Effects of calcium entry blockers on contractions evoked by endothelin-1, [Ala^{3,11}]endothelin-1 and [Ala^{1,15}]endothelin-1 in rat isolated aorta

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1 The aim of the present study was to examine the contractile responses evoked by the recently characterized vasoactive peptide endothelin-1 (ET-1) and by two of its structural analogues, [Ala^{3,11}]ET-1 and [Ala^{1,15}]ET-1 in endothelium-denuded rat isolated aorta, and also to assess the extent of dependence of these responses on extracellular calcium entry.

2 ET-1 (0.3 to 10 nM), [Ala^{3,11}]ET-1 (2.25 to 225 nM) and [Ala^{1,15}]ET-1 (0.04 to 1.36 μ M) evoked concentration-dependent contractions in normal, calcium-containing medium with the order of potency: ET-1 > [Ala^{3,11}]ET-1 > [Ala^{1,15}]ET-1.

3 Preincubation of tissues for 60 min with diltiazem (1 μ M) induced a significant 3 fold rightward shift of concentration-effect curves to ET-1 without affecting maximal responses elicited by 10 nM of this peptide, whereas the same treatment failed to modify concentration-effect curves to [Ala^{3,11}]ET-1.

4 Preincubation of tissues for 60 min with nifedipine (0.1 or 1 μ M) markedly inhibited contractions elicited by either ET-1 (10 nM) or [Ala^{1,15}]ET-1 (0.41 μ M). Furthermore, when added cumulatively to tissues maximally contracted by ET-1 (10 nM), nifedipine (3 nM to 1 μ M) induced concentration-dependent relaxations with an IC₅₀ value of 21.8 ± 5.9 nM. Maximal relaxation to nifedipine, 1 μ M, amounted to $56.9 \pm 11.5\%$.

5 Submaximal concentrations of ET-1 (3 nM), [Ala^{3,11}]ET-1 (75 nM) and [Ala^{1,15}]ET-1 (0.41 μ M), gave about 85% of maximal contractions elicited by noradrenaline (1 μ M) in normal, calcium-containing medium. These contractile responses were all reduced by about 70% in calcium-free medium. Pretreatment of tissues with diltiazem (1 μ M) or with nifedipine (0.1 or 1 μ M) did not affect these residual contractions.

6 Upon readdition of calcium (10 μ M to 10 mM) to tissues in calcium-free medium, preincubated with submaximal concentrations of one or the other of the peptides, concentration-dependent contractions were elicited with EC₅₀ values of 1.21 ± 1.1 mM (ET-1-exposed rings), 74.6 ± 9.1 μ M ([Ala^{3,11}]ET-1-exposed rings) and 102 ± 27 μ M ([Ala^{1,15}]ET-1-exposed rings). Calcium-induced responses were significantly inhibited by diltiazem (1 μ M) in both ET-1- and [Ala^{3,11}]ET-1-exposed tissues and by nifedipine (0.1 and 1 μ M) in ET-1-exposed tissues. However, nifedipine did not significantly affect calcium induced responses in [Ala^{1,15}]ET-1-exposed rings.

7 Readdition of calcium to a calcium-free medium containing 40 mM K⁺, evoked concentration-dependent responses that were unaffected by the presence of ET-1 (3 nM).

8 Overall, these results indicate that contractions to ET-1 and its analogues in rat aorta are to some extent dependent on extracellular calcium. However, the mode of antagonism by diltiazem and nifedipine of responses to the peptides in normal medium, as well as of contractions induced by readdition of calcium in peptide-exposed tissues in calcium-free medium, argue against a direct activation by endothelin or its analogues of L-type calcium channels. Finally, removal of one or the other of the disulphide bonds of ET-1 reduces the potency of the peptide.

Introduction

A potent vasoactive peptide was identified in the medium of porcine endothelial cells in culture and

named endothelin (Yanagisawa *et al.*, 1988). Recently it has been reported that there is a family of related peptides and the peptide that was originally described has been designated endothelin-1 (ET-1;

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Inoue *et al.*, 1989). However, ET-1 has since been demonstrated to exert multiple actions. It is a potent secretagogue, releasing atrial natriuretic factor from rat myocytes (Fukuda *et al.*, 1988), inhibits renin release from rat glomeruli (Rakugi *et al.*, 1988), has positive inotropic effects on guinea-pig atria (Ishikawa *et al.*, 1988), contracts guinea-pig ileal smooth muscle, interferes with sympathetically-induced twitch responses in the rat vas deferens (Hiley *et al.*, 1989) and possesses bronchoconstrictor qualities (Uchida *et al.*, 1988). Early reports suggested that its vasoconstrictor effects were dependent to a large extent on extracellular calcium and were inhibited by the dihydropyridine calcium entry blockers nifedipine and nicardipine (Hirata *et al.*, 1988; Yanagisawa *et al.*, 1988). These observations correlate with the reported failure of the peptide to induce accumulation of phosphoinositides in rat vascular smooth muscle cells in culture (Hirata *et al.*, 1988). More recent data however, suggest that calcium entry through dihydropyridine-sensitive calcium channels might play only a minor role in the vasoconstrictor responses to the peptide (Auguet *et al.*, 1988; Hagiwara *et al.*, 1988; Miasiro *et al.*, 1988).

Therefore, it was of interest to study the dependence on extracellular calcium of responses to ET-1 in rat aorta *in vitro*. The role of intracellular and extracellular calcium in agonist-induced contractile responses and the effects of calcium entry blockers thereon have been extensively studied in this artery (Godfraind *et al.*, 1986). Few structure-activity studies of the bicyclic peptide ET-1 have been undertaken, but disulphide bonds in cyclic peptides such as somatostatin, oxytocin and atrial natriuretic factor for example are required for maintaining their biologically active conformations (Veber & Saperstein, 1979; Misono *et al.*, 1984; Hraby, 1985). The relative importance of the disulphide bonds for the activity of ET-1 is therefore an interesting question, and this has been assessed by comparing its activity with that of two analogues, [Ala^{3,11}]- and [Ala^{1,15}] ET-1. The results show that contractions elicited by ET-1 and some of its vasoactive analogues can be partially antagonized by the calcium entry blockers diltiazem and nifedipine. Elimination of one or other of the disulphide bonds by replacement of the appropriate cysteine residues with alanine, reduced potency and changed some of the functional characteristics of the peptide in this tissue. A preliminary account of this work has been published (Topouzis *et al.*, 1989).

Methods

Aortae were obtained from male Sprague-Dawley rats weighing 200–225 g, taking care not to damage

the endothelium. Four rings were taken from each aorta and stripped of their endothelium by rubbing with a wooden match stick. Tissues were placed in a physiological solution (composition in mM: NaCl 112, KCl 5, NaHCO₃ 25, KH₂PO₄ 1, MgSO₄ 1.2, CaCl₂ 1.25 and glucose 11.5), gassed with 95% O₂ and 5% CO₂. The tissues were allowed to equilibrate for 60 min then contracted with noradrenaline, 1 µM. This maximal noradrenaline-induced contraction served as a reference for all subsequent contractile responses. After the response had reached a plateau, acetylcholine, 1 µM, was added to the bath, to test for the absence or presence of endothelium (Furchgott & Zawadzki, 1980). Preparations were then washed and left to equilibrate for 2 h, during which period the medium was changed 4 times. During the second hour tissues were incubated with either one of the calcium-entry blockers or vehicle. The blockers were then present for the duration of the experimental protocol. At the end of the incubation period, concentrations of ET-1 or an analogue of ET-1 were added to the bath in a cumulative fashion. Only one concentration-effect curve to the peptides was constructed in each tissue. Alternatively, in some experiments nifedipine (3 nM to 1 µM) or diltiazem (1 µM) was added cumulatively to preparations precontracted with 10 nM ET-1.

It has been suggested that ET-1 may activate calcium channels (Yanagisawa *et al.*, 1988) and to examine this possibility contractions induced by readmission of calcium to tissues in the presence of the peptides or noradrenaline were examined. At the end of the 1 h incubation period in the presence or absence of a calcium entry blocker, tissues were washed 4 times with calcium-free medium over a 10 min period. For the first 2 washes the medium contained EGTA 0.2 mM. The calcium-free solutions were obtained by omitting calcium and contained a calcium-entry blocker when necessary. A sub-maximal concentration of ET-1, one of its analogues or noradrenaline was then added to the bath. When the contraction elicited reached a plateau, Ca²⁺ (10 µM to 10 mM) was added cumulatively to contract the tissues further. These responses to calcium are expressed as a percentage of the maximal response to noradrenaline (1 µM). In some of these latter experiments 2 min before the addition of ET-1 the calcium-free solution was substituted by one containing 40 mM K⁺ with an equivalent reduction in Na⁺ content.

Materials

Noradrenaline bitartrate (Boyer, France), acetylcholine HCl (Sigma, France) and diltiazem HCl (Synthelabo, France) were dissolved in deionized water. Nifedipine HCl (Sigma, France) was dissolved

in ethanol to a concentration of 1 mM. Solutions were prepared daily. Care was taken when using nifedipine to work in conditions of diminished light. Human/porcine endothelin (Peninsula Laboratories, U.K.), [Ala^{3,11}]ET-1 and [Ala^{1,15}]ET-1 (synthesized at Merrell Dow Research Institute, Strasbourg, France) were prepared as 0.1 mM stock solutions in deionized water and kept at 4°C. Dilutions were prepared daily in deionized water.

Statistics

Comparisons were made by the unpaired Student's *t* test or analysis of variance (StatView 512 + programme for Macintosh computers, Brain Power Inc.). *P* < 0.05 was taken as denoting significant differences.

Results

ET-1, [Ala^{3,11}]ET-1 and [Ala^{1,15}]ET-1 all contracted rat aortic preparations without endothelium in a concentration-dependent manner (Figure 1). Contractions to all three peptides developed slowly. ET-1 was about 13 and 200 fold more potent than the [Ala^{3,11}]- and [Ala^{1,15}]-ET-1 derivatives respectively. Concentrations of the peptides eliciting a contraction equal to 50% of that elicited by noradrenaline (1 µM) in the same tissue and the maximal responses obtained are shown in Table 1.

Preincubation of tissues with diltiazem (1 µM) for 60 min did not significantly affect maximal responses elicited by ET-1 (Figure 2a) but increased EC₅₀ values, from 0.88 ± 0.24 nM to 2.95 ± 1.0 nM (*t* test, *P* = 0.013; *n* = 4). However, neither maximal responses evoked by [Ala^{3,11}]ET-1 nor EC₅₀ values (39.4 ± 14.5 nM and 28.2 ± 10.3 nM respectively) were significantly altered (Figure 2b).

When rat aortic rings were preincubated with nifedipine (0.1 µM) for 60 min, contractile responses induced by ET-1 3 and 10 nM were significantly reduced (Figure 3a) as compared to responses elicited in parallel in tissues not exposed to nifedipine. A

Figure 1 Contractile responses evoked by endothelin (○, *n* = 8), [Ala^{3,11}]endothelin (●, *n* = 7) and [Ala^{1,15}]endothelin (□, *n* = 8) in rat isolated aorta. Contractions are expressed as a percentage of responses induced by noradrenaline (NA, 1 µM) in the same tissues. Vertical bars represent s.e.mean.

10 fold higher concentration of nifedipine had no greater effect (Figure 3a). Similarly, nifedipine (0.1 µM) inhibited responses elicited by [Ala^{1,15}]ET-1 0.41 µM (Figure 3b).

When added to endothelium-denuded tissues precontracted maximally by ET-1, 10 nM, nifedipine (3 nM to 1 µM) elicited a concentration-dependent relaxation (Figure 4). At 1 µM, nifedipine relaxed ET-1-induced contractions maximally by 56.9 ± 11.5% and the IC₅₀ value was 21.8 ± 5.9 nM (*n* = 4). Diltiazem (1 µM) relaxed precontracted tissues maximally by only 29.8 ± 8.7% (*n* = 5).

In a calcium-free medium, addition of equieffective concentrations of the three peptides (producing about 85% of the maximal response to noradrenaline in normal medium), ET-1 (3 nM), [Ala^{3,11}]ET-1

Table 1 Concentrations of endothelin and endothelin analogues that evoke a response equal to 50% of the maximal contraction evoked by noradrenaline (EC_{50,NA}), and the maximal effects (E_{max,NA}) of the peptides at the highest concentrations tested (EP_{max}) relative to those of noradrenaline in rat isolated aorta

Peptide	EC _{50,NA} (nM)	EP _{max} (nM)	E _{max,NA} (%)
Endothelin	1.4 ± 0.3	10	109.2 ± 22.3
[Ala ^{3,11}]endothelin	18.3 ± 4.0*	225	101.1 ± 18.6
[Ala ^{1,15}]endothelin	299 ± 71*†	1360	125.2 ± 7.4

Values are the means ± s.e.mean of 7 or 8 observations.

* Significantly different from endothelin value (*P* ≤ 0.006).

† Significantly different from [Ala^{3,11}]endothelin value (*t* test, *P* = 0.002).

Figure 2 Contractile responses elicited by (a) endothelin and (b) [Ala^{3,11}]endothelin in rat isolated aorta in the absence (○, *n* = 4) and in the presence (●, *n* = 4) of diltiazem (1 μM). Contractions are expressed as a percentage of responses induced by noradrenaline (NA, 1 μM) in the same tissues. Vertical bars represent s.e.mean of 4 observations.

(75 nM) or [Ala^{1,15}]ET-1 (0.41 μM) evoked slowly developing responses that were sustained for at least 20 min and amounted to $17.35 \pm 2.5\%$ (*n* = 16), $40.9 \pm 15.5\%$ (*n* = 7) and $18.8 \pm 1.9\%$ (*n* = 7) respec-

Figure 3 Contractile responses elicited by (a) endothelin and (b) [Ala^{1,15}] endothelin in rat isolated aorta. (a) Responses elicited in the absence (○, *n* = 10) and in the presence of 0.1 (●, *n* = 6) or 1 (□, *n* = 4) μM nifedipine. (b) Responses elicited in the absence (○, *n* = 4) and in the presence (●, *n* = 4) of 1 μM nifedipine. Contractions are expressed as a percentage of responses induced by noradrenaline (NA, 1 μM) in the same tissues. Vertical bars represent s.e.mean.

tively of maximal responses evoked by noradrenaline (1 μM) (Figure 5). These responses were not significantly different from each other and were approximately 35%, 45% and 24% respectively of responses

Figure 4 Relaxation evoked by addition of nifedipine (1 nM to 1 μ M) to rat isolated aorta maximally precontracted with endothelin, 10 nM. Results are the means of 4 observations. Vertical bars represent s.e.mean.

elicited by the same concentrations of the peptides in normal medium. These residual contractions to ET-1 were not affected by either diltiazem (1 μ M) or nifedipine (1 μ M), those to [Ala^{3,11}]ET-1 were not affected by diltiazem (1 μ M) and those to [Ala^{1,15}]ET-1 were not affected by nifedipine (0.1 μ M) (Figure 5).

Readdition of calcium (30 μ M to 10 mM) to tissues washed with Ca²⁺-free medium elicited no significant change in tension. However, in tissues washed in Ca²⁺-free medium and then exposed to 3 nM ET-1, readdition of Ca²⁺ evoked a concentration-dependent response with an EC₅₀ value of 1.21 $\pm$ 1.1 mM. In the presence of diltiazem (1 μ M), these ET-1-dependent concentration-effect curves to calcium were significantly modified (analysis of variance, $P = 0.0009$) but neither maximal responses to calcium nor the EC₅₀ values (Figure 5a) were significantly different (t test). Nifedipine (0.1 μ M) also antagonized responses elicited by 10 μ M to 10 mM calcium in tissues pre-exposed to ET-1 3 nM (analysis of variance, $P = 0.0001$) but EC₅₀ values were not altered. A ten fold higher concentration of nifedipine had no greater effect (Figure 5b).

Readdition of calcium (10 μ M to 10 mM) to tissues partially precontracted with 75 nM [Ala^{3,11}]ET-1 elicited concentration-dependent responses, EC₅₀ 74.6 $\pm$ 9.1 μ M ($n = 7$). This EC₅₀ value is significantly

less (t test, $P = 0.042$) than that found in ET-1-exposed rings. Preincubation with diltiazem (1 μ M) significantly ($P = 0.0073$) shifted the calcium concentration-effect curves to the right (EC₅₀ 348.3 $\pm$ 169.7 μ M, $n = 7$) with a concomitant significant increase of maximal contractions (Figure 5c). Analysis of variance indicates a significant difference between these concentration-effect curves ($P = 0.0007$). Concentrations of 0.1 mM and 0.3 mM calcium were equieffective in the absence and presence respectively of diltiazem and an apparent K_b value for diltiazem of about 0.5 μ M was calculated.

Readdition of calcium (10 μ M to 10 mM) to tissues partially precontracted with 0.41 μ M [Ala^{1,15}]ET-1 in the absence of Ca²⁺, elicited concentration-dependent responses, EC₅₀ 102 $\pm$ 27 μ M (Figure 5d, $n = 4$). This EC₅₀ value is significantly less than that found in ET-1-exposed rings (t -test, $P < 0.0078$), but is not significantly different from the EC₅₀ value for Ca²⁺ determined in the presence of [Ala^{3,11}]ET-1. Preincubation with nifedipine, 0.1 μ M, did not significantly alter these maximal responses or EC₅₀ values for Ca²⁺ (Figure 5d) and analysis of variance indicated no significant difference between these 2 concentration-effect curves ($P = 0.498$). Readdition of calcium to tissues exposed to 0.3 μ M noradrenaline in the absence of Ca²⁺ also elicited concentration-dependent responses (Figure 6). In the presence of diltiazem, 1 μ M, these concentration-effect curves were significantly shifted to the right, the EC₅₀ values were increased by about 3 fold ($P < 0.012$) and the maximal responses depressed ($P = 0.046$).

In the presence of 40 mM K⁺ and the absence of calcium, residual responses to 3 nM ET-1 were unchanged compared to those in a Ca²⁺-free medium with a normal concentration of K⁺. Readdition of calcium (10 μ M to 10 mM) to tissues in Ca²⁺-free medium containing 40 mM K⁺, induced concentration-dependent contractions with an EC₅₀ value of 0.7 $\pm$ 0.2 mM ($n = 4$). In the presence of both 3 nM ET-1 and 40 mM K⁺, EC₅₀ values for Ca²⁺ (0.4 $\pm$ 0.1 mM, $n = 4$) were not significantly altered and neither were maximal responses (t -test; Figure 7). Analysis of variance did not demonstrate any significant difference ($P = 0.301$).

Discussion

Endothelin (ET-1) was recently described as a vasoconstrictor peptide released from endothelial cells in culture (Yanagisawa *et al.*, 1988). This peptide and two of its analogues [Ala^{3,11}] and [Ala^{1,15}]ET-1 elicited concentration-dependent contractions of rat isolated aorta preparations (Figure 1). It is noteworthy that removal of one or the other of the disulphide bridges reduced potency but did not

Figure 5 Contractions evoked by either 3 nM endothelin (a, b), 75 nM $[\text{Ala}^{3,11}]$ endothelin (c) or 0.41 μM $[\text{Ala}^{1,15}]$ endothelin (d) in Ca^{2+} -free medium (▲) and responses to the subsequent addition of Ca^{2+} (○). Inhibitory effects of 1 μM diltiazem (●; a and c) and 0.1 (●) or 1 μM (□) nifedipine (b and d) are also shown. Results are expressed as percentage of contraction evoked by 1 μM noradrenaline (NA). Vertical bars represent s.e.mean of 4 to 10 observations.

markedly affect maximal evoked responses. Alanine was chosen to replace the cysteine residues because this substitution retains a chiral amino acid, it is also a neutral residue and does not introduce any new charge at the substituted position and is pseudo isosteric with cysteine. Evidently, the presence of both disulphide bonds is not essential for activity of ET-1. However, inhibition of the formation of one or other

of these bonds by the inclusion of blocking groups on the sulphur of the appropriate cysteine residues results in reduction (Kimura *et al.*, 1988) or loss of activity (Hirata *et al.*, 1989). This probably indicates that steric hindrance from the large blocking groups distorts the tertiary structure of the peptide while simple removal of one or other of the bridges has a relatively small effect.

Figure 6 Contractions induced by calcium in rat isolated aortic rings exposed to $0.3 \mu\text{M}$ noradrenaline in the absence (○) and presence (●) of $1 \mu\text{M}$ diltiazem. Results are means of 4 observations. Vertical bars represent s.e.mean.

Removal of extracellular calcium, a procedure that abolished responses to 100 mM K^+ , reduced, but did not abolish, responses to equieffective submaximal concentrations of ET-1 and its analogues. Results of this type, obtained with a variety of other agonists, have been interpreted as indicating that contractions are partially dependent on intracellular and partially on extracellular calcium (Godfraind *et al.*, 1986). Diltiazem antagonized contractions elicited by ET-1 in normal calcium-containing medium. However, the concentration-effect curves in the presence of diltiazem were parallel to those obtained in its absence and maximal responses were unchanged (Figure 2a). This sort of antagonism by calcium entry blockers of agonist-induced responses that are partly dependent on extracellular calcium in this tissue is not typical. It was expected that maximal concentrations would be reduced with little or no change in EC_{50} values (see for example Godfraind & Dieu, 1981; Godfraind & Miller, 1983). However, diltiazem failed to antagonize responses elicited by $[\text{Ala}^{3,11}]\text{ET-1}$. Since ET-1, $[\text{Ala}^{3,11}]\text{ET-1}$ and $[\text{Ala}^{1,15}]\text{ET-1}$ all have affinity for the same binding site, at least in the central nervous system (Jones *et al.*, 1989), the results indicate that the antagonism by diltiazem of ET-1-induced responses is unlikely to be due to an effect at ET-1 receptors. Also, diltiazem and ET-1 do not interact with each others specific binding sites (Hirata *et al.*, 1988; Van Renterghem *et al.*, 1988).

Responses elicited by the readdition of calcium in the presence of ET-1 and $[\text{Ala}^{3,11}]\text{ET-1}$ were significantly affected by diltiazem although the type of inhibition did not seem to be the same in each case. This is surprising if the peptides activate the same receptors (Jones *et al.*, 1989). These results (Figures 5a, c) are also not characteristic of antagonism of calcium-induced contractions in the presence of other agonists such as noradrenaline in rat aorta by high concentrations of calcium entry blockers (Figure 6).

On the other hand nifedipine inhibited responses elicited by both ET-1 and $[\text{Ala}^{1,15}]\text{ET-1}$ in a manner typical of its antagonism of other agonists such as noradrenaline, whose contractile responses are partly dependent on extracellular calcium (Godfraind *et al.*, 1986). This inhibition is unlikely to be due to an effect on ET-1 receptors since dihydropyridines do not interact with ET-1 binding sites (Hirata *et al.*, 1988; Van Renterghem *et al.*, 1988). Furthermore, nifedipine relaxed tissues precontracted with ET-1 with an IC_{50} value of about 22 nM . The maximal relaxation elicited by nifedipine was comparable to the reduction in maximal responses to ET-1 in the absence of extracellular calcium. The IC_{50} value for nifedipine was similar to that of 19 nM determined for inhibition of noradrenaline-induced contractions in rat aorta (Godfraind & Miller, 1983). Contractions induced by readdition of calcium to tissues in calcium-free medium in the presence of ET-1 were also antagonized by nifedipine in a

Figure 7 Contractions induced by calcium in rat isolated aorta in a solution containing 40 mM K^+ in the absence (○) and presence (●) of 3 nM endothelin. Vertical bars represent s.e.mean of 4 observations.

manner that would be expected if dihydropyridine sensitive calcium channels were being activated (Godfraind, 1983). Overall, these experiments using nifedipine are in agreement with reports of antagonism of ET-1-mediated responses by dihydropyridines and therefore with the idea that ET-1 activates dihydropyridine-sensitive calcium channels (Yanagisawa *et al.*, 1988). However, it has been reported that ET-1-induced contractions in rat aorta were not antagonized by diltiazem, D600 or nifedipine (Auguet *et al.*, 1988). Also, ET-1 did not potentiate calcium-induced contractions in depolarized tissues (Figure 7). The calcium channel activator, Bay K 8644, enhances contractions (Hwang & van Breemen, 1985) and ^{45}Ca influx (Chiu *et al.*, 1986) under these conditions and thus it seems unlikely that ET-1 directly activates dihydropyridine-sensitive calcium channels.

The sensitivity of ET-1-exposed tissues to calcium,

as indicated by the EC_{50} values of the Ca^{2+} -curves, was significantly lower than in tissues exposed to equipotent concentrations of $[\text{Ala}^{3,11}]$ or $[\text{Ala}^{1,15}]\text{ET-1}$ (Figure 5). The reason for this difference is unknown at present but it might indicate that ET-1 is a less effective activator of membrane channels that permit the passage of calcium than are the other analogues. Alternatively the analogues might reduce calcium extrusion from the cells to a greater extent than ET-1.

To summarize, the results obtained in this study using diltiazem and nifedipine suggest that there is a limited role for extracellular calcium entry via L-type calcium channels in the responses to ET-1 and to its analogues. This may be because ET-1 and its analogues only activate these channels secondarily to the activation of other channels that permit the entry of extracellular calcium (Van Renterghem *et al.*, 1988).

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