

Endothelin-1-Induced Vasoconstriction in Humans

Reversal by Calcium Channel Blockade but Not by Nitrovasodilators or Endothelium-Derived Relaxing Factor

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The vascular effects of endothelin-1 (ET) in humans were investigated by brachial artery infusions of ET into 25 healthy volunteers. Forearm blood flow increased from a mean $\pm$ SD value of 2.3 ± 1.5 to 2.5 ± 1.5 ml/min/100 ml forearm tissue ($n=25$, $p<0.05$) in response to low dose (0.5 ng/min/100 ml forearm tissue) ET infusion and decreased to 1.78 ± 1.3 and 1.1 ± 0.9 ml/min/100 ml forearm tissue ($p<0.001$) during higher dosages (25 and 50 ng/min/100 ml forearm tissue). Sodium nitroprusside (0.6 μ g/min/100 ml forearm tissue, $n=6$), acetylcholine (16 μ g/min/100 ml forearm tissue, $n=7$), nifedipine (6 μ g/min/100 ml forearm tissue, $n=6$), and verapamil (80 μ g/min/100 ml forearm tissue, $n=6$) were infused alone and in combination with ET to evaluate the interactions between ET-induced vasoconstriction and stimulation of vascular muscle cyclic GMP levels by sodium nitroprusside, release of endothelium-derived relaxing factor by acetylcholine, and blockade of voltage-operated calcium channels by nifedipine and verapamil. Neither the vasodilator nor the vasoconstrictor response to ET was influenced by sodium nitroprusside or acetylcholine. In contrast, both calcium antagonists converted ET-induced vasoconstriction (e.g., Δ forearm vascular resistance to ET 50 ng/min/100 ml forearm tissue, $151 \pm 100\%$ and $164 \pm 92\%$ in verapamil and nifedipine groups, respectively) to vasodilation ($-35 \pm 12\%$ and $-21 \pm 16\%$, $p<0.05$). Our results demonstrate both ET-induced vasodilation (at low dosages) and vasoconstriction (at high dosages) in resistance vessels of normal humans. Blockade of voltage-operated calcium channels prevented ET-induced vasoconstriction and unmasked the vasodilator effect of high ET dosages. In human resistance vessels, blockade of voltage-operated Ca^{2+} channels but not cyclic GMP-dependent vasodilation may be an effective tool to inhibit ET-induced vasoconstriction. (*Circulation* 1991;83:469-475)

The observation that removal of the endothelium of canine femoral arteries reduced potassium chloride-induced vasoconstriction led to the suggestion that the endothelium may be capable of secreting a vasoconstrictor substance.¹ Subsequent experiments demonstrated hypoxia- or anoxia-mediated release of a diffusible, non-protease-sensitive vasoconstrictor substance from endothelial cells^{2,3} as well as protease-sensitive contractile activ-

ity in supernatants from cultured endothelial cells.⁴ Endothelin is a 21-amino-acid polypeptide that has been isolated from supernatants of cultured porcine endothelial cells.⁵ Endothelial cells produce and release endothelin-1 (formerly human or porcine endothelin),⁶ which has potent and long-lasting vasoconstrictor activity in vivo and in vitro.⁷⁻¹⁰ The peptide produces sustained increases of blood pressure in rats pretreated with atropine, propranolol, and bunazosin,⁵ but systemic vasodilation and a decrease of blood pressure have also been described after left atrial injection of endothelin-1 into cats.¹¹ The precise cellular mechanism of action of endothelin-1 is not known, and differing results have been obtained with respect to the reversibility of endothelin-1-induced vasoconstriction. The contention that endothelin-1 may act as an endogenous Ca^{2+} channel agonist⁵ has been disputed based on observations that endothelin-1-induced vasoconstriction is not

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fully reversible by organic calcium antagonists.¹²⁻¹⁴ In contrast, release of endothelium-derived relaxing factor by acetylcholine or bradykinin and nitrovasodilators abolished endothelin-1 induced contractions in animal preparations¹⁵ and normal human arteries.¹⁶ Data on the *in vivo* effects of endothelin-1 in humans are scarce.^{17,18} The present study was designed to evaluate the vascular effects of endothelin-1 in humans and to investigate the influence of endothelium-derived relaxing factor, the nitrovasodilator sodium nitroprusside, and calcium channel blockade on endothelin-1-induced vasoconstriction.

Methods

Twenty-five healthy, normotensive (casual sitting blood pressure, less than 140/90 mm Hg) volunteers (18 men and seven women) 19–39 years old (mean, 26 years) participated in the study. The study protocol was approved by the hospital ethical committee on the use of human subjects in clinical investigations, and written informed consent was obtained from all subjects.

Forearm Blood Flow Measurements

Forearm blood flow was measured bilaterally by venous occlusion plethysmography.^{19,20} A mercury-in-silastic strain gauge was placed at the upper third of the forearm, which rested comfortably on a support slightly above the level of the heart. The strain gauge was coupled to an electronically calibrated plethysmograph (EC3, Hokanson, Issaquah, Wash.). Venous occlusion was achieved by a blood pressure cuff applied proximal to the elbow and inflated to 40 mm Hg by a rapid cuff inflator (EC10, Hokanson). The hand was excluded from the circulation by inflating a pediatric blood pressure cuff placed around the wrist to 50 mm Hg above systolic pressure 1 minute before and during forearm blood flow measurements to eliminate the unpredictable influence of arteriovenous shunts in the hand. Experiments were done on the left (experimental) forearm while blood flow measurements on the right (control) arm served as a continuous control. Determinations of forearm blood flow were made by analyzing four to six consecutive recordings using a digitizing board and a suitably programmed computer. The mean value was taken for statistical evaluation. Forearm vascular resistance was calculated by dividing mean arterial pressure by forearm blood flow and is expressed in arbitrary units. The electrocardiogram was monitored throughout the study.

Preparation of Solutions for Infusions

The forearm volume of each subject was measured by water displacement using the Archimedes principle (range, 700–1,200 ml), and drug doses were adjusted accordingly. All solutions were freshly prepared before each study. Endothelin-1, (endothelin, CIBA-GEIGY, Basel, Switzerland) was diluted in Physiogel (gelatin solution 4%; MW 22,000; Swiss Red Cross, Berne, Switzerland) to avoid binding to

syringes or tubing. Infusions of Physiogel at volumes of as much as 6 ml/min have been shown not to influence forearm blood flow to a measurable degree.²¹ Biological activity of endothelin was tested for each stock solution by measuring the contractile response of artery strips *in vitro*.¹⁶ Sodium nitroprusside (Hoffmann-La Roche, Basel, Switzerland) and verapamil (Knoll, Ludwigshafen, FRG) were dissolved in 0.9% NaCl solution, and sodium nitroprusside solutions were protected from light. Nifedipine was administered using commercially available (Bayer, Leverkusen, FRG) solutions in black-coated syringes and tubing for light protection. Intra-arterial infusions were performed with volumes between 0.5 and 2.0 ml/min using a constant-speed infusion pump (Sage Instruments Inc., Syracuse, N.Y.).

Study Protocol

All studies were performed in the morning with the subjects recumbent and comfortably resting after a light breakfast. All subjects had been asked to refrain from smoking and consuming caffeinated beverages for 8 hours before the study; none was taking any medicine. The studies were performed in a quiet, air-conditioned room at an ambient temperature of 20–22°C and lasted for approximately 5 hours. Under local anesthesia (lidocaine 1%), an 18-gauge catheter (Abbocath-T, Abbott, Sligo, Ireland) was inserted into the left brachial artery for regional drug infusion and recording of arterial pressure using a Statham P23 Pb pressure transducer. The subjects were allowed to rest for 30 minutes after instrumentation before measurement of basal forearm blood flow, intra-arterial blood pressure, and heart rate. Next, endothelin was infused in three doses (0.5, 25, and 50 ng/min/100 ml forearm tissue) in all subjects. Analysis of the time course of flow changes in the first eight subjects showed that the onset of action usually occurred in the second minute and was maximal from the third minute on. Thus, in the remaining subjects, measurements were obtained during the fifth minute of endothelin infusions. After completion of the endothelin infusions, forearm blood flow was allowed to return to normal, which took between 60 and 90 minutes. Sodium nitroprusside (0.6 µg/min/100 ml forearm tissue, *n*=6), acetylcholine (16 µg/min/100 ml forearm tissue, *n*=7), verapamil (80 µg/min/100 ml forearm tissue, *n*=6), and nifedipine (6 µg/min/100 ml forearm tissue, *n*=6) were then infused for 3 minutes each, and forearm blood flow was measured in the third minute of each infusion. The doses were chosen based on experiments demonstrating that they produced maximal regional vasodilator responses without systemic hemodynamic effects.²¹⁻²⁴ Finally, combined infusions of the vasodilator compounds and the three endothelin doses were performed.

Statistical Analysis

Values are given as mean ± SD. One-factor analysis of variance was used to test for differences attribut-

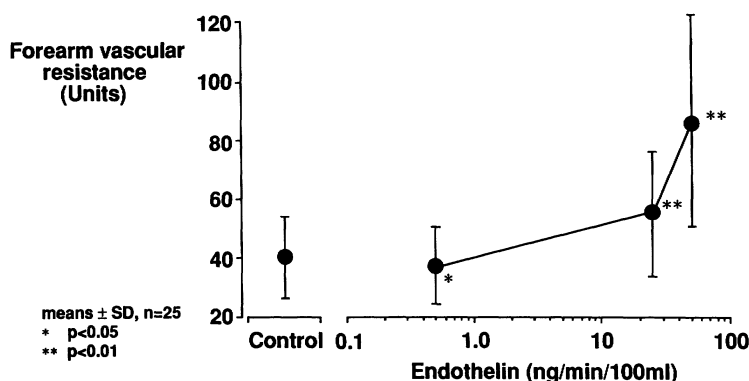


FIGURE 1. Plot of changes of calculated forearm vascular resistance to increasing doses of brachial artery infusions of endothelin in normal subjects. Forearm vascular resistance decreased significantly during infusion of the smallest dose of endothelin but increased dose dependently for the higher doses.

able to the various vasodilator compounds. The *t* test for paired observations was used to compare results obtained after a particular intervention with the corresponding control values. For evaluation of the effects of the vasodilator compounds on endothelin-induced vasoconstriction, percent changes from the corresponding control values were used. A two-tailed probability value of less than 0.05 was considered to indicate a significant difference. All calculations were performed using the STATVIEW II (Abacus, Berkeley, Calif.) statistical program.

Results

Vascular Effects of Endothelin

Intra-arterial infusions of endothelin were well tolerated and did not result in any untoward effect. Blood pressure, heart rate, and forearm blood flow in the contralateral arm did not change throughout the study, indicating that the drug infusions had no systemic effects. Although the lowest dose of endothelin (0.5 ng/min/100 ml forearm tissue) caused a small but significant increase of forearm blood flow (2.3 ± 1.5 versus 2.5 ± 1.5 ml/min/100 ml forearm tissue, $n=25$, $p<0.05$), the higher doses resulted in reductions of forearm blood flow to 1.78 ± 1.3 and 1.1 ± 0.9 ml/min/100 ml forearm tissue, respectively ($p<0.001$). Figure 1 shows the corresponding

changes of forearm vascular resistance—decrease of $-6.2 \pm 26.9\%$ for the lowest endothelin dose and increases of $40.1 \pm 38.2\%$ and $130.3 \pm 85.3\%$, respectively, for the higher doses.

Vasodilator Drugs and Endothelin-Induced Vasoconstriction

Forearm blood flow increased to 14.9 ± 4.4 with sodium nitroprusside, 27.6 ± 12.6 with acetylcholine, 16.9 ± 6.9 with verapamil, and 22.2 ± 7.1 ml/min/100 ml forearm tissue with nifedipine. The effects on forearm blood flow of the vasodilators in combination with endothelin are shown in Figure 2. Neither sodium nitroprusside nor acetylcholine prevented endothelin-induced decreases of forearm blood flow. In contrast, verapamil as well as nifedipine not only prevented endothelin-induced decreases of forearm blood flow but also resulted in significant ($p<0.05$) increases to values of more than those of the corresponding controls. The percent changes of forearm vascular resistance in response to endothelin alone and in combination with the vasodilator compounds are shown in Figure 3. The response to endothelin alone was similar in the four groups of subjects with maximal increases of forearm vascular resistance between $90 \pm 85\%$ (subjects assigned to sodium nitroprusside) and $164 \pm 92\%$ (subjects assigned to nifed-

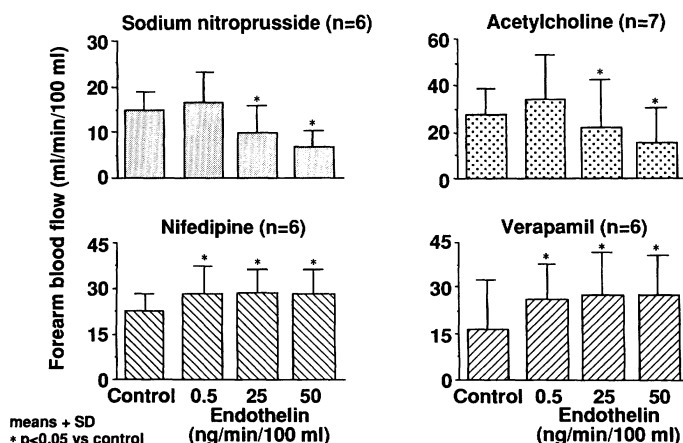


FIGURE 2. Bar graphs of forearm blood flow during infusion of sodium nitroprusside, acetylcholine, nifedipine, and verapamil alone (control) and during combined infusions of vasodilators and endothelin. Endothelin resulted in vasoconstriction during coadministration of sodium nitroprusside and acetylcholine, whereas the calcium antagonists nifedipine and verapamil not only abolished the vasoconstrictor activity of the polypeptide but also resulted in higher forearm blood flow values compared with control.

ipine) ($p=NS$ by analysis of variance). Changes of forearm vascular resistance to the lowest dose of endothelin were not significantly altered by any of the vasodilators. However, the influences of nifedipine and verapamil on the vasoconstrictor effects of the two highest doses were significantly different from those of sodium nitroprusside and acetylcholine ($p<0.05$ by analysis of variance). Thus, the increase of forearm vascular resistance to, for example, the highest dose of endothelin (Figure 3) was not influenced by sodium nitroprusside ($90\pm 85\%$ versus $204\pm 200\%$, $p=NS$) or acetylcholine ($113\pm 66\%$ versus $249\pm 248\%$, $p=NS$) but was converted from a vasoconstrictor to a vasodilator response by both verapamil ($151\pm 100\%$ versus $-35\pm 12\%$, $p<0.05$) and nifedipine ($164\pm 92\%$ versus $-21\pm 16\%$, $p<0.05$).

Discussion

Our study demonstrates for the first time in humans that endothelin has a dual action on resistance vessels (e.g., a small vasodilator effect at a low dose and marked vasoconstriction at higher doses). Moreover, calcium channel blockade by either a dihydropyridine calcium antagonist, nifedipine, or a nondihydropyridine calcium antagonist, verapamil, not only prevented endothelin-induced vasoconstriction but also unmasked the vasodilator effects of the peptide. In contrast, neither sodium nitroprusside, which evokes vascular muscle relaxation by increasing cyclic GMP (cGMP) levels,²⁶ nor acetylcholine, which stimulates the release of endothelium-derived relaxing factor and subsequently increases cGMP levels in vascular muscle,^{23,27-29} influenced the vasoconstrictor response to endothelin.

The finding of endothelin-induced vasodilation at a low dose (0.5 ng/min/100 ml forearm tissue) is compatible with in vitro and animal experiments. Thus, intravenous injection of endothelin in rats causes an initial decrease of blood pressure^{5,30} and an increase of hindquarter vascular conductance.³¹ Systemic vasodilation has also been observed in cats after left atrial injection of endothelin.¹⁰ The mechanisms leading to vascular relaxation remain unclear. Endothelin releases prostacyclin³²⁻³⁴ and endothelium-derived relaxing factor from vascular endothelium.³² Moreover, removal of the endothelium enhances the vasoconstrictor response to endothelin, suggesting that endothelium-derived relaxing factor may be physiologically counteracting the vasoconstrictor effects of endothelin.¹⁶ Thus, it is likely that endothelin stimulates the release of these vasodilator substances and in turn causes vasodilation at low concentrations, which may not produce either vasoconstriction or the vasoconstrictor effects may be overcome by the endogenous release of the relaxing factors. The finding that endothelin consistently induced vasodilation during coadministration of verapamil and nifedipine at doses that caused profound vasoconstriction when administered alone further supports the view that the peptide has a dual action in the vasculature. Slow calcium channel blockade apparently removes the vasoconstrictor effects of the peptide, thereby unmasking the vasodilator activity of even high doses of endothelin.

The observation that both nifedipine and verapamil inhibited endothelin-induced vasoconstriction and endothelin attenuated the vasodilator effects of sodium nitroprusside and endothelium-derived relax-

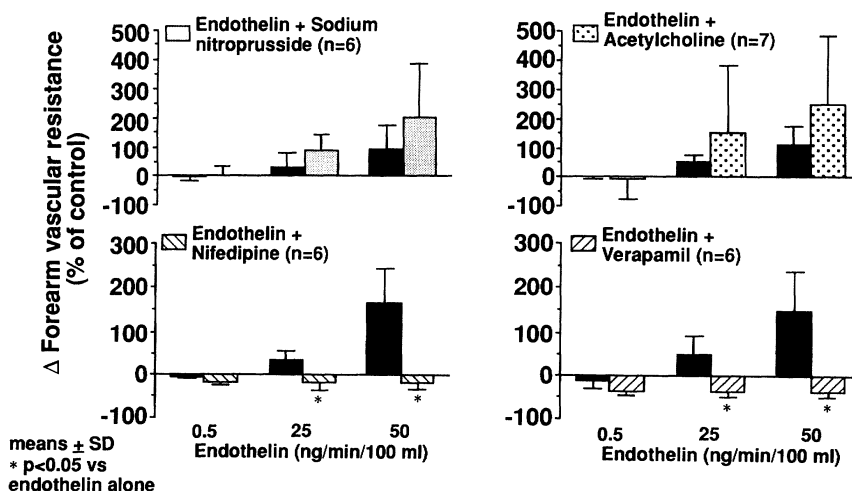


FIGURE 3. Bar graphs of percent changes of calculated forearm vascular resistance in response to intra-arterial infusion of endothelin alone and during coadministration of sodium nitroprusside, acetylcholine, nifedipine, and verapamil. Endothelin-induced changes of forearm vascular resistance were similar in the absence and presence of sodium nitroprusside and acetylcholine, respectively (top panels). Nifedipine and verapamil converted the vasoconstrictor response to the two highest doses of endothelin into a vasodilator response (bottom panels).

ing factor release by acetylcholine is in contrast to most in vitro findings. Thus, in rings of canine arteries contracted with endothelin, acetylcholine caused rapid and complete relaxation,³⁵ whereas calcium antagonists were ineffective in canine veins,³⁵ rabbit and rat artery preparations,⁸ or isolated rat aorta.¹⁴ As endothelium-derived relaxing factor release by acetylcholine and administration of sodium nitroprusside also abolished endothelin-induced contractions in human internal mammary artery rings,¹⁶ the discrepancies cannot be entirely related to species differences. These contrasting results are not readily explained but may be related to the following.

First, our experimental model investigates vascular effects of endothelin in resistance vessels, whereas in vitro experiments usually use large conduit vessels. Differences with respect to the vascular effects of endothelin may exist between large arteries and resistance vessels. Although the comparative vasodilating potencies of the vasodilators used along the vascular tree are not known, it is also conceivable that they may act differently on large arteries or capacitance vessels compared with resistance vessels. Such an effect might contribute to the discrepancies between our results and those obtained in vitro. The fact that forearm blood flow was highest on average after acetylcholine precludes, however, the possibility that differing vasodilator potencies of the vasodilators in forearm resistance vessels accounted for the lack of effect of sodium nitroprusside and acetylcholine on endothelin-induced vasoconstriction.

Second, the precise mechanism of action of endothelin in vascular muscle is not clear but appears to involve a component sensitive to blockade of voltage-operated Ca^{2+} channels^{36,37} as well as a nonsensitive component.^{8,13,34,37} The nonblockable component is associated with an increase of intracellular free cytosolic calcium due to phospholipase C activation and generation of inositol trisphosphate (IP_3)^{12,37-39} and possibly also to direct activation of protein kinase C.⁴⁰ The resulting membrane depolarization then appears to activate voltage-operated, calcium antagonist blockable L-type Ca^{2+} channels, leading to sustained contractions.^{37,41} In addition, in certain vascular preparations such as the porcine coronary artery, endothelin may interact with the Ca^{2+} channel complex through a transducer macromolecule such as the membrane guanosine trisphosphate-binding proteins.³⁶ It has also been shown that the development of vascular tone depends more on calcium influx through slow Ca^{2+} channels in small than in large arteries.⁴² Even though the initiation of the cascade of events leading to sustained vasoconstriction may not be related to activation of voltage-operated Ca^{2+} channels, it is therefore conceivable that the marked dependence of vascular tone on the activity of voltage-operated Ca^{2+} channels may account for the abolition of sustained endothelin-induced vasoconstriction in resistance vessels. The similarity of the responses to nifedipine and verapamil demonstrates that the reversal of endothelin-

induced vasoconstriction is not specific for dihydropyridine calcium antagonists but rather relates to the activity of voltage-operated slow Ca^{2+} channels.

Methodological Aspects

The model of intra-arterial infusions was chosen to assess the vascular effects of endothelin and the influences of various vasodilators. The approach offers the advantage that high regional drug concentrations can be achieved without evoking systemic hemodynamic effects. None of the interventions caused any measurable systemic hemodynamic effect, indicating that changes of forearm blood flow reflected changes of forearm vascular resistance independent from reflex mechanisms triggered by, for example, changes of blood pressure. Because flow is regulated by small resistance vessels, our results cannot be directly compared with those of in vitro studies of large conduit arteries. Furthermore, forearm blood flow goes mainly to muscle and skin; nothing is known about possible differences with respect to endothelin effects in these vascular beds. It is also obvious that the forearm may not be representative for other vascular beds, such as the renal or splanchnic circulation.

We did not measure systemic plasma endothelin concentrations during infusions. Although endothelin infusions did not change blood pressure or heart rate, subsystemic doses of endothelin might have acted on the kidney or atrial myocytes and resulted in reduced renal perfusion⁴³ with an increase of renin or a release of atrial natriuretic peptide.⁴⁴ If one estimates systemic plasma endothelin concentrations in our subjects (based on a forearm volume of 1,000 ml and a plasma volume of approximately 3,000 ml for a 70-kg subject and disregarding the rapid clearance of endothelin,¹⁷ the concentration during the highest endothelin infusion (50 ng/min/100 ml forearm tissue) would be approximately 170 pg/ml, or 68 pmol/l. Although an effect on the kidney or atrial myocytes cannot be ruled out, an average endothelin concentration of 57 pmol/l during intravenous infusions did not influence plasma renin activity or plasma aldosterone and atrial natriuretic peptide concentrations in normal subjects.¹⁷ Thus, it appears unlikely that reduced renal perfusion⁴³ with an increase of renin or a release of atrial natriuretic peptide⁴⁴ contributed to the vasoconstrictor and vasodilator responses to endothelin, respectively.

We used Physiogel as a solvent for endothelin infusions. In previous experiments, we have shown that Physiogel in volumes threefold more than in our studies did not induce a measurable degree of vasodilation.²¹ Therefore, the increase in forearm blood flow in the endothelin-infused arm in the face of unchanged blood pressure as well as blood flow in the contralateral control arm must be interpreted as endothelin-induced vasodilation.

The concentrations of vasodilator drugs were high and led to maximal regional vasodilator responses in our model. The absolute forearm blood flow values

obtained after sodium nitroprusside, acetylcholine, verapamil, and nifedipine were comparable to those described previously.²²⁻²⁵ It remains to be seen whether the blocking effects of calcium antagonists on endothelin-induced vasoconstriction are demonstrable with lower drug doses. Furthermore, the potential heterogeneity of responses of different vascular beds to endothelin may yield different results when the effects of calcium antagonists are evaluated during systemic endothelin administration.

Potential Implications

The physiological role of endothelin is not yet defined, and plasma levels in healthy humans are low.^{12,17,45-47} However, endothelin may be involved in the local regulation of vascular tone,^{16,48} and its vasoconstrictor effects may be of importance in the pathophysiology of vasospasms,^{49,50} acute renal failure,⁵¹ or myocardial infarction.⁵² Our findings suggest that blockade of voltage-operated Ca^{2+} channels might have a role in the prevention or reversal of endothelin-induced increases in human vascular tone.

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References

- De Mey JG, Vanhoutte PM: Role of the intima in cholinergic and purinergic relaxation of the isolated canine femoral artery. *J Physiol (Lond)* 1981;316:347-355
- Rubayani GM, Vanhoutte PM: Hypoxia releases a vasoconstrictor substance from the canine vascular endothelium. *J Physiol (Lond)* 1985;364:45-56
- De Mey JH, Vanhoutte PM: Anoxia and endothelium dependent reactivity of the canine femoral artery. *J Physiol (Lond)* 1983;335:65-74
- Hickey KA, Rubanyi G, Paul RJ, Highsmith RF: Characterization of a coronary vasoconstrictor produced by cultured endothelial cells. *Am J Physiol* 1985;248:C550-C556
- Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, Masaki T: A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 1988;332:411-415
- Itoh Y, Yanagisawa M, Ohkubo S, Kimura C, Kosaka T, Inoue A, Ishid N, Mitsui Y, Onda H, Fujino M, Masaki T: Cloning and sequence analysis of cDNA encoding the precursor of a human endothelium-derived vasoconstrictor peptide, endothelin: Identity of human and porcine endothelin. *FEBS Lett* 1988;231:440-444
- Tomobe Y, Miyauchi T, Saito A, Yanagisawa M, Kimura S, Goto K, Masaki T: Effect of endothelin on the renal artery from spontaneously hypertensive and Wistar Kyoto rats. *Eur J Pharmacol* 1988;152:373-374
- D'Orleans-Juste P, Finet M, de Nucci G, Vane JR: Pharmacology of endothelin-1 in isolated vessels: Effect of nicardipine, methylene blue, hemoglobin and gossypol. *J Cardiovasc Pharmacol* 1989;13(suppl 5):19-22
- Han SP, Trapani AJ, Fok KF, Westfall TC, Knuepfer MM: Effects of endothelin on regional hemodynamics in conscious rats. *Eur J Pharmacol* 1989;159:303-305
- Minkes RF, MacMillan LA, Bellan JA, Kerstein MD, McNamara DB, Kadowitz PJ: Analysis of regional responses to endothelin in the hindquarter vascular bed of cats. *Am J Physiol* 1989;256:H598-H602
- Lippton HL, Hauth TA, Summer WR, Hyman AL: Endothelin produces pulmonary vasoconstriction and systemic vasodilation. *J Appl Physiol* 1989;66:1008-1012
- Wallnöfer A, Weir S, Ruegg U, Cauvin C: The mechanism of action of endothelin-1 as compared with other agonists in vascular smooth muscle. *J Cardiovasc Pharmacol* 1989;13:S23-S31
- Watanabe T, Kusumoto K, Kitayoshi T, Shimamoto N: Positive inotropic and vasoconstrictive effects of endothelin-1 in vivo and in vitro experiments: Characteristics and role of l-type calcium channels. *J Cardiovasc Pharmacol* 1989;13(suppl 5):108-111
- Chabrier PE, Auguet M, Roubert P, Onchampt MO, Gillard V, Guillon J-M, Delafloffe S, Braquet P: Vascular mechanism of action of endothelin-1: Effect of Ca^{2+} antagonists. *J Cardiovasc Pharmacol* 1989;13(suppl 5):218-219
- Fortes ZB, de Nucci G, Garcia-Leme J: Effect of endothelin-1 on arterioles and venules in vivo. *J Cardiovasc Pharmacol* 1989;13(suppl 5):200-201
- Lüscher TF, Yang Z, Tschudi M, von Segesser L, Stulz P, Boulanger C, Siebenmann R, Turina M, Bühler FR: Interaction between endothelin-1 and endothelium-derived relaxing factor in human arteries and veins. *Circ Res* 1990;66:1088-1094
- Vierhapper H, Wagner O, Nowothny P, Waldhäusl W: Effect of endothelin-1 in man. *Circulation* 1990;81:1415-1418
- Hughes AD, Thom SAM, Woodall N, Schachter M, Hair WM, Martin GN, Sever PS: Human vascular responses to endothelin-1: Observations in vivo and in vitro. *J Cardiovasc Pharmacol* 1989;13(suppl 5):225-228
- Greenfield ADM, Whitney RJ, Mowbray JF: Methods for the investigation of peripheral blood flow. *Br Med Bull* 1963;19:101-109
- Kiowski W, Bühler FR, van Brummelen P, Amann FW: Plasma noradrenaline concentration and adrenoceptor-mediated vasoconstriction in normotensive and hypertensive man. *Clin Sci* 1981;60:483-489
- Bolli P, Müller FB, Linder L, Raine AEG, Resink TJ, Erne P, Kiowski W, Ritz R, Bühler FR: The vasodilator potency of atrial natriuretic peptide in man. *Circulation* 1987;75:221-228
- Amann FW, Bolli P, Kiowski W, Bühler FR: Enhanced alpha-adrenoceptor-mediated vasoconstriction in essential hypertension. *Hypertension* 1981;3(suppl 1):I-119-I-123
- Linder L, Kiowski W, Bühler FR, Lüscher TF: Indirect evidence for the release of endothelium-derived relaxing factor in the human forearm circulation in vivo: Blunted response in essential hypertension. *Circulation* 1990;81:1762-1767
- Hulthén UL, Bolli P, Amann FW, Kiowski W, Bühler FR: Enhanced vasodilation in essential hypertension by calcium channel blockade with verapamil. *Hypertension* 1982;4(suppl II):II-26-II-31
- Kiowski W, Bertel O, Erne P, Hulthén UL, Bolli P, Ritz R, Bühler FR: Hemodynamic and reflex mechanisms of acute and chronic antihypertensive therapy with the calcium channel blocker nifedipine. *Hypertension* 1983;5(suppl 1):I-70-I-74
- Schultz KD, Schultz K, Schultz G: Sodium nitroprusside and other smooth muscle relaxants increase cyclic GMP levels in rat ductus deferens. *Nature* 1977;265:750-751
- Rapoport RM, Draznin MB, Murad F: Endothelium-dependent relaxation in rat aorta may be mediated through cyclic GMP-dependent protein phosphorylation. *Nature* 1983;306:174-176
- Rapoport RM, Murad F: Agonist-induced endothelium-dependent relaxation in rat thoracic aorta may be mediated through cGMP. *Circ Res* 1983;52:352-357
- Lüscher TF: *Endothelial Vasoactive Substances and Cardiovascular Disease*. Basel, S Karger AG, 1988, pp 1-133
- Watanabe TX, Kumagaye SI, Nishio H, Nakajima K, Kimura T, Sakakibara S: Effects of endothelin-1 and endothelin-3 on blood pressure in conscious hypertensive rats. *J Cardiovasc Pharmacol* 1989;13(suppl 5):207-208
- Wright CE, Fozard JR: Regional vasodilation is a prominent feature of the haemodynamic response to endothelin in anaes-

- thetized, spontaneously hypertensive rats. *Eur J Pharmacol* 1988;155:201-203
32. De Nucci G, Thomas R, D'Orleans-Juste P, Walder C, Antunes E, Warner D, Vane JR: Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelium-derived relaxing factor. *Proc Natl Acad Sci USA* 1988;85:9797-9800
33. Rae GA, Trybulec M, de Nucci G, Vane JR: Endothelin-1 releases eicosanoids from rabbit isolated perfused kidney and spleen. *J Cardiovasc Pharmacol* 1989;13(suppl 5):89-92
34. Thiemermann C, Lidbury PS, Thomas GR, Vane JR: Endothelin inhibits ex vivo platelet aggregation in the rabbit. *Eur J Pharmacol* 1988;158:181-182
35. Vanhoutte PM, Auch-schwelek W, Boulanger C, Janssen PA, Katusic ZS, Komori K, Miller VM, Schini VB, Vidal M: Does endothelin-1 mediate endothelium dependent contractions during anoxia? *J Cardiovasc Pharmacol* 1989;13(suppl 5):124-128
36. Goto K, Kasuya Y, Matsuki N, Takuwa Y, Kurihara H, Ishikawa T, Kimura S, Yanisagawa M, Masaki T: Endothelin activates the dihydropyridine-sensitive, voltage-dependent Ca^{2+} channel in vascular smooth muscle. *Proc Natl Acad Sci USA* 1989;86:3915-3918
37. Van Renterghem C, Vigne P, Barhanin J, Schmid-Alliana A, Frelin C, Ladzunski M: Molecular mechanism of action of the vasoconstrictor peptide endothelin. *Biochem Biophys Res Commun* 1988;157:977-985
38. Resink TJ, Scott-Burden T, Bühler FR: Endothelin stimulates phospholipase C in cultured vascular smooth muscle cells. *Biochem Biophys Res Commun* 1988;157:1360-1368
39. Kasuya Y, Takuwa Y, Yanagisawa M, Kimura S, Goto K, Masaki T: Endothelin-1 induces vasoconstriction through two functionally distinct pathways in porcine coronary artery: Contribution of phosphoinositide turnover. *Biochem Biophys Res Commun* 1989;161:1049-1055
40. Yang Z, Bauer E, von Segesser L, Stulz P, Turina M, Lüscher TF: Different mobilization of calcium in endothelin-1 induced contractions in human arteries and veins: Effects of calcium antagonists. *J Cardiovasc Pharmacol* 1990;16:654-660
41. Masaki T: The discovery, the present state and the future prospects of endothelin. *J Cardiovasc Pharmacol* 1989;13(suppl 5):1-4
42. Cauvin C, Lukeman S, Cameron J, Hwang O, van Bremen C: Differences in norepinephrine activation and diltiazem inhibition of Ca^{2+} channels in isolated rabbit aorta and mesenteric resistance vessels. *Circ Res* 1985;56:822-828
43. Miller WL, Redfield MM, Burnett JC Jr: Integrated cardiac, renal, and endocrine actions of endothelin. *J Clin Invest* 1989;83:317-320
44. Fukuda Y, Hirata Y, Yoshimi H: Endothelin is potent secretagogue for atrial natriuretic peptide in cultured rat atrial myocytes. *Biochem Biophys Res Commun* 1988;155:1244-1247
45. Suzuki N, Matsumoto H, Kitada C, Yanagisawa M, Miyauchi T, Masaki T, Fujino M: Immunoreactive endothelin-1 in plasma detected by a sandwich-type enzyme immunoassay. *J Cardiovasc Pharmacol* 1989;13(suppl 5):155-152
46. Hartter E, Woloszczuk W: Radioimmunoassay of endothelin. *Lancet* 1989;1:909
47. Ando K, Hirata Y, Shichiri M, Emori T, Marumo F: Presence of immunoreactive endothelin in human plasma. *FEBS Lett* 1989;245:164-166
48. Boulanger C, Lüscher TF: Release of endothelin from the porcine aorta: Inhibition by endothelium derived relaxing factor. *J Clin Invest* 1990;85:587-590
49. Yang Z, Richard V, von Segesser L, Bauer E, Stulz P, Turina M, Lüscher TF: Threshold concentrations of endothelin-1 potentiate contractions to norepinephrine and serotonin in human arteries: A new mechanism of vasospasm? *Circulation* 1990 (in press)
50. Kurihara H, Yoshizumi M, Sugiyama T, Yamaoki K, Nagai R, Takaku F, Satoh H, Inui J, Yanisagawa M, Masaki T, Yasaki Y: The possible role of endothelin-1 in the pathogenesis of vasospasm. *J Cardiovasc Pharmacol* 1989;13(suppl 5):132-137
51. Firth JD, Ratcliffe PJ, Raine AEG, Ledingham JGG: Endothelin: An important factor in acute renal failure? *Lancet* 1988;2:1179-1181
52. Miyauchi T, Yanagisawa M, Tomizawa T, Sugishita YN, Fujino M, Ajisaka R, Goto K, Masaki T: Increased plasma concentration of endothelin-1 and big endothelin-1 in acute myocardial infarction. *Lancet* 1989;2:53-54

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