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In vivo pharmacological characterization of the non-peptide endothelin receptor antagonist SB 209670

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1 The aim of the present study was to assess the ability of SB 209670, a high affinity non-peptide endothelin receptor antagonist (0.4 and 18 nM K_is at human cloned ET_A and ET_B receptors, respectively), to inhibit the haemodynamic actions of endothelin-1 *in vivo*.

2 Systemic administration of (±)-SB 209670, given either as a bolus i.v. injection or as a continuous i.v. infusion, did not alter basal haemodynamic parameters in the anaesthetized rat.

3 Infusion of (±)-SB 209670 ($10\ \mu g\ kg^{-1}\ min^{-1}$) selectively inhibited the depressor and carotid vasodilator response to exogenous endothelin-1: $100\ \mu g\ kg^{-1}\ min^{-1}$ was required to inhibit significantly the biphasic haemodynamic actions of endothelin-1. The haemodynamic actions of angiotensin II and calcitonin gene-related peptide were unaltered by $100\ \mu g\ kg^{-1}\ min^{-1}$ (±)-SB 209670.

4 Bolus i.v. administration of (±)-SB 209670 ($1\ mg\ kg^{-1}$) selectively inhibited the depressor and carotid vasodilator actions of endothelin-1: $10\ mg\ kg^{-1}$ (±)-SB 209670 was required to inhibit the secondary vasoconstrictor actions of endothelin-1.

5 (±)-SB 209670 ($10\ mg\ kg^{-1}$) was also effective at antagonizing the pressor actions of endothelin-1 in the conscious rat for up to 3 h after intraduodenal administration thereby demonstrating that the antagonist was bioavailable upon enteric administration. This dose of (±)-SB 209670 did not alter basal haemodynamic parameters in the conscious rat.

6 Thus, (±)-SB 209670 is an effective endothelin receptor antagonist *in vivo*. Using the doses defined in this study, SB 209670 may, therefore, serve as a useful tool for understanding the role of endogenous endothelin-1 in the control of cardiovascular function under both physiological and pathophysiological conditions.

Keywords: Endothelin receptor antagonist; endothelin-1; SB 209670; regional haemodynamics; ET_A-receptor subtype; ET_B-receptor subtype

Introduction

The identification of a novel non-peptide endothelin receptor antagonist, SB 209670, has recently been reported by Ohlstein *et al.* (1994b,c). This molecule, designed using a conformational model of endothelin-1 (Elliott *et al.*, 1994b), is a high affinity ligand at human cloned ET_A and ET_B receptors (0.4 and 18 nM K_is, respectively; Nambi *et al.*, 1994). *In vitro* studies have demonstrated that SB 209670 is a potent, competitive endothelin receptor antagonist, producing concentration-dependent, parallel rightward shifts in the endothelin-1 concentration-response curves in rat isolated aortae (endothelin-1-induced ET_A receptor-mediated vascular contraction; K_b of 0.4 nM) and rabbit pulmonary arteries (sarafotoxin S6c-induced ET_B receptor-mediated vascular contraction; K_b of 52 nM; Ohlstein *et al.*, 1994a,b,c). SB 209670 is 45, 180 and 775 fold more potent than BQ-123, Ro 47-0203 (bosentan) and PD142893, respectively, in inhibiting ET_A receptor-mediated vascular contraction (Ohlstein *et al.*, 1994a). *In vitro*, the functional antagonism produced by SB 209670 is specific for endothelin-1: 10 μM SB 209670 does not inhibit noradrenaline-, arginine vasopressin- or KCl-induced contraction in rat isolated aortae, or angiotensin II-induced contraction in rabbit isolated aortae (Ohlstein *et al.*, 1994a). SB 209670 is a chiral molecule with the (+)-antipode being at least three orders of magnitude more potent than the (−)-antipode: (±)-SB 209670 inhibits endothelin-1-mediated contraction in human isolated circumflex coronary arteries with a K_b of 7 ± 3 nM (Ohlstein *et al.*, 1994a). Since the development of efficacious endothelin receptor antagonists has the potential to define directly the precise role of endogenous endothelin-1 in the control of cardiovascular physiology and pathophysiology, the aim of the present study was to assess the ability of (±)-SB 209670 to inhibit selectively the haemodynamic actions of exogenously administered endothelin-1 in the rat. Furthermore, in order to demonstrate that the antagonist was bioavailable, studies were designed to assess the ability of (±)-SB 209670 to inhibit the vasopressor actions of endothelin-1 following intraduodenal administration of antagonist. A preliminary account of some of the work described in this study was presented to the Federation of American Societies for Experimental Biology and the XIIth International Congress of Pharmacology (Douglas *et al.*, 1994a,b; Ohlstein *et al.*, 1994b).

Methods

Surgical preparation of anaesthetized rats

Male Sprague-Dawley rats (Charles River) were prepared for haemodynamic evaluation as previously described (Douglas *et al.*, 1992). Briefly, rats were anaesthetized with sodium pentobarbitone ($65\ mg\ kg^{-1}$, i.p.) allowing cannulation of the left jugular vein and right femoral artery and vein (PE50 polyethylene tubing; Clay Adams) for bolus i.v. agonist administration, measurement of arterial blood pressure and continuous infusion of antagonist, respectively. Instantaneous arterial blood pressure was recorded with a miniature Gould P231D pressure transducer (Grass Instrument Co.) linked to a Model 79D Grass polygraph; this signal was used to derive mean arterial pressure and heart rate. Body temperature was maintained at 37 ± 1°C using a K-20-F water blanket

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(American Hamilton). In addition, a PE240 tracheal cannula was inserted to allow the animal to be artificially respired with room air (54 cycles min^{-1}; 10 ml kg^{-1}; Harvard Apparatus).

Effect of continuous i.v. infusion of (±)-SB 209670 on the systemic haemodynamic actions of endothelin-1, angiotensin II and human calcitonin gene-related peptide in the anaesthetized rat

Once basal haemodynamic parameters had stabilized following completion of the initial surgical preparation, an infusion of either saline vehicle (150 µl min^{-1}) or (±)-SB 209670 (1–100 µg kg^{-1} min^{-1}) was initiated via the right femoral vein cannula. This infusion was maintained throughout the duration of the experiment. Following a 30 min equilibration period, the rats received a bolus i.v. injection of endothelin-1 (300 pmol kg^{-1} via the jugular vein). In order to demonstrate that (±)-SB 209670 selectively inhibited the vascular actions of endothelin-1, rats also received 300 pmol kg^{-1} doses of angiotensin II and human calcitonin gene-related peptide. Angiotensin II and human calcitonin gene-related peptide were studied because, like endothelin-1, they are potent vasoactive peptides producing pronounced pressor and depressor responses, respectively. The doses of the peptides employed were approximately equivalent to their ED_{50}s as vasoactive agonists in the anaesthetized rat (Douglas & Hiley, 1991; Douglas *et al.*, 1992). All agonists were washed through the venous cannula with 100 µl saline. Changes in mean arterial pressure and heart rate were monitored following agonist administration. Each animal was used to study the effects of a single dose of antagonist or vehicle only. Throughout the duration of these studies animals received supplemental i.v. doses of sodium pentobarbitone (approximately 3 mg kg^{-1} at appropriate time points) in order to maintain a satisfactory level of anaesthesia.

Effect of continuous i.v. infusion of (±)-SB 209670 on the carotid haemodynamic actions of endothelin-1 in the carotid bed of the anaesthetized rat

In a separate set of rats which had been prepared for blood pressure measurement, a pulsed Doppler flow probe, linked to the polygraph via a 545C-4 directional flowmeter (Bioengineering, The University of Iowa, U.S.A.), was placed around the right carotid artery for measurement of carotid blood flow and vascular resistance. This vascular bed was chosen since endothelin-1 is known to act as both a vasodilator and vasoconstrictor in this resistance bed (Gardiner *et al.*, 1989). Following a 30 min continuous i.v. infusion of either saline vehicle or (±)-SB 209670 (10–100 µg kg^{-1} min^{-1}), a dose-response curve was constructed to endothelin-1 (1–1000 pmol kg^{-1}). Thus, it was possible to establish whether or not the inhibitory effects of (±)-SB 209670 on the systemic haemodynamic actions of endothelin-1 were paralleled by similar inhibitory actions in a regional vascular bed.

Effect of (±)-SB 209670 administered as a bolus i.v. injection on the systemic and carotid haemodynamic actions of endothelin-1 in the anaesthetized rat

In a separate group of experiments, the effect of a bolus i.v. dose of (±)-SB 209670 on the systemic and carotid haemodynamic responses to endothelin-1 were studied. Once basal haemodynamic parameters had stabilized, changes in resting haemodynamic parameters were recorded over a 5 min period following the bolus i.v. administration of either (±)-SB 209670 (1–10 mg kg^{-1}) or saline vehicle (equal volume 1 ml kg^{-1}). Once this initial 5 min period was complete, the animals then received a bolus i.v. dose of endothelin-1 (300 pmol kg^{-1} via the jugular vein). Changes in mean arterial pressure, heart rate and carotid blood flow (and, therefore, vascular resistance) were monitored following agonist administration. Each animal was used to study the effects of a single dose of antagonist or vehicle only.

Effect of (±)-SB 209670 administered as a bolus intraduodenal injection on the systemic pressor actions of endothelin-1 in the conscious rat

Male Sprague-Dawley rats were prepared with chronic indwelling catheters following the protocol of Ohlstein *et al.* (1992). Briefly, rats were anaesthetized with sodium methohexitone (100 mg kg^{-1}, i.p.) allowing catheters to be placed in the abdominal aorta (to record systemic arterial blood pressure and heart rate) and vena cava (for i.v. bolus administration of endothelin-1, 200 pmol kg^{-1}) via the left femoral artery and vein. An additional length of Tygon tubing was sutured into the duodenum for bolus intraduodenal administration of saline vehicle or 3–10 mg kg^{-1} (±)-SB 209670 (this route of administration was chosen over gastric lavage since it was less stressful to the conscious rats and therefore, facilitated accurate repeated haemodynamic recordings). All tubing was tunneled under the skin and exited at the midscapular region. Tubing was filled with a dextrose:heparin solution (1 ml contained 0.5 g dextrose and 1000 units ml^{-1} heparin) to prevent obstructive thrombus formation. Following completion of the surgery, animals were housed in Plexiglass cages under a 12 h light-dark cycle with access to standard laboratory chow and drinking water *ad libitum*. Animals were allowed at least 3 days recovery from surgical intervention prior to undergoing experimentation, during which time they were trained to become accustomed to placement in a restraining cage.

Table 1 Continuous i.v. infusion of (±)-SB 209670 does not alter basal systemic haemodynamic parameters in the anaesthetized rat

		Dose of (±)-SB 209670		
	Saline (*n* = 7)	*1 µg kg^{-1} min^{-1}* (*n* = 4)	*10 µg kg^{-1} min^{-1}* (*n* = 8)	*100 µg kg^{-1} min^{-1}* (*n* = 8)
Body weight (g)	407 ± 21	435 ± 13	386 ± 10	402 ± 12
Prior to infusion				
Mean arterial pressure (mmHg)	98 ± 5	105 ± 12	104 ± 9	99 ± 5
Heart rate (min^{-1})	331 ± 28	322 ± 25	304 ± 17	304 ± 5
After 30 min infusion				
Mean arterial pressure (mmHg)	88 ± 8	91 ± 12	101 ± 6	91 ± 7
Heart rate (min^{-1})	306 ± 21	282 ± 24	283 ± 16	282 ± 15

Statistical comparisons between saline-treated rats and those which received (±)-SB 209670 (1–100 µg kg^{-1} min^{-1}) performed by analysis of variance (ANOVA; Fisher's protected least squares difference): no statistical differences were identified. Values shown are the mean ± s.e.mean; *n* represents the number of animals examined in each group.

On the day of experimentation animals were placed in a restraining cage following which they received a bolus i.v. dose of endothelin-1 (control response). Thirty minutes later, immediately prior to a repeat dose of endothelin-1, either saline vehicle or (±)-SB 209670 was administered as an intraduodenal bolus dose. Repeat doses of endothelin-1 were administered every 30 min over a 4 h period following administration of (±)-SB 209670 or saline.

Materials

All drugs and peptides were made freshly each day and stored on ice throughout the duration of the experiment in a light tight container. Endothelin-1 was purchased from American Peptide Co., Inc. (Santa Clara, CA, U.S.A.) and angiotensin II acetate and human calcitonin gene-related peptide were obtained from Sigma Chemical Co. (St. Louis, MO,

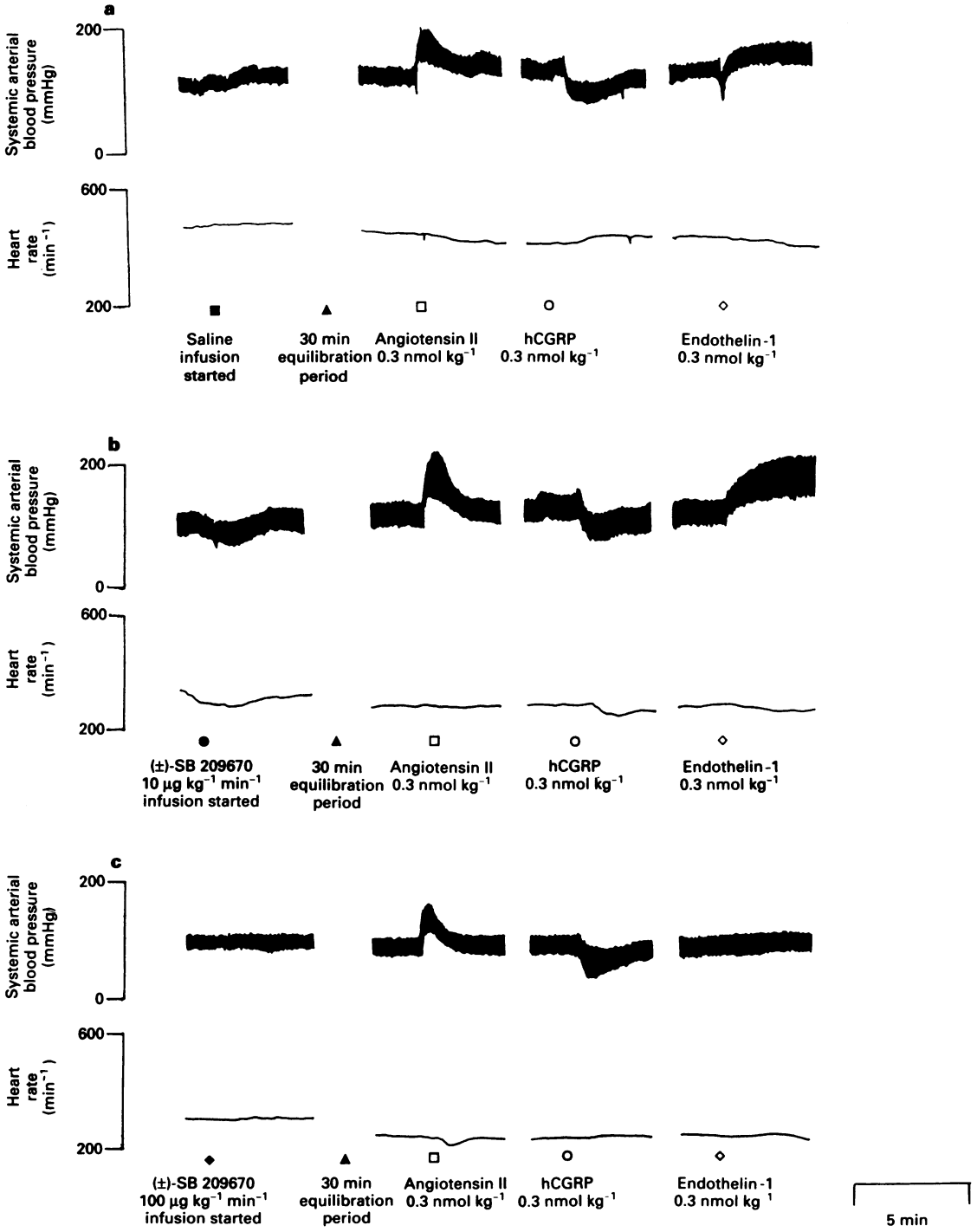

Figure 1 Representative experimental traces showing the effects of 0.3 nmol kg^{-1} angiotensin II (□), human calcitonin gene-related peptide (○) and endothelin-1 (◇) on systemic arterial pressure and heart rate in the anaesthetized rat during continuous i.v. of either (a) saline (■) or (b) 10 (●) or (c) 100 (◆) μg kg^{-1} min^{-1} (±)-SB 209670 infusion. After initiating the infusion of (±)-SB 209670, a 30 min equilibration period (▲) was allowed prior to administration of agonists. The figure (b) illustrates that the continuous infusion of 10 μg kg^{-1} min^{-1} (±)-SB 209670 selectively attenuates the initial hypotensive actions of bolus i.v. endothelin-1. The secondary hypertensive response to endothelin-1 is antagonized only by a higher dose of 100 μg kg^{-1} min^{-1} (±)-SB 209670 (c). Although 100 μg kg^{-1} min^{-1} (±)-SB 209670 inhibits the haemodynamic actions of endothelin-1, the responses to angiotensin II and calcitonin gene-related peptide remain unaltered following administration of antagonist demonstrating the selectivity of (±)-SB 209670 for endothelin-1.

U.S.A.). Stock solutions of peptides (40 μM) were dissolved in distilled water: all serial dilutions were made using saline as the diluent. (±)-SB 209670 ([(±)-(1S, 2R, 3S)-3-(2-carboxymethoxy-4-methoxyphenyl)-1-(3, 4-methylenedioxy-phenyl)-5-(prop-1-yloxy) indane 2-carboxylic acid]) was synthesized in the Department of Medicinal Chemistry, SmithKline Beecham Pharmaceuticals and was dissolved in saline vehicle (0.9% NaCl, w/v).

Statistical analysis

Values are expressed as the mean ± s.e.mean and *n* represents the number of animals used in a particular group. Statistical analysis was performed by analysis of variance (ANOVA; Fisher's protected least squares difference). Differences were considered to be significant where $P<0.05$.

All experiments were performed in specific accordance with the guidelines of the Animal Care and Use Committee, SmithKline Beecham Pharmaceuticals and the American Association for Laboratory Animal Care.

Results

Continuous i.v. infusion of (±)-SB 209670 selectively inhibits the systemic and carotid haemodynamic actions of endothelin-1 in the anaesthetized rat

Relative to saline-treated animals, a 30 min i.v. infusion of (±)-SB 209670 (1–100 μg kg^{-1} min^{-1}) had no effect on basal mean arterial blood pressure or heart rate (Table 1, Figure 1).

The lowest dose of antagonist studied (1 μg kg^{-1} min^{-1}) failed to produce a significant inhibition of either the systemic vasodepressor or vasopressor response to endothelin-1. However, infusion of a ten fold higher dose of (±)-SB 209670 (10 μg kg^{-1} min^{-1}) selectively inhibited the initial hypotensive response to bolus i.v. endothelin-1 administration (Figures 1b and 2a). This dose of antagonist did not alter the secondary hypertensive response to endothelin-1 (Figure 1b, 2b). Indeed, significant inhibition of the secondary increase in blood pressure to endothelin-1 was observed only during the infusion of 100 μg kg^{-1} min^{-1} (±)-SB 209670 (Figure 1c, 2b).

(±)-SB 209670, at 100 μg kg^{-1} min^{-1}, a dose which produced ~80% inhibition of both endothelin-1-induced initial systemic hypotension and secondary systemic hypertension, failed to antagonize the vasopressor response to angiotensin II or the depressor response to human calcitonin gene-related peptide (Figures 1c, 2c and 2d). Thus, the inhibitory action of (±)-SB 209670 was selective for endothelin-1 since no antagonism was observed with other unrelated vasoactive peptides.

In animals with similar basal mean arterial blood pressures, heart rates and carotid blood flow and vascular resistance (Table 2), the dose of (±)-SB 209670 (10 μg kg^{-1} min^{-1}) which selectively inhibited the systemic vasodepressor response to bolus i.v. administration of endothelin-1 was also found to inhibit selectively the vasodilatation observed following bolus i.v. endothelin-1 administration in the carotid vascular bed (Figure 3a). This dose of (±)-SB 209670 failed

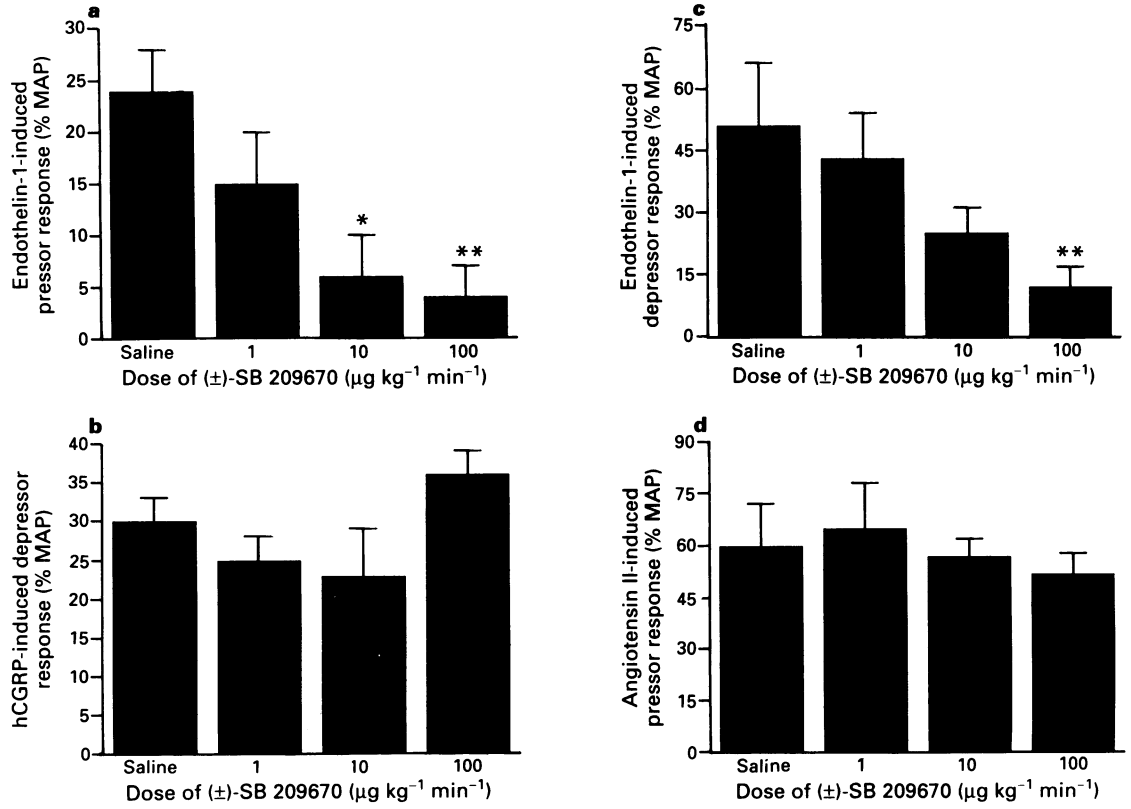

Figure 2 Effect of continuous i.v. infusion of 1 ($n=4$), 10 ($n=8$) or 100 ($n=8$) μg kg^{-1} min (±)-SB 209670 on the (a) initial vasodepressor and (b) secondary vasopressor actions of endothelin-1 (0.3 nmol kg^{-1}, i.v. bolus) compared to (c) the vasodepressor actions of human cacitonin gene-related peptide (hCGRP, 0.3 nmol kg^{-1}, i.v. bolus) and (d) the vasopressor actions of angiotensin II (0.3 nmol kg^{-1}; i.v. bolus). Low dose infusions of (±)-SB 209670 (10 μg kg^{-1} min^{-1}) selectively inhibit the initial vasodepressor actions of endothelin-1: significant inhibition of the secondary pressor actions of endothelin-1 is observed only with 100 μg kg^{-1} min^{-1}. (±)-SB 209670 did not alter the haemodynamic actions of the other vasoactive peptides examined. In addition, 30 min i.v. infusion of (±)-SB 209670 did not alter basal haemodynamic parameters. Statistical comparisons performed by analysis of variance (ANOVA: Fisher's protected least squares difference): $^{*}P<0.05$ and $^{**}P<0.01$ for animals which received (±)-SB 209670 compared to saline-treated control rats ($n=7$). Values are expressed as the mean ± s.e.mean; *n* is the range of the number of animals used at the different doses studied.

Table 2 Continuous i.v. infusion of (±)-SB 209670 does not alter basal regional haemodynamic parameters in the anaesthetized rat

	Dose of (±)-SB 209670		
	Saline (n = 6)	10 μg kg^{-1} min^{-1} (n = 5)	100 μg kg^{-1} min^{-1} (n = 6)
Body weight (g)	314 ± 5	329 ± 16	311 ± 5
Basal haemodynamic parameters			
Mean arterial pressure (mmHg)	97 ± 7	92 ± 5	93 ± 3
Heart rate (min^{-1})	340 ± 12	337 ± 8	346 ± 6
Carotid flow (kHz)	6.28 ± 1.23	8.72 ± 0.78	7.11 ± 0.54

Statistical comparisons between saline-treated rats and those which received (±)-SB 209670 (1–100 μg kg^{-1} min^{-1}) performed by analysis of variance (ANOVA; Fisher's protected least squares difference): no statistical differences were identified. Values shown are the mean ± s.e.mean; *n* represents the number of animals examined in each group.

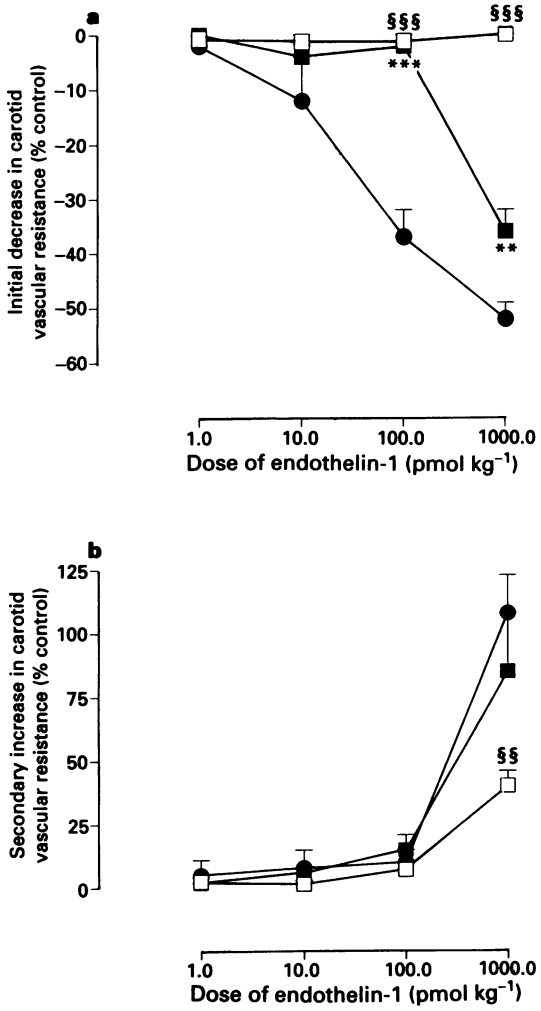

Figure 3 Dose-response curves for the (a) initial vasodilator and (b) secondary vasoconstrictor actions of endothelin-1 (1–1000 pmol kg^{-1}, i.v. bolus) in the carotid vascular bed of the anaesthetized rat during the continuous infusion of either 10 (■; *n* = 5) or 100 (□; *n* = 6) μg kg^{-1} min^{-1} (±)-SB 209670. Control responses (●; *n* = 6) were obtained during the continuous infusion of saline vehicle. Low dose infusions of (±)-SB 209670 (10 μg kg^{-1} min^{-1}) selectively inhibit the initial regional vasodilator actions of endothelin-1 in the carotid bed: significant inhibition of the endothelin-1-induced secondary vasoconstriction is observed only using 100 μg kg^{-1} min^{-1} reflecting the observations made on the endothelin-1-induced haemodynamic changes. Statistical comparisons performed by use of analysis of variance (ANOVA; Fisher's protected least squares difference): $^{**}P < 0.01$ and $^{***}P < 0.001$ and §§$P < 0.01$ and §§§$P < 0.001$ for animals which received 10 and 100 μg kg^{-1} min^{-1} (±)-SB 209670, respectively, compared to saline-treated control rats. Values are expressed as the mean ± s.e.mean; *n* is the range of the number of animals used at the different doses.

to inhibit the secondary vasoconstrictor actions of endothelin-1 (Figure 3b) and, as was the case in the systemic circulation, the carotid vasoconstrictor actions of endothelin-1 could be inhibited only by a ten fold higher dose of antagonist (100 μg kg^{-1} min^{-1}; Figures 3a and b).

Bolus i.v. injection of (±)-SB 209670 selectively inhibits the systemic and carotid haemodynamic actions of endothelin-1 in the anaesthetized rat

Relative to saline-treated animals, the bolus i.v. administration of (±)-SB 209670 (1–10 mg kg^{-1}) had no effect on basal mean arterial blood pressure, heart rate or carotid haemodynamics (Table 3 and Figure 4).

In a similar fashion to those observations made during the i.v. infusion of antagonist, (±)-SB 209670 (1 mg kg^{-1}) selectively inhibited the initial systemic vasodepressor response and associated decrease in carotid vascular resistance to endothelin-1 administration: it did not alter the secondary hypertensive response to endothelin-1 (Figure 5). Significant inhibition of the secondary increase in blood pressure and carotid vasoconstriction to endothelin-1 could be inhibited only using 10 mg kg^{-1} (±)-SB 209670 (Figures 4, 5).

Bolus intraduodenal administration of (±)-SB 209670 inhibits the systemic pressor actions of endothelin-1 in the conscious rat

Mean arterial pressure in rats was not altered 30 min after the bolus intraduodenal administration of (±)-SB 209670 (systemic blood pressure went from 104 ± 4 to 103 ± 5 mmHg following intraduodenal saline administration, from 104 ± 4 to 103 ± 7 mmHg following intraduodenal administration of 3 mg kg^{-1} (±)-SB 209670 and from 97 ± 2 to 99 ± 3 mmHg following intraduodenal administration of 10 mg kg^{-1} (±)-SB 209670; *n* = 5 in each group).

Intraduodenal administration of 10 mg kg^{-1} (±)-SB 209670 produced a significant inhibition of the hypertensive responses to repeated endothelin-1 injections (200 pmol kg^{-1}, i.v. bolus every 30 min). Significant inhibition was observed 60 min after the administration of (±)-SB 209670, an effect which persisted for the next 2 h (Figure 6). Significant inhibition could not be observed with a lower dose of 3 mg kg^{-1} (±)-SB 209670. Due to the susceptibility of the initial, transient endothelin-1-induced depressor response to desensitization (Le Monnier de Gouville *et al.*, 1990), similar comparisons could not be made for the vasodepressor actions of endothelin-1 using this repeat dosing procedure. Nevertheless, the data clearly indicated that (±)-SB 209670 was bioavailable following intraduodenal administration.

Discussion

SB 209670 is a high affinity, functional and competitive non-peptide endothelin receptor antagonist *in vitro* (K_is of 0.4

Table 3 Bolus i.v. administration of (±)-SB 209670 does not alter basal systemic haemodynamic parameters in the anaesthetized rat

	Saline ($n = 9$)	Dose of (±)-SB 209670 1 mg kg^{-1} ($n = 6$)	10 mg kg^{-1} ($n = 4$)
Body weight (g)	315 ± 14	329 ± 16	316 ± 5
Prior to bolus administration			
Mean arterial pressure (mmHg)	88 ± 6	88 ± 5	81 ± 9
Heart rate (min^{-1})	378 ± 7	361 ± 18	375 ± 15
Carotid flow (kHz)	4.16 ± 0.88	2.94 ± 0.28	3.60 ± 0.88
Carotid resistance (mmHg kHz^{-1})	28.6 ± 5.5	25.3 ± 5.4	32.3 ± 14.7
5 min after bolus administration			
Mean arterial pressure (mmHg)	89 ± 6	86 ± 6	80 ± 7
Heart rate (min^{-1})	380 ± 7	358 ± 17	369 ± 13
Carotid flow (kHz)	4.25 ± 0.88	3.13 ± 0.27	3.90 ± 1.04
Carotid resistance (mmHg kHz^{-1})	28.2 ± 5.5	29.0 ± 3.7	30.2 ± 1.4

Statistical comparisons between saline-treated rats and those which received (±)-SB 209670 (1–10 mg kg^{-1}) performed by analysis of variance (ANOVA; Fisher's protected least squares difference): no statistical differences were identified. Values shown are the mean ± s.e.mean; *n* represents the number of animals examined in each group.

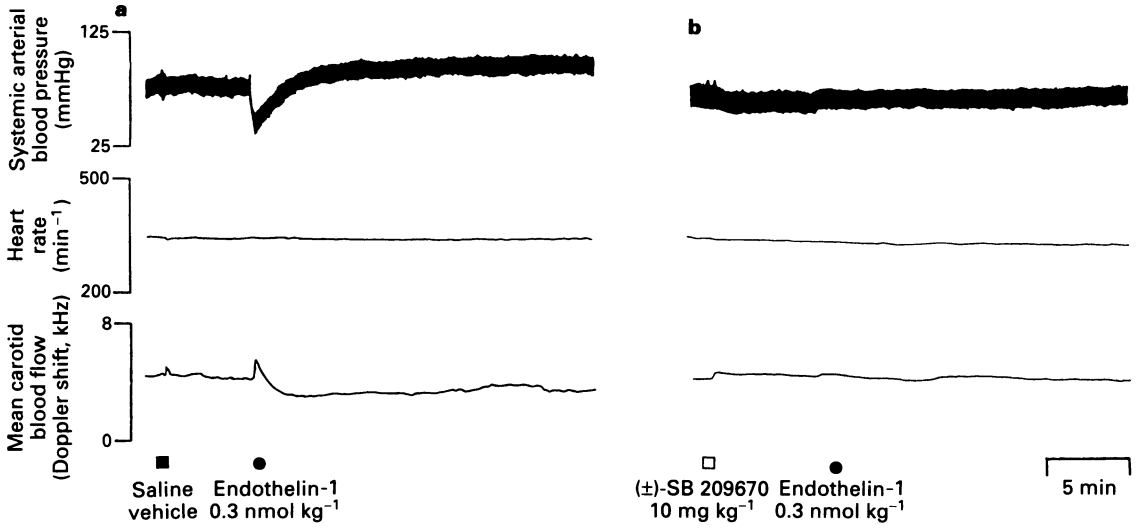

Figure 4 Representative experimental traces showing the effects of 0.3 nmol kg^{-1} endothelin-1 (●) on mean arterial pressure, heart rate and carotid artery blood flow in the anaesthetized rat following the bolus i.v. administration of either (a) saline (■) or (b) 10 mg kg^{-1} (±)-SB 209670 (□). The figure illustrates that bolus i.v. administration of 10 mg kg^{-1} (±)-SB 209670 inhibits both the vasodilator and vasoconstrictor actions of endothelin-1. In addition, (±)-SB 209670 did not alter basal haemodynamic parameters.

and 18 nM at human cloned ET_A and ET_B receptors, respectively; Ohlstein *et al.*, 1994b,c; Elliott *et al.*, 1994b, Nambi *et al.*, 1994). Relative to other endothelin receptor antagonists reported in the literature, SB 209670 is extremely potent being 45, 180 and 775 fold more potent than BQ-123, bosentan (Ro 47-0203) and PD142893 at inhibiting ET_A receptor-mediated vascular contraction, respectively (Ihara *et al.*, 1991; Cody *et al.*, 1992; Doherty *et al.*, 1993; Roux *et al.*, 1993; Ohlstein *et al.*, 1994a).

This study demonstrated that continuous i.v. infusion or bolus i.v. administration of (±)-SB 209670 was effective in antagonizing both the systemic haemodynamic changes and the alterations in carotid blood flow associated with the i.v. bolus administration of endothelin-1. Such findings are consistent with SB 209670 acting as a functional endothelin receptor antagonist *in vitro* (Ohlstein *et al.*, 1994a; Nambi *et al.*, 1994). *In vitro*, the functional antagonism produced by SB 209670 is specific for endothelin-1: 10 μM SB 209670 does not inhibit noradrenaline-, arginine vasopressin- or KCl-induced contraction in rat isolated aortae, or angiotensin II-induced contraction in rabbit isolated aortae. Similarly, a dose of (±)-SB 209670 which inhibited both the initial vasodepressor and secondary vasopressor actions of endothelin-1 did not alter the *in vivo* vasodilator and vasoconstrictor responses to human calcitonin gene-related peptide and angiotensin II, respectively. SB 209670 is a chiral molecule with the (+)-antipode being at least three orders of magnitude more potent than the (−)-antipode *in vitro* (Ohlstein *et al.*, 1994a). Therefore, the pharmacological antagonism of the endothelin-1 induced haemodynamic changes observed in this study is likely to reflect the affinity of the (+)-antipode (rather than the (−)-antipode) present in racemic SB 209670. Indeed, the antagonism observed *in vivo* with 10 mg kg^{-1} (±)-SB 209670 is indistinguishable from that observed with the same dose of (+)-SB 209670 (Ohlstein *et al.*, 1994c).

Systemic administration of (±)-SB 209670 (either bolus i.v. injection or continuous i.v. infusion), did not alter basal haemodynamic parameters in the anaesthetized rat. This is in accord with previous observations made with the ET_A-selective endothelin receptor antagonist, BQ-123, supporting the hypothesis that, at least acutely, endothelin plays a relatively insignificant role in the maintenance/control of basal haemodynamics in the normotensive Sprague-Dawley rat. This contrasts the haemodynamic role of endogenous

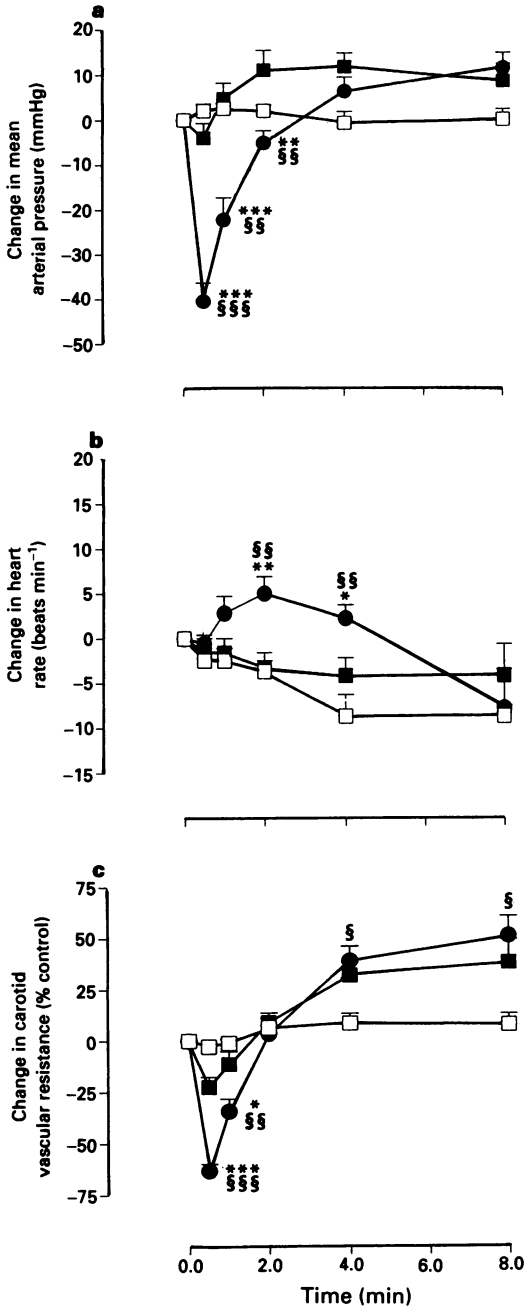

Figure 5 Effect of pretreatment with 1 (■; $n = 6$) or 10 (□; $n = 4$) mg kg^{-1} (±)-SB 209670 on the changes in (a) mean arterial pressure, (b) heart rate and (c) carotid vascular resistance observed in the anaesthetized rat following i.v. administration of 0.3 nmol kg^{-1} endothelin-1. (●; $n = 9$) represents the changes observed in control animals pretreated with an equal volume of saline vehicle. Low dose administration of (±)-SB 209670 (1 mg kg^{-1}) selectively inhibited the initial depressor actions of endothelin-1: significant inhibition of the endothelin-1-induced secondary pressor and regional vasoconstriction is observed only with 10 mg kg^{-1} (±)-SB 209670 reflecting the observations made during the continuous i.v. infusion of antagonist. Statistical comparisons performed by use of analysis of variance (ANOVA; Fisher's protected least squares difference): $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ and §$P < 0.01$, §§$P < 0.01$ and §§§$P < 0.001$ for animals which received 1 and 10 mg kg^{-1} (±)-SB 209670, respectively, compared to saline-treated control rats. Values shown are the mean ± s.e.mean; n represents the number of animals examined in each group.

endothelin-1 in hypertensive strains of rat where this peptide may be involved in the abnormal elevation of total peripheral resistance (Douglas *et al.*, 1992; 1994c; 1995; Elliott *et al.*, 1994a).

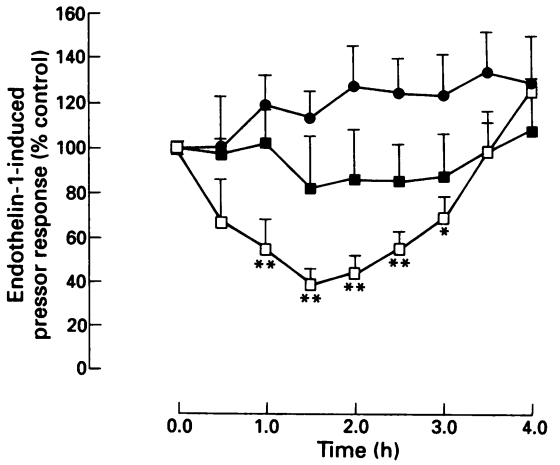

Figure 6 Effect of bolus intraduodenal administration of 3 (■; $n = 5$) or 10 (□; $n = 5$) mg kg^{-1} (±)-SB 209670 on the increases in mean arterial pressure observed in the conscious rat following repeated i.v. administration of 0.3 nmol kg^{-1} endothelin-1; (●; $n = 5$) represents the changes observed in control animals pretreated with an equal volume of saline vehicle. The figure demonstrates that (±)-SB 209670 is bioavailable following intraduodenal administration. Statistical comparisons performed by use of analysis of variance (ANOVA; Fisher's protected least squares difference): $^{*}P < 0.05$ and $^{**}P < 0.01$ for animals which received 10 mg kg^{-1} (±)-SB 209670 compared to saline-treated control rats. Values shown are the mean ± s.e. mean; n represents the number of animals examined in each group.

Infusion of 10 μg kg^{-1} min^{-1} (±)-SB 209670 selectively inhibited the depressor and carotid vasodilator responses to exogenous endothelin-1: a higher dose of 100 μg kg^{-1} min^{-1} was required to inhibit significantly both components of the biphasic haemodynamic response to endothelin-1. Similarly, bolus i.v. administration of (±)-SB 209670 (1 mg kg^{-1}) selectively inhibited the depressor and carotid vasodilator actions of endothelin-1: 10 mg kg^{-1} (±)-SB 209670 was required to inhibit the secondary vasoconstrictor actions of endothelin-1. Whilst (±)-SB 209670 selectively inhibited the vasodepressor and carotid vasodilator actions of endothelin-1, it is interesting to note that neither 10 μg kg^{-1} min^{-1} (i.v. infusion) nor 1 mg kg^{-1} (i.v. bolus) potentiated the secondary pressor actions of endothelin-1 significantly. However, although not significantly different from control responses, the peak increase in mean arterial pressure was observed sooner following i.v. bolus endothelin-1 administration in the presence of 1 mg kg^{-1} (±)-SB 209670 (at approximately 1–2 min after agonist administration) relative to vehicle-treated rats (at approximately 4–8 min after agonist administration). Thus, it is possible that at these doses, (±)-SB 209670 was having some antagonistic effect against the pressor/vasoconstrictor component of the haemodynamic response to endothelin-1. However, an i.v. infusion of a dose of (±)-SB 209670 greater than the 'no effect dose' of antagonist (1 μg kg^{-1} min^{-1}) and smaller than the 'minimum' dose required to inhibit the vasodilator actions of endothelin-1 (10 μg kg^{-1} min^{-1}) was not studied in order to validate such a hypothesis i.e. an i.v. infusion of 3 μg kg^{-1} min^{-1}. Similarly, a bolus i.v. dose of less than 1 mg kg^{-1} (±)-SB 209670 was not studied.

It has been suggested that the pressor actions of endothelin-1 are mediated by the ET_A receptor subtype since endothelin-1 is more potent than endothelin-3 as a pressor agonist (Spokes *et al.*, 1989; Douglas & Hiley, 1991; Ohlstein & Douglas, 1993). In contrast, the potencies of endothelin-1 and endothelin-3 as vasodepressor agonists are not significantly different, raising the possibility that this response is mediated by the ET_B receptor. This proposition is supported by the observation that the pressor actions of endothelin-1

are inhibited selectively by BQ-123 over a dose-range which does not attenuate the depressor actions of endothelin-1 (Douglas *et al.*, 1992). However, such a classification is now recognized as being an oversimplification. Several ET_B-selective agonists, including BQ-3020, sarafotoxin S6c and [$Ala^{1,3,11,15}$]endothelin-1, increase vascular tone *in vivo* e.g. BQ-3020 increases renal, hindquarter and mesenteric vascular resistances in the conscious rat via a mechanism which is insensitive to the ET_A-selective antagonist FR 139317 (Gardiner *et al.*, 1994b). Indeed, it is now appreciated that ET_B-receptors make a significant contribution to the contractile actions of endothelin-1 both *in vitro* (Harrison *et al.*, 1992; Moreland *et al.*, 1992; Panek *et al.*, 1992; Sumner *et al.*, 1992; Warner *et al.*, 1993) and *in vivo* (Hiley *et al.*, 1989; Douglas & Hiley, 1991; Bigaud & Pelton, 1992; Gardiner *et al.*, 1994b). Furthermore, although radioligand binding studies and functional assays demonstrate that SB 209670 exhibits approximately 30 fold selectively for the ET_A receptor subtype over the ET_B receptor subtype (Nambi *et al.*, 1994; Ohlstein *et al.*, 1994a), the observation that (±)-SB 209670 selectively inhibits the depressor and carotid vasodilator actions of endothelin-1 in the anaesthetized rat demonstrates that the haemodynamic actions of endothelin-1 are not simply the function of ET_B-mediated vasodilatation followed by ET_A-mediated vasoconstriction.

Similar observations to those reported here with SB 209670 have been made with a chemically distinct endothelin receptor antagonist: although less potent than SB 209670, the ET_A/ET_B receptor antagonist, Ro 46-2005 (10 mg kg^{-1}, i.v. bolus) selectively inhibits the vasodepressor actions of endothelin-1 (this dose of Ro 46-2005 did not inhibit the secondary pressor actions of endothelin-1; Clozel *et al.*, 1993a,b). Indeed, a dose of 30 mg kg^{-1} bosentan (Ro 47-0203) is only effective at blocking the secondary pressor actions of endothelin-1 if the dose of endothelin-1 is lowered from 500 to 50 pmol kg^{-1} (Gardiner *et al.*, 1994a). Similarly, bolus i.v. administration of either PD 145065 or PD 142893 selectively inhibits the depressor response to endothelin-1 in the anaesthetized rat (Doherty *et al.*, 1993). Clozel *et al.* (1993b) postulated that the selective inhibition of the dilator actions of endothelin-1 by Ro 46-2005 was the result of a pharmacokinetic phenomenon: since the dilator actions of endothelin-1 are mediated *via* an ET_B-receptor subtype linked to endothelium-derived nitric oxide release *in vitro* (De Nucci *et al.*, 1988; Hiley *et al.*, 1989; Douglas & Hiley, 1990), it was suggested that Ro 46-2005 gained preferential access to this endothelial receptor over the ET_A and ET_B receptor subtypes located on the smooth muscle deeper within the tunica media. The observations reported in this study, where i.v. bolus administration of (±)-SB 209670 selectively inhibits the depressor and carotid vasodilator actions of endothelin-1, would lend support to such a hypothesis. However, since several chemically diverse antagonists with different physico-chemical properties (PD 145065 and PD 142893, linear C-terminal hexapeptide analogues; SB 209670, an indane carboxylate; Ro 46-2005, a pyrimidyl sulphonamide) exhibit this phenomenon, a pharmacokinetic explanation is questionable. Furthermore, the present study demonstrated that this phenomenon was also observed during the sustained i.v. infusion of (±)-SB 209670, during which time the levels of antagonist at the tunica intima and media would have been expected to have approached some type of equilibrium. Several alternative pharmacodynamic explanations exist to explain the differences in sensitivity to endothelin receptor antagonism including differences in ET_A/ET_B spare receptor reserves, receptor-coupling mechanisms or binding kinetics. Finally, it is also possible that in the intact animal, vasodilatation may be mediated by an hitherto uncharacterized non-ET_A (possibly a subtype of the ET_B receptor) which is more sensitive than the ET_A/ET_B receptors mediating vasoconstriction to SB 209670, Ro 46-2005, PD 145065 and PD 142893 (Douglas *et al.*, 1992; Warner *et al.*, 1993).

In summary, (±)-SB 209670 is a bioavailable, efficacious selective endothelin receptor antagonist in the intact rat and may, therefore, serve as a useful pharmacological tool for understanding the pathophysiological role of endogenous endothelin-1 in the control of numerous disease processes including essential hypertension, cerebral vasospasms, vascular restenosis and arterial vasospasm following angioplasty and acute renal dysfunction (Brooks *et al.*, 1994; Douglas *et al.*, 1994d,e; 1995; Elliott *et al.*, 1994a; Ohlstein *et al.*, 1994c; Sulpizio *et al.*, 1994).

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References

BIGAUD, M. & PELTON, J.T. (1992). Discrimination between ET_A- and ET_B-receptor-mediated effects of endothelin-1 and [$Ala^{1,3,11,15}$] endothelin-1 by BQ-123 in the anaesthetized rat. *Br. J. Pharmacol.*, **107**, 912–918.

BROOKS, D.P., DEPALMA, P.D., GELLAI, M., NAMBI, P., OHLSTEIN, E.H., ELLIOTT, J.D., GLEASON, J. & RUFFOLO, R.R. (1994). Nonpeptide endothelin receptor antagonists. III: effect of SB 209670 and BQ-123 on acute renal failure in anesthetized dogs. *J. Pharmacol. Exp. Ther.* (in press).

CLOZEL, M., BREU, V., BURRI, K., CASSAL, K.M., FISCHLI, W., GRAY, G.A., HIRTH, G., LÖFFLER, B.M., MÜLLER, M., NEIDHART, W. & RAMUZ, H. (1993a). Pathophysiological role of endothelin revealed by the first orally active endothelin receptor antagonist. *Nature*, **365**, 759–761.

CLOZEL, M., BREU, V., GRAY, G.A. & LÖFFLER, B.M. (1993b). *In vivo* pharmacology of Ro 46-2005, the first synthetic nonpeptide endothelin receptor antagonist: implications for endothelin physiology. *J. Cardiovasc. Pharmacol.*, **22** (Suppl. 8), S377–S379.

CODY, W.L., DOHERTY, A.M., HE, J.-X., DEPUE, P.L., RAPUNDALO, S.T., HINGORANI, G.A., MAJOR, T.C., PANEK, R.L., DUDLEY, D.T., HALEEN, S.J., LADOUCEUR, D., HILL, K.E., FLYNN, M.A. & REYNOLDS, E.E. (1992). Design of a functional antagonist of endothelin. *J. Med. Chem.*, **35**, 3301–3303.

DE NUCCI, G., THOMAS, R., D'ORLEANS-JUSTE, P., ANTUNES, E., WALDER, C., WARNER, T.D. & VANE, J.R. (1988). Pressor effects of circulating endothelin are limited by its removal from the pulmonary circulation and by the release of prostacyclin and endothelium-derived relaxing factor. *Proc. Natl Acad. Sci. U.S.A.*, **85**, 9797–9800.

DOHERTY, A.M., CODY, W.L., HE, J.X., DEPUE, P.L., CHANG, X.M., WELCH, K.M., FLYNN, M.A., REYNOLDS, E.E., LADOUCEUR, D., DAVIS, L.S., KEISER, J.A. & HALEEN, S.J. (1993). *In vitro* and *in vivo* studies with a series of hexapeptide endothelin antagonists. *J. Cardiovasc. Pharmacol.*, **22** (Suppl. 8), S98–S102.

DOUGLAS, S.A., ELLIOTT, J.D. & OHLSTEIN, E.H. (1992). Regional vasodilation to endothelin-1 is mediated by a non-ET_A receptor subtype in the anaesthetized rat: effect of BQ-123 on systemic haemodynamic responses. *Eur. J. Pharmacol.*, **221**, 315–324.

DOUGLAS, S.A., ELLIOTT, J.D. & OHLSTEIN, E.H. (1994a). SB 209670 inhibits the hemodynamic effects of endothelin-1 (ET-1) in the anesthetized rat. *FASEB J.*, **8**, A42.

DOUGLAS, S.A., ELLIOTT, J.D. & OHLSTEIN, E.H. (1994b). Pharmacological characterization of the nonpeptide endothelin receptor antagonist SB 209670. *Can J. Physiol. Pharmacol.*, **72** (Suppl. 1), 110.

DOUGLAS, S.A., EZEKIEL, M., GELLAI, M. & OHLSTEIN, E.H. (1994c). BQ-123, a selective endothelin (ETA) receptor antagonist, lowers blood pressure in different rat models of hypertension. *J. Hypertension*, **12**, 561–567.

DOUGLAS, S.A., GELLAI, M., EZEKIEL, M., FEUERSTEIN, G.Z., ELLIOTT, J.D. & OHLSTEIN, E.H. (1995). Antihypertensive actions of the novel, non-peptide endothelin receptor antagonist SB 209670. *Hypertension*, (in press).

DOUGLAS, S.A. & HILEY, C.R. (1990). Endothelium-dependent vascular activities of endothelin-like isopeptides in the isolated superior mesenteric arterial bed of the rat. *Br. J. Pharmacol.*, **101**, 81–88.

DOUGLAS, S.A. & HILEY, C.R. (1991). Endothelium-dependent mesenteric vasorelaxant effects and systemic actions of endothelin (16-21) and other endothelin-related peptides in the rat. *Br. J. Pharmacol.*, **104**, 311–320.

DOUGLAS, S.A., LOUDEN, C., VICKERY-CLARK, L.M., STORER, B.L., HART, T., FEUERSTEIN, G.Z., ELLIOTT, J.D. & OHLSTEIN, E.H. (1994d). A role for endogenous endothelin-1 in neointima formation following rat carotid artery balloon angioplasty: protective effects of the novel nonpeptide endothelin receptor antagonist SB 209670. *Circ. Res.*, **75**, 190–197.

DOUGLAS, S.A., VICKERY-CLARK, L.M. & OHLSTEIN, E.H. (1994e). Functional evidence that balloon angioplasty results in transient nitric oxide synthase induction. *Eur. J. Pharmacol.*, **255**, 81–89.

ELLIOTT, J.D., EZEKIEL, M., GELLAI, M., DOUGLAS, S.A. & OHLSTEIN, E.H. (1994a). Antihypertensive effect of the endothelin receptor antagonist SB 209670 in conscious spontaneously hypertensive rats (SHR). *FASEB J.*, **8**, A7.

ELLIOTT, J.D., LAGO, A., COUSINS, R.D., GAO, A., LEBER, J.D., ERHARD, K., EGGLESTON, D., NAMBI, P., LEE, J.A., BROOKS, D.P., FEUERSTEIN, G., RUFFOLO, R.R., Jr, WEINSTOCK, J., GLEASON, J.G., PEISHOFF, C.E. & OHLSTEIN, E.H. (1994b). (1S, 2R, 2S)-3-(2-carboxymethoxy-4-methoxyphenyl)-1-(3,4-methylenedioxy phenyl)-5-(prop-1-yloxy)indane-2-carboxylic acid, a potent and selective non-peptide endothelin receptor antagonist. *J. Med. Chem.*, **37**, 1553–1557.

GARDINER, S.M., COMPTON, A.M. & BENNETT, T. (1989). Regional haemodynamic effects of endothelin-1 in conscious, unrestrained Wistar rats. *J. Cardiovasc. Pharmacol.*, **13** (Suppl. 5), S202–S204.

GARDINER, S.M., KEMP, P.A. & BENNETT, T. (1994a). Effects of bosentan (Ro 47-020), an ET_A-, ET_B-receptor antagonist, on regional haemodynamic responses to endothelins in conscious rats. *Br. J. Pharmacol.*, **112**, 823–830.

GARDINER, S.M., KEMP, P.A., MARCH, J.E., BENNETT, T., DAVENPORT, A.P. & EDVINSSON, L. (1994b). Effects of an ET_A-receptor antagonist, FR 139317, on regional haemodynamic responses to endothelin-1 and [$Ala^{11,15}$]Ac-endothelin-1 (6-21) in conscious rats. *Br. J. Pharmacol.*, **112**, 477–486.

HARRISON, V.J., RANDRIANTSOA, A. & SCHOEFFER, P. (1992). Heterogeneity of endothelin-sarafotoxin receptors mediating contraction of pig coronary artery. *Br. J. Pharmacol.*, **105**, 511–513.

HILEY, C.R., DOUGLAS, S.A. & RANDALL, M.D. (1989). Pressor effects of endothelin-1 and some analogs in the perfused superior mesenteric arterial bed of the rat. *J. Cardiovasc. Pharmacol.*, **13** (Suppl. 5), S197–S199.

IHARA, M., NOGUCHI, K., SAEKI, T., FUKURODA, S., TSUCHIDA, S., KIMURA, T., FUKAMI, K. & YANO, M. (1991). Biological profiles of highly potent novel endothelin antagonists selective for the ET_A receptor. *Life Sci.*, **50**, 247–255.

LE MONNIER DE GOUVILLE, A.-C., LIPPTON, H., COHEN, G., CAVERO, I. & HYMAN, A. (1990). Vasodilator activity of endothelin-1 and endothelin-3: rapid development of cross-tachyphylaxis and dependence on the rate of endothelin administration. *J. Pharmacol. Exp. Ther.*, **254**, 1024–1028.

MORELAND, S, MCMULLEN, D.M., DELANEY, C.L., LEE, V.G. & HUNT, J.T. (1992). Venous smooth muscle contains vasoconstrictor ET_B-like receptors. *Biochem. Biophys. Res. Commun.*, **184**, 100–106.

NAMBI, J., WU, H.L., PULLEN, M., ELSHOURBAGY, N., OHLSTEIN, E.H., BROOKS, D.P., ELLIOTT, J.D., GLEASON, J. & RUFFOLO, R.R. Jr. (1994). Non-peptide endothelin receptor antagonists. I: effects on binding and signal transduction in human ET_A and ET_B receptors. *J. Pharmacol. Exp. Ther.*, (in press).

OHLSTEIN, E.H., BECK, G., DOUGLAS, S.A., NAMBI, P., GLEASON, J.G., RUFFOLO, R.R., Jr, FEUERSTEIN, G.Z. & ELLIOTT, J.D. (1994a). Non-peptide endothelin receptor antagonists. II: pharmacological characterization of SB 209670. *J. Pharmacol. Exp. Ther.*, (in press).

OHLSTEIN, E.H. & DOUGLAS, S.A. (1993). Endothelin-1 modulates vascular smooth muscle structure and vasomotion: implications in cardiovascular pathology. *Drug Develop. Res.*, **29**, 108–128.

OHLSTEIN, E.H., GELLAI, M., BROOKS, D.P., VICKERY, L., JUGUS, J., SULPIZIO, A., RUFFOLO, R.R., Jr, WEINSTOCK, J. & EDWARDS, R.M. (1992). The antihypertensive effect of the angiotensin II receptor antagonist DuP 753 may not be due solely to angiotensin II receptor antagonism. *J. Pharmacol. Exp. Ther.*, **262**, 595–601.

OHLSTEIN, E.H., NAMBI, P., DOUGLAS, S.A., BECK, G., GLEASON, J., RUFFOLO, R.R., Jr, FEUERSTEIN, G. & ELLIOTT, J.D. (1994b). SB 209670, a rationally designed potent non-peptide endothelin receptor antagonist. *FASEB J.*, **8**, A803.

OHLSTEIN, E.H., NAMBI, P., DOUGLAS, S.A., EDWARDS, R.M., GELLAI, M., LAGO, A., LEBER, J.D., COUSINS, R.D., GAO, A., PEISHOFF, C.E., BEAN, J.W., EGGLESTON, D., ELSHOURBAGY, N., KUMAR, C., LEE, J.A., YUE, T.-L., LOUDEN, C., BROOKS, D.P., RUFFOLO, R.R. Jr, FEUERSTEIN, G., WEINSTOCK, J., GLEASON, J.G. & ELLIOTT, J.D. (1994c). SB 209670, a rationally designed nonpeptide endothelin receptor agonist. *Proc. Natl Acad. Sci U.S.A.*, **91**, 8052–8056.

PANEK, R.L., MAJOR, T.C., HINGORANI, G.P., DOHERTY, A.M., TAYLOR, D.G. & RAPUNDALO, S.T. (1992). Endothelin and structurally related analogs distinguish between endothelin receptor subtypes. *Biochem. Biophys. Res. Commun.*, **184**, 566–571.

ROUZ, S.P., CLOZEL, M., SPRECHER, Y., GRAY, G. & CLOZEL, J.-P. (1993). Ro 47-0203, a new endothelin receptor antagonist, reverses chronic vasospasm in experimental subarachnoid hemorrhage. *Circulation*, **88**, I–170.

SPOKES, R.A., GHATEI, M.A. & BLOOM, S.R. (1989). Studies with endothelin-3 and endothelin-1 on rat blood pressure and isolated tissues: evidence for multiple endothelin receptor subtypes. *J. Cardiovasc. Pharmacol.*, **13** (Suppl. 5), S191–S192.

SULPIZIO, A.C., HIEBLE, J.P., MITCHELL, M., SAUERMELCH, C. & WILLETTE, R.N. (1994). Nonpeptide endothelin receptor antagonists: cerebrovascular characterization and effects on delayed cerebral vasospasm. *Can. J. Physiol. Pharmacol.*, **72** (Suppl. 1), 169.

SUMNER, M.J., CANNON, T.R., MUNDIN, J.W., WHITE, D.G. & WATTS, I.S. (1992). Endothelin ETA and ETB receptors mediate vascular smooth muscle contraction. *Br. J. Pharmacol.*, **107**, 858–860.

WARNER, T.D., ALLCOCK, G.H., MICKLEY, E.J., CORDER, R. & VANE, J.R. (1993). Comparative studies with the endothelin receptor antagonist BQ-123 and PD 142893 indicate at least three endothelin receptors. *J. Cardiovasc. Pharmacol.*, **22** (Suppl. 8), S117–S120.

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