through an action on the ETA receptor (Haynes & Webb, 1994). These observations have since been confirmed by others (Berrazueta *et al.*, 1997; Verhaar *et al.*, 1998). Responses in the forearm resistance vessels are usually predictive of those in the systemic circulation (Webb, 1995), so these data suggested that systemic ETA receptor antagonism would produce systemic vasodilatation. Recently, however, acute systemic administration of the selective ETA antagonist, BQ-123, was reported to have no effect on systemic haemodynamics (Schmetterer *et al.*, 1998; Montanari *et al.*, 2000).

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We hypothesized that a systemic haemodynamic effect of ETA receptor blockade was not seen in these studies because the doses of BQ-123 used provided insufficient ETA receptor blockade to affect blood pressure. In addition, because healthy subjects have a number of reflex mechanisms that serve to defend blood pressure, we hypothesized that an important effect on systemic vascular resistance might have been missed by measurement of blood pressure alone. We also hypothesized that systemically effective ETA antagonism with BQ-123 might be associated with inhibition of the vasoconstriction to exogenous doses of infused ET-1 sufficient to cause modest effects on forearm vascular resistance. We, therefore, undertook two studies. First, we assessed the haemodynamic effects of increasing doses of BQ-123, using bioimpedance cardiography, with the aim of achieving a high degree of ETA selective receptor blockade. Second, we examined whether haemodynamically active doses of BQ-123 would antagonize the response to exogenous ET-1, by infusion of local doses of ET-1 into the forearm circulation, after administration of BQ-123 systemically, and measuring responses using forearm plethysmography.

Methods

Subjects

Five healthy men (age range 18–30 years) were recruited to each of the two studies, which were performed in the Clinical Research Centre at the Western General Hospital, Edinburgh, with the approval of the local research ethics committee and the written informed consent of each subject. The investigations conformed to the principles outlined in the Declaration of Helsinki. No subject received vasoactive medication in the week before each phase of the study, and all subjects were asked to abstain from alcohol, nicotine and caffeine-containing products for 24 h and from food for at least 4 h before any measurements were made. All studies were performed from 08.30 h, in a quiet room kept at a controlled temperature (22–24°C).

Drugs

BQ-123 (Clinalfa AG, Laufelfingen, Switzerland, molecular weight 632.7) at doses ranging from 100–3000 nmol min^{-1} was used as a selective ETA receptor antagonist, with 2500 lower affinity for the ETB receptor (IC_{50} ETA of 7.3 nM against IC_{50} ETB of 18 μM) (Ihara *et al*., 1992). The dose range was selected from previous studies investigating the local effects of BQ-123, which suggested that 100 nmol min^{-1} is the threshold around which systemic effects might be observed (Haynes & Webb, 1994). BQ-123 was dissolved in physiological saline (0.9%, Baxter Healthcare Ltd, Thetford, U.K.). Saline was also used as placebo. BQ-123 and placebo were administered in a double-blind manner and infused intravenously at a constant rate of 1 ml min^{-1} for 15 min *via* an 18 standard wire gauge (SWG) cannula sited in the right antecubital fossa (Study 1) or right distal forearm (Study 2).

ET-1 (Clinalfa AG, molecular weight 2492) was dissolved in physiological saline to a concentration of 5 pmol min^{-1} (Haynes & Webb, 1994) and infused into the left brachial artery *via* a 27 SWG steel cannula at a constant rate of 1 ml min^{-1} for a total of 90 min.

All solutions were prepared from sterile stock solutions on the day of the study. For the forearm studies 1% lignocaine (Astra Pharmaceuticals, Stockholm, Sweden) was used as a local anaesthetic.

Measurements

Systemic haemodynamics Haemodynamic measurements were made at 10 min intervals from 30 min pre-dose until 60 min post-dose, then at 30 min intervals until 2 h, then hourly until 4 h post-dose. Blood pressure was recorded in duplicate at each time-point using a well-validated semi-automated non-invasive oscillometric sphygmomanometer (Takeda UA-751 sphygmomanometer, Takeda Medical Inc) (Wiinberg *et al*., 1988), in the right arm. Cardiac output (CO) and heart rate (HR) were recorded using a well validated non-invasive bioimpedance technique as previously described (NCCOM 3; BoMed Medical Manufacturer Ltd) (Goldstein *et al*., 1986).

Forearm blood flow Blood flow was measured in both forearms by venous occlusion plethysmography using mercury-in-silastic gauges (Benjamin *et al*., 1995; Webb, 1995) that were securely applied to the widest part of each forearm. The hands were excluded from the circulation during each measurement period by inflation of a wrist cuff to 220 mmHg. Upper arm cuffs were intermittently inflated to 40 mmHg for 10 in every 15 s to temporarily prevent venous outflow from the forearm and thus obtain plethysmographic recordings. Recordings of forearm blood flow were made repeatedly at 10 min intervals over 3-min periods. Voltage output from a dual-channel Vasculab SPG 16 strain gauge plethysmograph (Medasonics Inc) was transferred to a Macintosh personal computer (Performa 475, Apple Computer Inc, Cupertino, CA, U.S.A.) using a MacLab analogue digital converter and Chart software (v. 3.2.8; both from AD Instruments, Castle Hill, NSW, Australia). Calibration was achieved using the internal standard of the Vasculab plethysmography units.

Plasma ET-1, big ET-1 and BQ-123 During Study 1, 10 ml of venous blood was obtained from a cannula inserted in the left antecubital vein before and at 5, 15 and 240 min after BQ-123 infusion for measurement of plasma ET-1 and big ET-1. In addition, during the 300 and 1000 nmol min^{-1} phases, sub-aliquots of the samples were used for plasma BQ-123 assay. Samples were collected into sterile EDTA tubes (K3 EDTA, Vacutainer, Becton Dickinson) centrifuged at $2000 \times g$ for 20 min and stored in plain tubes at -80°C prior to assay. Plasma ET-1 and big ET-1 concentrations were determined by standard radioimmunoassay (Peninsula Laboratories), as previously described (Newby *et al*., 1998a).

BQ-123 assay BQ-123 concentrations in plasma were measured by high performance liquid chromatography (HPLC) with fluorescence detection. One volume of plasma was precipitated with 4 volumes of ethanol, ultracentrifuged at 4°C for 15 min at $10{,}000 \times g$, and the resulting supernatant injected into the HPLC column. The HPLC system consisted

of a Waters 510 HPLC pump, WISP (Waters Intelligent Sample Processor) and Spherisorb S5 ODS column (Waters Ltd, Watford, Herts. U.K.) with detection by an LS-5 fluorometric detector (Perkin-Elmer Ltd, Beaconsfield, Bucks, U.K.), with excitation and emission wavelengths of 284 and 348 nm respectively. The mobile phase consisted of 60 : 40 acetonitrile: de-ionized water with tri-fluoroacetic acid at a concentration of 0.1%. The peptide TAK-044 was found to fluoresce at identical wavelengths to BQ-123 and was eluted from the column with a retention time similar to but not identical with BQ-123, allowing its separate measurement. Hence, TAK-044 was used as a standard in this assay. Recovery of BQ-123 from plasma was found to be 107% and the intra- and inter-assay variations were 5.8 and 9.6% respectively.

Study design

Study 1: Systemic haemodynamic study This was a double-blind, placebo-controlled, balanced, 5-way crossover study in five subjects, investigating the responses to four doses of BQ-123 (100, 300, 1000 and 3000 nmol min^{-1}) and placebo (0.9% saline). An ascending dose regimen was followed, allowing safety and tolerability of lower doses to be assessed before proceeding. Total doses of BQ-123 administered were 1.5, 4.5, 15 and 45 μmol (or 0.95, 2.84, 9.5 and 28.4 mg). The order of the placebo dose was randomly allocated so that each subject received it on a different visit. Each visit was separated by at least 5 days. Subjects rested supine for 20 min before any haemodynamic measurements, and baseline measures were then made in the 30 min before study drug administration.

Study 2: ET-1 challenge study This was a double-blind, placebo-controlled, 3-way crossover study in five subjects (three of whom participated in the systemic study), investigating the effects of intra-arterial ET-1 on forearm blood flow (FBF), after treatment with either 300 or 1000 nmol min^{-1} of BQ-123 or placebo. After baseline infusion of saline for 30 min, subjects received a 15 min intravenous infusion of BQ-123 (300 or 1000 nmol min^{-1}) or placebo *via* a cannula in the right forearm, followed immediately by an intra-arterial infusion of ET-1 at a dose of 5 pmol min^{-1} for a total of 90 min *via* a left brachial artery cannula.

Data analysis

Study 1: Systemic haemodynamics Data were stored and analysed using the Microsoft Excel data analysis package (Excel 5.0, Microsoft Ltd). Blood pressure data at each time point were calculated as the mean of two recordings and represented as mean arterial pressure (MAP), calculated as diastolic BP + 1/3 pulse pressure. Bioimpedance data at each time point were calculated as the mean of four recordings. Data were corrected using body surface area to give cardiac index (CI) for direct comparison of subjects. Systemic vascular resistance index (SVRI) was calculated by dividing MAP by CI and expressed in arbitrary units. Baseline data were calculated as the mean of -10 and 0 min recordings. Haemodynamic data are expressed as placebo-corrected percentage change from baseline $\pm$ s.e.mean. Statistical analysis was performed on untransformed data. Responses were examined by repeated measures analysis of variance (ANOVA) and Bonferroni correction was applied to examine significance at each time point. Statistical significance was taken at the 5% level.

Using MAP & SVRI measurements from a previous placebo-controlled study over 4 h (Strachan *et al*., 1999), the study was calculated to have a power of $\sim$90% to detect a 15% change in MAP and 20% change in SVRI ($P = 0.05$) with five subjects. The number of subjects was agreed with the local ethics committee on that basis.

Study 2: ET-1 challenge Plethysmographic data listings were extracted from the chart data files and forearm blood flows calculated for individual venous occlusion cuff inflations using a template spreadsheet (Excel 4.0; Microsoft Ltd). As flow only stabilizes after 60 s of wrist cuff inflation, recordings made in the first 60 s were not used for analysis. The last five flow recordings in each measurement period were calculated and averaged for the infused and non-infused arms (Webb, 1995). To reduce the variability of blood flow data, the ratio of flows in the two arms was calculated for each time point, in effect using the non-infused arm as a contemporaneous control for the active treatment arm (Benjamin *et al*., 1995). Forearm blood flow results are shown as the percentage change from basal values in the ratio of blood flow between infused and non-infused arm. Data were examined by repeated measures analysis of variance (ANOVA) and Bonferroni correction was applied to examine significance at each time point. Statistical significance was taken at the 5% level.

From FBF measurements in a previous study using ET-1 at 5 pmol min^{-1} (Newby *et al*., 1998b), the study was calculated to have a power of 99% to detect abolition of the vasoconstriction response to ET-1 by BQ-123, and a power of $\sim$80% to detect a 66% attenuation of this response ($P = 0.05$) with five subjects.

Results

Study 1: Systemic haemodynamics

All five subjects (mean age 26 ± 2 years) completed all parts of the study. No adverse effects of treatment were reported.

Plasma ET-1 and big ET-1 Baseline values of plasma ET-1 and big ET-1 concentrations ranged from 4.4 to 5.2 pg ml^{-1} and 25 to 42 pg ml^{-1} respectively. There were no significant differences between baseline plasma ET-1 or big ET-1 concentrations in any phase of the study. Neither ET-1 nor big ET-1 changed significantly following treatment with any dose of BQ-123 or placebo (Table 1A,B).

Plasma BQ-123 concentrations Plasma concentrations of BQ-123 were undetectable with both doses at baseline. For 300 nmol min^{-1} BQ-123, mean plasma concentrations were 126 ± 11 nmol l^{-1} at 5 min rising to 174 ± 20 nmol l^{-1} at 15 min. For 1000 nmol min^{-1} BQ-123, they were 424 ± 33 nmol l^{-1} and 510 ± 64 nmol l^{-1} respectively (ETA receptor IC_{50} 7.3 nM; ETB receptor IC_{50} 18 μM) (Ihara *et al*.,

Table 1 (A) Plasma ET-1 (pg ml^{-1}) levels at baseline, 5, 15 and 240 min following infusion of BQ-123. (B) Plasma big ET-1 (pg ml^{-1}) levels at baseline, 5, 15 and 240 min following infusion of BQ-123

BQ-123 (nmol min^{-1})	*Baseline* (pg ml^{-1})	*5 min* (pg ml^{-1})	*15 min* (pg ml^{-1})	*240 min* (pg ml^{-1})
(A)				
Placebo	4.91±0.69	5.16±0.74	5.81±0.50	5.77±0.75
100	4.57±0.48	4.08±0.39	3.95±0.65	4.44±0.29
300	5.23±0.52	5.29±0.49	5.49±0.95	5.88±0.65
1000	4.43±0.28	6.07±0.49	5.56±0.95	5.80±0.65
3000	2.88±0.10	4.08±0.49	4.03±0.63	4.22±0.41
(B)				
Placebo	45.7±14.4	49.3±10.7	66.2±14.4	38.8±7.1
100	37.8±7.4	29.2±1.9	26.0±2.4	35.8±5.2
300	40.2±6.5	38.5±6.3	49.5±14.1	48.4±6.0
1000	57.5±9.1	51.9±8.5	51.4±9.4	51.5±9.2
3000	26.6±6.7	34.7±0.6	30.7±3.1	30.1±4.6

Table 2 Baseline data – absolute values

BQ-123 (nmol min^{-1})	*MAP* (mmHg)	*SVRI*	*CI* (L min^{-1} m^{-2})	*HR* (b.p.m.)
Placebo	78.9±1.5	22.7±1.3	3.52±0.21	64.2±6.6
100	79.2±2.3	21.9±1.7	3.69±0.25	57.1±5.2
300	80.8±3.5	21.8±1.8	3.80±0.31	63.9±6.6
1000	78.2±2.1	22.5±2.9	3.63±0.38	54.5±4.8
3000	78.6±3.3	23.5±2.2	3.45±0.35	57.3±5.9
ANOVA				
Baseline data	$P=0.96$	$P=0.98$	$P=0.89$	$P=0.69$

1992). BQ-123 was no longer detectable in the plasma by 4 h at either dose.

Haemodynamic parameters Baseline measurements for haemodynamic parameters were similar during all treatment periods (Table 2). After BQ-123 administration, changes were apparent in all parameters by the first measurement at 10 min. Maximal changes occurred between 40 and 60 min, with a prolonged effect occurring at the two highest doses, excepting changes of heart rate, which were maximal at 15 min.

MAP decreased in a dose-dependent fashion. This was statistically significant at 300, 1000 and 3000 nmol min^{-1} BQ-123 (300 nmol min^{-1}: ANOVA $P<0.05$ vs placebo, 1000 and 3000 nmol min^{-1}: $P<0.01$ vs placebo) with a maximum mean placebo-corrected reduction of 12.4±3.5% after 3000 nmol min^{-1}. Placebo corrected SVRI also decreased in a dose dependent fashion. This decrease was significant for all doses of BQ-123 (ANOVA $P<0.01$ vs placebo). The maximum decrease in SVRI (23.3±4.3%) occurred with 3000 nmol min^{-1} of BQ-123 (Table 3 and Figure 1).

CI and HR increased significantly at all doses ($P<0.01$; ANOVA, Table 3 and Figure 1).

Study 2: ET-1 challenge

Subjects who received placebo followed by local infusion of ET-1 developed a slow onset progressive vasoconstriction in the infused arm compared to the non-infused arm (maximum reduction in FBF: −48±10% at 90 min). This response was attenuated by 300 nmol min^{-1} BQ-123 (−27±8% at 90 min $P>0.5$ vs placebo) and abolished by 1000 nmol min^{-1} BQ-123 (−8%±3%, $P<0.01$ vs placebo, Figure 2).

Discussion

We have shown, in healthy humans, that BQ-123 causes substantial systemic vasodilatation, associated with a small but significant reduction in arterial blood pressure. A dose-dependent effect was observed, with little additional effect occurring above 1000 nmol min^{-1}. This work confirms the importance of the endothelin system, and of the vascular ETA receptor in controlling vascular tone and blood pressure (Haynes & Webb, 1998), and is in accord with the results of previous local forearm infusion studies (Haynes & Webb, 1994; Berrazueta *et al*., 1997; Verhaar *et al*., 1998).

We have several reasons for concluding that the effects on vascular tone and blood pressure are mediated by the ETA receptor. Measured BQ-123 concentrations in plasma at both 300 and 1000 nmol min^{-1} were substantially greater than the IC_{50} for BQ-123 at the ETA receptor. Even so, at 1000 nmol min^{-1} the plasma concentration of 510 nmol l^{-1}, was more than 35 fold lower than the IC_{50} for the ETB receptor (18 μM), consistent with effective but selective ETA receptor blockade (Ihara *et al*., 1992). In addition, there is a substantial body of evidence that the ETB receptor is a clearance receptor for ET-1 (Fukuroda *et al*., 1994; Ozaki *et al*., 1995; Dupuis *et al*., 1996) and that agents that block the ETB receptor *in vivo* cause increases in plasma ET-1 concentrations (Haynes *et al*., 1996; Weber *et al*., 1996; Sutsch *et al*., 1998; Strachan *et al*., 1999). In contrast, in this

Table 3 Haemodynamic changes after BA-123 administration

BQ-123 (nmol min^{-1})	*MAP* (mmHg)	*SVRI*	*CI* (L min^{-1} m^{-2})	*HR* (b.p.m.)
100	−4.8±2.6%	−15.8±7.6%	14.3±8.9%	23.8±13.4%†
	(−4.0±2.5)	(−3.9±2.1)	(0.49±0.32)	(12.1±6.5)
300	−6.8±3.6%	−20.6±3.0%*	13.0±2.2%*	11.8±4.4%
	(−5.4±2.8)	(−4.5±0.7)	(0.47±0.07)	(6.8±2.4)
1000	−8.2±3.1%†	−22.7±5.2%†	17.9±5.7%†	20.0±5.9%†
	(−6.4±2.4)	(−5.4±1.9)	(0.59±0.16)	(11.6±3.0)
3000	−12.4±3.5%†	−23.3±4.3%†	12.7±1.6%	18.7±6.8%*
	(−10.1±3.2)	(−5.6±2.6)	(0.43±0.04)	(10.7±3.1)

Results given are maximum placebo corrected percentage change from baseline±s.e.mean. *$P>0.05$ vs placebo, †$P>0.01$ vs placebo: ANOVA+Bonferroni correction.

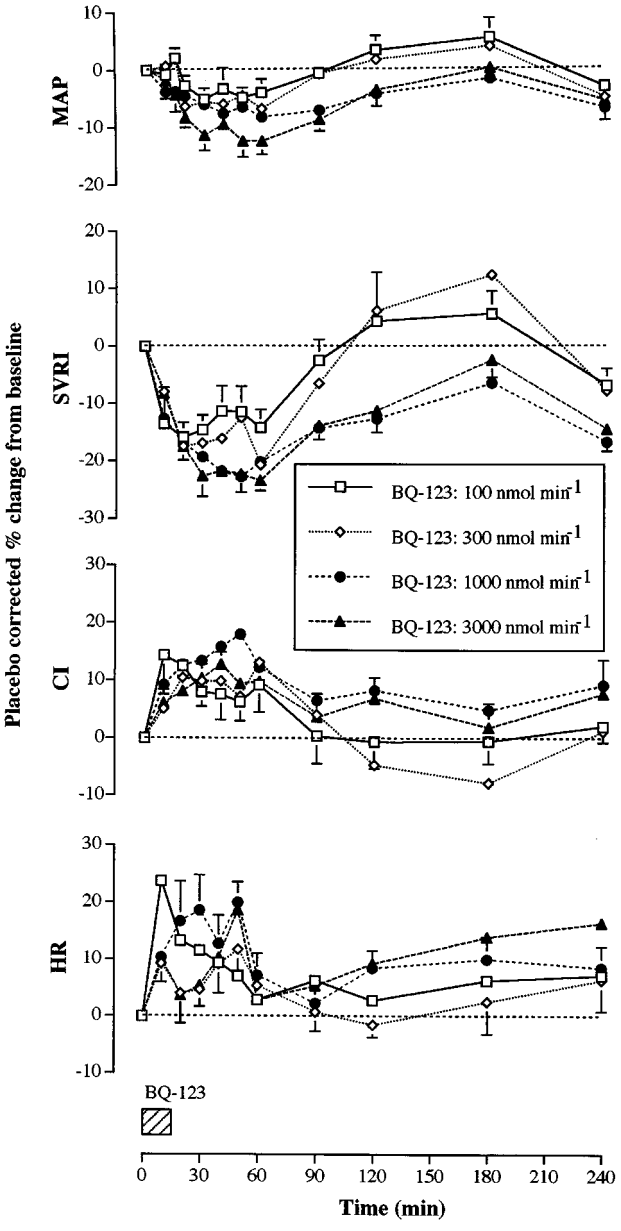

Figure 1 Change in MAP against time for ascending doses of BQ-123; change in SVRI against time for ascending doses of BQ-123; change in CI against time for ascending doses of BQ-123; change in HR against time for ascending doses of BQ-123.

study, there was no significant increase in either big ET or ET-1 plasma concentration at any dose of BQ-123 (Table 1A,B). Finally, we have previously shown that the net effect of systemic ETB receptor blockade is to cause systemic vasoconstriction, and therefore, we would anticipate that any ETB blockade would attenuate the vasodilatation associated with ETA blockade. Indeed, this effect may contribute to the lack of further vasodilatation at the highest dose of BQ-123, which was associated with a tendency for a rise in plasma ET-1 concentration, also consistent with a threshold effect on the ETB receptor at this dose.

Doses sufficient to lower blood pressure (300 and 1000 nmol min^{-1}) also antagonized the forearm vasoconstriction to brachial artery administration of ET-1 and, in keeping with its submaximal effect on SVRI, the lower dose of BQ-123 (300 nmol min^{-1}) only partially antagonized forearm vasoconstriction to ET-1. Of note, however, in the presence of a higher degree of ETA blockade, exogenous ET-1 failed to produce vasodilatation. This possibly reflects the local balance of dilator and constrictor effects mediated by endothelial and vascular smooth muscle ETB receptors. However it is also possible that the locally administered ET-1 was washed out by an increase in forearm blood flow consequent upon the vasodilatation induced by systemic BQ-123. Comparison with a constrictor agent unaffected by ETA antagonism would be needed to clarify this further.

Previous studies in healthy men (Schmetterer *et al*., 1998; Montanari *et al*., 2000) failed to demonstrate a significant effect of BQ-123 on basal haemodynamics. However, the doses used were substantially lower, at 23.7 nmol min^{-1} for 60 min, followed by 94.8 nmol min^{-1} for 60 min (Schmetterer *et al*., 1998) and ~9 nmol min^{-1} for 90 min (Montanari *et al*., 2000). These should be compared with 100 nmol min^{-1} BQ-123 for 15 min as the threshold dose for a systemic haemodynamic effect in our studies. In addition, both other studies measured blood pressure but not systemic vascular resistance, whereas, from the current study, the latter was a more powerful measure of the vascular effect of BQ-123, underlining the importance of this measurement in detecting modest haemodynamic influences. In this regard, other published studies in humans with endothelin antagonists do appear to show modest (~10 mmHg) reductions in blood pressure, with both bosentan (mixed ETA/ETB) (Weber *et al*., 1996) and ABT-627 (ETA selective) (Verhaar *et al*., 2000). In the latter study, although systemic haemodynamics were only recorded at 30 min and 8 h after dosing, systemic

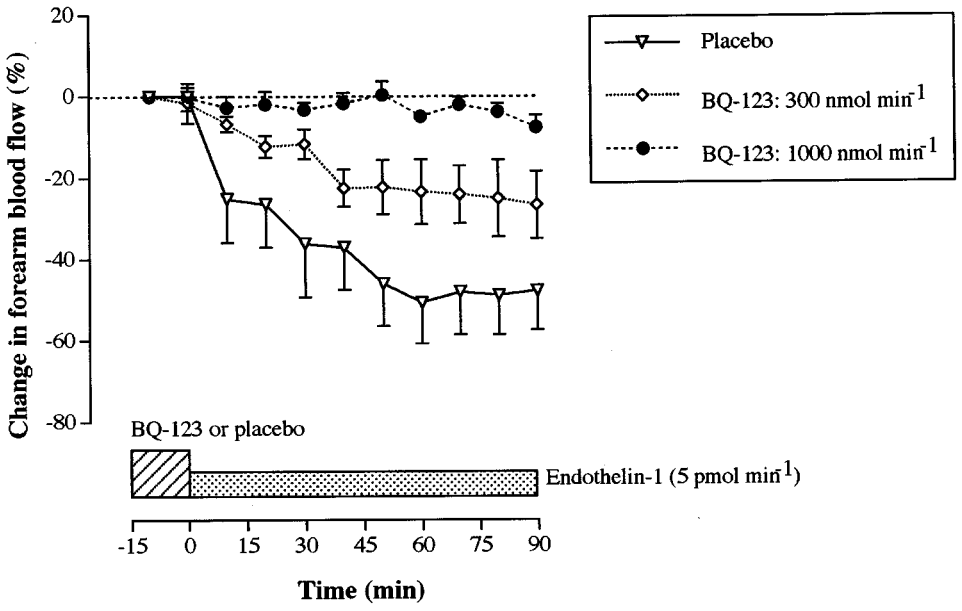

Figure 2 Graph illustrating response in forearm blood flow to intra-arterial ET-1, when pre-treated with placebo or BQ-123 (either 300 or 1000 nmol min^{-1} for 15 min).

vascular resistance was measured and significant effects on this parameter were found in both acute and chronic dosing.

It should be noted that we have also previously failed to detect significant systemic effects, as measured by blood pressure and heart rate, at our lowest dose of BQ-123 (100 nmol min^{-1}) when administered into the brachial artery (Haynes & Webb, 1994). These current results suggest that 100 nmol min^{-1} does have a small systemic action, most apparent in its effects on systemic vascular resistance, that was not detected in forearm studies, perhaps because the major vasodilatation is in other vascular beds. In recognition of this potential problem, we have more recently used a 10 fold lower dose of 10 nmol min^{-1} BQ-123, (Verhaar *et al.*, 1998), as a local dose for forearm studies. The current study confirms the rationale for this approach.

It is also interesting to note that a 15 min infusion of BQ-123 produces haemodynamic effects for up to 4 h at the higher doses. Although we have plasma estimations of BQ-123 concentrations only at 0, 15 and 240 min in the current study, subsequent experiments, with identical dosing schedules of BQ-123, demonstrate that the peak concentration is achieved at the end of the infusion, falls to $\sim$10% by 30 min and is undetectable by 75 min post infusion (unpublished data). This suggests that the observed responses are a pharmacodynamic effect rather that a reflection of the plasma half-life of BQ-123. This is similar to our experience with the non-selective ET antagonist TAK 044 where the systemic haemodynamic effects of a 15 min bolus were still observable at 24 h, whereas the peptide had a plasma half-life of 30–60 min (Haynes *et al.*, 1996).

In the current study, there was an increase in heart rate similar to that observed in other acute studies (Weber *et al.*, 1996; Wenzel *et al.*, 1998). These effects are not generally seen in chronic dosing studies with endothelin antagonists in patients with either hypertension or heart failure (Krum *et al.*, 1998; Sutsch *et al.*, 1998). For this reason, the effects are probably mediated through the activation of a cardiopulmonary reflex response to systemic vasodilatation rather a direct chronotropic effect on the heart.

Although the total number of subjects studied was low ($n=5$), the power of the study was sufficient to allow clear conclusions to be drawn. Given the limited experience with BQ-123 at these systemic doses, there were safety reasons for keeping the number of subjects to a minimum. In this regard, it is reassuring to note that, despite substantial systemic vasodilatation, and significant lowering of the mean arterial pressure, no side effects were observed or reported by the subjects.

In conclusion, this study with BQ-123 demonstrates that systemic ETA receptor antagonism causes substantial peripheral vasodilatation and modest lowering of blood pressure, consistent with an important role for the endothelin system in the maintenance of vascular tone in man. It remains to be seen, in direct comparison between selective ETA and mixed ETA/B receptor antagonists, which of the therapeutic approaches will offer the greater haemodynamic benefit in specific clinical indications.

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