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Endothelin Inhibition as a Biologic Target for Treating Hypertension

Hans R. Brunner

Endothelin, a 21-amino-acid peptide, binds to a specific receptor on vascular smooth muscle cells, thereby inducing vasoconstriction. Although plasma levels are not consistently elevated in hypertension, there is evidence that endothelin has an important role in its pathogenesis. Administration of endothelin antagonists has lowered blood pressure and reduced end-organ damage in some animal models. It has also reduced the cross-sectional area of neointima due both to hypertension and vascular injury. Coadministration of endothelin and angiotensin II to rats produced a synergistic hypertensive effect. Similarly, coadministration of an endothelin antagonist with an angiotensin converting enzyme inhibitor resulted in a synergistic lowering of blood pressure. Several preliminary clinical studies have been done. The endothelin antagonist bosentan has decreased vascular resistance and blood pressure and increased cardiac index in patients with congestive heart failure. Plasma endothelin levels are elevated in the acute phase of myocardial infarction and in chronic heart failure. The magnitude of this increase, measured 3 days after patients experienced myocardial infarction, had a significance at least equal to known risk factors in predicting 1 year survival. Thus, there are reasons to believe that endothelin antagonists may become a useful tool in the management of various cardiovascular disorders. Am J Hypertens 1998;11:103S–109S © 1998 American Journal of Hypertension, Ltd.

KEY WORDS: **Endothelin antagonist, hypertension, endothelin.**

The endothelium plays a role in regulating vascular resistance by releasing substances that dilate or constrict blood vessels. These substances, endothelins, are a family of peptides having potent vasoconstrictive effects. There are basically three types of endothelin: endothelin-1, endothelin-2, and endothelin-3. They are all formed from precursors (big endothelin) by enzymatic cleavage and are all 21–amino-acid peptides with disulfide bridges (Figure 1). Endothelin-1 and endothelin-2 act on the endothelin-A (ET_A) receptor of smooth muscle to produce a prolonged contraction and vasoconstriction. Endothelin-3 can also induce vasoconstriction by binding to the endothelin-B (ET_B) receptor on smooth muscle, but it also acts on the ET_B receptors of endothelial cells, where it can actually have the opposite effect. It has been suggested that, under normal conditions, low concentrations of endothelin stimulate ET_B receptors, thus contributing to the release of endothelium-derived relaxing factors.[1] Endothelin-1 is probably the best studied of this family. When administered to animals, endothelin-1 causes severe vasoconstriction.[2] The contraction of vascular smooth muscle produced by endothelin-1 may be mediated by an increase in intracellular calcium, but the details have not been established.[3] Infusion of endothelin-1 into healthy human volunteers resulted in a 2.5-fold increase in plasma endothelin-1 concentration and a rise

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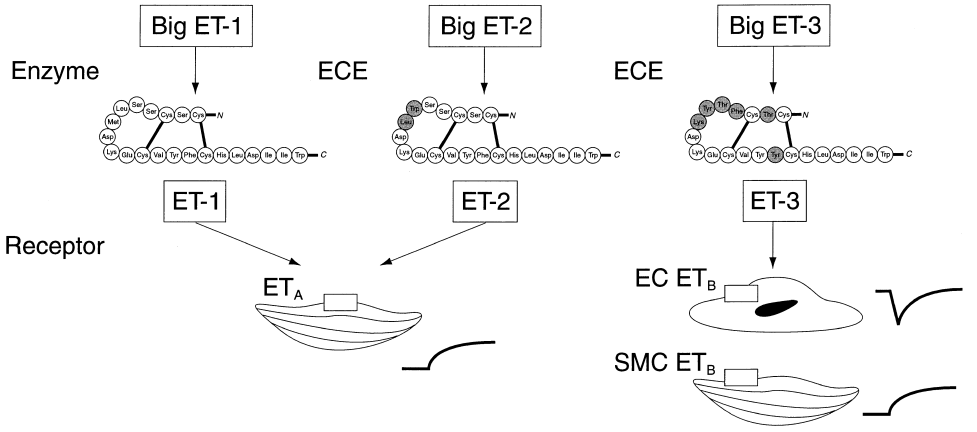

FIGURE 1. *The endothelin system. Big ET-1, precursor of endothelin-1; Big ET-2, precursor of endothelin-2; Big ET-3, precursor of endothelin-3; ET-1, endothelin-1; ET-2, endothelin-2; ET-3, endothelin-3; ECE, endothelin converting enzyme; ET_A, endothelin-A receptor; ET_B, endothelin-B receptor; EC, endothelial cell; SMC, smooth muscle cell. Precursors of endothelin-1, -2, and -3 are enzymatically converted to endothelin-1, -2, and -3, which bind at receptor sites ET_A and ET_B as shown.*

in blood pressure of approximately 6 mm Hg; in contrast, infusion of the same molar concentration of endothelin-3 raised plasma levels of endothelin-3 2.5-fold, but had no effect on blood pressure.[4] Like angiotensin II and norepinephrine, endothelin-1 is now thought to play a role in hemodynamic control. It has not been proven, however, that endothelin normally participates in the control of vascular tone, and its role in hypertension has not been established. Plasma levels of endothelin-1 do not increase significantly during hypertension in humans or animals.[5,6] However, endothelin-1 is increased in the aorta and in mesenteric arteries of deoxycorticosterone acetate (DOCA)-salt hypertensive rats, despite normal blood levels. In contrast, the vascular tissue of spontaneously hypertensive rats does not have a higher endothelin-1 content.[7] There is also substantial vascular hypertrophy in DOCA-salt hypertensive rats, compared to limited hypertrophy of blood vessels in spontaneously hypertensive rats.[8] It is therefore possible that endothelin-1, which has powerful hypertrophic and mitogenic properties, could have a causal role in this vascular hypertrophy. Endothelin-1 is thought to interact with the sympathetic nervous system and the renin-angiotensin system in the regulation of blood pressure and sodium metabolism, but the mechanisms of these interactions are unknown. Endothelin-1 may also help regulate vascular tone by modulating angiotensin converting enzyme levels, since it has been reported that endothelin stimulates the release of angiotensin II from mesenteric arteries.[9]

EFFECTS OF ENDOTHELIN ON BLOOD PRESSURE

Exogenous endothelin-1 alone in sufficiently high concentrations has been reported to increase blood pressure in both humans and experimental animals. Continuous infusion of endothelin-1 in rats at a rate of 60 μg/kg of body weight/day resulted in a significant increase in the systolic blood pressure, while 6 μg/kg/day had no significant effect.[2] Yoshida et al[10] studied the effects of endothelin-1 and angiotensin II, separately and in combination, on blood pressure and physiologic parameters of Sprague-Dawley rats. Intraperitoneal administration of angiotensin II at the rate of 400 μg/kg/day did not induce a significant change in systolic blood pressure compared to that of controls (saline infusion). Nor did continuous infusion of 3 μg/kg/day of endothelin-1 affect systolic blood pressure significantly. However, the combined infusion of endothelin-1 and angiotensin II at these same concentrations and rates produced a significant increase in systolic blood pressure (Figure 2). The increase was statistically significant 1 day after the start of the infusion and increased thereafter. After 6 days, systolic blood pressure was 148.0, 142.7, and 143.5 mm Hg in rats given angiotensin II, endothelin-1, or saline, respectively, whereas it was 189.0 mm Hg in angiotensin II plus endothelin-1–infused rats ($P < .05$ *v* all other groups); this is an increase of 32% compared to controls. Angiotensin II, endothelin-1, and the combination did not result in significant changes in body weight, fluid retention, urine volume, urinary sodium excretion, or urinary potassium excretion, suggesting that the effect on blood pressure was not due to renal effects. In contrast, in the same study, continuous infusion of norepinephrine (360 μg/kg/day), endothelin-1 (3 μg/kg/day), and the combination norepinephrine plus endothelin-1 did not result in any significant changes in systolic blood pressure. However, sufficiently high infusions of norepinephrine (1.8 mg/kg/day) have been shown to induce a sustained in-

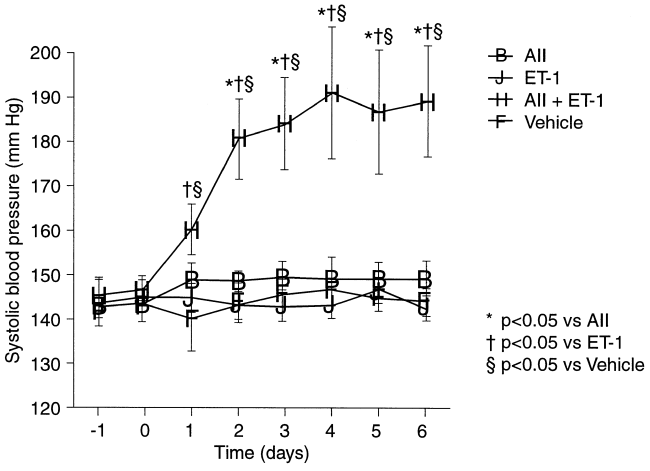

FIGURE 2. *Effects of infusion of angiotensin II (AII), endothelin-1 (ET-1), and the combination AII plus ET-1 on systolic blood pressure in conscious rats. Control vehicle (physiologic saline) is also shown. Reprinted with permission from Yoshida K et al.*[10]

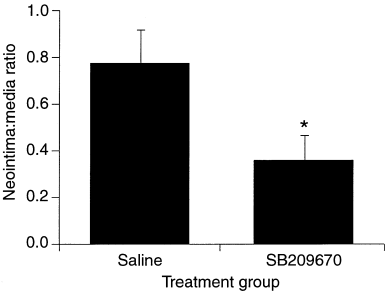

FIGURE 3. *Neointima:media ratio in rats that received either saline or the endothelin receptor antagonist SB 209670. Results are given as mean ± SEM.* *P < .05 *compared with control (saline) by ANOVA and Fisher's protected least-squares difference. Reprinted with permission from Douglas SA et al.*[14]

crease in systolic blood pressure in rats.[11] There have been reports that endothelin-1 can amplify the hypertensive effect of norepinephrine under certain conditions,[12] so the interaction of these peptides is still unresolved.

ENDOTHELIN AND NEOINTIMA FORMATION

The major problem limiting the long-term effectiveness of percutaneous transluminal coronary angioplasty (PTCA) is the high incidence of neointima formation and vascular restenosis.[13,14] Endothelin-1 levels are elevated after PTCA, suggesting that endothelin-1 is implicated in restenosis. In general, advanced atherosclerotic lesions are characterized by large numbers of smooth muscle cells, macrophages, and T lymphocytes. Angiotensin II and norepinephrine are thought to be involved in the development of neointima, and endothelin-1, a potent mitogen, may also play a role in the process.

Douglas et al[14] investigated the effects of exogenous endothelin-1, and of blockage of endothelin-1, on neointima formation in both an in vitro model and in vivo. The addition of endothelin-1 (1 nmol/L) increased neointima formation, as evidenced by a ninefold increase in thymidine incorporation in cultured rat aortic vascular smooth muscle cells. This effect was inhibited in a dose-dependent manner by the simultaneous addition of the endothelin receptor antagonist SB 209670. Endothelin-1-induced proliferation, as measured by thymidine incorporation, was completely inhibited by 0.1 μmol/L of SB 209670. However, even the maximum concentration of SB 209670 used did not alter cell viability, nor did it change basal proliferation, implying that the effect was not due to a general inhibition. Furthermore, addition of SB 209670 in a concentration that completely blocked endothelin-1-induced proliferation had no effect on proliferation induced by angiotensin II, fibroblast growth factor, or platelet-derived growth factor. As part of the same study, the in vivo effects of endothelin-1 and SB 209670 were determined using balloon angioplasty performed on the left common carotid artery of Sprague-Dawley rats. In one set of experiments, rats were administered endothelin-1 by intraarterial infusion over a 30-min period immediately after balloon angioplasty. Endothelin-1 infusion (500 pmol/kg body weight over a 30-min period) significantly increased the amount of neointima formation relative to controls. The cross-sectional area of the neointima was 73% greater in the endothelin-1-treated group, and there was a corresponding increase of 53% in the neointima-to-media ratio. This effect was dose dependent. Other than size, the morphology of lesions from endothelin-1-treated rats was similar to those treated with saline. The morphology of undamaged contralateral carotid arteries was not affected by endothelin-1.

In a second experiment, rats were treated either with SB 209670 or saline beginning 3 days before and continuing 2 weeks after angioplasty. The arteries were removed at the end of this period and examined for neointima formation. Treatment with SB 209670 reduced formation of neointima after balloon angioplasty. It did not affect the tunica media, but decreased the neointima cross-sectional area by 53% and the ratio of neointima-to-medial cross-sectional area by 47% (Figure 3). All animals treated with SB 209670 appeared healthy on gross physical examination, and there were no differences in body weights between treated rats and controls, indicating that the inhibitory effect of SB 209670 was not due to nonselective cytotoxicity.[14]

Several studies have reported that cultured rat and human vascular smooth muscle proliferation is mediated by the ET_A receptor.[15,16] Since endothelin-1 levels

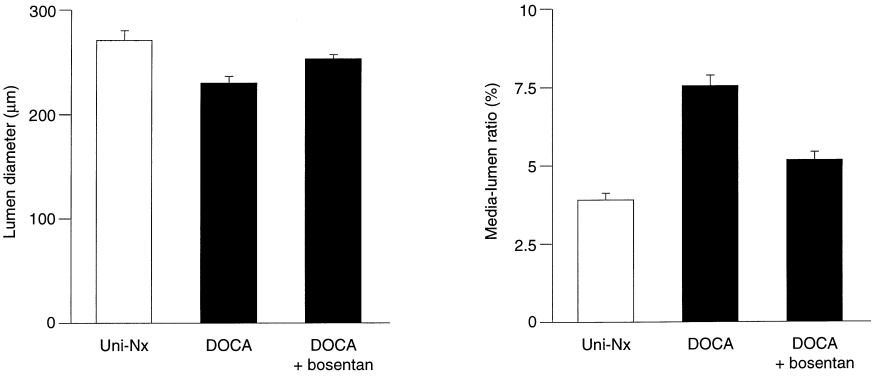

FIGURE 4. *Lumen diameter (μm) and media:lumen ratio (expressed as a percentage) of resistance arteries in uninephrectomized control rats (Uni-Nx), DOCA-salt hypertensive rats (DOCA), and DOCA-salt hypertensive rats administered bosentan.*

are sharply elevated in the human coronary sinus after PTCA, it has been suggested that enhanced exposure of smooth muscle to endothelin-1 may stimulate neointima formation. Thus, endothelin-1 may be derived from circulating blood or come from damaged endothelial or smooth muscle cells in the vicinity of the lesion. While the evidence implicates such a mechanism, it has not been definitely established that endothelin-1 has a causal role in restenosis. Because endothelin-1 induces the release of several growth factors, such as platelet-derived growth factor (PDGF), it may function as an indirect or comitogen. The increase in neointima area in response to endothelin-1 is also interesting considering its short plasma half-life, which is about 1 min.[17] However, unlike angiotensin II and norepinephrine, the effects of endothelin-1 are prolonged; the half-life for endothelin-1-receptor dissociation is over 100 h.[18]

EFFECTS OF BOSENTAN

Most studies with ET_A receptor antagonists, such as BQ-123, found that they produce only moderate lowering of blood pressure.[19] Bosentan is a recently developed, long-acting, nonselective endothelin receptor antagonist. It blocks both ET_A and ET_B receptors. A study of DOCA-salt hypertensive rats used bosentan to examine whether the increase in vascular endothelin contributes to elevated blood pressure and vascular hypertrophy.[1] The rats received bosentan (100 mg/kg body weight/day) in their food for 3 weeks. The systolic blood pressure of DOCA-salt hypertensive rats increased to 197 mm Hg during the course of the study, whereas blood pressure of bosentan-treated rats increased to only 177 mm Hg ($P < .01$). The mesenteric resistance arteries of DOCA-salt hypertensive rats had a smaller lumen diameter than those in uninephrectomized control animals (230 *v* 271 μm, $P < .01$). The arterial lumen diameter in bosentan-treated DOCA rats was 253 μm, which was significantly larger ($P < .05$) than the diameter (230 μm) in untreated DOCA rats (Figure 4). The media:lumen ratio was significantly greater in DOCA rats than in the controls (7.6% *v* 3.9%, $P < .01$); in bosentan-treated DOCA rats, this parameter was 5.2%, which was significantly higher than that of uninephrectomized controls ($P < .05$) and untreated DOCA rats ($P < .01$) (Figure 4). The width of the media and cross-sectional area of the media of resistance arteries were significantly smaller in bosentan-treated rats compared to untreated DOCA rats. There were no significant differences in lumen diameter or the cross-sectional area of the media of vessels between bosentan-treated rats and uninephrectomized control rats. The vasoconstrictor responses of bosentan-treated rats were nearly those of controls. Plasma endothelin-1 was significantly higher in bosentan-treated rats, possibly indicating that it was being produced but not binding to its receptor in these animals. These results strongly suggest that endothelin-1 plays a role in the elevation of blood pressure and in vascular hypertrophy in the DOCA-salt hypertensive rat model.

Further evidence that blockade of endothelin receptors by bosentan decreases blood pressure and inhibits vascular hypertrophy came from a study of spontaneously hypertensive stroke-prone rats (SHRSP).[20] SHRSP were administered bosentan in their diet starting at 3 months of age. Untreated SHRSP and untreated Wistar-Kyoto (WKY) rats served as controls. Bosentan-treated rats had lower mean systemic blood pressure, and lower systolic, diastolic, pulse, and mean pressures in arterioles in the cerebrum than did untreated SHRSP. These blood pressures were still significantly higher than those in WKY rats (Table 1). Measurements of first-order arterioles in the cerebrum were made when the animals reached 6 months of age. The cross-sectional area of the vessel wall was significantly larger in SHRSP than in WKY, but in bosentan-treated SHRSP the area was not significantly different from the area in WKY rats (Table 1). That is, treatment with bosentan prevented hypertrophy in cerebral arterioles without normalizing either mean systemic pressure or pulse blood pressure. However, bosentan did not have any significant effects on internal or external diameters of arterioles in SHRSP. A number

TABLE 1. MEAN SYSTEMIC ARTERIAL PRESSURE, PULSE PRESSURE, AND VESSEL CROSS-SECTIONAL AREA IN BOSENTAN-TREATED RATS AND CONTROLS

Parameter	WKY	SHRSP-U	SHRSP-B
Systemic arterial mean pressure, mm Hg	90 ± 3	183 ± 3*	152 ± 5*†
Pulse pressure, mm Hg	25 ± 3	40 ± 2*	33 ± 2*†
Cross-sectional area of vessel wall, μm^2	1299 ± 65	1627 ± 173*	1287 ± 78†

Values are mean ± SEM

WKY, Wistar-Kyoto rats; SHRSP-U, spontaneously hypertensive stroke-prone rats, untreated; SHRSP-B, spontaneously hypertensive stroke-prone rats, bosentan-treated.

* $P < .05$ v *WKY;* † $P < .05$ v *untreated SHRSP.*

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of factors have been postulated as contributing to the cerebral vascular hypertrophy and remodeling that occurs during hypertension, including the increased blood pressure itself, angiotensin II and other neurohormonal factors, and genetic factors. This study shows that bosentan, an antagonist of both ET_A and ET_B, prevents hypertrophy of arterioles in the cerebrum in SHRSP, which suggests that endothelin-1 contributes to the hypertrophy of cerebral arteries occurring during hypertension. This effect may not apply to all arteries under all conditions. In a study by Li and Schiffrin, endothelin receptor blockade did not significantly affect hypertrophy in small mesenteric, coronary, renal, or femoral arteries.[21] This could be due to several differences in the studies. Endothelin-1 may contribute to hypertrophy of cerebral arteries, but not to all other arteries. It is also possible that the effect may only be significant in blood vessels above a certain size, or when blood pressure rises above some threshold.

Bosentan has also been studied in patients with heart failure, in whom plasma endothelin-1 levels are commonly elevated.[22] These patients all had congestive heart failure of >3 months' duration. Endothelin-1 concentrations were above normal in all patients and correlated with the extent of pulmonary hypertension, with left and right heart-filling pressures, with pulmonary vascular resistance, and, inversely with cardiac index. Plasma big endothelin-1 levels were also above normal. This was a randomized, double-blind study in which patients received two intravenous infusions of either placebo or bosentan (100 mg followed 60 min later by 200 mg). All patients were previously on regimens consisting of angiotensin converting enzyme inhibitors (24), diuretics (20),

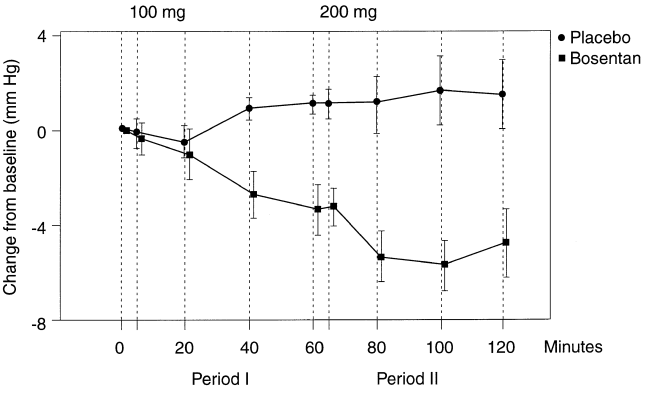

FIGURE 5. *Change from baseline in pulmonary arterial pressure (mean ± SEM) after infusion with bosentan or placebo in patients with CHF (congestive heart failure). Reprinted with permission from Kiowski W et al.*[22]

digoxin (15), calcium antagonists (2), low-dose β-blockers (3), long-acting nitrates (9), and antiarrhythmic drugs, which were withheld prior to infusions. Hemodynamics and plasma endothelin-1 concentrations were determined before and during the 120-min infusion. Bosentan reduced mean pulmonary artery pressure by 13.7% compared to the effects of placebo (Figure 5). It also lowered mean arterial blood pressure by 7.7%, right atrial pressure by 18.2%, and pulmonary artery wedge pressure by 8.6%, all compared to results shown by placebo. It increased the cardiac index by 13.6% (Figure 6), but did not change the heart rate. Consequently, calculated systemic vascular resistance fell by 16.5% and pulmonary vascular resistance by 33.2% ($P < .01$ for both). Most of the effect was seen after the first (100 mg) infusion. Plasma endothelin-1 levels rose significantly in the patients receiving bosentan from a mean of 38.9 pg/mL to 89.6 pg/mL. Big endothelin-1, norepinephrine, angiotensin

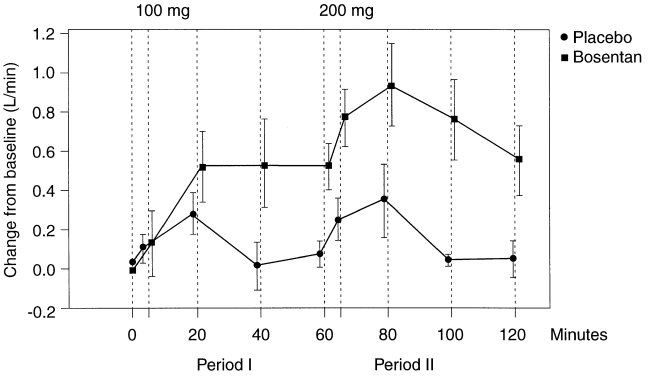

FIGURE 6. *Change from baseline in cardiac output (mean ± SEM) after infusion with bosentan or placebo in patients with CHF (congestive heart failure). Reprinted with permission from Kiowski W et al.*[22]

II, and renin levels did not change, either in the placebo group or in patients receiving bosentan. These results seem to provide strong evidence that endothelin-1 contributes to vascular tone in patients with congestive heart failure.

PLASMA ENDOTHELIN AS A PROGNOSTIC INDICATOR

Plasma endothelin levels are elevated in the acute phase of myocardial infarction (MI) and in chronic heart failure. To test whether plasma endothelin concentration is predictive of subsequent mortality, Omland et al[23] related it to 1-year mortality after documented myocardial infarction. Blood samples from 142 patients in the subacute phase following myocardial infarction were assayed for endothelin 3 days after the onset of symptoms. Plasma endothelin was 5.6 ± 0.3 pg/mL (mean ± SEM) in the patients with myocardial infarction compared to 3.7 ± 0.3 pg/mL in controls ($P = .05$), who were patients admitted to hospital with acute chest pain but without evidence of myocardial necrosis. Of the 142 MI patients, those who had clinical heart failure during their hospitalization had significantly higher plasma endothelin concentrations than those without evidence of heart failure (7.2 ± 0.8 *v* 4.9 ± 0.2 pg/mL, $P < .001$). At the conclusion of the 1-year follow-up, the day 3 plasma endothelin level of survivors was found to have been 5.1 ± 0.2 pg/mL (mean ± SEM). In comparison, patients who died of cardiac causes during this 1-year period had endothelin levels of 9.2 ± 1.5 pg/mL, which was significantly higher ($P < .001$). Analysis by a Cox proportional-hazards model revealed that plasma endothelin level was strongly related to survival ($P < .0001$). Comparison with variables previously determined to be associated with a poor prognosis, eg, age, male sex, and previous history of angina, systemic hypertension, or MI, found that, with the exception of male sex, none of these variables provided additional information after introduction of plasma endothelin in a multivariate model (Table 2).

TABLE 2. MULTIVARIATE RELATION BETWEEN VARIOUS DEMOGRAPHIC, CLINICAL, AND BIOCHEMICAL VARIABLES AND 1-YEAR MORTALITY AFTER MYOCARDIAL INFARCTION ACCORDING TO A COX PROPORTIONAL-HAZARDS MODEL

Variable	Coefficient	*P*
Endothelin	3.1608	<.0001
Atrial natriuretic factor		.2808*
Age		.8271*
Male sex	−1.4625	.0066
Systemic hypertension		.1483*
In-hospital heart failure		.4945*

** Factors not included in the model.*

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SUMMARY

Endothelins are a family of 21–amino-acid peptides having potent vasoconstrictive effects. They bind to specific receptors, ET_A and ET_B, on smooth muscle and endothelial cells. Endothelin-1 is the best studied of this family, but its role in hypertension and other pathologies is still not well understood. When administered to animals it causes severe vasoconstriction, but plasma levels are not significantly elevated in hypertension. Recent studies have reported a synergistic hypertensive effect of endothelin-1 and angiotensin II, but not between endothelin-1 and norepinephrine. Endothelin receptor blockade has lowered blood pressure and reduced the cross-sectional area of neointima both in hypertensive rats and after vascular injury. Infusion of the nonspecific endothelin receptor antagonist bosentan in patients with congestive heart failure decreased blood pressure, increased cardiac index, and reduced vascular resistance. Plasma endothelin-1 levels were found to have a significance at least equal to known risk factors in predicting the 1-year survival rate of patients after myocardial infarction. While endothelin-1 alone is not thought to be an independent factor in the development of hypertension or associated end-organ damage and pathology, there is increasing evidence that it may play a significant role in such processes and may offer an important pathway for therapeutic intervention. The recently developed endothelin receptor antagonist bosentan appears promising in this regard.

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