The effect of diltiazem on the coronary haemodynamic and cardiac functional effects produced by intracoronary administration of endothelin-1 in the anaesthetized dog

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1 We have studied the effect of the calcium channel antagonist, diltiazem, on the coronary haemodynamic and cardiac functional responses produced by intracoronary (i.c.) administration of endothelin-1 (ET-1) in anaesthetized dogs.

2 ET-1, 1, 3 and 10 ng kg⁻¹ i.c., produced dose-related increases in coronary blood flow with no cardiac functional or systemic haemodynamic changes. ET-1, 30, 100 and 300 ng kg⁻¹ i.c., produced dose-related reductions in coronary artery blood flow. The reduction in coronary blood flow was accompanied by dose-related falls in cardiac output, mean arterial pressure, $+dP/dt$ and $-dP/dt$ and increases in left ventricular end-diastolic pressure. However, there was no reflex tachycardia in response to the fall in blood pressure and at 300 ng kg⁻¹, ET-1 produced a 22% reduction in heart rate.

3 Following a series of abnormal ECG changes, four out of five dogs died of ventricular fibrillation at 13 ± 2 min after 300 ng kg⁻¹ ET-1.

4 The administration of diltiazem (15 µg kg⁻¹ min⁻¹, i.v.) reduced mean arterial pressure by 10% and heart rate by 15%, and increased coronary blood flow by 39%. Diltiazem did not have any significant effect on the coronary dilator response to low doses of ET-1. Although there was a general trend for diltiazem to inhibit the coronary vasoconstrictor responses to ET-1, diltiazem significantly attenuated only the reduction in coronary blood flow produced by 100 ng kg⁻¹ ET-1 by 60% but not the response to 30 or 300 ng kg⁻¹ ET-1. Two out of five diltiazem-treated dogs died of ventricular fibrillation with a mean time to death of 20 min following treatment with ET-1 (300 ng kg⁻¹).

5 ET-1 is a very potent coronary vasodilator. At slightly higher doses ET-1 is also a coronary vasoconstrictor. ET-1 also appears to have direct cardiotoxicity independent of myocardial ischaemia. The vasoconstrictor activity and direct cardiotoxicity are only weakly inhibited by diltiazem.

Introduction

It has been known for several years that the vascular endothelium can produce vasodilator substances that have profound effects on the underlying vascular smooth muscle, e.g. prostacyclin and endothelium-derived relaxing factor (Vanhoutte *et al.*, 1986). More recent studies have shown that the vascular endothelium can also produce vasoconstrictor substances. The demonstrations by Hickey *et al.* (1985), Gillespie *et al.* (1986) and O'Brien *et al.* (1987) that cultured vascular endothelial cells can produce a stable, potent vasoconstrictor substance was rapidly followed by the isolation, purification and characterization of this substance as a 21 amino acid peptide that was termed endothelin (Yanagisawa *et al.*, 1988). It is now known that there are genes for three forms of endothelin, termed ET-1, ET-2 and ET-3 (Inoue *et al.*, 1989). ET-1, the originally described endothelin and formerly called porcine/human endothelin, is expressed by endothelial cells, whereas ET-2 and ET-3, which differ from ET-1 by two and six amino acid residues, respectively, are not (Inoue *et al.*, 1989). It is possible that ET-3 is produced in neural tissue, but at the present time no tissue has been found to express ET-2 (Inoue *et al.*, 1989).

ET-1 was shown by Yanagisawa *et al.* (1988) to be a potent constrictor of the porcine coronary artery *in vitro* and to produce a long-lasting pressor effect in ganglion-blocked rats. These vasoconstrictor responses were insensitive to a wide variety of receptor antagonists but were sensitive to blockade by voltage-operated calcium channel antagonists. Thus, it was postulated that ET-1 may be acting as an agonist to activate voltage-operated calcium channels in vascular smooth muscle (Yanagisawa *et al.*, 1988). It is now known that ET-1 probably

does not directly activate voltage-operated calcium channels (Chabrier *et al.*, 1989) but, similar to a wide variety of other vasoconstrictor substances, does activate phospholipase C to increase the production of inositol phosphates (Pang *et al.*, 1989; Ohlstein *et al.*, 1989) and cause the release of intracellular calcium (Van Renterghem *et al.*, 1989) and the influx of extracellular calcium through receptor-operated calcium channels (Pang *et al.*, 1989). However, it has been shown in rat cultured aortic smooth muscle cells that conditioned medium from bovine cultured aortic endothelial cells and synthetic ET-1 both cause membrane depolarization and generate verapamil-sensitive action potentials (Highsmith *et al.*, 1989). Thus, it is possible that ET-1 may produce different effects on different blood vessels, with the relative importance of the influx of extracellular calcium through voltage-operated calcium channels varying from negligible to very high.

In view of the potent, calcium channel blocker-sensitive, coronary vasoconstrictor activity of ET-1 *in vitro* and the well-documented *in vivo* coronary vasodilator activity of calcium antagonists such as diltiazem (Nagao *et al.*, 1972), we have studied the effects of ET-1 on the coronary circulation and cardiac function following direct intracoronary administration in the anaesthetized dog and the effects of diltiazem on those responses.

Methods

Anaesthetized dogs

Adult male mongrel dogs, 10–15 kg, were anaesthetized with sodium pentobarbitone (35 mg kg⁻¹, i.v.). An endotracheal tube was inserted and the animals were artificially ventilated with a positive-pressure respirator (Harvard Apparatus Co.) at a frequency of 12 cycles min⁻¹ with a tidal volume of 20 ml kg⁻¹. Body temperature was measured with a rectal

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probe and maintained at 38°C with a heating pad. A femoral artery and vein were cannulated for the measurement of blood pressure with a Statham P231D pressure transducer and the administration of supplemental anaesthesia and fluids, respectively. The left cephalic vein was cannulated for the administration of saline or diltiazem. Needle electrodes were inserted subcutaneously to monitor the lead II ECG with a Grass 7PGD ECG amplifier. Heart rate was measured by a Grass 7P4H tachograph triggered by the Q wave of the ECG.

A left thoracotomy was performed at the fifth intercostal space and the heart was suspended in a pericardial cradle. The left atrium was retracted and a segment of the left circumflex coronary artery distal to the atrial branch and proximal to any major ventricular branches was dissected free and an electromagnetic flow probe, 6 mm in circumference and connected to a CME model FM501 flowmeter (Carolina Medical Electronics), was placed on the artery to measure coronary blood flow. A snare occluder was placed around the artery distal to the flowprobe for the determination of zero flow. A 25 gauge L-shaped needle catheter was inserted into the circumflex coronary artery distal to the flowprobe for the intracoronary (i.c.) administration of ET-1. The root of the aortic arch was cleaned of adhering fat and an electromagnetic flowprobe, 35–45 mm in circumference, connected to a CME model FM501 flowmeter (Carolina Medical Electronics) was placed around it to measure pulsatile and mean aortic blood flow. The pulsatile diastolic blood flow was used to set zero flow on the flowmeter and was checked periodically throughout the experiment for baseline drift.

The right carotid artery was exposed and a 5 French Millar Mikro-Tip catheter pressure transducer (model SPC-350) was passed down the carotid artery into the left ventricle for the measurement of left ventricular pressure. The maximal rate of change of left ventricular pressure during systole ($+dP/dt$) and during diastole ($-dP/dt$) were obtained by differentiating the left ventricular pressure signal with a Grass 7P20C differentiator. Left ventricular end-diastolic pressure was measured by amplifying the left ventricular pressure signal. All parameters were recorded on a Grass Model 7E Polygraph.

Experimental protocol

Following the completion of surgery the animals were allowed to stabilize for at least 30 min before the start of the experiment. After stabilization baseline haemodynamic parameters were recorded and an intravenous infusion of either 0.9% saline or diltiazem ($15 \mu\text{g kg}^{-1} \text{min}^{-1}$) was started at a rate of $0.1 \text{ ml kg}^{-1} \text{min}^{-1}$. Ten minutes after the start of the infusion, when the haemodynamic parameters had stabilised, the dose-response curve to ET-1 was started. Bolus doses of endothelin were given over approximately 30 s directly into the coronary artery in volumes of less than 0.5 ml and were flushed in with 1 ml 0.9% NaCl. Each dose of ET-1 was administered when the response to the previous dose had subsided and the haemodynamic parameters had returned to baseline. Measurements were taken at the peak coronary blood flow response.

All procedures were performed in accordance with the 'Guide for the care and use of laboratory animals', NIH Pub-

lication No:86-23 and were approved by the Institutional Animal Care and Use Committee.

Drugs

Stock solutions of ET-1 (Peninsula Labs) were made in distilled water and stored at 4°C. Subsequent dilutions were made in 0.9% NaCl. ET-1 has a molecular weight of 2419.8, therefore the dose-range of endothelin studied, 1 to 300 ng kg^{-1} , corresponds on a mole basis to 0.4 to 120 pmol kg^{-1} , respectively. Diltiazem hydrochloride (Sigma Chemical Co.) was dissolved in 0.9% NaCl.

Statistical analysis

All data are expressed as the mean $\pm$ s.e.mean of n observations. The statistical significance of the effect of diltiazem and saline on basal haemodynamics was determined by Student's paired t test. The statistical significance of the difference between the effects of ET-1 in saline- and diltiazem-treated dogs was determined by Student's unpaired t test.

Results

Table 1 shows that an infusion of saline had no significant effects on coronary or systemic haemodynamics. However, diltiazem ($15 \mu\text{g kg}^{-1} \text{min}^{-1}$, i.v.) lowered blood pressure by $10 \pm 2\%$, heart rate by $14 \pm 4\%$, coronary vascular resistance by $31 \pm 9\%$, total peripheral resistance by $11 \pm 3\%$ and increased coronary blood flow by $39 \pm 19\%$ (Table 1).

At low doses ($1\text{--}10 \text{ ng kg}^{-1}$, i.c.) ET-1 produced a dose-related increase in coronary blood flow with no concomitant changes in cardiac function or systemic haemodynamics (Figure 1). The coronary vasodilator response produced by low doses of ET-1 was very short-lived (see Figure 1). Although this dilator response was observed in all animals studied there was a very large variation in the magnitude of this effect between animals. This effect was not due to an injection artifact since equal volumes of ET-1 vehicle did not produce any coronary vascular or cardiac functional effects. The coronary vasodilator response produced by ET-1 was observed in both saline- and diltiazem-treated animals and as a result of the wide variability of this response there was no significant difference between the two groups of animals.

Higher doses of ET-1 ($30\text{--}300 \text{ ng kg}^{-1}$, i.c.) produced dose-dependent reductions in coronary blood flow (Figure 2). In many cases these reductions in coronary blood flow were preceded by transient and variable increases in coronary blood flow. In contrast to the vasodilator responses seen at low doses, the constrictor responses to ET-1 were of a much longer duration, such that following 100 ng kg^{-1} it took approximately 30 min for coronary blood flow to return to normal. The coronary vasoconstrictor response curve for ET-1 was very steep such that 30 ng kg^{-1} reduced coronary flow by $9 \pm 2\%$ and 300 ng kg^{-1} reduced coronary flow by $89 \pm 4\%$. Diltiazem ($15 \mu\text{g kg}^{-1} \text{min}^{-1}$, i.v.) produced a small rightward shift in the dose-response curve to ET-1 such that the response to 100 ng kg^{-1} ET-1 was significantly reduced (Figure 2). The reduction in coronary blood flow was accom-

Table 1 The effect of saline ($0.1 \text{ ml kg}^{-1} \text{min}^{-1}$, i.v.) (a) and diltiazem ($15 \mu\text{g kg}^{-1} \text{min}^{-1}$, i.v.) (b) on resting coronary and systemic haemodynamics

		Mean arterial pressure (mmHg)	Heart rate (beats min^{-1})	Total peripheral resistance (mmHg $\text{l}^{-1} \text{min}^{-1}$)	Coronary blood flow (ml min^{-1})	Coronary vascular resistance (mmHg $\text{ml}^{-1} \text{min}^{-1}$)
(a)	Control	104 ± 6	150 ± 9	90.5 ± 11.3	23.0 ± 2.4	4.64 ± 0.30
	+ Saline	104 ± 6	153 ± 9	88.9 ± 12.1	24.2 ± 2.6	4.40 ± 0.29
(b)	Control	106 ± 4	144 ± 8	95.0 ± 4.5	23.0 ± 3.4	5.20 ± 0.88
	+ Diltiazem	$95 \pm 4^*$	$123 \pm 4^*$	$84.6 \pm 5.7^*$	32.8 ± 7.2	$3.68 \pm 0.87^*$

Values are expressed as the mean $\pm$ s.e.mean of 5 observations per group. $^*P < 0.05$ (Student's one-tailed paired t test).

Figure 1 Original recording of the coronary vasodilator effect of low doses of endothelin 1 in the anaesthetized dog.

Figure 2 The effect of endothelin-1 (ET-1, 30–300 ng kg⁻¹, i.c.) on left circumflex coronary blood flow in the anaesthetized dog. (○) Responses in saline (0.1 ml kg⁻¹ min⁻¹, i.v.)-treated animals (*n* = 5); (●) responses in diltiazem (15 µg kg⁻¹ min⁻¹, i.v.)-treated animals (*n* = 5). Each point represents the mean percentage change from control values obtained immediately before administration of ET-1 and the vertical bars represent 1 s.e.mean. ****P* < 0.001 (Student's unpaired *t* test).

Figure 3 The effect of coronary vasoconstrictor doses of endothelin-1 (ET-1) on (a) +*dP/dt*, (b) -*dP/dt* and (c) left ventricular end-diastolic pressure. (○, S) Responses in saline (0.1 ml kg⁻¹ min⁻¹, i.v.)-treated animals (*n* = 5); (●, D) responses in diltiazem (15 µg kg⁻¹ min⁻¹, i.v.)-treated animals (*n* = 5). Each point represents the mean percentage change from control values obtained immediately before administration of ET-1 and the vertical bars represent 1 s.e.mean. **P* < 0.05, ****P* < 0.001 (Student's unpaired *t* test).

panied by systemic haemodynamic changes. ET-1 produced dose-related reductions in +*dP/dt* and -*dP/dt* with an increase in left ventricular end-diastolic pressure (Figure 3). Cardiac output and mean arterial pressure were reduced (Figure 4). However, there was no reflex tachycardia in response to the fall in blood pressure. Indeed, 300 ng kg⁻¹ ET-1 reduced heart rate by 22 ± 7% (Figure 4). Diltiazem tended to reduce the haemodynamic responses produced by intracoronary ET-1. However, these inhibitions, like the inhibition of the coronary blood flow response, were slight.

Four out of five saline-treated dogs died of ventricular fibrillation at 13 ± 2 min after receiving 300 ng kg⁻¹ ET-1 following a series of ECG changes that are not normally associated with myocardial ischaemia. Figure 5 shows that within 1 min of the end of the injection of 300 ng kg⁻¹ ET-1, there was a marked elevation of the ST segment and a very small prolongation of the PR interval. Two minutes later there was a marked prolongation of the PR interval and marked prolongation of the RR interval, as reflected in the bradycardia. Approximately 10 min later the animal went into ventricular flutter and at 15 min following the administration of ET-1 died of ventricular fibrillation (Figure 5). Two out of five diltiazem-treated dogs died of ventricular fibrillation with a mean time to death of 20 min following 300 ng kg⁻¹ ET-1.

Discussion

This study shows that ET-1 has profound vasoconstrictor effects in the coronary circulation and effects, both direct and indirect, on myocardial function in the anaesthetized dog and that diltiazem can reduce, but not abolish, these responses to ET-1. These studies also clearly show that in addition to the well characterized vasoconstrictor response, ET-1 also produces a potent coronary vasodilator response.

Kurihara *et al.* (1989) have also shown that ET-1, in the same dose-range used in this study, produces potent coronary

Figure 4 The effect of coronary vasoconstrictor doses of endothelin-1 (ET-1) on (a) cardiac output, (b) mean arterial pressure and (c) heart rate. (○, S) Responses in saline (0.1 ml kg⁻¹ min⁻¹, i.v.)-treated animals (*n* = 5); (●, D) responses in diltiazem (15 µg kg⁻¹ min⁻¹, i.v.)-treated animals (*n* = 5). Each point represents the mean percentage change from control values obtained immediately before administration of ET-1 and the vertical bars represent 1 s.e.mean. **P* < 0.05 (Student's unpaired *t* test).

Figure 5 The effects of a high dose of endothelin-1 (300 ng kg^{-1} , i.c.) on coronary and systemic haemodynamics leading to death at 15 min.

vasoconstriction when administered directly into the coronary circulation of the anaesthetized dog. However, in contrast to our findings, Kurihara *et al.* (1989) did not observe a coronary vasodilator response to ET-1 at low doses. This discrepancy may arise from the fact that in the study of Kurihara *et al.* (1989) the coronary circulation was autoperfused with femoral arterial blood, whereas in our experiments the coronary circulation was left intact. It is noteworthy, however, that low doses of ET-1 have been shown to produce vasodilatation in other vascular beds including the renal (Lippton *et al.*, 1988), hindquarter (Wright & Fozard, 1988) and cerebral (R.N. Willette, personal communication) vascular beds. The fact that ET-1 produces direct coronary vasoconstriction, as shown in this study and by Kurihara *et al.* (1989), and that ET-1 gene expression in porcine aortic endothelial cells is induced by agents related to thrombus formation, e.g. thrombin, has led Kurihara *et al.* (1989) to suggest that ET-1 may play a role in the pathogenesis of coronary vasospasm associated with endothelial injury and subsequent thrombus formation. However, the nature of the response to physiologically or pathophysiologically released ET-1, if it does indeed occur *in vivo*, would appear to depend upon the amount of ET-1 released. If ET-1 is only released in very small amounts, then it is possible that the physiological or pathophysiological role of ET-1 is the production of vasodilatation.

Concomitant with the reductions in coronary blood flow, ET-1 produced marked cardiac functional effects. The increase in left ventricular end-diastolic pressure that was produced is commonly seen during myocardial ischaemia following coronary artery occlusion. However, the reductions in cardiac contractility, as assessed from $+dP/dt$, left ventricular diastolic relaxation, as assessed from $-dP/dt$, and cardiac output leading to a profound fall in systemic arterial pressure were much greater and more prolonged than those commonly associated with myocardial ischaemia. This suggests that endothelin may have some direct effect on myocardial function in addition to the myocardial dysfunction that would occur as a result of the ischaemia produced by the reduction in coronary blood flow. Interestingly, it has been shown that myocardium from rat, pig and man possesses binding sites for ET-1 (Davenport *et al.*, 1989), supporting the hypothesis that

ET-1 can have direct actions on the myocardium. Previous *in vitro* studies have shown that ET-1 produces a positive inotropic effect in rabbit isolated papillary muscles (Watanabe *et al.*, 1989) and Langendorff perfused hearts of the rat (Baydoun *et al.*, 1989). However, it was found that the initial positive inotropic response to ET-1 observed in the rat perfused heart became a negative inotropic response upon repeated administration (Baydoun *et al.*, 1989). Thus, ET-1 appears to have both positive and negative inotropic actions *in vitro* and predominantly or exclusively negative inotropic effects *in vivo*.

The electrocardiographic changes that occurred following 300 ng kg^{-1} ET-1 were very unusual in that they were not identical to those produced merely by myocardial ischaemia. Myocardial ischaemia produced by occlusion of a coronary artery is associated with ST segment elevation and the frequent occurrence of premature ventricular contractions (PVCs) and occasionally ventricular tachycardia and fibrillation. Myocardial ischaemia *per se* does not produce prolongation of the PR interval or bradycardia similar to that seen with ET-1. Moreover, the incidence of ventricular fibrillation following coronary artery occlusion in mongrel dogs in our laboratory is low (approximately 10%, A.J. Nichols, unpublished observations), in contrast to the 80% incidence following ET-1. Thus, it would appear that ET-1 has direct effects on the electrical properties of the myocardium and its conduction system. It is interesting to note that the snake venom, sarafotoxin S6b, which has a peptide structure very similar to that of ET-1 (Takasaki *et al.*, 1988a), is believed to act as a direct cardiotoxin (Takasaki *et al.*, 1988b) and has been shown to produce ST segment elevation and severe atrioventricular block in mice (Wollberg *et al.*, 1988). Thus, if ET-1 can indeed be produced and released in large quantities from coronary arterial endothelial cells under pathophysiological conditions, then it may not only cause coronary artery vasoconstriction, but may also produce cardiac arrhythmias that may ultimately lead to ventricular fibrillation and death.

The inhibitory effect of diltiazem on the responses to ET-1 suggests that ET-1 may be acting, either directly or indirectly, *via* activation of voltage-operated calcium channels. However, the degree of inhibition produced by diltiazem was not large

despite the large dose of diltiazem which has previously been shown to inhibit myocardial calcium influx under pathological conditions (Bush *et al.*, 1982). Moreover, the evidence from *in vitro* experiments would suggest that ET-1 does not activate voltage-operated calcium channels to produce vasoconstriction (Chabrier *et al.*, 1989; Ohlstein *et al.*, 1989). However, ET-1 has been shown to depolarize vascular smooth muscle cells and generate action potentials that are sensitive to inhibition by calcium channel antagonists (Highsmith *et al.*, 1989). It is possible, therefore, that at least a small component of the response to ET-1 in the coronary vasculature may be mediated by such a process and consequently is sensitive to inhibition by diltiazem. It is also possible that diltiazem is acting as a functional antagonist to inhibit the response to ET-1 by a mechanism that is unrelated to the mechanism of action of ET-1. The infusion of diltiazem produced coronary and systemic haemodynamic changes that are commonly seen with calcium channel antagonists, e.g. vasodilatation, which suggests that intracellular calcium levels were reduced by diltiazem. Thus, the diltiazem-treated dogs had a lower vascular tone and possibly reduced intracellular calcium levels that may have attenuated the responses to ET-1.

Although the incidence of ventricular fibrillation and death was lower in the diltiazem-treated animals relative to the saline-treated animals, diltiazem did not abolish the cardiotoxicity produced by ET-1. Thus, although this dose of diltiazem has been shown to have a profound effect in reducing

calcium influx and be cardioprotective in canine ischaemic/reperfused myocardium *in vivo* (Bush *et al.*, 1982) it was, at most, only partially effective at inhibiting ET-1 mediated cardiotoxicity. Thus, the mechanism of the direct cardiotoxic response to ET-1 is not likely to be mediated by calcium influx through voltage-operated calcium channels. If this were the case, it would be expected that there would be at least some evidence of a positive inotropic response, similar to that seen with the calcium channel activator Bay K 8644 (Schramm *et al.*, 1983). Thus, the mechanism of the direct cardiotoxic response to ET-1 remains unclear.

In conclusion, we have shown that ET-1, when administered directly into the left circumflex coronary artery of the anaesthetized dog, produces coronary vasodilatation at low doses and vasoconstriction at high doses. The vasoconstrictor responses are accompanied by cardiac functional responses, e.g. profound negative inotropic effects and electrocardiographic changes, that are not normally associated with myocardial ischaemia, and thus suggest a direct cardiotoxic effect of ET-1. The responses to ET-1 are weakly attenuated by diltiazem, but this may be the result of functional antagonism rather than inhibition of ET-1-mediated calcium influx through voltage-operated calcium channels.

The authors would like to thank Ms Rose Pimpinella for her expert secretarial assistance.

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(Received August 9, 1989

Revised November, 1989

Accepted November 3, 1989)