Constriction to ETB receptor agonists, BQ-3020 and sarafotoxin S6c, in human resistance and capacitance vessels *in vivo*

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Aims The aim of the study was to examine the effects of the ETB receptor selective agonists sarafotoxin S6c (SFTX6c) and BQ-3020 on the forearm resistance and capacitance vessels in healthy subjects *in vivo*.

Methods The local response to intra-arterial or intravenous infusion of SFTX6c (5 pmol min⁻¹) or BQ-3020 (50 pmol min⁻¹) was assessed, on separate occasions, in eight healthy men (aged 20–28 years). Data (mean $\pm$ s.e.mean) were examined by ANOVA. Results are expressed as percentage change from baseline at 90 min.

Results SFTX6c and BQ-3020 reduced forearm blood flow, following local intra-arterial infusion ($-25 \pm 7\%$ and $-27 \pm 7\%$, respectively; $P < 0.001$) and reduced hand vein diameter, following local intravenous infusion ($-30 \pm 8\%$ and $-16 \pm 7\%$, respectively; $P < 0.001$).

Conclusions We have shown that locally active infusions of the selective ETB receptor agonists SFTX6c and BQ-3020 cause arterial constriction and venoconstriction in healthy human blood vessels *in vivo*. These results indicate that ETB receptor stimulation may mediate vasoconstriction in humans.

Keywords: endothelin-1, ETB receptor agonist, vasoconstriction

Introduction

The powerful vasoconstrictor and vasopressor effects of endothelin-1 (ET-1) [1, 2] are mediated via the ET-1 selective, vascular smooth muscle cell ETA receptor [3], while the predominant effect of the nonisopeptide selective ETB receptor [4] would appear to be vasodilatation [5–7] mediated by the endothelial cell ETB receptor. However, ETB receptors have also been described on vascular smooth muscle cells [8] and may contribute to vasoconstriction [9]. Indeed, we have previously demonstrated venoconstriction and vasoconstriction in response to locally active doses of the ETB receptor selective agonist sarafotoxin S6c (SFTX6c) [10] in healthy subjects [11–13] and in patients with heart failure [14].

To address whether the vasoconstrictor effects of SFTX6c [11–14] could also be demonstrated with

another, structurally distinct, ETB receptor agonist, we investigated the response to infusion of locally active doses of SFTX6c and BQ-3020, a selective ETB agonist [15, 16], in the forearm resistance and capacitance vessels of healthy subjects *in vivo*. BQ-3020 is a linear analogue of ET-1 with closer structural similarity to ET-1 than SFTX6c [15].

Methods

Subjects and protocol

Eight healthy men, within the age range of 18–40 years, were recruited to the study, which was conducted with the approval of the local Research Ethics Committee and with the written informed consent of each subject.

The effects of local intra-arterial and intravenous infusion of BQ-3020 and SFTX6c were investigated in eight healthy men in a single-blind, randomised, four way crossover study. On separate occasions, each separated by at least 1 week, each subject received either intra-arterial infusion or intravenous infusion of SFTX6c or BQ-3020. Subjects rested recumbent throughout each study in a

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Figure 1 Response of forearm blood flow and hand vein diameter to local intra-arterial infusion and local intravenous infusion of SFTX6c (5 pmol min^{-1} ; open circles) and BQ-3020 (50 pmol min^{-1} ; closed circles), respectively. Responses are expressed as mean percentage change $\pm$ s.e.mean.

quiet temperature-controlled room ($23\text{--}25^\circ\text{C}$). Saline (0.9%) was infused for at least 30 min before active drug infusion to allow recording of baseline measurements.

Drugs, administration and measurements

Sarafotoxin S6c and BQ-3020 (both Calbiochem-Novabiochem, Nottingham, UK) were administered by continuous infusion for 90 min. SFTX6c was administered at an infusion rate of 5 pmol min^{-1} as described previously [11–14]. BQ-3020 was administered at an infusion rate of 50 pmol min^{-1} , based on results from a pilot study (data not shown).

The brachial artery of the nondominant arm was cannulated under local anaesthetic (1% lignocaine; Astra Pharmaceuticals, Kings Langley, England) with a 27 SWG steel cannula (Cooper's Needle Works). The infusion rate was kept constant at 1 ml min^{-1} throughout. The

response to intra-arterial infusion was assessed by measurement of forearm blood flow using a standard plethysmographic technique [17].

A 23-gauge butterfly needle was sited in a selected dorsal hand vein in the direction of blood flow without the use of local anaesthetic. The same vein was cannulated for each study in that individual. The infusion rate was kept constant throughout at 0.25 ml min^{-1} . The response to intravenous infusion was assessed by measurement of the diameter of the cannulated dorsal hand vein using a standard displacement technique [17, 18].

Blood pressure and heart rate were measured in the noninfused arm using a well-validated semiautomated method [19] at 30 min intervals throughout the infusions.

Statistical analysis

Analysis was performed as described previously [17]. All results are expressed as mean $\pm$ standard error of the mean (s.e.mean) at 90 min. Blood pressure, heart rate and baseline measurements were compared using the Student's paired *t*-test. The forearm blood flow and vein diameter responses were examined by repeated-measures analysis of variance (ANOVA) (Excel 5.0, Microsoft Ltd, Wokingham, UK). Statistical significance was accepted at the 5% level.

Results

All subjects, aged 20–28 years, completed the study. There was no significant difference between baseline measurements on each of the study visits. All drug effects were confined to the infused arm [Table 1].

Sarafotoxin S6c and BQ-3020 reduced forearm blood flow ($-25 \pm 7\%$ and $-27 \pm 7\%$, respectively; $P < 0.001$), following intra-arterial infusion, indicating vasoconstriction in the infused arm (Figure 1). Both SFTX6c and BQ-3020 caused a reduction in vein diameter following intravenous infusion ($-30 \pm 8\%$ and $-16 \pm 7\%$, respectively; $P < 0.001$) (Figure 1).

Discussion

We have confirmed that infusion of a locally active dose of the ETB receptor agonist SFTX6c causes arterial constriction and venoconstriction in healthy human blood vessels *in vivo* [11–13]. We have also extended these observations to show, for the first time, that similar effects are seen with the structurally distinct ETB receptor selective agonist, BQ-3020. These findings support the view that vasoconstriction can occur in response to stimulation of ETB receptors *in vivo*. It has been suggested that this vasoconstriction results from displacement of endogenous ET-1 onto unoccupied ETA receptors [6]. However, this seems unlikely, given that we have shown

Table 1 Mean arterial pressure (MAP), heart rate (HR), forearm blood flow (FBF) and vein diameter at baseline and at 90 min following the start of each infusion. Values are mean $\pm$ s.e.mean.

	Intra-arterial infusion		Intravenous infusion	
	BQ-3020 (50 pmol min ⁻¹)	SFTX6c (5 pmol min ⁻¹)	BQ-3020 (50 pmol min ⁻¹)	SFTX6c (5 pmol min ⁻¹)
MAP (mmHg)				
Basal	93 $\pm$ 3	86 $\pm$ 2	87 $\pm$ 2	89 $\pm$ 2
90 min	96 $\pm$ 5	92 $\pm$ 3	86 $\pm$ 3	90 $\pm$ 3
HR (beats min ⁻¹)				
Basal	56 $\pm$ 3	54 $\pm$ 2	57 $\pm$ 3	57 $\pm$ 3
90 min	56 $\pm$ 2	54 $\pm$ 3	55 $\pm$ 3	58 $\pm$ 4
FBF (ml 100 ml ⁻¹ min ⁻¹)				
Control arm				
Basal	4.0 $\pm$ 0.8	4.4 $\pm$ 1.0		
90 min	5.0 $\pm$ 1.4	5.2 $\pm$ 1.0		
Infused arm				
Basal	4.6 $\pm$ 1.0	3.9 $\pm$ 0.7		
90 min	3.6 $\pm$ 0.7	3.2 $\pm$ 0.1		
Vein diameter (arbitrary units)				
Basal			2.7 $\pm$ 0.4	2.6 $\pm$ 0.3
90 min			2.3 $\pm$ 0.3	1.8 $\pm$ 0.4

previously that the constrictor effects of SFTX6c could be blocked by the selective ETB receptor antagonist BQ-788 [13] but not by the selective ETA receptor antagonist BQ-123 (unpublished observations).

Although vasoconstriction does appear to occur with ETB receptor selective agonists, the integrated role of endothelial cell dilator and vascular smooth muscle constrictor receptors only emerges from studies with endothelin receptor antagonists. Indeed, we have recently demonstrated local [7] and systemic [5] vasoconstriction in response to the ETB receptor selective antagonist BQ-788 [20], indicating that the balance of effects of endogenous ET-1 acting at vascular smooth muscle and endothelial ETB receptors in healthy resistance vessels favours vasodilatation. Further investigation of the role of the ETB receptor in health and in cardiovascular disease is important to distinguish whether combined ETA/ETB antagonists or selective ETA antagonists will be more effective as vasodilator treatments in the clinical setting.

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