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European Journal of Pharmacology 397 (2000) 367–371

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Endothelin-1 attenuates bradykinin-induced hypotension in rats

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Received 19 December 1999; received in revised form 15 March 2000; accepted 21 March 2000

Abstract

Endothelin-1 has vasoconstrictor and mitogenic properties and may contribute to the pathogenesis of hypertension by enhancing vasoconstrictor mechanisms. In this study, we investigated the ability of endothelin-1 decrease the hypotensive effects of the vasodilator bradykinin in anesthetized rats. We also studied the effects a two-week oral pre-treatment with losartan (10 mg/kg/day) or enalapril (25 mg/kg/day) on endothelin-1-induced changes in the hypotensive responses to bradykinin. Bradykinin (0.4, 1.6, 6.4, and 25 μg/kg, i.v.) induced dose-dependent hypotensive responses which were attenuated ($P < 0.05$) by endothelin-1 (2 μg/kg, i.v.). This effect of endothelin-1 was abolished by the mixed endothelin receptor antagonist *N*-Acetyl-alpha-[10,11-Dihydro-5*H*-dibenzo[*a*,*d*]cycloheptadien-5-yl]-D-Gly-Leu-Asp-Ile-Ile-Trp (PD145065, 1 mg/kg, i.v.). Endothelin-1 also decreased ($P < 0.05$) the responses to bradykinin in rats pre-treated with losartan, but had no effect in rats pre-treated with enalapril. These results suggest that endothelin-1 may contribute to the development of hypertension by decreasing the responses to bradykinin through a mechanism not involving angiotensin AT_1 receptors, although the inhibition of angiotensin converting enzyme blunted the effect of endothelin-1.

Keywords: Blood pressure; Endothelin-1; Bradykinin; Enalapril; Losartan; (Rat)

1. Introduction

Concomitant with the discovery of the endothelins, it was suggested that disturbances in the regulation of these peptides might contribute to the pathogenesis of a number of cardiovascular diseases (Yanagisawa et al., 1988), including hypertension. Endothelin-1, the major isoform produced by endothelial cells, interacts with endothelin ET_A and ET_B receptors on vascular smooth muscle cells to cause potent vasoconstriction (Haynes et al., 1995), whereas endothelin ET_B receptors found on the endothelium mediate vasodilatation through the production of endothelium-dependent vasodilators (Rubanyi and Polokoff, 1994). Basal levels of endothelin-1 have a role in the maintenance of vascular tone and blood pressure (Haynes and Webb, 1994; Li et al., 1994). Although plasma endothelin-1 levels may not reflect vascular production of the peptide because most of it is released towards the vascular smooth muscle, the plasma concentrations of endothelin-1 are normal or increased in hypertensive patients (Khono et al., 1990; Saito et al., 1990; Levin, 1995). Furthermore, endothelin-1 is overexpressed in the endothelium of small arteries of some severely hypertensive patients (Schiffrin et al., 1997). In addition to its vasoconstrictor and mitogenic properties (Battistini et al., 1993), several interactions of endothelin-1 with other mechanisms involved in the regulation of cardiovascular function have been described (Rossi et al., 1999). Thus, endothelin-1 potentiates the postjunctional action of norepinephrine (Wong-Dusting et al., 1991), augments the vasoconstriction to angiotensin II (Clavell et al., 1996) and activates the renin–angiotensin system (Rakugi et al., 1990). These findings suggest that endothelin-1 plays a complex role in the pathophysiology of hypertension, partly by enhancing vasoconstrictive mechanisms. However, the possibility that increased endothelin-1 levels may decrease the vasodilator effects of kinins, thereby contributing to the development and maintenance of hypertension, has not been investigated.

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0014-2999/00/$ - see front matter
PII: S0014-2999(00)00239-9

Bradykinin causes arterial hypotension through the stimulation of endothelial B_2 kinin receptors, thereby increasing the release of vasodilators such as nitric oxide, prostacyclin and endothelium-derived hyperpolarizing factor (Linz et al., 1999). In the present study, we investigated whether endothelin-1 changes the hypotensive responses to bradykinin in rats. Since endothelin-1 exerts important effects on the renin–angiotensin system (Rossi et al., 1999), we also examined whether pre-treatment with losartan, an angiotensin II type-1 (AT_1) receptor antagonist, or enalapril, an angiotensin converting enzyme inhibitor, affects the endothelin-1-induced changes in the hypotensive responses to bradykinin.

2. Material and methods

2.1. Protocol I: effect of endothelin-1 on the hypotensive responses to bradykinin

Male Wistar rats (250–300 g) provided by CEMIB-UNICAMP were anesthetized with sodium pentobarbital (Hypnol, Cristália, SP, Brazil; 40 mg/kg, i.p.). Controlled ventilation with a Harvard ventilator for small animals (model 683) was initiated after cannulation of the trachea with a Gelco tube. The right carotid artery and left femoral vein were cannulated for the measurement of arterial blood pressure and drug administration, respectively. The arterial catheter was connected to a COBE transducer (Arvada), and the signal was amplified with a GP4A-general purpose amplifier (Stemtech). The amplifier outputs were connected to an A/D board and this to a computer loaded with CODAS data acquisition software (AT-CODAS; DATAQ Instruments). The pulsatile arterial pressure was continuously recorded at a sample rate of 200 Hz throughout the experiment. The experiments were initiated after allowing at least 20 min for stabilization.

All drugs were dissolved in saline and were given in a 100 μl intravenous (i.v.) bolus, and were washed in with a further 100 μl of saline. All rats received saline followed by bradykinin in doses of 0.4, 1.6, 6.4, and 25 μg/kg. Each dose of bradykinin was given when the mean arterial pressure had returned to baseline after the previous injection (usually 2–7 min). Thirty minutes after the last dose of bradykinin, the rats were randomly assigned to one of three experimental groups ($n = 8$/group): (1) group PD + ET, which received the mixed endothelin ET_A/ET_B receptor antagonist *N*-Acetyl-alpha-[10,11-Dihydro-5*H*-dibenzo[*a*,*d*]cycloheptadien-5-yl]-D-Gly-Leu-Asp-Ile-Ile-Trp (PD145065, 1 mg/kg) followed, 5 min later, by endothelin-1 (2 μg/kg); (2) group ET, which received endothelin-1 (2 μg/kg) and saline; and (3) control group, which received only saline. Ten minutes after endothelin-1 (or saline) injection, all rats received saline followed by the same doses of bradykinin as described above.

2.2. Protocol II: effect of endothelin-1 on the hypotensive responses to bradykinin in rats pre-treated with losartan or enalapril

2.2.1. Pre-treatment with losartan or enalapril and blood pressure measurements

Male Wistar rats (230–270 g) provided by CEMIB-UNICAMP were maintained under controlled light and temperature conditions. The rats were trained twice a week for 2 weeks for the measurement of blood pressure by a tail-cuff method. Thereafter, two groups of rats were treated for two additional weeks either with losartan (10 mg/kg/day; Rodrigo et al., 1997) or enalapril maleate (25 mg/kg/day; Moreno et al., 1995) administered via the drinking water. Another group of rats that received tap water served as the control.

2.2.2. Effect of endothelin-1 on the hypotensive responses to bradykinin in rats pre-treated with losartan

Rats pre-treated with losartan were anesthetized and underwent the same general procedures described in protocol I. Thereafter, all of the animals received saline followed by the same doses (0.4, 1.6, 6.4, and 25 μg/kg) of bradykinin used in the protocol I. Thirty minutes after the last dose of bradykinin, the rats randomly received endothelin-1 (2 μg/kg, group ET, $n = 7$) or saline (control group, $n = 7$). Finally, 10 min after endothelin-1 (or saline) injection, all of the rats received saline followed by the same doses of bradykinin described in the protocol I.

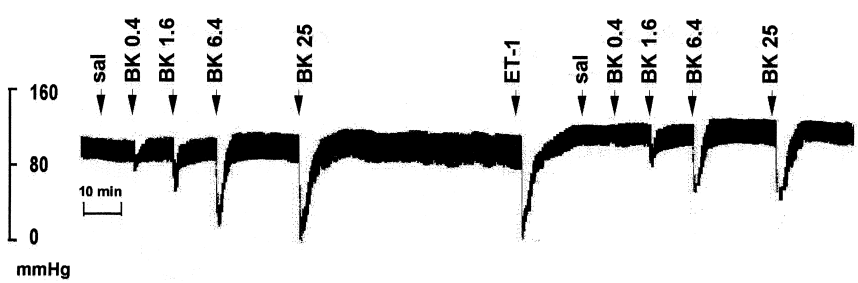

Fig. 1. Representative traces showing arterial blood pressure changes produced by saline (sal) and bradykinin (BK; 0.4, 1.6, 6.4, 25 μg/kg i.v.) in anesthetized rats before and after the injection of endothelin-1 (2 μg/kg, group ET).

2.2.3. Effect of endothelin-1 on the hypotensive responses to bradykinin in rats pre-treated with enalapril

Rats pre-treated with enalapril received the same treatment as that described for rats pre-treated with losartan, except that the highest dose (25 μg/kg) of bradykinin was not given because enalapril potentiates the hypotensive effects of bradykinin.

2.3. Drugs

Bradykinin, endothelin-1 and PD 145065 were purchased from Sigma (St. Louis, MO, USA). Losartan and enalapril maleate were provided by Biosintética (São Paulo, SP, Brazil)

2.4. Statistical analysis

The results were expressed as means ± S.E.M. The changes in mean arterial pressure were calculated as the difference between the baseline value and those recorded at the lowest values of mean arterial pressure after each dose of bradykinin. Comparisons between groups were analyzed using one-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls test and the unpaired Student's two-tailed *t* test in the first and second protocol, respectively. A probability (P) value < 0.05 was the minimum level for statistical significance.

3. Results

3.1. Effect of endothelin-1 on the hypotensive responses to bradykinin

The initial i.v. injections of bradykinin produced similar dose-dependent hypotensive responses in the three groups of rats and these responses were not affected by saline (data not shown). The administration of endothelin-1 reduced ($P < 0.05$) the hypotensive responses to the same doses of bradykinin (Figs. 1 and 2). In the PD + ET group, the mixed endothelin receptor antagonist PD145065 abolished the endothelin-1-induced attenuation of the hypotensive effects of bradykinin (Fig. 2).

Table 1
Effect of 2-week treatment with losartan (10 mg/kg) per day or enalapril maleate (25 mg/kg) per day on systolic blood pressure (SBP) in male rats. Control rats received tap water
Values are the mean ± S.E.M.

Group	SBP (mm Hg)
Control (n = 24)	
Basal	117 ± 5
Treatment	119 ± 3
Losartan (n = 14)	
Basal	116 ± 3
Treatment	99 ± 3[a]
Enalapril (n = 12)	
Basal	119 ± 8
Treatment	96 ± 5[a]

[a] $P < 0.05$ versus the respective basal values.

3.2. Effect of endothelin-1 on the hypotensive responses to bradykinin in rats pre-treated with oral losartan or enalapril

There were no significant differences in the baseline systolic blood pressures among the experimental groups (Table 1). The oral administration of losartan or captopril significantly reduced blood pressure levels compared to the respective basal values ($P < 0.05$, Table 1).

The dose-dependent hypotensive responses to bradykinin in rats pre-treated with losartan (Fig. 3) were

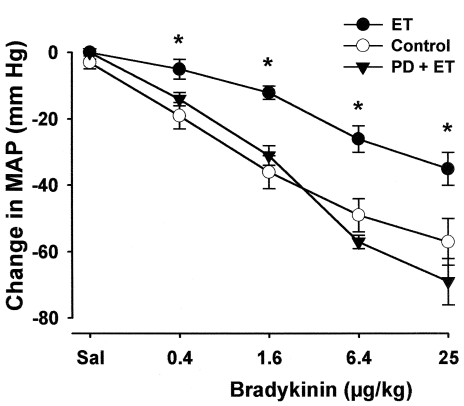

Fig. 2. Changes in mean arterial pressure (MAP) caused by the i.v. injection of saline (sal) or bradykinin (0.4, 1.6, 6.4, 25 μg/kg) in anesthetized rats after the injection of PD 145065 (1 mg/kg) plus endothelin-1 (2 μg/kg) (group PD + ET), endothelin-1 (2 μg/kg) plus saline (group ET), or saline plus saline (control group). $n = 8$ in each group. Values are the mean ± S.E.M. * $P < 0.05$ versus the control group.

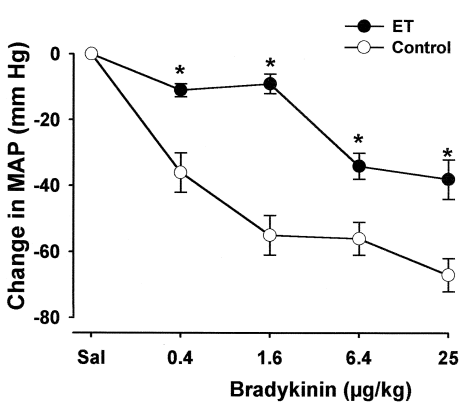

Fig. 3. Change in mean arterial pressure (MAP) caused by the injection of saline (sal) or bradykinin (0.4, 1.6, 6.4, 25 μg/kg) in anesthetized rats pre-treated with losartan (10 mg/kg per day) after the injection of endothelin-1 (2 μg/kg, group ET) or saline (control group). $n = 7$ in each group. Values are the mean ± S.E.M. * $P < 0.05$ versus the control group.

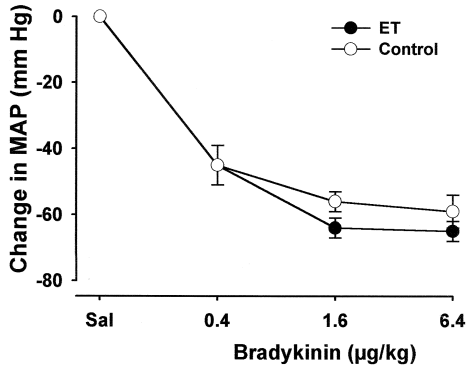

Fig. 4. Change in mean arterial pressure (MAP) caused by the injection of saline (sal) or bradykinin (0.4, 1.6, 6.4 μg/kg) in anesthetized rats pretreated with enalapril maleate (25 mg/kg per day) after the injection of endothelin-1 (2 μg/kg, group ET) or saline (control group). $n = 6$ in each group. Values are the mean ± S.E.M.

not affected by the i.v. administration of saline but were attenuated ($P < 0.05$) by endothelin-1 (Fig. 3). In contrast, the bradykinin-induced dose-dependent hypotensive responses in rats pre-treated with enalapril (Fig. 4A) were unaltered by saline or endothelin-1 (Fig. 4).

4. Discussion

The major findings of this study were that (i) acute administration of endothelin-1 decreased the hypotensive responses to bradykinin, (ii) antagonism of angiotensin AT_1 receptors with losartan had no influence on this effect of endothelin-1, whereas (iii) inhibition of angiotensin converting enzyme by enalapril protected against this effect of endothelin-1.

Hypertensive patients have an abnormal endothelium-dependent vascular function (Panza et al., 1990) which probably involves alterations in the synthesis and interaction of vasoconstrictor and vasodilator factors. Previous studies, which have focused on endothelin-1-induced potentiation of the responses to vasoconstrictors such as norepinephrine, serotonin and angiotensin II (Clavell et al., 1996; Wong-Dusting et al., 1991; Yang et al., 1990), have suggested that endothelin-1 may have a role in hypertension. The present study indicates that endothelin-1 may change endothelial function and contribute to the development of hypertension by decreasing the vascular responses to bradykinin. This conclusion agrees with previous observations that low concentrations of endothelin-1 inhibit substance P- and acetylcholine-induced vasodilation in dog middle cerebral artery (Kaito et al., 1995). As shown here, the selective endothelin receptor antagonist PD 145065 abolished the action of endothelin-1, a finding which supports the idea that endothelin receptor antagonists may benefit patients with hypertension and cardiovascular diseases (Benigni and Remuzzi, 1999; Gray and Webb, 1996).

Bradykinin causes vasodilation by acting on the B_2 kinin receptors of endothelial cells, thereby stimulating the release of nitric oxide, prostacyclin and endothelium-derived hyperpolarizing factor (Regoli et al., 1998; Linz et al., 1999). Thus, although we did not provide any biochemical evidence, we could speculate that interactions between endothelin-1 and B_2 kinin receptors may have occurred and affected intracellular signal transduction pathways. As a result, an impairment of bradykinin-stimulated release of, or the response to, any of these vasodilator substances may have occurred.

Endothelin-1 did not affect the hypotensive responses to bradykinin in rats pre-treated with enalapril. These results agree with a previous study in human volunteers which demonstrated that enalaprilat blunted endothelin-1-induced vasoconstriction when infused locally in the forearm (Abernethy et al., 1995). The ability of enalapril to protect against the endothelin-1-induced attenuation of the hypotensive responses to bradykinin is probably related to the inhibition of angiotensin converting enzyme which in turn would reduce the production of angiotensin and the breakdown of bradykinin (Carretero et al., 1981; Vanhoutte, 1998). In addition, angiotensin converting enzyme inhibitors can affect endothelial B_2 kinin receptor signalling to amplify the responses to bradykinin (Hecker et al., 1997; Linz et al., 1999) and may also enhance bradykinin binding sites (Minshall et al., 1997). Taken together, these facts suggest that enalapril may have interacted with bradykinin receptors in such a way that bradykinin receptors were protected against the impairment caused by endothelin-1.

Pre-treatment with losartan had no effect on the endothelin-1-induced attenuation of the hypotensive responses to bradykinin, suggesting that endothelin-1 reduces the responses to bradykinin via a mechanism not involving angiotensin AT_1 receptors. In contrast to enalapril, losartan has no effect on angiotensin converting enzyme activity. Additionally, losartan probably does not interfere with B_2 kinin receptors as do angiotensin converting enzyme inhibitors (Linz et al., 1999; Minshall et al., 1997).

In conclusion, endothelin-1 may exert an autocrine bradykinin-inhibiting effect on endothelial cells. A strategy targeted at this interaction may thus offer relevant improvement in the outcomes of hypertension therapy. Further studies are needed to define the clinical relevance of these findings.

Acknowledgements

J.E. Tanus-Santos, W.M. Gordo, and RC Sampaio were supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, SP, Brazil). The authors thank Dr. Stephen Hyslop for reviewing the manuscript. H. Moreno is supported by CNPq (Brazil).

References

Abernethy, D.R., Laurie, N., Andrawis, N.S., 1995. Local angiotensin-converting enzyme inhibition blunts endothelin-1-induced increase in forearm vascular resistance. Clin. Pharm. Ther. 58, 328–334.

Battistini, B., Chailler, P., D'Orleans-Juste, P., Briere, N., Sirois, P., 1993. Growth regulatory properties of endothelins. Peptides 14, 385–399.

Benigni, A., Remuzzi, G., 1999. Endothelin antagonists. Lancet 353, 133–138.

Carretero, O.A., Miyazaki, S., Scicli, A.G., 1981. Role of kinins in the acute antihypertensive effect of the converting enzyme inhibitor, captopril. Hypertension 3, 18–22.

Clavell, A.L., Mattingly, M.T., Stevens, T.L., Nir, A., Wright, S., Aarhus, L.L., Heublein, D.M., Burnett, J.C. Jr., 1996. Angiotensin converting enzyme inhibition modulates endogenous endothelin in chronic canine thoracic inferior vena caval constriction. J. Clin. Invest. 97, 1286–1292.

Gray, G.A., Webb, D.J., 1996. The endothelin system and its potential as a therapeutic target in cardiovascular disease. Pharmacol. Ther. 72, 109–148.

Haynes, W.G., Webb, D.J., 1994. Contribuition of endogenous generation of endothelin-1 to basal vascular tone. Lancet 344, 852–854.

Haynes, W.G., Strachan, F.E., Webb, D.J., 1995. Endothelin ET_A and ET_B receptors cause vasoconstriction of human resistance and capacitance vessels in vivo. Circulation 92, 357–363.

Hecker, M., Blaukat, A., Bara, A.T., Müller-Esterl, W., Busse, R., 1997. ACE inhibitor potentiation of bradykinin-induced venoconstriction. Br. J. Pharmacol. 121, 1475–1481.

Kaito, N., Onoue, H., Abe, T., 1995. Suppression of cerebral vasodilation with endothelin-1. Peptides 16, 1127–1132.

Khono, M., Yasunari, K., Murakawa, K., Yokokawa, K., Horio, T., Fukui, T., Takeda, T., 1990. Plasma immunoreactive endothelin in essential hypertension. Am. J. Med. 88, 614–618.

Levin, E.R., 1995. Endothelins. N. Engl. J. Med. 333, 356–363.

Li, J.S., Larivière, R., Schiffrin, E.I., 1994. Effect of a nonselective endothelin antagonist on vascular remodelling in deoxycorticosterone acetate-salt hypertensive rats: evidence for a role of endothelin in vascular hypertrophy. Hypertension 24, 183–188.

Linz, W., Wohlfart, P., Schölkens, B.A., Malinski, T., Wiemer, G., 1999. Interactions among ACE, kinins and NO. Cardiovasc. Res. 43, 549–561.

Minshall, R.D., Tan, F., Nakamura, F., Rabito, S.F., Becker, R.P., Marcic, B., Erdos, E.G., 1997. Potentiation of the actions of bradykinin by angiotensin I-converting enzyme inhibitors. The role of expressed human bradykinin B_2 receptors and angiotensin I-converting enzyme in CHO cells. Circ. Res. 81, 848–856.

Moreno, H. Jr., Nathan, L.P., Costa, S.K.P., Metze, K., Antunes, E., Zatz, R., de Nucci, G., 1995. Enalapril does not prevent the myocardial ischemia caused by chronic inhibition of nitric oxide synthesis. Eur. J. Pharmacol. 287, 93–96.

Panza, J.A., Quyyumi, A.A., Brush, J.E., Epstein, S.E., 1990. Abnormal endothelium-dependent relaxation in patients with essential hypertension. N. Engl. J. Med. 323, 22–27.

Rakugi, H., Tabuchi, Y., Nakamaru, M., Nagano, M., Higashimoroi, K., Mikami, H., Ogihara, T., Suzuki, N., 1990. Endothelin activates the vascular renin–angiotensin system in rat mesenteric arteries. Biochem. Int. 21, 867–872.

Regoli, D., Allogho, S.N., Rizzi, A., Gobeil, F.J., 1998. Bradykinin receptors and their antagonists. Eur. J. Pharmacol. 348, 1–10.

Rodrigo, E., Maeso, R., Muñoz-García, R., Navarro-Cid, J., Ruilope, L.M., Cachofeiro, V., Lahera, V., 1997. Endothelial dysfunction in spontaneously rats: consequences of chronic treatment with losartan or captopril. J. Hypertens. 15, 613–618.

Rossi, G.P., Sacchetto, A., Cesari, M., Pessina, A.C., 1999. Interaction between endothelin-1 and the renin–angiotensin–aldosterone system. Cardiovasc. Res. 43, 300–307.

Rubanyi, G.M., Polokoff, M.A., 1994. Endothelins: molecular biology, biochemistry, pharmacology, physiology, and pathophysiology. Pharmacol. Rev. 46, 325–415.

Saito, Y., Nakao, K., Mukoyama, M., Imura, H., 1990. Increased plasma endothelin levels in patients with essential hypertension. N. Engl. J. Med. 322, 205.

Schiffrin, E.L., Deng, L.Y., Sventek, P., Day, R., 1997. Enhanced expression of endothelin-1 gene in endothelium of resistance arteries in severe human essential hypertension. J. Hypertens. 15, 57–63.

Vanhoutte, P.M., 1998. Endothelial dysfunction and inhibition of converting enzyme. Eur. Heart J. 19 (Suppl. J), J7–J15.

Wong-Dusting, H.K., La, M., Rand, M.J., 1991. Endothelin-1 enhances vasoconstrictor responses to sympathetic nerve stimulation and noradrenaline in the rabbit ear artery. Clin. Exp. Pharmacol. Physiol. 18, 131–136.

Yanagisawa, M., Kurihara, H., Kimura, S., Tomobe, Y., Kobayashi, M., Mitsui, Y., Yasaki, Y., Goto, K., Masaki, T., 1988. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 332, 411–415.

Yang, Z., Richard, V., Von Segesser, L., Bauer, E., Stulz, P., Turina, M., Luscher, T.F., 1990. Threshold concentrations of endothelin-1 potentiate contractions to norepinephrine and serotonin in human arteries. A new mechanism of vasospasm?. Circulation 82, 188–195.