Br. J. Pharmacol. (1994), **112**, 963–971

Endothelin-1-induced myocardial ischaemia and oedema in the rat: involvement of the ET_A receptor, platelet-activating factor and thromboxane A_2

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1 The objectives of the present experiments were to assess the role of ET_A receptors in mediating endothelin-1 (ET-1)-induced myocardial ischaemia and oedema and to study the involvement of platelet-activating factor (PAF) and thromboxane A_2 (TxA_2) in these actions of ET-1 in rats.

2 Intravenous bolus injection of ET-1 (0.1–2 nmol kg^{-1}) into anaesthetized rats induced ST segment elevation of the electrocardiogram in a dose-dependent manner without causing arrhythmias. ST segment elevation developed within 20–90 s and persisted for at least 10–20 min following administration of ET-1.

3 Pretreatment of the animals with the selective endothelin ET_A receptor antagonist, FR 139317 (2.5 mg kg^{-1}, i.v.) inhibited by 86% the ST segment elevation elicited by ET-1 (1 nmol kg^{-1}). Pretreatment with intravenous administration of BM 13505 (1 mg kg^{-1}), a TxA_2 receptor antagonist, OKY-046 (10 mg kg^{-1}), a thromboxane synthase inhibitor or the specific PAF receptor antagonist, WEB 2086 (1 mg kg^{-1}) or BN 52021 (10 mg kg^{-1}) markedly suppressed ST segment elevation in response to ET-1. Infusion of indomethacin (3 mg kg^{-1} bolus plus 2 mg kg^{-1} h^{-1}) did not significantly affect ET-1-induced ST segment elevation.

4 Bolus injection of ET-1 (1 nmol kg^{-1}, i.v.) to conscious rats resulted in a prolonged pressor effect preceded by a transient depressor response. Corresponding to changes in blood pressure, a small transient tachycardia was followed by a sustained bradycardia. ET-1 enhanced albumin leakage by 87 and 120% in the left ventricle and right atrium, respectively, as measured by the extravasation of Evans blue dye.

5 The selective ET_A receptor antagonist, FR 139317 (2.5 mg kg^{-1}) significantly blunted the pressor action of ET-1 and the accompanying bradycardia without affecting the depressor response. Furthermore, FR 139317 almost completely abolished the permeability effect of ET-1 in both vascular beds studied.

6 Pretreatment of the animals with BM 13505 (1 mg kg^{-1}), OKY-046 (10 mg kg^{-1}), WEB 2086 (1 mg kg^{-1}) or BN 52021 (10 mg kg^{-1}) significantly reduced ET-1 (1 nmol kg^{-1})-induced albumin extravasation both in the left ventricle and right atrium. The PAF receptor antagonists, WEB 2086 and BN 52021 were equally potent inhibitors in the left ventricle, whereas BN 52021 appeared to be a more potent inhibitor than WEB 2086 in the right atrium. Pretreatment with indomethacin (3 mg kg^{-1} plus 2 mg kg^{-1} h^{-1}) did not modify the permeability response to ET-1. None of these compounds affected significantly ET-1-induced changes in mean arterial blood pressure and heart rate.

7 These results indicate that intravenous administration of ET-1 provokes ST segment elevation and myocardial oedema and suggest that these events are mediated, in part, through release of secondary mediators, such as PAF and TxA_2 via the activation of ET_A receptors.

Keywords: Endothelin; ET_A receptor; FR139317; ST segment elevation; protein extravasation; myocardial ischaemia; PAF; thromboxane; rat heart

Introduction

An increasing body of evidence suggests that endothelin-1 (ET-1) plays an important role in coronary ischaemic diseases. Elevated plasma concentrations of ET-1 can be detected in the coronary circulation both in patients with ischaemic heart diseases (Yasuda *et al.*, 1990; Matsuyama *et al.*, 1991; Toyo-oka *et al.*, 1991; Ray *et al.*, 1992) and in laboratory animals during experimental myocardial ischaemia and reperfusion (Tsuji *et al.*, 1991; Watanabe *et al.*, 1991). Coronary arteries from man and other species are very sensitive to the vasoconstrictor action of ET-1 *in vitro* (Yanagisawa *et al.*, 1988; Chester *et al.*, 1989; Cocks *et al.*, 1989; Franco-Cereceda, 1989). Administration of ET-1 directly into the coronary artery of dogs, pigs and rats dramatically increased coronary resistance and ST segment elevations of the electrocardiogram, similar to the clinical phenomenon of Prinzmetal angina, were observed (Ezra *et al.*, 1989; Kurihara *et al.*, 1989; Nichols *et al.*, 1990; Hom *et al.*, 1992; Harada *et al.*, 1993). Furthermore, intravenous injection of ET-1 has been reported to enhance albumin extravasation in the rat coronary circulation (Filep *et al.*, 1992) and therefore contributing to oedema formation, a characteristic feature of the inflammatory reaction associated with acute myocardial ischaemia (Entman *et al.*, 1991).

The cardiac actions of ET-1 may be mediated through activation of one or more endothelin receptor subtypes. Both ET_A (which is highly selective for ET-1) and ET_B (non isopeptide-selective) receptor subtypes are expressed in cardiac tissues (Arai *et al.*, 1990; Sakurai *et al.*, 1990; Lin *et al.*, 1991; Molenaar *et al.*, 1993). In addition, pharmacological studies have suggested the existence of a third, non ET_A/ET_B receptor subtype in the pig coronary artery, which, like ET_A receptors, may mediate the vasoconstrictor action of ET-1 (Harrison *et al.*, 1992). It remains to be determined whether

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this ET receptor subtype is similar to those described on bovine cultured endothelial cells (Emori *et al.*, 1990) or rat pituitary gland (Samson *et al.*, 1990). The mechanism of action of ET-1 on the coronary vasculature *in vivo* is far from being fully understood. Results from studies on isolated coronary arteries have implicated the involvement of voltage-gated calcium channels (Yanagisawa *et al.*, 1988) and stimulation of phosphoinositide hydrolysis with subsequent release of intracellular calcium (Kasuya *et al.*, 1989; Pang *et al.*, 1989) in mediating the contractile action of ET-1. The findings that the *in vivo* coronary vasoconstrictor action of ET-1 can be only partially antagonized by calcium channel blockers (Hom *et al.*, 1992; Harada *et al.*, 1993) indicate that mechanisms other than calcium influx through voltage-operated channels are also involved. Indeed, recent evidence suggests that secondary mediators, such as platelet-activating factor (PAF) and thromboxane A_2 (TxA_2) may mediate certain actions of ET-1. Both PAF and TxA_2 have been implicated in ET-1-induced mobilization of intracellular Ca^{2+} in cultured vascular smooth muscle cells (Takayashu *et al.*, 1989). PAF receptor antagonists have been reported to attenuate albumin extravasation elicited by ET-1 in the rat pulmonary and gastrointestinal circulation (Filep *et al.*, 1991b) and to protect against ET-1-induced sudden-death in mice and rats (Terashita *et al.*, 1989). In the present study, we examined the effects of a selective ET_A receptor antagonist, FR 139317 (Aramori *et al.*, 1993; Sogabe *et al.*, 1993) on the coronary vascular responses to ET-1 and studied the involvement of PAF and TxA_2 in mediating the coronary vascular actions of ET-1 in the rat.

Methods

Vascular permeability measurements

The experiments were performed on conscious, chronically catheterized male Wistar rats weighing 205–290 g. The animals were housed in individual metabolic cages and catheters were implanted into the abdominal aorta and vena cava as described previously (Filep *et al.*, 1987). During the experiments the animals could move freely and had free access to food and water. Mean arterial blood pressure (MABP) and heart rate were monitored continuously by a blood pressure analyzer (Micro-Med, Louisville, KY, U.S.A.) using a COBE CDX III pressure transducer.

On the day of the experiment, following an equilibrium period of 1 h, basal cardiovascular parameters were measured for 20 min before drug administration. To measure protein extravasation, Evans blue dye (20 mg kg^{-1}), which binds to plasma albumin (Rawson, 1943), was injected i.v. together with ET-1 (1 nmol kg^{-1}). Previous experiments showed that at this dose, ET-1 markedly enhances albumin extravasation in the rat coronary circulation (Filep *et al.*, 1992). The animals were divided into six groups and were pretreated with one of the following drugs: FR 139317 (2.5 mg kg^{-1}, i.v., 5 min prior to ET-1), a selective ET_A receptor antagonist (Aramori *et al.*, 1993; Sogabe *et al.*, 1993); indomethacin (3 mg kg^{-1} bolus followed by an infusion of 2 mg kg^{-1} h^{-1} started 60 min before injection of ET-1); OKY-046, a thromboxane synthase inhibitor (10 mg kg^{-1}, i.v., 60 min prior to ET-1) (Garcia-Szabo *et al.*, 1984); the TxA_2 receptor antagonist, BM 13505 (1 mg kg^{-1}, i.v., 5 min before injection of ET-1) (Patscheke *et al.*, 1987); the platelet-activating factor receptor antagonists, WEB 2086 (1 mg kg^{-1}, i.v.) (Casals-Stenzel *et al.*, 1987) or BN 52021 (10 mg kg^{-1}, i.v.) (Braquet *et al.*, 1985; Földes-Filep *et al.*, 1987) 10 min before administration of ET-1. Previous studies have demonstrated that indomethacin and OKY-046 at the dose employed inhibited prostaglandin (Filep *et al.*, 1987) and thromboxane A_2 formation (Garcia-Szabo *et al.*, 1984), BM 13505 inhibited the effects of the thromboxane A_2-mimetic, U 44069 (Patscheke *et al.*, 1984) and both WEB 2086 and BN 52021 blocked the hypotensive effect of exogenous platelet-activating factor in rats (Filep *et al.*, 1991b). Ten minutes after injection of ET-1, the animals were anaesthetized with sodium pentobarbitone (50 mg kg^{-1}), and the heart was perfused with 40 ml 0.9% NaCl solution through a catheter inserted into the abdominal aorta. Portions of the anterior wall of the left ventricle and right atrium were excised and weighed. Tissue Evans blue content was measured by spectrophotometry following extraction with formamide (4 ml per g wet tissue weight). The Evans blue content of each sample was expressed as μg dye per g dry weight of tissue to avoid underestimation of changes due to plasma fluid extravasation.

Electrocardiogram measurements

Male Wistar rats weighing 210–295 g were anaesthetized with sodium pentobarbitone (50 mg kg^{-1}). Catheters were inserted into the right femoral artery and vein and electrodes were placed on the left and right forlegs and left hindleg. The change in ST-segment of the Lead II electrocardiogram (ECG) was used to monitor coronary ischaemia. Following control cardiovascular and ECG measurements, ET-1 (0.1, 1 or 2 nmol kg^{-1}) was injected i.v. in a volume of 25 μl kg^{-1} body weight followed by 30 μl 0.9% NaCl. In another series of experiments, the animals were pretreated with FR 139317, indomethacin, OKY-046, BM 13505, WEB 2086 or BN 52021 as described above before injection of ET-1 (1 nmol kg^{-1}, i.v.). The animals were monitored for 20 min following the injection of ET-1. Each animal received only one dose of ET-1 and one type of pretreatment. Lead II ECG was recorded using a Siemens Sirecust 341 electrocardiograph (Germany).

Drugs and chemicals

ET-1 was synthesized in our laboratories by solid-phase methodology. The purity of the preparation was greater than 97% as measured by high performance liquid chromatography. ET-1 was dissolved in distilled water and stored at −20°C. On the day of the experiments, an aliquot was removed and diluted further with 0.9% NaCl. Other drugs were freshly prepared each day and includeed FR 139317 ((**R**)2-[(**R**)-2-[(**S**)-2-[[1-(hexahydro-1*H*-azepinyl)]-carbonyl] amino-4-methylpentanoyl] amino-3-[3-(1-methyl-1*H*-indoyl)] propionyl]amino-3-(2-pyridyl) propionic acid, Fujisawa Pharmaceutical Co., Osaka, Japan); OKY-046 ((E)-3-[4-(1-imidazolylmethyl)phenyl]-2-propenoic acid hydrochloride, ONO Pharmaceuticals, Japan); BM 13505 (4-[2-(4-chlorobenzene sulphonylamino) ethyl) benzene] acetic acid, Boehringer-Mannheim GmbH, Mannheim, Germany); WEB 2086 (3-[4-(2-chlorphenyl)-9-methyl-6H-thienol [3,2-f] [1,2,4] triazolo-[4,3-a] [1,4]-diazepine-2-yl]-1-(morpholinyl)-1-propanon, Boehringer-Ingelheim KG, Ingelheim, Germany) and BN 52021 (ginkgolide B, 9H-1,7a-(epoxymethanol)-1H,6aH,cyclopenta [c] [2-3-b] furo-[3,2':3,4] cyclopenta-[1,2-d]furan 5,9,12-[4H] trione, 3 *tert*-butyl-hexahydro 4,7b,11, hydroxy-8-methyl, Institut Henri Beaufour, Le Plessis Robinson, France). Drugs were dissolved in 0.9% saline with the exception of indomethacin and BM 13505 which were dissolved in ethanol, and BN 52021 which was dissolved in dimethylsulphoxide and were diluted further with 0.9% NaCl as appropriate. Indomethacin and Evans blue dye were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A.

Statistical analysis

Results are expressed as means ± s.e.mean. Statistical analysis of the data was performed by two-way analysis of variance using ranks (Friedman's test) followed by a Wilcoxon-Wilcox test (Wilcoxon & Wilcox, 1964) to identify differences between control and repeated measurements on the same animals; by one-way analysis of variance using

ranks (Kruskal-Wallis test) followed by Dunn's multiple contrast hypothesis test (Dunn, 1964), when various treatments were compared to the same control group; or by Wilcoxon signed rank test and Mann-Whitney U test, for paired and unpaired observations, respectively. A level of $P<0.05$ was considered significant for all tests.

Results

Effects of FR 139317, indomethacin, thromboxane and PAF receptor blockade on ET-1-induced changes in blood pressure and heart rate in conscious and anaesthetized rats

In agreement with previous studies, intravenous bolus injection of ET-1 (1 nmol kg^{-1}) to conscious rats evoked biphasic effects on MABP with a transient decrease followed by a prolonged pressor action. Corresponding to the changes in MABP, a small transient tachycardia was followed by a sustained bradycardia (Figure 1). Administration of FR 139317 (2.5 mg kg^{-1}) by itself did not produce significant changes in MABP and heart rate, whereas it inhibited by 76% the pressor action of 1 nmol kg^{-1} ET-1 and the concomitant bradycardia, without affecting the depressor response to ET-1 (Figure 1). Increasing the dose of FR 139317 did not cause further inhibition of the pressor effect of ET-1.

Pretreatment of the animals with indomethacin (3 mg kg^{-1} plus 2 mg kg^{-1} h^{-1}), WEB 2086 (1 mg kg^{-1}) or BN 52021 (10 mg kg^{-1}) caused neither significant changes in MABP and heart rate nor modified the depressor and pressor actions of ET-1 (1 nmol kg^{-1}) (Table 1). Administration of BM 13505 (1 mg kg^{-1}) 5 min before injection of ET-1, increased MABP from 112 ± 4 to 125 ± 4 mmHg ($n = 6$, $P<0.05$). BM 13505 treatment prolonged the duration of the depressor action of 1 nmol kg^{-1} ET-1 up to 75 s without affecting its magnitude. The pressor response to ET-1 was similar in control and BM 13505-treated animals (Table 1). MABP rose from 111 ± 3 to 123 ± 4 mmHg ($n = 6$, $P<0.05$) following OKY-046 (10 mg kg^{-1}) treatment. However, OKY-046 failed to affect the depressor and pressor responses to ET-1 (Table 1). None of these inhibitors and antagonists affected significantly ET-1-induced changes in heart rate (Table 1).

The basal values for MABP and heart rate were significantly higher in anaesthetized than in conscious animals

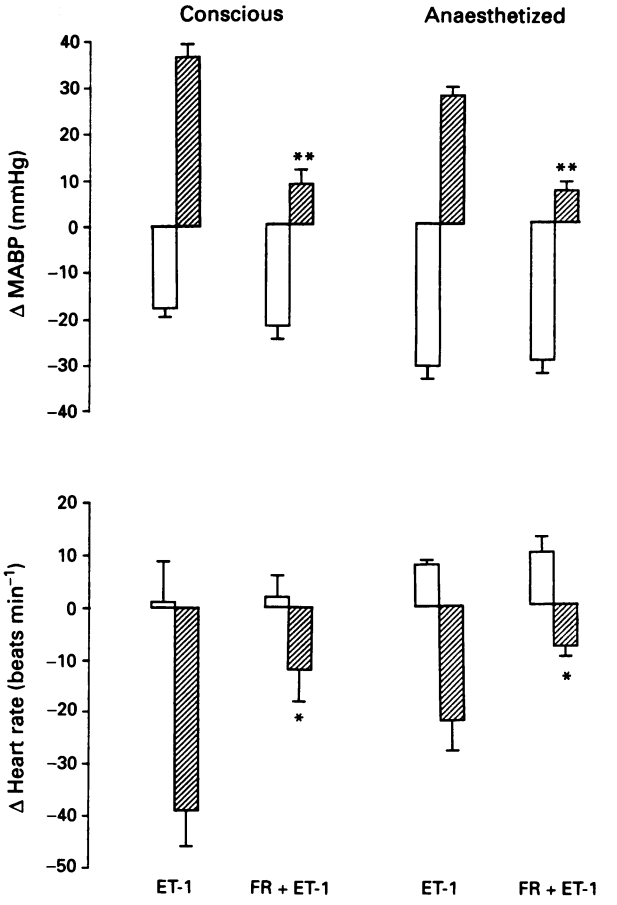

Figure 1 Effect of FR 139317 (FR, 2.5 mg kg^{-1}, i.v.) on the peak depressor (open columns) and pressor (hatched columns) responses elicited by endothelin-1 (ET-1, 1 nmol kg^{-1}, i.v.) and accompanying changes in heart rate in conscious and anaesthetized rats. The basal values for mean arterial blood pressure (MABP) and heart rate in conscious rats were 107 ± 2 mmHg and 335 ± 8 beats min^{-1} ($n = 10$), respectively, and in anaesthetized rats were 124 ± 2 mmHg and 426 ± 10 beats min^{-1} ($n = 10$), respectively. Values are means with s.e.mean for five experiments. $^*P<0.05$, $^{**}P<0.01$ (compared to ET-1 by the Mann-Whitney U test).

Table 1 Endothelin-1 (ET-1)-induced maximum decrease and increase in mean arterial blood pressure (MABP) and accompanying changes in heart rate in conscious and anaesthetized rats

		Basal		*Maximum depressor response*		*Maximum pressor response*	
		MABP (mmHg)	*Heart rate* (beats min^{-1})	*ΔMABP* (mmHg)	*ΔHeart rate* (beats min^{-1})	*ΔMABP* (mmHg)	*ΔHeart rate* (beats min^{-1})
Conscious rats							
ET-1	8	112 ± 3	343 ± 9	-18 ± 2	2 ± 6	39 ± 3	-42 ± 9
Indomethacin + ET-1	5	115 ± 3	342 ± 12	-18 ± 3	-2 ± 6	47 ± 4	-45 ± 8
OKY-034 + ET-1	6	111 ± 3	326 ± 11	-22 ± 2	0 ± 7	36 ± 6	-32 ± 9
BM 13505 + ET-1	6	112 ± 4	338 ± 12	-29 ± 4	3 ± 8	34 ± 3	-31 ± 5
WEB 2086 + ET-1	6	111 ± 2	312 ± 9	-22 ± 4	5 ± 7	41 ± 2	-41 ± 10
BN 52021 + ET-1	6	118 ± 2	334 ± 12	-25 ± 3	3 ± 7	30 ± 3	-29 ± 5
Anaesthetized rats							
ET-1	5	$125 \pm 2^{**}$	$440 \pm 15^{**}$	$-31 \pm 3^{**}$	8 ± 1	$28 \pm 2^{*}$	$-22 \pm 6^{*}$
Indomethacin + ET-1	4	124 ± 5	455 ± 10	-31 ± 2	12 ± 8	37 ± 7	-29 ± 11
OKY-034 + ET-1	5	122 ± 5	445 ± 15	-34 ± 5	10 ± 6	28 ± 2	-35 ± 4
BM 13505 + ET-1	6	126 ± 2	428 ± 22	-27 ± 3	5 ± 3	22 ± 3	-16 ± 5
WEB 2086 + ET-1	5	126 ± 4	439 ± 16	-29 ± 5	6 ± 5	23 ± 6	-24 ± 12
BN 52021 + ET-1	4	126 ± 3	416 ± 26	-32 ± 3	12 ± 3	28 ± 6	-27 ± 8

Following control measurements, the animals were pretreated with indomethacin (3 mg kg^{-1} plus 2 mg kg^{-1} h^{-1} for 60 min), OKY-046 (10 mg kg^{-1} for 60 min), BM 13505 (1 mg kg^{-1} for 5 min), WEB 2086 (1 mg kg^{-1} for 10 min) or BN 52021 (10 mg kg^{-1} for 10 min) before injection of ET-1 (1 nmol kg^{-1}, i.v.). The values are means ± s.e.mean for n experiments. Kruskal-Wallis test indicated that variation among median values for all parameters in various treatment groups was not significantly greater (P values were between 0.08 and 0.83) than expected by chance both in conscious and anaesthetized rats. $^*P<0.05$; $^{**}P<0.01$ (compared to ET-1 injection in conscious rats by the Mann-Whitney U test).

(MABP were 113 ± 1 mmHg, $n = 37$ and 124 ± 1 mmHg, $n = 34$ in conscious and anaesthetized animals, respectively, $P < 0.05$; heart rate were 333 ± 5 and 433 ± 6 beats min^{-1}, respectively, $P < 0.01$). Both MABP and heart rate remained stable for 80 min following control measurements. The magnitude of the transient fall in MABP following injection of ET-1 (1 nmol kg^{-1}) was significantly greater in anaesthetized than in conscious rats, whereas the pressor effect of ET-1 was more pronounced in conscious than in anaesthetized animals (Table 1). As observed in conscious rats, FR 139317 significantly attenuated, but did not completely prevent the pressor response to ET-1 and the accompanying bradycardia without affecting the depressor response (Figure 1). Intravenous administration of BM 13505 or OKY-046 resulted in significant increases in MABP in anaesthetized rats. For instance, MABP rose from 126 ± 2 mmHg to 135 ± 5 mmHg ($n = 6$, $P < 0.05$) and from 122 ± 5 mmHg to 127 ± 7 mmHg ($n = 5$, $P < 0.05$) following BM 13505 and OKY-046 treatment, respectively. However, neither BM 13505 nor OKY-046 affected the depressor and pressor responses to ET-1 (1 nmol kg^{-1}) (Table 1). In addition, indomethacin, WEB 2086 or BN 52021 also failed to modify the effects of ET-1 on MABP (Table 1). None of the TxA_2 and PAF receptor antagonists and TxA_2 synthase inhibitors affected the ET-1-induced changes in heart rate (Table 1).

Effects of ET_A, thromboxane and PAF receptor blockade on ET-1-induced albumin extravasation

In agreement with our previous observations, ET-1 at 1 nmol kg^{-1} increased the Evans blue content in the left ventricle and right atrium by 87 and 120%, respectively (Figure 2). These increases were almost completely abolished by FR 139317 (Figure 2). BM 13505 and OKY-046 reduced ET-1-induced albumin extravasation in the left ventricle by 85 and 71%, respectively and in the right atrium by 82 and 47%, respectively (Figure 3). Pretreatment of animals with indomethacin did not modify the increase in permeability elicited by ET-1 (Figure 3). The PAF receptor antagonists, WEB 2086 and BN 52021, appeared to be equally potent inhibitors of the permeability effect of ET-1 in the left ventricle (73 and 86% inhibition, respectively), whereas BN 52021 was a more potent inhibitor of ET-1-induced albumin extravasation than WEB 2086 in the right atrium (99% versus 69% inhibition) (Figure 3). None of the drugs alone affected tissue Evans blue content (data not shown).

Effects of intravenous administration of ET-1 on ECG

Bolus i.v. injections of ET-1 (0.1, 1 or 2 nmol kg^{-1}) produced ST segment elevation in a dose-dependent manner (Figure 4). Statistically significant, albeit small, elevations were observed following ET-1 at a dose as low as 0.1 nmol kg^{-1}. ST segment elevations were observed within 60–90, 30–50 and 20–40 s corresponding to the three doses of ET-1 used. ST segment elevation persisted for 10–14 min following 0.1 nmol kg^{-1} ET-1, whereas no complete recovery to control levels was observed within 20 min following the injection of the two highest doses of ET-1 (Figure 4). Administration of ET-1 up to a dose of 2 nmol kg^{-1} did not produce arrhythmias.

Effects of ET_A, thromboxane and PAF receptor blockade on ET-1-induced ST segment elevation

The ET_A receptor antagonist, FR 139317 (2.5 mg kg^{-1}) attenuated the ST segment elevation induced by ET-1 (1 nmol kg^{-1}, i.v.) by 86% (Figure 5). Pretreatment of the animals with indomethacin (3 mg kg^{-1} plus 2 mg kg^{-1} h^{-1}) did not affect the ST segment elevation elicited by ET-1 (Figure 5). On the other hand, both the thromboxane synthase inhibitor OKY-046 (10 mg kg^{-1}) and the TxA_2 receptor antagonist BM 13505 (1 mg kg^{-1}) suppressed ST segment elevation elicited by ET-1 (Figure 5). Similarly, pretreatment of the animals with the PAF receptor antagonist, WEB 2086 (1 mg kg^{-1}) or BN 52021 (10 mg kg^{-1}) markedly attenuated the ET-1-induced ST segment elevation (Figure 5). None of the inhibitors or antagonists alone caused significant changes in ST segment (data not shown).

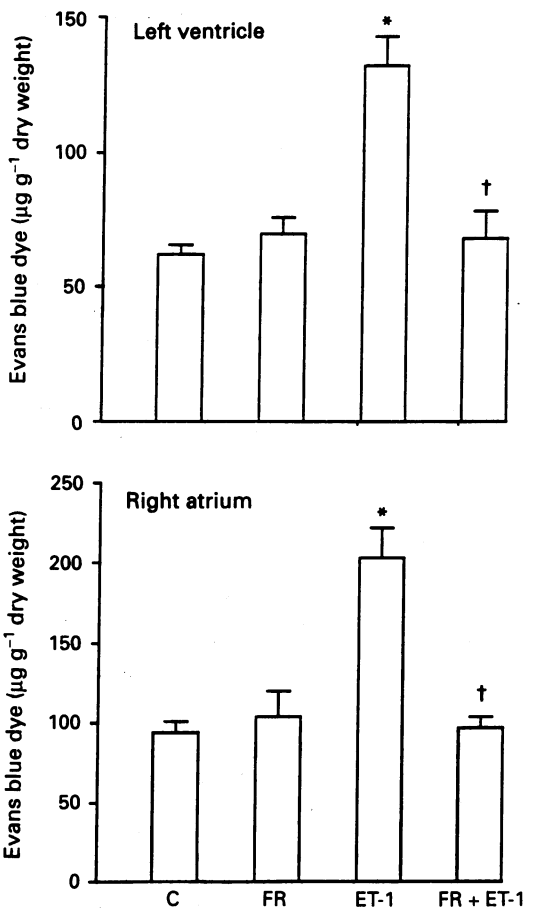

Figure 2 Effects of FR 139317 on endothelin-1 (ET-1)-induced albumin extravasation in the coronary circulation of conscious rats. The animals were pretreated with 0.9% NaCl (C, control) or FR 139317 (FR, 2.5 mg kg^{-1}, i.v. for 5 min), then ET-1 (1 nmol kg^{-1}) or its vehicle (C) were injected together with Evans blue dye (20 mg kg^{-1}). Ten minutes later, the animals were anaesthetized and the heart was perfused with 0.9% NaCl. The permeability measurements were made 15 min after injection of ET-1. Values are means with s.e.mean. $n = 4$ for FR 139317 and FR 139317 plus ET-1, $n = 5$ for control and ET-1. $^*P < 0.05$ (compared to control), $^\dagger P < 0.05$ (compared to ET-1 by Dunn's multiple contrast hypothesis test).

Discussion

The present results showed that i.v. injection of ET-1 produced ST segment elevation and myocardial oedema in the rat. These effects of ET-1 were significantly attenuated by a selective ET_A receptor antagonist, a thromboxane synthase inhibitor, and specific TxA_2 or PAF receptor antagonists.

Since we are not equipped to perform ECG measurements on freely moving rats, and anaesthesia is known to alter cardiovascular control mechanisms (Cox & Bagshaw, 1980), we compared the effects of the above mentioned inhibitors on the haemodynamic effects of ET-1 in conscious and anaesthetized rats. In confirmation of earlier observations, the present study also shows a biphasic change of MABP in response to i.v. bolus injection of ET-1 in both conscious (Gardiner *et al.*, 1989; Le Monnier de Gouville *et al.*, 1990) and anaesthetized rats (Yanagisawa *et al.*, 1988; De Nucci *et*

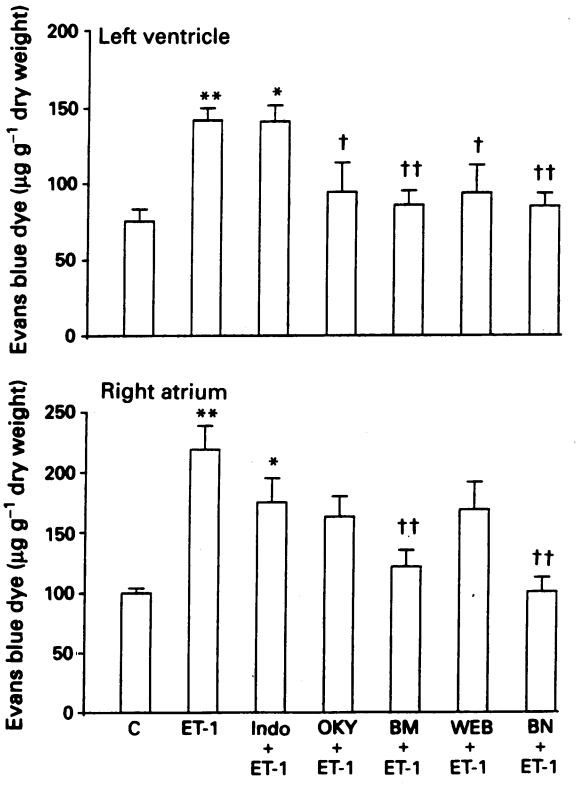

Figure 3 Pharmacological modulation of endothelin-1 (ET-1)-induced albumin extravasation in the coronary circulation of conscious rats. The animals were pretreated with 0.9% NaCl (C, control), indomethacin (Indo, 3 mg kg^{-1} plus 2 mg kg^{-1} h^{-1} for 60 min), OKY-046 (OKY, 10 mg kg^{-1} for 60 min), BM 13505 (BM, 1 mg kg^{-1} for 5 min), WEB 2086 (WEB, 1 mg kg^{-1} for 10 min) or BN 52021 (BN, 10 mg kg^{-1} for 10 min) before i.v. bolus injection of ET-1 (1 nmol kg^{-1}) or its vehicle (C) plus Evans blue dye (20 mg kg^{-1}). Ten minutes later, the rats were anaesthetized and the heart was perfused with 0.9% NaCl. The permeability measurements were made 15 min after the injection of ET-1. Values are mean with s.e.mean, $n = 8$ for control and ET-1, $n = 5$ for indomethacin plus ET-1 and $n = 6$ for all other treatments. $^*P<0.05$; $^{**}P<0.01$ (compared to control), $\dagger P<0.05$; $\dagger\dagger P<0.01$ (compared to ET-1 by Dunn's multiple contrast hypothesis test).

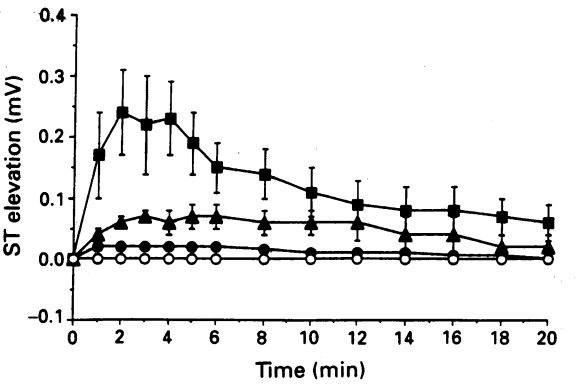

Figure 4 ST segment elevation elicited by intravenous bolus injection of endothelin-1 (ET-1) in anaesthetized rats. The animals received 0.9% NaCl (vehicle of ET-1, $n = 5$) (○), ET-1, 0.1 nmol kg^{-1} (●, $n = 4$), 1 nmol kg^{-1} (▲, $n = 6$) or 2 nmol kg^{-1} (■, $n = 5$) at 0 min. Each point represents mean ± s.e.mean.

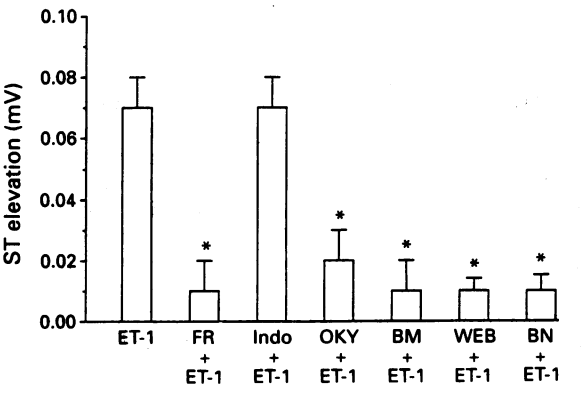

Figure 5 Effects of FR 139317, indomethacin, OKY-046, BM 13505, WEB 2086 and BN 52021 on ST segment elevation induced by intravenous administration of endothelin-1 (ET-1) in anaesthetized rats. The animals were pretreated with 0.9% NaCl, FR 139317 (FR, 2.5 mg kg^{-1} for 5 min, $n = 5$), indomethacin (Indo, 3 mg kg^{-1} plus 2 mg kg^{-1} h^{-1} for 60 min, $n = 4$), OKY-046 (OKY, 10 mg kg^{-1} for 60 min, $n = 5$), BM 13505 (BM, 1 mg kg^{-1} for 5 min, $n = 6$), WEB 2086 (WEB, 1 mg kg^{-1} for 10 min, $n = 5$) or BN 52021 (BN, 10 mg kg^{-1} for 10 min, $n = 5$) before injection of ET-1 (1 nmol kg^{-1}). Values are means ± s.e.mean. $^*P<0.05$ (compared to ET-1 by Dunn's multiple contrast hypothesis test).

al., 1988; Le Monnier de Gouville *et al.*, 1990). The depressor and pressor responses were accompanied by a transient reflex tachycardia and a prolonged reflex bradycardia, respectively. However, the magnitude of the changes in MABP and heart rate were different in anaesthetized and conscious rats. ET-1 produced significantly greater decreases in MABP in anaesthetized than in conscious rats, whereas it evoked greater increases in MABP in conscious than in anaesthetized rats. These observations can be explained by differences in the resting MABP of conscious and anaesthetized rats. Elevations in basal MABP (anaesthetized rats relative to conscious rats) would result in augmentation of the apparent vasodepressor activity and attenuation of the apparent vasopressor activity of various substances.

FR 139317 significantly attenuated the pressor response to ET-1 without affecting its depressor action in both conscious and anaesthetized rats (Sogabe *et al.*, 1993 and the present study). FR 139317 has been reported to be 7000 times more potent in inhibiting the binding of ET-1 to ET_A than ET_B receptors *in vitro* (Aramori *et al.*, 1993). Since ET_B receptors located on the vascular endothelium have been implicated in the mediation of the transient depressor action of ET-1 (Saeki *et al.*, 1991; Douglas & Hiley, 1991), it might be concluded that at the dose used, FR 139317 does not block ET_B receptors *in vivo*. It should be noted, however, that the degree of inhibition of the pressor action of ET-1 by FR 139317 did not exceed 80% either in anaesthetized or conscious rats confirming previous results (Sogabe *et al.*, 1993). Increasing of the dose of FR 139317 did not reduce further the maximum increase in MABP evoked by ET-1 (Sogabe *et al.*, 1993 and the present study). These observations lend further support to the notion that more than one subtype of endothelin receptor is involved in the generation of the pressor effect of ET-1 in the rat (Ihara *et al.*, 1992; Filep *et al.*, 1992, 1993b; Cristol *et al.*, 1993; McMurdo *et al.*, 1993a). The observations that ET-1-induced bradycardia was also markedly attenuated by FR 139317 suggest that ET-1-induced changes in heart rate were secondary to changes in MABP (i.e. a pressor response resulted in reflex bradycardia).

Pretreatment of the animals with indomethacin, OKY-046, BM 13505, WEB 2086 or BN 52021 failed to affect the ET-1-induced changes in MABP and heart rate in both conscious and anaesthetized rats. These findings indicate that neither cyclo-oxygenase products nor PAF are involved in mediating these actions of ET-1. In contrast to these observations, indomethacin was found to potentiate the pressor effect of ET-1 in pithed rats with resting blood pressure of about 50 mmHg (De Nucci *et al.*, 1988). This apparent discrepancy might be, in part, attributed to differences in cardiovascular

control mechanisms operant in conscious and pithed rats. Thus, one may speculate that mechanisms other than release of vasodilator prostanoids are involved in limiting the pressor activity of ET-1 in conscious rats. One of these mechanisms may be endothelium-derived nitric oxide, as inhibition of nitric oxide synthesis markedly potentiated the pressor effect of ET-1 in conscious rats (Filep *et al.*, 1993a). Another possibility might be that ET-1 is less active in releasing vasodilator prostaglandins in conscious than pithed rats, and consequently prostaglandins might be more important in limiting the pressor effects of ET-1 in pithed than conscious rats. The observations that cyclo-oxygenase inhibitors can attenuate the depressor response to ET-1 in anaesthetized dogs (Hermán *et al.*, 1989), and either inhibit or potentiate the pressor action of ET-1 in anaesthetized guinea-pigs (Whittle *et al.*, 1989) or rabbits (Thiemermann *et al.*, 1990; Rogerson *et al.*, 1993), respectively, indicate important species differences in the mechanisms mediating the vascular actions of ET-1. It is uncertain at present which receptor subtypes mediate the ET-1-induced release of vasodilator prostanoids in the rat. The finding that FR 139317 did not modify the depressor response elicited by ET-1, which is thought to be partly mediated by prostacyclin (De Nucci *et al.*, 1988), would argue against the involvement of ET_A receptors. This suggestion is further supported by the observations that ET-1 induces prostacyclin release from bovine cultured aortic endothelial cells (Filep *et al.*, 1991a) which do not possess ET_A receptors (Saeki *et al.*, 1991) and that the prostacyclin releasing properties of ET-1 were not affected by ET_A receptor antagonists in the anaesthetized dog (Hermán *et al.*, 1993) and rabbit (McMurdo *et al.*, 1993b). On the other hand, the supposedly selective ET_A receptor antagonist, BQ-123 (Ihara *et al.*, 1992) was found to inhibit the vasoconstrictor effects of ET-1 and its ability to release prostacyclin from the isolated perfused rat lung (D'Orleans-Juste *et al.*, 1992). However, these latter experiments did not exclude the possibility that prostacyclin release was a consequence of a non-specific defence mechanism of the vasculature in response to drastic elevations in perfusion pressure following administration of ET-1. Furthermore, the selectivity of BQ-123 has recently been questioned (see below). It is also possible that both ET_A and ET_B receptors could mediate prostacyclin release, but their involvement may differ from vascular region to vascular region.

Previous studies demonstrated that intracoronary administration of ET-1 dramatically increases coronary resistance and causes ST segment elevations and arrhythmias in pigs (Ezra *et al.*, 1989), dogs (Kurihara *et al.*, 1989; Nichols *et al.*, 1990; Hom *et al.*, 1992) and rats (Harada *et al.*, 1993). The present results extend these observations by demonstrating that i.v. administration of ET-1 can also produce ST segment elevation without associated arrhythmias. Following i.v. injection of 2 nmol kg^{-1} ET-1, the peak plasma concentration of the peptide in the coronary circulation might be about 100 fold lower than those levels that might have been achieved by intracoronary injections of 6–7 nmol kg^{-1} ET-1 in rats (Harada *et al.*, 1993). The doses of ET-1 used in the present study result in peak plasma ET-1 levels that are two-to-three orders of magnitude higher than those detected under pathological conditions (Yasuda *et al.*, 1990; Matsuyama *et al.*, 1991; Toyo-oka *et al.*, 1991; Tsuji *et al.*, 1991; Watanabe *et al.*, 1991; Ray *et al.*, 1992). It should, however, be noted that plasma ET-1 levels may not necessarily reflect local production and/or concentration of the peptide. The ST segment elevation by endothelin-1 is thought to be due to myocardial ischaemia related to coronary vasoconstriction, whereas a direct action on the myocardium has been implicated in inducing arrhythmias (Harada *et al.*, 1993). Therefore, one may assume that under the present experimental conditions (i.e. at lower coronary plasma concentrations) ET-1 acted primarily on the coronary vascular smooth muscle. The selective ET_A receptor antagonist, FR 139317 protected the heart from ST segment elevation. The observations that FR 139317 did not inhibit completely this action of ET-1 are consistent with the hypothesis that in addition to ET_A receptors, non ET_A/ET_B receptors are also involved in the generation of the vasoconstriction in the coronary circulation (Harrison *et al.*, 1992).

ET-1-induced ST segment elevation was significantly attenuated by the TxA_2 synthesis inhibitor, OKY-046, the TxA_2 receptor blocker, BM 13505 or by the PAF receptor antagonists, WEB 2086 or BN 52021. Numerous studies have shown that ET-1 could activate phospholipase A_2 via various intracellular signalling pathways, leading to release of arachidonic acid and PAF (for recent reviews see Simonson & Dunn, 1992; Sokolovsky, 1992). Arterial tissue and the heart actively convert arachidonic acid to cyclo-oxygenase products including TxA_2 (Hirsh *et al.*, 1981). Both TxA_2 and PAF are potent coronary vasoconstrictors (Hirsh *et al.*, 1981; Feuerstein *et al.*, 1984). Although we did not measure TxA_2 and PAF levels in the coronary circulation in the present experiments, previous studies have documented the capability of ET-1 to release TxA_2 from various tissues (cf. Simonson & Dunn, 1992; Sokolovsky, 1992) and ET-1 has also been reported to enhance PAF release from glomerular mesangial cells (López-Farré *et al.*, 1991). Interestingly, the cyclo-oxygenase inhibitor, indomethacin, failed to inhibit ST segment elevation produced by ET-1. The reason for this observation is not known at present. One possible explanation might be that cyclo-oxygenase blockade diverted arachidonic acid to the lipoxygenase pathway, leading to formation of, for example, sulphidopeptide leukotrienes, which are also known to provoke coronary vasoconstriction (cf. Piper, 1984). Taken together, the protective effects of TxA_2 and PAF receptor blockade on ST segment elevation appear to be due to the inhibitory action on the coronary vasoconstrictor action of ET-1.

In addition to inducing vasoconstriction, ET-1 also enhanced albumin extravasation in the coronary vascular bed of conscious rats. Pretreatment of the animals with FR 139317 almost completely abolished the ET-1-induced increase in albumin extravasation in both the left ventricle and right atrium. These results are consistent with our previous findings with another supposedly selective ET_A receptor antagonist, BQ-123 (Filep *et al.*, 1992). However, recent studies have questioned the selectivity of BQ-123 as it had a preferential antagonistic effect on the ET-3 response in the rat vas deferens (Eglezos *et al.*, 1993). These observations raised the possibility that BQ-123 may also antagonize a non ET_A/ET_B receptor. Whether the atypical endothelin receptor described in the rat vas deferens might be similar to that found in the coronary arteries (Harrison *et al.*, 1992), is not known at present. The findings that FR 139317 markedly attenuated, but did not inhibit completely the ET-1-induced ST segment elevation suggest that FR 139317 may not be an antagonist of this receptor subtype.

The presents results also showed that the permeability effect of ET-1 is mediated through release of secondary mediators, such as TxA_2 and PAF. As with coronary vasoconstriction, indomethacin failed to inhibit albumin extravasation evoked by ET-1, whereas tissue Evans blue accumulation was significantly attenuated by the TxA_2 synthase inhibitor, OKY-046 and the TxA_2 receptor antagonist, BM 13505. However, in addition to inhibition of TxA_2 production, cyclo-oxygenase blockade might have led to enhanced formation of other arachidonic acid metabolites, e.g. leukotrienes (see above), which are known to promote protein extravasation (Dahlén *et al.*, 1981). The PAF receptor antagonists, WEB 2086 and BN 52021 at the doses employed were equally potent in attenuating ET-1-induced accumulation of Evans blue dye in the left ventricle, whereas BN 52021 appeared to be a more potent inhibitor than WEB 2086 in the right atrium. We have found similar differences in the inhibitory action of these PAF antagonists in the lower bronchi and spleen of rats (Filep *et al.*, 1991b). These differences occurred despite the fact that WEB 2086 and BN 52021 at the doses used, caused similar inhibition of the

hypotensive action of exogenous PAF in the conscious rat (Filep *et al.*, 1991b). The different responses to WEB 2086 and BN 52021 suggest that one of the antagonists might act on a different PAF receptor population (Hwang, 1988). An increase in MABP, *per se*, could not be the basis for the observed albumin extravasation as TxA_2 and PAF receptor antagonists were highly effective in inhibiting albumin leakage elicited by ET-1 without affecting the pressor action of this peptide. Indeed, mediator-stimulated increase in protein extravasation is primarily attributable to interendothelial cells gap formation in the venules (Grega *et al.*, 1986). Vasoconstrictors, like noradrenaline that can elevate capillary hydrostatic pressure, but do not elicit gap formation could not promote protein efflux (Grega *et al.*, 1986). ET-1 may induce gap formation directly or more likely through release of secondary mediators, including TxA_2 and PAF. An increase in systemic blood pressure and/or elevation in capillary hydrostatic pressure would, however, facilitate protein extravasation and oedema formation provided that gaps are formed. Thus, attenuation of ET-1-induced coronary vasoconstriction by FR 139317 would lead to a reduction in hydrostatic pressure in the coronary circulation, which, in turn, could contribute to the decrease in albumin extravasation.

In conclusion, the present results demonstrates that i.v. injection of ET-1 provokes ST segment elevation and enhances myocardial albumin extravasation in the rat and suggest that these actions of ET-1 are mediated, in part, through release of secondary mediators, such as TxA_2 and PAF via the activation of ET_A receptors. These data also suggest a therapeutic potential for ET_A receptor antagonists in the treatment of acute ischaemic heart diseases where ET-1 production is enhanced.

FR 139317 was a generous gift of Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan. We thank Drs P. Braquet (Institut Henri Beaufour, Le Plessis Robinson, France), H. Heuer (Boehringer-Ingelheim GmbH, Ingelheim, Germany) and K. Stegmeier (Boehringer-Mannheim KG, Mannheim, Germany) for generously supplying us with BN 52021, WEB 2086 and BM 13505, respectively. This study was supported by the Foundation of the Maisonneuve-Rosemont Hospital. A.F. and J.G.F. are scholars of the Fonds de la Recherche en Santé du Québec (FRSQ).

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(Received December 7, 1993
Revised March 17, 1994
Accepted March 22, 1994)