British Journal of Pharmacology (2000) 131, 1732–1738

www.nature.com/bjp

Cardiovascular effects of endothelin-1 and endothelin antagonists in conscious, hypertensive ((mRen-2)27) rats

***,[1]S.M. Gardiner, [1]J.E. March, [1]P.A. Kemp & [1]T. Bennett**

[1]School of Biomedical Sciences, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH

1 SB 209670 is a potent antagonist of the vasoconstrictor (ET_A- and ET_B-receptor-mediated) and vasodilator (ET_B-receptor-mediated) effects of endothelin, whereas SB 234551 is relatively selective for the constrictor (ET_A-receptor-mediated) effects. Since we had previously found SB 209670 exerted antihypertensive, vasodilator effects in conscious, heterozygous, transgenic ((mRen-2)27) (abbreviated to TG) rats, here we compared the two antagonists in that model, and assessed their chronic effects on responses to exogenous endothelin-1. We did this to test our global hypothesis, namely, that SB 209670, but not SB 234551, would cause inhibition of the depressor effects of exogenous endothelin-1 *in vivo*, and that this differential effect would be associated with a more marked antihypertensive action of SB 234551 in TG rats.

2 SB 209670 and SB 234551 (infused for 50 h) exerted similar, sustained, antihypertensive effects in TG rats.

3 The antihypertensive effects of the antagonists occurred at times when the pressor effects of exogenous endothelin-1 were not significantly inhibited. Furthermore, SB 234551 did not exert a greater antihypertensive effect than SB 209670 at a time (i.e., 2–4 h) when the depressor effects of endothelin-1 were abolished by the latter, but not by the former (although this differential action was lost after 24 h infusion).

4 The results caused us to reject the hypothesis that selective antagonism of the vasoconstrictor effects of endothelin-1 would result in SB 234551 exerting a greater antihypertensive effect than SB 209670 in TG rats.

British Journal of Pharmacology (2000) **131,** 1732–1738

Keywords: Transgenic rats; endothelin; endothelin antagonists

Abbreviations: SD rats, Hannover Sprague Dawley rats; TG rats, heterozygous transgenic ((mRen-2)27) rats

Introduction

In 1990, Mullins and co-workers (Mullins *et al*., 1990) described hypertension induced by inserting the mouse Ren-2 gene into the genome of the Sprague Dawley (SD) rat. Not surprisingly, the hypertension in such transgenic ((mRen-2)27) (abbreviated to TG) rats is, in part, angiotensin-dependent, since it is clearly responsive to treatment with angiotensin converting enzyme inhibitors or angiotensin receptor antagonists (for review see Lee *et al*., 1996). Unexpectedly, however, we obtained functional evidence for a substantial involvement of endothelin, in addition to angiotensin, in maintaining the hypertension in conscious, male, heterozygous TG rats, since the non-peptide, non-selective (i.e., ET_A- and ET_B-receptor) endothelin receptor antagonist, SB 209670, caused a significant lowering of blood pressure (Gardiner *et al*., 1995). However, in a contemporaneous study, Whitworth *et al*. (1995) reported no effect of bosentan, which is also a non-peptide, non-selective endothelin receptor antagonist (Clozel *et al*., 1993), in male heterozygous TG rats. Some possible explanations for this apparent discrepancy have recently been offered (Rossi *et al*., 1999a; Gardiner & Bennett, 1999; Rossi & Pessina, 1999).

A notable difference between our experiments, and those of Whitworth and colleagues, was the choice of antagonist, particularly since there is evidence to suggest that the properties of SB 209670 and bosentan may differ. Thus, in rat and human pulmonary vessels, endothelin-induced contractions are inhibited by SB 209670, but not by bosentan (McCulloch *et al*., 1998; Ohlstein *et al*., 1998). In this regard, the antagonist profile of the novel, non-peptide, endothelin receptor antagonist, SB 234551 (Ohlstein *et al*., 1998), is of interest. Like SB 209670 (Ohlstein *et al*., 1994) and bosentan (Clozel *et al*., 1994), SB 234551 causes functional antagonism of endothelin-induced contractions (ET_A-receptor-mediated) of rat aorta, and like SB 209670, but unlike bosentan, SB 234551 inhibits endothelin-induced contractions in isolated human pulmonary artery (Ohlstein *et al*., 1998; MacLean *et al*., 1998). However, the most important distinguishing property of SB 234551 is that it is considerably less potent (IC_{50} = 7000 nM) than SB 209670 (IC_{50} = 4 nM) against endothelin-mediated dilatation (ET_B-receptor-mediated) in the rabbit saphenous vein (Ohlstein *et al*., 1998). Thus, SB 209670 is a potent antagonist of the constrictor and dilator effects of endothelin, whereas SB 234551 is potent against endothelin-mediated constrictor, but not dilator, effects (Ohlstein *et al*., 1998). Bosentan differs from both SB 209670 and SB 234552, being ineffective against some endothelin-induced constrictions (Ohlstein *et al*., 1998).

Therefore, against this background it was of interest to compare the antihypertensive activities of SB 209670 and SB 234551 in conscious TG rats, our hypothesis being that the reported lesser activity of SB 234551 against endothelin-induced (ET_B-receptor-mediated) vasodilator effects would result in a greater antihypertensive action, especially because it has been suggested that the predominant role for endogenous endothelin in the rat is vasodilatation (Gellai *et al*., 1996).

*Author for correspondence.

The question of antagonist doses was an important one. It is difficult to perform full, interpretable dose-response studies in conscious animals, particularly using an agonist with such a complex haemodynamic profile as endothelin (Gardiner *et al*., 1994). It might be suggested that abolition of cardiovascular reflexes and neurohumoral mechanisms with appropriate antagonists would simplify the system. However, such interventions in conscious rats produce very low and labile blood pressures (e.g., Gardiner & Bennett, 1985), and these interventions could not be maintained (ethically) for the period of the experimental protocol planned (see Methods). Although testing a range of antagonist doses would have been ideal, this would have required the use of large numbers of additional animals, which we considered ethically unjustifiable. Therefore, we chose the dose of SB 209670 which we had found to lower blood pressure in TG rats (Gardiner *et al*., 1995), and which has also been shown to exert antihypertensive effects in spontaneously hypertensive rats, and in rats with renin-dependent hypertension (Douglas *et al*., 1995b). For SB 234551, we chose a dose which has been reported to cause marked inhibition of endothelin-induced (ET_A-receptor-mediated) pressor effects in conscious rats (Ohlstein *et al*., 1998). In that study, only one submaximal dose of endothelin was used; therefore, we elected to assess the influence of SB 209670 or SB 234551 against a range of endothelin doses.

Methods

Untreated, male, heterozygous TG rats (4–5-months-old), and homozygous, age-matched, male Hannover Sprague Dawley (SD) rats (i.e., the strain from which the TG rats were originally derived (Mullins *et al*., 1990)) were bred in the Biomedical Services Unit, University of Nottingham, from animals supplied by Dr J.J. Mullins (University of Edinburgh). To date, all our recovery surgery for cardiovascular studies has been performed under sodium methohexitone anaesthesia. However, recently sodium methohexitone became unavailable, and hence we needed to find an alternative. The currently recommended anaesthetic regimes for recovery surgery in rats are either fentanyl/fluanisone (Hypnorm) and midazolam, reversed with naloxone, or fentanyl and medetomidine, reversed with nalbuphine and atipamezole (Flecknell, 1996). Therefore, prior to embarking on the main investigations, we performed the following pilot studies to assess the competence of cardiac baroreflexes 24 h after surgery using different anaesthetic regimes, as an index of any differential hangover effects.

Cardiac baroreflex sensitivities in SD and TG rats using different anaesthetic regimes

Groups of TG and SD rats ($n=6$ or 7 in each) were anaesthetized with either sodium methohexitone (60 mg kg^{-1} i.p., supplemented as required), or Hypnorm (fentanyl citrate 0.126 mg kg^{-1}; fluanisone 4 mg kg^{-1}, i.p.) and midazolam (5 mg kg^{-1}, i.p.), reversed with naloxone (0.1 mg kg^{-1}, i.p.), or fentanyl and meditomidine (300 μg kg^{-1} of each, i.p.), reversed with nalbuphine and atipamezole (1 mg kg^{-1} of each, s.c.). Catheters were implanted in the distal abdominal aorta (*via* the caudal artery) for recording blood pressures, and in the right jugular vein for the administration of drugs.

Starting 24 h after surgery, when the animals were fully conscious and freely-moving, blood pressures were measured using a fluid-filled pressure transducer (Bell & Howell, type 4–442) connected, through a Gould transducer amplifier (model 13–4615–50) to a custom-designed microprocessor (University of Limburg, Maastricht), sampling at 2 ms intervals, and averaging and storing to disc every cardiac cycle. To assess cardiac baroreflex sensitivities, each rat received an i.v. bolus injection of sodium nitroprusside (100 μg ml^{-1} in 0.1 ml), or infusion of methoxamine (400 μg ml^{-1} at 0.2 ml min^{-1}), in random order. The slope of the line relating mean arterial blood pressure to the succeeding pulse interval, whilst both variables were changing, was taken as an index of the cardiac baroreflex sensitivity (Gardiner *et al*., 1990).

Effects of SB 209670 or SB 234551 on blood pressure and responses to endothelin-1 in TG and SD rats

On the basis of the results of the pilot studies (see Results), the anaesthetic regime chosen for the full experiments was fentanyl and medetomidine, reversed with nalbuphine and atipamezole (for doses see above). Intravascular catheters were implanted under anaesthesia, and experiments were started 24 h after surgery. Blood pressures were measured for at least 60 min, in conscious, freely moving animals (see above, data stored to disc every 5 s) to obtain baseline values. Thereafter, each animal received i.v. bolus doses of endothelin-1 (25, 50 and 250 pmol kg^{-1}) in ascending order, with at least 30 min between the first and second dose, and at least 60 min between the second and the third dose. Injections were given in a volume of 0.1 ml over a period of 5 s. Sixty minutes after the last dose of endothelin-1 had been given, animals were randomized to receive either SB 209670 (600 μg kg^{-1} bolus, 600 μg kg^{-1} h^{-1} infusion; SD rats $n=7$, TG rats $n=9$), SB 234551 (1 mg kg^{-1} bolus, 1 mg kg^{-1} h^{-1} infusion; SD rats $n=7$, TG rats $n=8$) or vehicle (0.1 ml bolus, 0.4 ml h^{-1} infusion; SD rats $n=7$, TG rats $n=8$). SB 209670 was dissolved in sterile saline (154 mmol l^{-1} NaCl), whereas SB 234551 was dissolved in 0.5% Na_2CO_3 (diluted 1:4 with dextrose for infusate); animals in the vehicle groups received one or other of those solutions. As the experiment progressed it became clear that there were no consistent differences between the effects of the two vehicles. Therefore, to minimize the use of animals, the vehicle groups included rats that had received vehicle for either SB 209670 (3 SD rats, 4 TG rats) or SB 234551 (4 SD rats, 4 TG rats).

Starting 2 h after the onset of the antagonist or vehicle infusions, i.v. bolus doses of endothelin-1 were administered (as above). The infusions were then continued for the remainder of the experimental period, using fluid-filled swivels for overnight administration (Blair *et al*., 1980), and the animals were re-challenged with endothelin in the mornings and afternoons of the following 2 days (i.e. 20–22 h, 24–26 h, 44–46 h and 48–50 h after the onset of antagonist or vehicle administration).

Data analysis

Within-group comparisons of variables or changes were made with Friedman's test (Theordorsson-Norheim, 1987), or Wilcoxon test, as appropriate. Between-group comparisons were made using the Mann–Whitney U-test or Kruskal–Wallis test, as appropriate, with the Holm-Bonferroni correction applied as described by Ludbrook (1998). A P value <0.05 was taken as significant. For the data shown in Figure 1, $m=6$ (3-way comparison at 2 h plus 3-way comparison of the areas (0–50 h). In the analysis of the

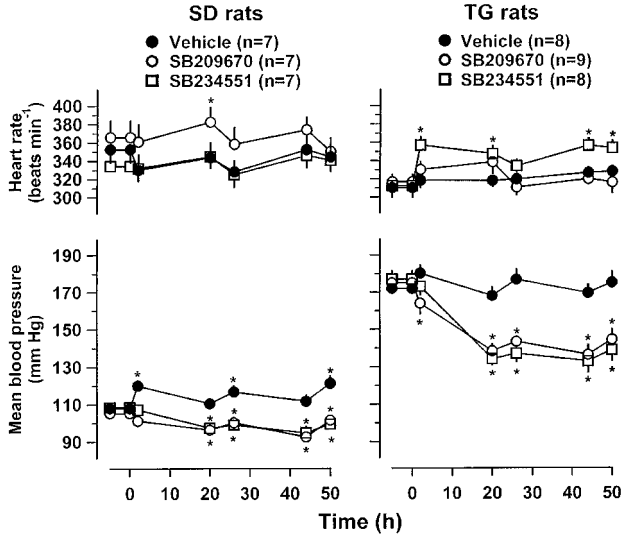

Figure 1 Mean blood pressure and heart rate before and during continuous infusions of vehicle, SB 209670 (600 μg kg^{-1} bolus, 600 μg kg^{-1} h^{-1}) or SB 234551 (1 mg kg^{-1} bolus, 1 mg kg^{-1} h^{-1} infusion) in conscious SD or TG rats. Values are mean and vertical bars show s.e.mean; *$P<0.05$ versus original baseline (Friedman's test). For each time point, the means shown represent the average of the baseline values preceding administration of endothelin-1.

data in Tables 1 and 2, $m=18$ (3-way comparison at each of six time points).

Materials

Endothelin-1 (human) was obtained from Bachem (U.K.). A stock solution (20 nmol ml^{-1}) was prepared in sterile water, and subsequent dilutions were made using sterile saline. SB 209670 ([(+)-(1S, 2R, 3S)-3-(2-carboxymethoxy-4-methoxyphenyl)-1-13,4-methylenedioxyphenyl)-5-(prop-1-yloxy)indane-2-carboxylic acid]), and SB 234551 ([(E)-alpha[[1-butyl-5-[2-[2-carboxyphenyl) methoxyl]-4-methoxyphenyl]-1H-pyrazol-4-yl] methylene]-6-methoxy-1,3-benzodioxole-5-propionic acid]) were gifts from Dr E. Ohlstein (SKB, U.S.A.).

Sodium nitroprusside and methoxamine hydrochloride were obtained from Sigma UK.

Anaesthetic and reversing agents were as follows: sodium methohexitone (Brietal, Lilly); fentanyl/fluanisone (Hypnorm, Janssen); midazolam (Antigen Pharmaceuticals); naloxone (Narcan, DuPont), fentanyl citrate (Martindale); medetomidine hydrochloride (Domitor, Pfizer); nalbuphine hydrochloride (Nubain, DuPont); atipamezole hydrochloride (Antisedan, Pfizer).

Results

Cardiac baroreflex sensitivities in SD and TG rats using different anaesthetic regimes

In conscious SD rats, prior to baroreflex tesing (i.e., 24 h after anaesthesia), there were no significant differences between the resting blood pressures of the animals which had been surgically prepared under anaesthesia with sodium methohexitone (109 ± 3 mmHg; $n=7$), with Hypnorm and midazolam (111 ± 2 mmHg; $n=6$) or with fentanyl and medetomidine (109 ± 1 mmHg; $n=7$). Resting heart rates in the three groups of SD rats were also not different (352 ± 6, 346 ± 9, and 333 ± 5 beats min^{-1}, respectively).

In conscious TG rats, prior to baroreflex testing (i.e., 24 h after anaesthesia), there were no significant differences between the resting blood pressures of the animals which had been surgically prepared with sodium methohexitone (169 ± 4 mmHg; $n=7$), with Hypnorm and midazolam (185 ± 8 mmHg; $n=7$), or with fentanyl and medetomidine (170 ± 4 mmHg; $n=7$) anaesthesia. Resting heart rates in the three groups of TG rats were also not different (311 ± 6, 319 ± 8 and 293 ± 7 beats min^{-1}, respectively).

Resting blood pressures were significantly higher in all groups of TG rats compared to the corresponding groups of SD rats. Resting heart rates tended to be lower in the TG rats than in the SD rats, although the difference was only significant for the groups prepared under fentanyl and medetomidine anaesthesia.

During baroreflex testing, all animals showed highly significant ($P<0.01$) linear correlations between pulse interval and mean blood pressure ($r>0.95$). The slopes of the regression lines (cardiac baroreflex sensitivities) were similar in the three groups of SD rats (1.33 ± 0.18, 1.05 ± 0.07 and 1.13 ± 0.15 ms mmHg^{-1} during methoxamine infusion, and 1.31 ± 0.16, 1.11 ± 0.10 and 1.32 ± 0.19 ms mmHg^{-1} following nitroprusside administration, in animals prepared under sodium methohexitone ($n=7$), Hypnorm and midazolam ($n=6$) and fentanyl and medetomidine ($n=7$), respectively).

In the TG rats, the slopes of the regression lines (cardiac baroreflex sensitivities) were similar in the groups prepared under sodium methohexitone or fentanyl and medetomidine (0.74 ± 0.15, 0.63 ± 0.17 ms mmHg^{-1} during methoxamine infusion and 0.71 ± 0.12, and 1.15 ± 0.11 ms mmHg^{-1} following nitroprusside administration, respectively ($n=7$ in each group)). However, in the group of TG rats prepared under Hypnorm and midazolam ($n=7$), the cardiac baroreflex sensitivity during the methoxamine test (0.29 ± 0.10 ms mmHg^{-1}) was significantly less than in the other two groups of TG rats. During the nitroprusside test, the cardiac baroreflex sensitivity (0.63 ± 0.17 ms mmHg^{-1}) was significantly less than in the group prepared under fentanyl and medetomidine, but not different from that of the group prepared under sodium methohexitone.

Cardiac baroreflex sensitivities in TG rats were significantly ($P<0.05$) less than in the corresponding group of SD rats, with the exception of the values obtained with the nitroprusside test in the groups prepared under fentanyl and medetomidine anaesthesia.

Effects of SB 209670 or SB 234551 on blood pressure and responses to endothelin-1 in TG and SD rats

Blood pressure For each time point, the average of the three values prior to each endothelin dose (see Methods), was used as the resting value, and the data are shown in Figure 1.

At the start of the experiment, resting arterial blood pressures in the three groups of SD rats were not different. Over the 50 h period of vehicle infusion, blood pressures were significantly higher than baseline (i.e., on the morning of day 1) at 2, 26 and 50 h (i.e., during the afternoon recordings). During infusion of SB 209670 or SB 234551, there were falls in arterial blood pressure in SD rats which were significant from 20 h (Figure 1) onwards. The integrated (area over the curve (0–50 h)) responses to SB 209670 and SB 234551 were not significantly different (385 ± 68 ($n=7$) and 465 ± 99 ($n=7$) mmHg h, respectively). There were no consistent changes in heart rate in the three groups of SD rats throughout the 50 h period of infusion (Figure 1).

Table 1 Responses to endothelin in SD rats

	Control	*2–4 h*	*20–22 h*	*24–26 h*	*44–46 h*	*48–50 h*
25 pmol kg^{-1}						
Vehicle	10±2	16±3	11±2	17±5	11±2	10±4
SB 209670	12±1	7±2	14±4	15±5	9±2	17±6
SB 234551	10±2	6±2	7±2	8±2	7±1	10±3
50 pmol kg^{-1}						
Vehicle	16±2	25±4	18±3	24±6	19±2	23±4
SB 209670	16±5	18±2	15±2	17±3	14±5	15±2
SB 234551	15±2	13±3	10±1	11±2	8±1	15±4
250 pmol kg^{-1} (depressor)						
Vehicle	−23±2	−23±4	−24±5	−20±4	−18±3	−15±2
SB 209670	−23±4	7±2*	5±1*	9±6*	6±1*	8±2*
SB 234551	−21±2	−17±3†	−9±2*†	−7±2*†	5±5*	5±4*
250 pmol kg^{-1} (pressor)						
Vehicle	33±5	35±4	38±5	35±3	39±5	33±4
SB 209670	38±5	34±4	36±6	38±5	39±3	36±3
SB 234551	30±4	17±3*†	24±2	24±3	27±2	25±4

Values (mean±s.e.mean; $n=7$ in each group) are the changes in mean arterial blood pressure (mmHg) from baseline to the peak of the response to endothelin, *$P<0.05$ versus corresponding vehicle infused group; †$P<0.05$ SB 234551 versus SB 209670 (Kruskal–Wallis test with Holm-Bonferroni correction).

Table 2 Responses to endothelin in TG rats

	Control	*2–4 h*	*20–22 h*	*24–26 h*	*44–46 h*	*48–50 h*
25 pmol kg^{-1}						
Vehicle	11±2	14±4	11±2	13±3	13±4	10±2
SB 209670	13±2	12±2	14±4	13±4	15±6	12±3
SB 234551	7±1	8±2	10±2	9±2	11±3	10±3
50 pmol kg^{-1}						
Vehicle	19±4	21±4	18±3	20±3	20±3	17±4
SB 209670	16±3	16±2	16±2	20±3	17±3	14±3
SB 234551	17±3	10±2	12±3	13±3	12±4	12±4
250 pmol kg^{-1} (depressor)						
Vehicle	−44±6	−57±8	−41±6	−48±7	−44±4	−36±5
SB 209670	−50±5	−3±2*	1±1*	3±2*	4±2*	7±2*
SB 234551	−48±3	−46±7†	−13±4*†	−12±4*	−7±6*	1±5*
250 pmol kg^{-1} (pressor)						
Vehicle	41±5	34±7	43±6	41±5	41±5	45±4
SB 209670	32±3	32±3	44±5	41±5	40±5	41±5
SB 234551	38±3	26±5	30±4	39±5	35±7	35±6

Values (mean±s.e.mean; $n=8$ or 9; see Figure 1) are the changes in mean arterial blood pressure (mmHg) from baseline to the peak of the response to endothelin, *$P<0.05$ versus corresponding vehicle infused group; †$P<0.05$ SB 234551 versus SB 209670 (Kruskal–Wallis test with Holm-Bonferroni correction).

Resting arterial blood pressures in the three groups of TG rats at the start of the experiment were not significantly different from each other, and were significantly higher than those of the SD rats (Figure 1).

During infusion of vehicle, there were no significant changes in arterial blood pressure in TG rats. Infusion of SB 209670 or SB 234551 caused falls in arterial blood pressure in TG rats which were significant from 2 h and 20 h, respectively, although at the 2 h time point there was no significant difference between the changes in the two groups. Moreover, the integrated (area over the curve (0–50 h)) responses to SB 209670 and SB 234551 were not significantly different (1492±159 ($n=9$) and 1689±244 ($n=8$) mmHg h, respectively), but in both cases were significantly ($P<0.05$) greater than the corresponding changes in the SD rats. In TG rats receiving vehicle or SB 209670, there were no significant changes in heart rate whereas, in the group receiving SB 234551, there was tachycardia (Figure 1). Over the entire experimental period, the integrated (area under the curve (0–50 h)) heart rate response to SB 234551 (1935±247 beats ($n=8$)), was significantly greater than the response to SB 209670 (778±190 beats ($n=9$)) or to vehicle (925±318 beats ($n=8$)).

Responses to endothelin-1 Measurements were made before and at the peak of the blood pressure changes following endothelin-1 administration. The two lower doses only caused pressor effects, whereas the highest dose caused a fall, followed by a rise, in arterial blood pressure.

Before the start of antagonist or vehicle infusion, the absolute pressor responses to endothelin-1 in all three groups of TG rats were similar to those in the corresponding groups of SD rats, but the depressor responses were significantly ($P<0.05$) greater in the former (Tables 1 and 2). However, when expressed in percentage terms, the depressor responses were not different, whereas the pressor responses were smaller in TG ($n=8$) than in SD ($n=7$) rats for the two lower doses of endothelin-1 (6±1% vs 10±1 and 11±2 vs 15±2%).

In the SD rats receiving vehicle or SB 209670 there were no changes in the pressor responses to endothelin-1, although depressor responses were consistently abolished by SB 209670 (Table 1). In contrast, SB 234551, caused significantly less inhibition (than SB 209670) of the depressor effects of endothelin-1 between 2 and 26 h after the onset of infusion of the antagonist. At the 2 h time point there was also a significant inhibition of the pressor actions of the highest dose of endothelin-1 (Table 1).

In TG rats receiving vehicle, SB 209670, or SB 234551, there were no significant changes in pressor responses to endothelin-1 (Table 2). However, the depressor responses to the highest dose of endothelin-1 was abolished at all time points by SB 209670, whereas SB 234551 had no effect on the depressor response at 2 h, and caused significantly less inhibition than SB 209670 up to 26 h (Table 2).

The heart rate responses to endothelin-1 administration were not analysed in detail, since the timing of the cardiovascular measurements was determined by the blood pressure profile, and hence did not necessarily reflect the maximum changes in heart rate. Furthermore, the differences in cardiac baroreflex sensitivities between SD and TG rats (above) confounded the interpretation of the results. In SD rats, before the start of infusion of vehicle, SB 209670, or SB 234551, there was a dose-dependent bradycardia in response to the two lower doses of endothelin-1 (-8 ± 3 ($n=7$), -9 ± 3 ($n=7$), -4 ± 3 ($n=7$) beats min^{-1}, respectively, following 25 pmol kg^{-1} and -30 ± 5 ($n=7$), -21 ± 2 ($n=7$), -30 ± 5 ($n=7$) beats min^{-1}, respectively, following 50 pmol kg^{-1}). The biphasic blood pressure response to the highest dose of endothelin-1 was associated with a rise ($+91\pm16$ ($n=7$), $+83\pm36$ ($n=7$), $+87\pm15$ ($n=7$) beats min^{-1}) followed by a fall (-79 ± 17 ($n=7$), -79 ± 18 ($n=7$), -53 ± 8 ($n=7$) beats min^{-1}) in heart rate in the three groups of SD rats (vehicle, SB 209670 and SB 234551, respectively).

In TG rats, the changes in heart rate following endothelin-1 administration were less consistent. Thus, before the start of vehicle, SB 209670, or SB 234551 infusion, the changes in heart rate in response to the two lower doses of endothelin-1 were: -1 ± 5 ($n=8$), -1 ± 5 ($n=9$), -3 ± 6 ($n=8$) and -15 ± 6 ($n=8$), -13 ± 3 ($n=9$), and -9 ± 5 ($n=8$) beats min^{-1}, respectively. The depressor response to the highest dose of endothelin-1 was associated with a rise in heart rate ($+75\pm10$ ($n=8$), $+67\pm9$ ($n=9$), $+89\pm5$ ($n=8$) beats min^{-1} in vehicle, SB 209670 and SB 234551 groups, respectively) but there were no consistent changes in heart rate accompanying the subsequent rise in blood pressure ($+13\pm5$ ($n=8$), -7 ± 6 ($n=9$), $+5\pm10$ ($n=8$) beats min^{-1} in vehicle, SB 209670 and SB 234551 groups, respectively).

Over the 50 h period of infusion of the antagonists, inhibition of the depressor response to the highest dose of endothelin-1 was associated with a loss of the accompanying tachycardia in both strains of rat (data not shown).

Discussion

The primary aim of the present study was to test the hypothesis that, in conscious, hypertensive, TG rats, the relatively selective ET_A-receptor antagonist, SB 234551 (Ohlstein *et al*., 1998), exerts a greater antihypertensive action than the non-selective (ET_A-ET_B-receptor) antagonist, SB 209670 (Ohlstein *et al*., 1994). On the basis of our results, this hypothesis must be rejected, since, at the doses used, we achieved an equivalent degree of blood pressure lowering with both antagonists.

An obvious question is to what extent were endothelin-mediated vasodilator effects preserved in the presence of the chosen dose of SB 234551, i.e., how selective was the effect of the antagonist? It was for this reason that we incorporated endothelin challenges into our experimental protocol, while being aware of the difficulty in quantitating functional antagonism of endothelin-mediated haemodynamic effects *in vivo*, due to its opposing vasoconstrictor and vasodilator actions (Clozel *et al*., 1994; Gardiner *et al*., 1994; Douglas *et al*., 1995a). We tried to diminish this problem by assessing the effects of a range of endothelin doses (Gardiner *et al*., 1994). The highest dose of endothelin we used caused an initial depressor effect which was uninfluenced by SB 234551 on the first day of administration but, thereafter, was increasingly inhibited. In contrast, SB 209670 abolished the depressor response from the first day onwards. This finding could, straightforwardly, be taken to indicate some degree of selectivity of SB 234551 for the vasoconstrictor effects of endothelin in the early stages of the experiment, but less so thereafter. However, it is notable that, at the time when depressor responses to endothelin were differentially affected by SB 234551 and SB 209670, the blood pressure profile was the opposite to that which we would have predicted, i.e., there was a small antihypertensive effect of SB 209670, but not of SB 234551, although the difference between the groups was not significant. This observation, we consider, is a solid reason for rejecting our hypothesis.

The pressor effects of endothelin were barely inhibited by SB 209670 in either SD or TG rats. These findings are consistent with studies in other models of experimental hypertension, which indicate that there is a clear separation between demonstrable antagonism of the pressor effects of bolus doses of exogenous endothelin and the antihypertensive effects of SB 209670 (Douglas *et al*., 1995a,b; Bunting & Widdop, 1999).

The effects of SB 234551 on the pressor responses to endothelin were also quite modest, and only apparent in SD rats. We know of only one study in which the effects of SB 234551 have been tested *in vivo* in rats (Ohlstein *et al*., 1998). There it was shown that a bolus dose of 1 mg kg^{-1} SB 234551 caused substantial acute inhibition of the pressor effects of a single bolus dose of endothelin (75% inhibition after 30 min, $\sim$40% inhibition after 2 h, $\sim$10% inhibition after 4 h), but infusions of SB 234551 were not given. Our results in SD rats, showing 40–50% inhibition of the pressor responses to endothelin 2 h after the onset of infusion of SB 234551, are consistent with those findings, but it is clear that, as for SB 209670, any functional antagonism by SB 234551 of the pressor effects of endothelin was not persistent.

The dose of endothelin used by Ohlstein *et al*. (1998) did not cause a depressor response; therefore, we believe we are the first to show the effects of SB 234551 in this context. In the early stages of the experiment, SB 234551 appeared to be selective for the pressor effects of endothelin (250 pmol kg^{-1}), but, as mentioned above, at the dose used here, the selectivity of SB 234551 for the vasoconstrictor effects of endothelin was lost with time. Notably, this was not accompanied by a waning of its antihypertensive effect. Thus, as for SB 209670, there was a disparity between the different *in vivo* actions of the antagonist.

Collectively, our findings indicate that the similar abilities of SB 209670 and SB 234551 to lower blood pressure in SD rats, and, to a greater extent in TG rats, occurred under conditions in which there was not necessarily a reduction in the pressor effects of bolus doses of endothelin. One interpretation of these findings is that the hypotensive effects of the antagonists depend on inhibition of the interaction between endogenous endothelin and the renin-angiotensin system (see review, Rossi *et al*., 1999a), rather than straightforward inhibition of endothelin-mediated vasoconstriction. Elsewhere, we have suggested that relative upregulation of the renin-angiotensin system in TG rats may promote an involvement of endothelin in the hypertension

(Gardiner *et al*., 1995; Gardiner & Bennett, 1999). However, it is equally feasible that the positive effect of endothelin on the renin-angiotensin system (see review, Rossi *et al*., 1999a) could contribute to the abnormal cardiovascular status in TG rats. One possibility raised by our previous findings was that TG rats, for various reasons (including structural), might be more sensitive to endothelin, and this could explain the antihypertensive responses to SB 209670, in the face of plasma levels of endothelin which are not elevated (Gardiner *et al*., 1995). However, here we did not find consistently greater pressor responses to endothelin in TG rats compared to SD rats, in spite of a depressed cardiac baroreflex sensitivity in the former. Hence, it appears that the involvement of endogenous endothelin in the maintenance of hypertension in TG rats, and, less so, in the maintenance of normotension in SD rats, is not due to differential sensitivity to the peptide.

How, then, are these findings to be reconciled with those of Whitworth *et al*. (1995), and those reported in a recent abstract by Rossi *et al*. (1999b), showing that bosentan did not lower blood pressure in TG rats? Elsewhere (Rossi & Pessina, 1999) it has been suggested that our measurements, 24 h after catheterization, may have been made under conditions in which endothelin release in TG rats was stimulated by stress and sympathetic activation; Rossi & Pessina (1999) claimed this was consistent with 'the high heart rate' in our recordings. However, our TG rats are relatively bradycardic (see Results), with resting heart rates not different from those recorded by telemetry (Witte & Lemmer (1995), and our own unpublished observations), and have plasma endothelin levels which are not elevated (Gardiner *et al*., 1995). Moreover, in the present study, the antihypertensive effects of the endothelin antagonists were maintained over 3 days. If it was the case that the previously reported antihypertensive effects of SB 209670 in TG rats were due to suppression of the acute actions of endothelin, stimulated by our experimental protocol, this effect should have waned with the passage of time.

The present results, showing a clear-cut and sustained antihypertensive effect of SB 234551 in TG rats, indicate that our earlier findings were not due to an idiosyncratic action of SB 209670. As indicated in the Introduction, the 'atypical' endothelin receptor mediating vasoconstriction in the human and rat pulmonary vasculature is antagonized by SB 209670 and by SB 234551, but not by bosentan or BMS 182874 (McCulloch *et al*., 1998; Ohlstein *et al*., 1998). Thus, it is possible that the different antagonist profiles of the drugs explains the failure of bosentan to lower blood pressure in TG rats (Whitworth *et al*., 1995; Rossi *et al*., 1999b).

An unexpected finding in the present study was the difference in the heart rate responses to SB 209670 and SB 234551 in TG rats. We (Gardiner *et al*., 1995) and others (Douglas *et al*., 1995b; Bunting & Widdop, 1999) have reported that the antihypertensive effects of SB 209670 were not associated with sustained tachycardia. Here it was shown that, during the 50 h period of infusion of SB 234551, there was tachycardia. We know of no studies in which the heart rate responses to chronic administration of ET_A-receptor antagonists in hypertensive rats have been reported over the time course studied here, although it is of interest that Douglas *et al*. (1994) reported inconsistent effects of BQ123 on heart rate following short term (6 h) administration in different models of hypertension. Notably, a recent study in rats with chronic heart failure (Mulder *et al*., 2000) reported similar effects on blood pressure, yet differential effects on heart rate, of combined ET_A/ET_B receptor antagonism compared to ET_A-selective antagonism, with a reduction in heart rate caused by the former, but not by the latter. These findings are consistent with the negative (ET_A-receptor mediated) and positive (ET_B-receptor mediated) chronotropic effects of endothelin *in vitro* (Ono *et al*., 1998). However, the mechanism(s) underlying the *in vivo* effects, and their potential therapeutic significance, require further investigation.

In conclusion, the present results show that, over a 50 h period of infusion, the endothelin receptor antagonists, SB 209670 and SB 234551, exert similar, sustained, antihypertensive effects in TG rats, despite initial differential effects on the depressor actions of exogenous endothelin-1. Moreover, the ability of the endothelin antagonists to lower blood pressure in TG rats is not accounted for by an increased sensitivity to the pressor action of exogenous endothelin-1 when compared to normotensive SD rats, which showed only small falls in blood pressure in response to SB 209670 or SB 234551.

References

BLAIR, R., FISHMAN, B., AMIT, Z. & WEEKS, J.R. (1980). A simple double channel swivel for infusions of fluids into unrestrained animals. *Pharmacol. Biochem. Behav.,* **12,** 463 – 466.

BUNTING, M.W. & WIDDOP, R.E. (1999). Differential haemodynamic effects of endothelin receptor antagonist, SB 209670, in conscious hypertensive and normotensive rats. *Eur. J. Pharmacol.,* **381,** 13 – 21.

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

CLOZEL, C., BREU, V., GRAY, G.A., KALINA, B., LÖFFLER, B.M., BURRI, K., CASSAL, J.M., HIRTH, G., MÜLLER, M., NEIDHART, W. & RAMUZ, H. (1994). Pharmacological characterization of bosentan, a new potent orally active nonpeptide endothelin receptor antagonist. *J. Pharmacol. Exp. Ther.,* **270,** 228 – 235.

DOUGLAS, S.A., EDWARDS, R.M., ELLIOTT, J.D. & OHLSTEIN, E.H. (1995a). *In vivo* pharmacological characterization of the nonpeptide receptor antagonist SB 209670. *Br. J. Pharmacol.,* **114,** 405 – 413.

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

DOUGLAS, S.A., GELLAI, M., EZEKIEL, M., FEUERSTEIN, G.Z., ELLIOTT, J.D. & OHLSTEIN, E.H. (1995b). Antihypertensive actions of the novel nonpeptide endothelin receptor antagonist SB 209670. *Hypertension,* **25,** 818 – 822.

FLECKNELL, P.A. (1996). *Laboratory Animal Anaesthesia* (Second Edition). London: Academic Press.

GARDINER, S.M. & BENNETT, T. (1985). Interactions between neural mechanisms, the renin-angiotensin system and vasopressin in the maintenance of blood pressure during water deprivation: studies in Long Evans and Brattleboro rats. *Clin. Sci.,* **68,** 647 – 657.

GARDINER, S.M. & BENNETT, T. (1999). Comment on 'Interactions between endothelin-1 and the renin-angiotensin-aldosterone system'. *Cardiovasc. Res.,* **44,** 449.

GARDINER, S.M., COMPTON, A.M., BENNETT, T. & HARTLEY, C.J. (1990). Can pulsed Doppler technique measure changes in aortic blood flow in conscious rats? *Am. J. Physiol.,* **259,** H448 – H456.

GARDINER, S.M., KEMP, P.A., MARCH, J.E. & BENNETT, T. (1994). Effects of bosentan (Ro 47-0203), 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., MARCH, J.E., KEMP, P.A., MULLINS, J.J. & BENNETT, T. (1995). Haemodynamic effects of losartan and the endothelin antagonist, SB 209670, in conscious, transgenic ((mRen-2)27), hypertensive rats. *Br. J. Pharmacol.,* **116,** 2237–2244.

GELLAI, M., FLETCHER, T., PULLEN, M. & NAMBI, P. (1996). Evidence for the existence of endothelin-B receptor subtypes and their physiological roles in the rat. *Am. J. Physiol.,* **40,** R254–R261.

LEE, M.A, BÖHM, M., PAUL, M., BADER, M., GANTEN, U. & GANTEN, D. (1996). Physiological characterization of the hypertensive transgenic rat TGR (mREN2)27. *Am. J. Physiol.,* **270,** E919–E929.

LUDBROOK, J. (1998). Multiple comparison procedures updated. *Clin. Exp. Pharmacol. Physiol.,* **25,** 1032–1037.

MACLEAN, M.R., DOCHERTY, C.C., MCCULLOCH, K.M. & MORECROFT, I. (1998). Effect of novel mixed ET_A/ET_B antagonists on responses to ET-1 in human small muscular pulmonary arteries. *Pulm. Pharmacol. Ther.,* **11,** 147–149.

MCCULLOCH, K.M., DOCHERTY, C. & MACLEAN, M. (1998). Endothelin receptors mediating contraction of rat and human pulmonary resistance arteries: effect of chronic hypoxia in the rat. *Br. J. Pharmacol.,* **123,** 1621–1630.

MULDER, P., BOUJEDAINI, H., RICHARD, V., DERUMEAUX, G., HENRY, J.P., RENET, S., WESSALE, J., OPGENORTH, T. & THUILLEZ, C. (2000). Selective endothelin-A versus combined endothelin-A/endothelin-B receptor blockade in rat chronic heart failure. *Circulation,* **102,** 491–493.

MULLINS, J.J., PETERS, J. & GANTEN, D. (1990). Fulminant hypertension in transgenic rats harbouring the mouse *Ren-2* gene. *Nature,* **344,** 541–544.

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

OHLSTEIN, E.H., NAMBI, P., HAY, D.W.P., GELLAI, M., BROOKS, D.P., LUENGO, J., XIANG, J.N. & ELLIOTT, J.D. (1998). Nonpeptide endothelin receptor antagonists XI. Pharmacological characterization of SB 234551, a high-affinity and selective nonpeptide ET_A receptor antagonist. *J. Pharmacol. Exp. Ther.,* **286,** 650–656.

ONO, K., SAKAMOTO, A., MASAKI, T. & SATAKE, M. (1998). Desensitization of ET_A endothelin receptor-mediated negative chronotropic response in right atria–species difference and intracellular mechanisms. *Br. J. Pharmacol.,* **125,** 787–797.

ROSSI, G.P. & PESSINA, A.C. (1999). Endothelin-1 in angiotensin II-dependent hypertension. *Cardiovasc. Res.,* **44,** 450–451.

ROSSI, G.P., SACCHETTO, A., CESARI, M. & PESSINA, A.C. (1999a). Interactions between endothelin-1 and the renin-angiotensin-aldosterone system. *Cardiovasc. Res.,* **43,** 300–307.

ROSSI, G.P., SACCHETTO, A., RIZZONI, D., BOVA, S., PORTERI, E., MAZZOCCHI, G., NUSSDORFER, G.G. & PESSINA, A.C. (1999b). Angiotensin II AT-1 and not endothelin receptor blockade prevents hypertension and related cardiovascular disease in renin (mREN-2) transgenic rats via adrenocortical steroids-independent mechanisms. *Circulation,* **100** (Suppl.), Abs. 2898.

THEODORSSON-NORHEIM, E. (1987). Friedman and Quade tests: BASIC computer program to perform non-parametric two-way analysis of variance and multiple comparisons on ranks of several related samples. *Comput. Biol. Med.,* **17,** 5–99.

WHITWORTH, C.E., VENIANT, M.M., FIRTH, J.D., CUMMING, A.D. & MULLINS, J.J. (1995). Endothelin in the kidney in malignant phase hypertension. *Hypertension,* **26,** 925–931.

WITTE, K. & LEMMER, B. (1995). Free-running rhythms in blood pressure and heart rate in normotensive and transgenic hypertensive rats. *Chronobiol. Int.,* **12,** 237–247.

(Received May 16, 2000
Revised September 11, 2000
Accepted October 12, 2000)