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Tissue selectivity of endothelin

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The effects of endothelin, a potent endogenous vasoconstrictor peptide, were examined in a range of vascular and non-vascular tissues. At concentrations that cause vasoconstricton in portal vein and aorta, the peptide strongly contracted rat uterus, trachea and vas deferens, but not guinea pig ileum. Nifedipine, a dihydropyridine calcium anatgonist, partially inhibited these contractions. Endothelin had no inotropic or chronotropic effect on the isolated rat heart. The peptide did not modulate secretion at the neuromusclar junction, from adrenal medullary cells or neutrophils, nor affect secretion or aggregation of platelets. The tissue responsiveness to endothelin was not the same as the tissue distribution of dihydropyridine receptors. This supports the idea that endothelin interacts with a specific receptor distinct from dihydropyridine sensitive calcium channels. The contractile effect of endothelin on non vascular smooth muscle suggests that the concept of endothelium dependent modulation of vascular smooth muscle tone may be extended to include epithelium dependent modulation of non vascular tissues.

Endothelin; Heart; Blood vessels; Uterus; Trachea; Vas deferens; Platelets; Chromaffin cells; Neuromuscular junction; Neutrophils

1. Introduction

Endothelin is a novel peptide vasoconstrictor derived from vascular endothelial cells (Yanagisawa et al., 1988). Its vasoconstrictor effect is dependent on extracellular calcium, but not sodium (Borges et al., 1988), and blocked by dihydropyridine-type calcium channel antagonists. Based on these findings and sequence similarities with biological toxins that are known to interact with ion channels, it has been suggested that endothelin might directly activate calcium channels.

More recent studies however have provided no evidence for competitive binding between endothelin and a range of calcium channel antagonists (Hirata et al., 1988). Endothelin is also strikingly similar to sarafotoxins from the snake *Atractaspis engaddensis* (Kloog et al., 1988) which acts on a specific receptor apparently distinct from any known vasoconstrictor receptors and the dihydropyridine sensitive calcium channel. It is therefore possible that endothelin also acts on a specific receptor distinct from calcium channels.

As endothelin shows a long lasting and potent effect on blood vessels, it could have a potential clinical use as a local vasoconstrictor, and the putative endothelin receptor might act as the target in the future development of therapeutic antagonistic drugs. Information on the nature and the tissue distribution of the endothelin receptor is a prerequisite for any such pharmacological applications. We have therefore investigated the effect of endothelin in various preparations.

This paper describes the effect on 14 different tissues to include vascular and non-vascular smooth muscle, heart, skeletal muscle and various secretory systems. The results indicate that the

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action of endothelin is not restricted to vascular smooth muscle, and is not correlated with the tissue distribution of dihydropyridine receptors.

2. Materials and methods

Pig endothelin was synthesised and supplied by Cambridge Research Biochemicals Ltd (UK), and the calcium channel antagonist, nifedipine, by Bayer A.G. (Wuppertal, FRG). Tissues were bathed in solutions as described in table 1.

2.1. Contraction experiments

Sprague Dawley rats (200-300 g) and guinea pigs (300-400 g) were killed by cervical dislocation, tissues were immediately removed, cleaned of connective tissue and mounted in tissue baths containing 2.5 ml of bathing solution. Drugs subsequently added to the bath did not change the volume by more than 1%.

TABLE 1

Composition (mM) of solutions used in experiments. Solutions a to d were equilibrated with 95% O_2-5% CO_2 to maintain the pH at 7.4.

	Tyrode [a]	Ringer [b]	De Jalon [c]	Krebs [d]	Locke [e]
NaCl	125.4	125	124	119	150
KCl	2.3	5.6	5.6	4.4	5
$CaCl_2$	2.5	1.1	0.27	2.5	2.5
$MgCl_2$	2.1	1.2	–	–	1.8
KH_2PO_4	0.4	–	–	1.2	–
$MgSO_4$	–	–	–	1.2	–
$NaHCO_3$	25	25	25	25	–
Glucose	11	11	11	11	11
HEPES	–	–	–	–	10
NaOH	–	–	–	–	5

[a] Tyrode solution was used for guinea-pig ileum experiments. Temperature 37 °C. [b] Ringer solution was used for rat right atrial and ventricular strip experiments. Temperature 30 °C. [c] De Jalon solution was used for rat uterus exeriments. Temperature 31 °C. [d] Krebs solutions was used for aorta and portal vein experiments at 37 °C, vas deferens experiments at 31 °C, and perfused kidney experiments at 35 °C, and trachea and rat diaphram experiments at 37 °C. A modified Krebs solution lacking $MgSO_4$, but containing 2.75 mM $MgCl_2$ and 0.5 mM $CaCl_2$ was used with the mouse soleus muscle-nerve preparation. [e] Locke solution was used for secretion experiments involving adrenal medullary cells and neutrophils.

2.1.1. Vascular tissue

Thoracic aorta and portal vein ring segments were used for vascular contraction experiments. The endothelium was mechanically abraded (Furchgott and Zawadzki, 1980), segments (2 mm) mounted on stirrups in the tissue baths, and the resting tensions set at 2 g and 1 g for aorta and vein respectively. Tensions were recorded isometrically (Grass FT 03 Transducer). The aortic segments were exposed to cumulative doses of drugs, whereas portal vein segments were exposed to a succession of doses of drugs, each one separated by 10 min recovery period in Krebs solution.

Rat kidneys were perfused with 35 °C Krebs solution through the renal artery at 4 ml/min using an LKB peristaltic pump (Model 2115). The inflow pressure was monitored continuously using a Statham Pressure Transducer (Model P23). Drugs were injected as a bolus (10 μl) into the perfusion fluid close to the kidney.

2.1.2. Heart

Right atria from rat were bathed in a Ringer solution (see table 1) and the tensions measured isometrically as described for the aorta and vein preparations, 1 g of resting tension being applied. The tension and frequency of the intrinsic beating were measured 10 min after application of the drugs which were applied cumulatively, as described for aortic ring experiments.

Strips, 3-4 mm wide, of right ventricle from rat were mounted in the tissue bath in direct contact with two stimulating silver electrodes. Square pulses, 0.5 ms duration at 2 Hz, were applied at a supramaximal voltage using a S-48 Grass stimulator; 3 g holding tension was applied and the tensions measured isometrically.

2.1.3. Skeletal muscle

Rat left hemidiaphrams were dissected, together with 2 cm of phrenic nerve, the tissue mounted in a tissue bath and the resting tension set at 3 g. The phrenic nerve was stimulated electrically by supramaximal square pulses of 0.5 ms duration. Muscle contraction was measured isometrically as described above (b). Two different stimulation protocols were employed: single twitches (0.1 Hz) applied continuously, inter-

spersed every 10 or 12 min with four pulses at 2 Hz i.e. a train of four (Ali and Savarese, 1976). Endothelin was applied cumulatively and contractions were recorded continuously.

2.1.4. Rat uterus

Virgin female rats received 0.1 mg/kg of stilboestrol 24 h prior to the experiment. Both uterine horns were mounted in De Jalon solution (see table 1) and the contraction measured isotonically at 500 mg tension using a low friction rotational Hall effect isotonic transducer. Drugs were added cumulatively.

2.1.5. Rat trachea

Rat tracheal rings were mounted and treated as described for aortic segments. The resting tension was set at 1 g and contractions were measured isometrically in response to cumulative concentrations of endothelin.

2.1.6. Vas deferens

Contractions of prostatic and epididymal portions of rat vas deferens were recorded isotonically at 500 mg tension. Drugs were applied cumulatively as described for the rat uterus.

2.1.7. Guinea pig ileum

Male adult guinea pigs were deprived of food for 24 h before the experiments; 2.5 cm pieces of intestine were mounted in a holder and contractions measured isotonically at 1 g holding tension. Endothelin was administered cumulatively.

2.2. Secretion experiments

2.2.1. Chromaffin cells

Cultured bovine adrenal medullary cells (Von Grafenstein et al., 1986) were washed once in Locke solution containing 0.1% bovine serum albumin (table 1) before being challenged for 10 min with 0.1 μM carbamylcholine at 23° C, the optimal temperature for carbamylcholine induced secretion (Knight and Baker, 1983), or at 37° C with a high potassium containing solution (isoosmotic replacement of NaCl by KCl). Endothelin or the calcium channel agonist BAY-K-8644 was included in the solutions as indicated in the legend of fig. 5. Catecholamine in the supernatant was assayed as described previously (Knight et al., 1985), and secretion was expressed as a percentage of total cellular content.

2.2.2. Neuromuscluar junction

The effect of endothelin on neurotransmission was determined by measuring the mean quantal content of endplate potentials in the mouse soleous muscle-nerve preparation before and after incubation with endothelin. In order to reduce the quantal content of the endplate potential to the equivalent of less than 10 miniature end plate potentials, the Krebs solution contained a low calcium concentration (table 1). A train of 60 endplate potentials, stimulated at 2 Hz, was recorded for each muscle fiber, and their mean amplitude and variance determined. Recordings were only made from fibers with a resting potential of at least − 60 mV and when the rise time of the endplate potential was less than 1.5 ms. The mean quantal content of the end plate potentials was calculated from the coefficient of variance of their amplitudes (Del Castillo and Katz, 1954). The mean quantal content for 17 fibers was measured (time taken being 1 h). The preparation was then immersed in solution containing 0.5 μM endothelin, and the mean quantal content of another 17 fibers was measured during the following hour.

2.2.3. Secretion from and aggregation of platelets

Platelet rich plasma was prepared from human blood taken into 0.1 volume of citrate/dextrose anticoagulant (Thompson et al., 1986). For secretion experiments, platelets were first loaded with [^{3}H]serotonin (0.1 μCi/ml) for 30 min at 37° C in the presence of 200 μM acetylsalicylate. Hundred microlitres of this cell suspension was added at 20° C to 100 μl of Locke solution either in the presence or absence of endothelin or in the presence or absence of thrombin, the final concentrations being 0-10 μM endothelin, and 0-1 units/ml thrombin. After 5 min the reaction was stopped by the addition of 1 ml of ice cold Locke solution containing 4% glutaraldehyde. The platelets were centrifuged and the [^{3}H]serotonin content in the supernatant was counted.

Aggregation of platelets was followed in a Born Aggregometer by changes in light transmission. Aliquots (600 μl) of platelet suspension were warmed to 37° C in siliconised glass cuvettes and stirred at 700 rpm. Additions (6 μl) of endothelin or ADP were made as described in the legend of fig. 5.

2.2.4. Synthesis and release of thromboxane and leukotriene from neutrophils

Rat peritoneal polymorphonuclear leucocytes were prepared from peritoneal lavage of Wistar rats (Moroney et al., 1988) and suspended in Locke solution at 5×10^6 ml. Hundred microlitres aliquots of the cell suspension were added to an equal volume of Locke solution containing various agonists. Endothelin was applied alone or together with N-formyl-met-leu-phe, or the calcium ionophore A-23187, or in a solution containing an elevated potassium concentration. The final concentrations were: endothelin, 0-10 μM; N-formyl-met-leu-phe, 0-10 μM; A-23187, 0-10 μM; K^+, 5-80 mM. After 10 min incubation at 37° C the cells were centrifuged at $12\,000 \times g$ for 2 min and the leukotriene B_4 and thromboxane B_2 content in the supernatant determined by radioimmunoassay (Moroney et al., 1988).

3. Results

Figure 1 shows the response of rat aorta and portal vein to endothelin. The peptide caused concentration dependent contractions in both tissues over the range 10^{-11}-10^{-7} M. The dihyropyridine calcium antagonist nifedipine (10^{-7} M) inhibited these responses (data not shown). A sustained pressor effect of endothelin was clearly seen in renal vascular bed (fig. 1c). Here a 10 μl bolus injection of phenylephrine brought about a transient rise in perfusion pressure whereas a similar bolus injection of endothelin elicited a sustained elevation in perfusion pressure apparent even after 1 h.

Figure 2 shows that the effect of endothelin is not restricted to vascular smooth muscle. For example, in the rhythmically quiescent rat uterus preparation endothelin (10^{-9}-10^{-7} M) both contracted and restored rhythmicity. Both these effects were inhibited by 0.1 μM nifedipine. Endothelin causes concentration dependent contractions in the rat trachea and in the prostate portion

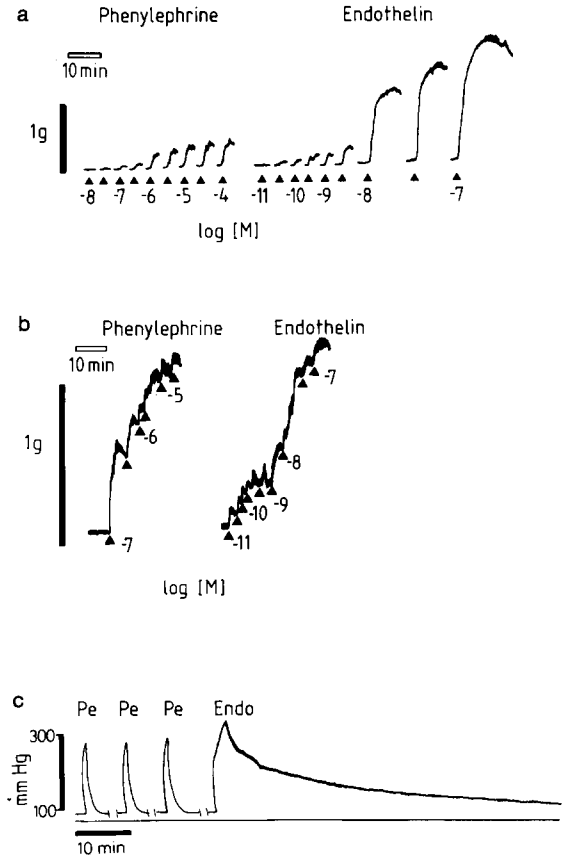

Fig. 1. Contractile response of vascular tissue to endothelin. (a) Responses of rat portal vein ring segments to phenylephrine and endothelin. The breaks in the traces indicate 10 min periods in which the tissue was allowed to recover from the earlier application of drug. The numbers are the Log of the concentration (M) of endothelin or phenylephrine used. The unnumbered arrow heads correspond to intermediate concentrations. (b) Responses of rat aortic ring segments to cumulative levels of phenylephrine or endothelin, doses being applied as described in (a). (c) Endothelin has a long lasting effect in kidney vascular bed. Perfusion pressure was measured for a constant flow of Krebs solution of 4 ml/min through kidney vasculature. Successive bolus injections of 10 nmol phenylephrine (Pe) were followed by a single bolus injection of 100 pmol endothelin. The increase in perfusion pressure induced by this peptide is longlasting compared to the transient increases seen with phenylephrine. The breaks in the traces indicate 10 min recovery periods following exposure to phenylephrine.

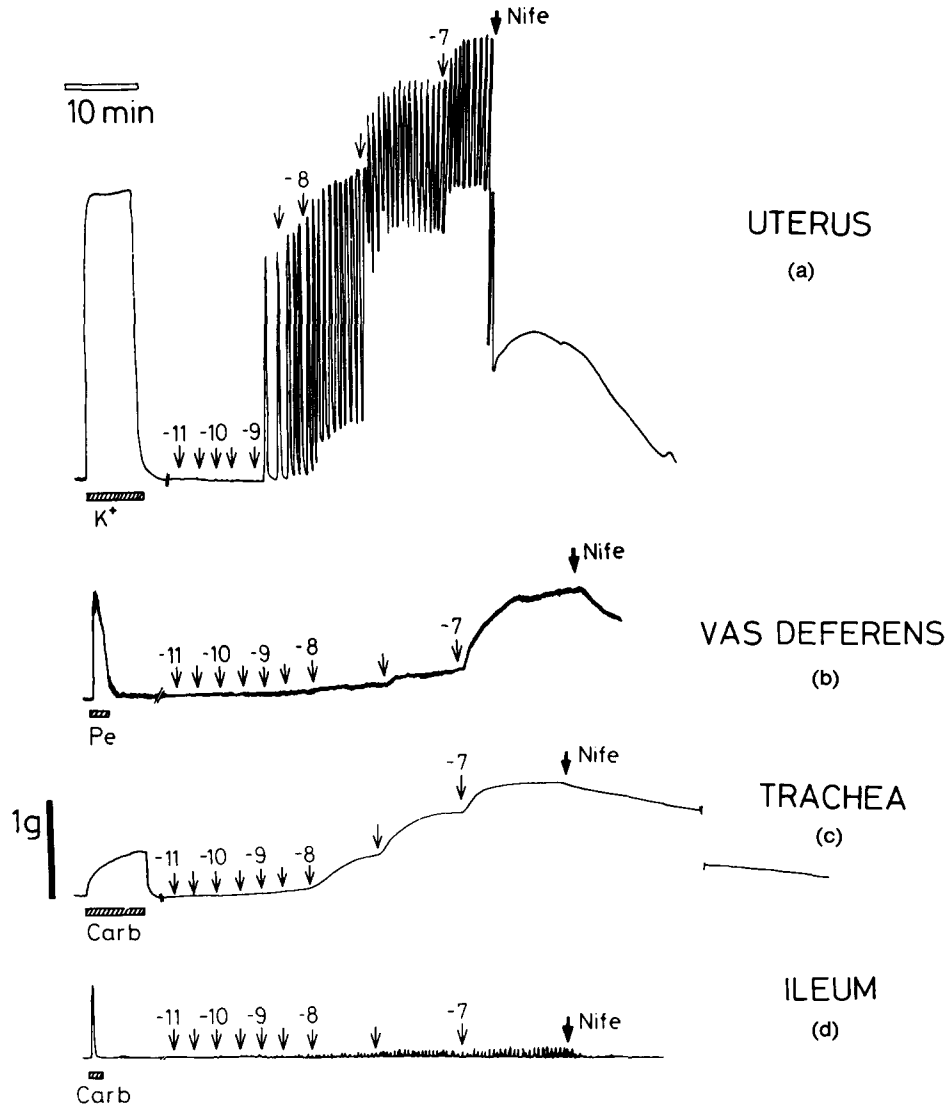

Fig. 2. Contractile response of non vascular smooth muscle preparations to endothelin. The concentrations of endothelin used were as described if fig. 1a. The calcium antagonist nifedipine (0.1 μM) was added at the times indicated (Nife). The traces shown here are from single experiments, but similar results have been obtained in four other experiments. (a) Isotonic recording of rat uterus tissue in response to a 59 mM KCl challenge (hatched bar, K^+), and to cumulative concentrations of endothelin applied at the times indicated by the arrows. (b) Isotonic recordings of the prostatic portion of vas deferens in response to 1 μM phenylephrine (hatched bar, Pe.) and to various concentrations of endothelin applied cumulatively as described in (a). (c) Isometric recordings of rat tracheal ring segments in response to 1 μM carbamylcholine (hatched bar, Carb.), and cumulative doses of endothelin. The vertical calibration bar corresponds to 1 g tension. The break in the tension trace corresponds to an interval of 20 min. (d) Isotonic recordings of guinea pig ileum in response to 1 μM carbamylcholine (hatched bar, Carb.), and cumulative doses of endothelin. Time bar corresponds to 10 min for all traces.

of the rat vas deferens. Endothelin was less effective however on the epididymal portion of vas deferens (data not shown). The tone in guinea-pig ileum was not increased by endothelin although there appeared to be a minor increase in rhythmic activity. Nifedipine (10^{-7} M) reduced the endothelin induced effects in each of these tissues (fig. 2).

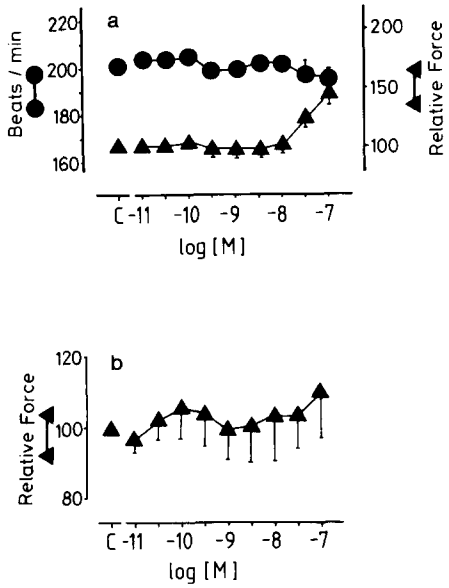

Fig. 3. The effect of endothelin on rat cardiac tissue. (a) The effect of endothelin on frequency (●) and strength (▲) of spontaneously beating rat right atrium. Endothelin was added cumulatively, and 10 min after each addition the frequency and strength of contraction were measured isometrically over 15 s. The force of contraction is expressed relative to the force measured at the begining of the experiment before endothelin was applied. Data are means (S.E.) for six determinations using six different animals. (b) The effect of endothelin on force of contraction of rat right ventricular strips. The procedure and expression of results are as described in (a) except that the ventricle was stimulated electrically at 2 Hz. Data are means (S.E.) of four determinations from four different animals.

If endothelin were a general calcium channel activator, it might be expected to also have a major effect on cardiac contractility as well as rhythm. Figure 3 however shows that endothelin had no effect on the rhythm of spontaneously beating atrium. The increase in the atrial force of contraction was very small compared to that seen with other drugs, such as β-adrenoceptor agonists. This small effect of endothelin was not seen in rat ventricle however, a preparation that is better suited to the study of positive inotrophic effects. Endothelin (10^{-11}-10^{-7} M) was also without effect on the contractile response of the nerve skeletal muscle preparation. The mean twitch tension measured from four rat diaphrams in the absence of endothelin was 1.313 g (S.E. 0.076) and in the presence of 10^{-7} M endothelin was 1.254 g (S.E. 0.079).

Secretion from paracrine, hormonal or neuronal cells is often triggered by a rise in intracellular free calcium brought about by entry of calcium through either voltage operated or chemically operated calcium channels. We have therefore extended our study to include the effect of endothelin on a variety of secretory cells. In the case of

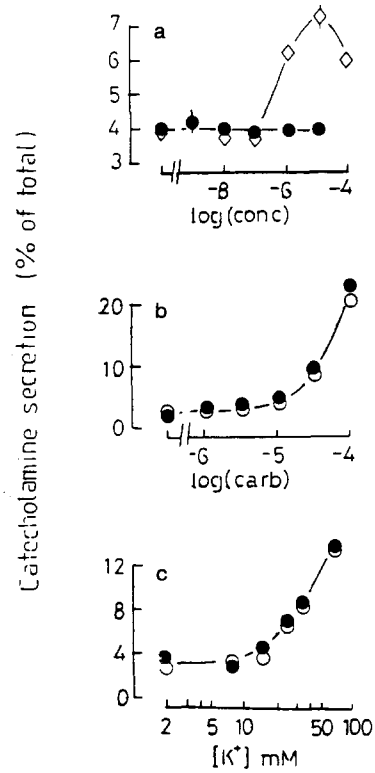

Fig. 4. The effect of endothelin on catecholamine secretion from isolated bovine adrenal medullary cells. Catecholamine in the supernatant, expressed as a percentage of the total cellular content, was measured after 10 min incubation with secretagogue. The effect of endothelin or the calcium channel agonist BAY-K-8644 was tested in the presence or absence of a carbamylcholine or potassium challenge. Data are the means and S.E.M. or four determinations. (a) BAY-K-8644, but not endothelin, enhances the potassium dependent secretory response. Cells were incubated in physiological saline containing either 2 mM K^+ or 25 mM K^+ together with the various concentrations shown of either endothelin (●) or BAY-K-8644 (◇). Catecholamine secretion in response to this increase in potassium is shown. (b, c). Endothelin does not modulate the K^+ or carbamylcholine evoked secretory response. Cells challenged with the various concentrations shown of (b) carbamylcholine, or (c) K^+, together with (●), or without (○) 1 μM endothelin.

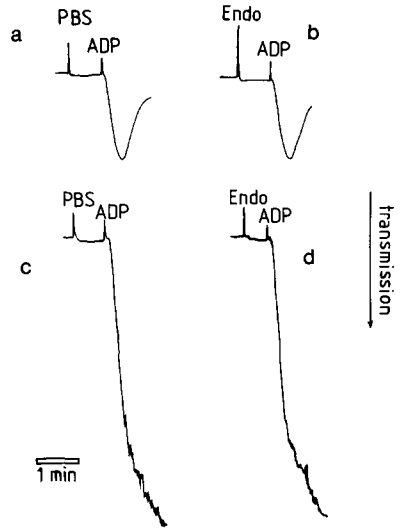

Fig. 5. Endothelin affects neither primary nor secondary aggregation of human platelets. The traces represent light transmission through a suspension of platelets in a Born Aggregometer. Aliquots (6 μl) of saline (PBS), endothelin (Endo) or ADP (ADP) were added to 0.6 ml of a stirred platelet suspension. (a, b) Primary aggregation triggered by 0.3 μM ADP in the absence (a) or presence (b) of 1 μM endothelin. (c, d) Secondary aggregation triggered by 10 μM ADP in the absence (c) or presence (d) of 1 μM endothelin.

bovine chromaffin cells, adrenaline and noradrenaline are secreted when calcium enters the cell through voltage operated, dihydropyridine sensitive calcium channels. Figure 4 shows quite clearly that the dihydropyridine type calcium channel agonist BAY-K-8644 enhanced the potassium induced secretory response. Endothelin however was without effect at concentrations that fully activated vascular smooth muscle, and had no effect on potassium or carbamylcholine evoked catecholamine release.

Endothelin was also without effect on acetylcholine release at the neuromuscular junction. Neither the miniature end plate potential nor the mean quantal content were altered by endothelin. The mean quantal content measured from 17 fibers was 6.3 (S.E. 0.5), and in the presence of 0.5 μM endothelin was, for another 17 fibers, 6.9 (S.E. 0.6).

Figure 5 shows that endothelin failed to promote platelet aggregation, and did not modulate the calcium dependent primary aggregation evoked by submaximal levels of ADP, nor the calcium independent secondary aggregation response induced by higher levels of ADP. Furthermore endothelin (up to 1 μM) failed to trigger serotonin secretion, and did not modulate the thrombin dose-response curve of serotonin secretion (EC_{50} for release being close to 0.01 units/ml in the presence or absence of endothelin. Endothelin was also without effect on the synthesis and release response of rat neutrophils. Endothelin (up to 1 μM) did not trigger synthesis and release of thromboxane or leukotriene from these cells, nor alter synthesis and release induced by either N-formyl-met-leu-phe or the calcium ionophore A-23187 (table 2).

4. Discussion

Endothelin induces a long-lasting effect on blood vessels, clearly illustrated by its effect on the perfusion pressure in the kidney. This may be the result of a slow dissociation of endothelin from its receptor, or due to a long-lasting intracellular event coupling receptor occupation to contraction.

TABLE 2

Secretion (ng) of leukotriene (LTB_4) and thromboxane (TXB_2) from 2.5×10^6 neutrophils in response to 10 min incubation with either 1 μM A-23187 or 1 μM N-formyl-met-leu-phe in the presence or absence of 1 μM endothelin. Data are means of three determinations, the values in parenthesis being the S.E.M.

	Control		1 μM A-23187		1 μM N-Formyl-met-leu-phe	
		+1 μM endo.		+1 μM endo.		+1 μM endo.
LTB_4	0.7 (0.1)	0.6 (0.1)	64.9 (2.9)	57.9 (1.1)	22.3 (0.1)	18.4 (0.4)
TXB_2	0.6 (0.1)	0.8 (0.1)	11.2 (0.1)	11.2 (0.1)	9.3 (1.5)	8.4 (0.5)

Our results show that endothelin is not only a potent vasoconstrictor but also contracts other non-vascular smooth muscle preparations such as uterus, vas deferens and trachea (see also Uchida et al., 1988). The effect of endothelin does not extend to all types of smooth muscle however. Ileum, for example, shows little or no response. Although these effects of endothelin can generally be blocked by nifedipine, the lack of effect of this peptide on other preparations rich in dihydropyridine sensitive calcium channels, e.g. cardiac muscle or chromaffin cells (Schramm et al., 1983, Garcia et al., 1984), suggests that endothelin does not generally and directly activate these dihydropyridine sensitive calcium channels. It is therefore possible that endothelin acts on a specific receptor that is distinct from, but coupled to a calcium channel. These data suggest that the tissue selectivity of endothelin is determined by the distribution of endothelin receptors rather than the distribution of a particular class of calcium channel. Our data provide no evidence that endothelin activates secretory tissue such as human platelets or neutrophils.

The physiological role of this peptide on non-vascular tissue is still unknown but the observation that endothelin activates smooth muscle of uterus, trachea and vas deferens suggest that the concept of endothelial mediated vascular tone may be extended to other types of tissue where interaction between epithelia and myocytes has not been extensively studied.

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References

Ali, H.H. and J.J. Savarese, 1976, Monitoring on neuromuscular function, Anaesthesiology 14, 215.

Borges, R., D.V. Carter, H.R.K. Von Grafenstein, J. Halliday and D.E. Knight, 1988, Activation of sodium channels is not essential for endothelin induced vasoconstriction, European J. Physiol. 413, 313.

Del Castillo, J. and B. Katz, 1954, The quantal components of the end-plate potential, J. Physiol. (London) 124, 560.

Furchgott, R.F. and J.V. Zawadzki, 1980, The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine, Nature 288, 373.

Garcia, A.G., F. Sala, J.A. Reig, S. Viniegra, R. Fonteriz and L. Gandia, 1984, Dihydropyridine BAY-K-8644 activates chromaffin cell calcium channels, Nature 309, 69.

Hirata, Y., H. Yoshimi, S. Takata, T.X. Watanabe, S. Kumagai, K. Nakajima and S. Sakakibara, 1988, Cellular Mechansism of action by a novel vasocontrictor endothelin in cultured rat vascular smooth muscle cells, Biochem. Biophys. Res. Comm. 154, 868.

Kloog, Y., I. Ambar, M. Sokolovsky, E. Kochva, Z. Wollberg and A. Bdolah, 1988, Sarafotoxin, a novel vasoconstrictor peptide: phosphoinositide hydrolysis in rat heart and brain, Science 242, 268.

Knight, D.E. and P.F. Baker, 1983, Stimulus secretion coupling in isolated bovine adrenal medullary cells, Quart. J. Experimental Physiol. 68, 123.

Knight, D.E., D.A. Tonge and P.F. Baker, 1985, Inhibition of exocytosis in bovine adrenal medullary cells by botulinum toxin type D, Nature 317, 719.

Moroney, M.A., M.J. Alcaraz, R.A. Forder, F. Carey and J.R.S. Hoult, 1988, Selectivity of neutrophil 5-Lipoxygenase and cyclo-oxygenase inhibition by an Anti-inflammatory Flavonoid Glycoside and Related Aglycone Flavonoids, J. Pharm. Pharmacol. 40, 787.

Schramm, M., G. Thomas, R. Towart and G. Franckowiak, 1983, Novel dihydropyridines with positive inotropic action through activation of Ca^{2+} channels, Nature 303, 535.

Thompson, N.T., M.C. Scrutton and R.B. Wallis, 1986, Synergistic responses in human platelets: Comparison between aggregation, secretion and cytosolic Ca^{2+} concentration, European J. Biochem. 161, 399.

Uchida, Y., H. Ninomiya, M. Saotome, A. Nomura, M. Ohtsuka, M. Yanagisawa, K. Goto, T. Masaki and S. Hasegawa, 1988, Endothelin, a novel vasoconstrictor peptide, as potent bronchoconstrictor, European J. Pharmacol. 154, 227.

Von Grafenstein, H., C.R. Roberts and P.F. Baker, 1986, Kinetic analysis of the triggered exocytosis endocytosis secretory cycle in cultured bovine adrenal medullary cells, J. Cell. Biol. 103, 2343.

Yanagisawa, M., H. Kurihara, S. Kimura, Y. Tomobe, M. Kobayashi, Y. Mitsui, Y. Yakaki, K. Goto and T. Masaki, 1988, A novel potent vasoconstrictor peptide produced by vascular endothelial cells, Nature 332, 411.