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**Research Article**

Endothelin-1 is a potent endothelium-derived vasoconstrictor peptide. Although circulating concentrations are not increased in essential hypertension, enhanced sensitivity to endothelin-1 has been observed in animal models of hypertension. We investigated dorsal hand vein responses to local infusion of endothelin-1 and norepinephrine in 12 patients with essential hypertension who had never received treatment and in 12 age and sex matched normotensive control subjects. The maximal venoconstriction and the geometric mean of the dose of norepinephrine that caused 50% of maximal venoconstriction were similar in hypertensive (mean +/- SE; 80 +/- 4%; 31 +/- 8 pmol/min) and normotensive subjects (87 +/- 5%, 22 +/- 9 pmol/min). In contrast, mean venoconstriction to endothelin-1 was significantly greater in hypertensive (49 +/- 5%) than in normotensive subjects (27 +/- 2%; P = 0.004). Sympathetically mediated venoconstriction elicited by deep breath was substantially potentiated by endothelin-1 in hypertensive (67 +/- 7% at 90 min) but not normotensive subjects (11 +/- 3% at 90 min; P = 0.001). Venoconstriction to endothelin-1 correlated positively with mean arterial pressure in the hypertensive subjects (r = 0.82; p = 0.001) but negatively in the normotensive subjects (r = -0.58; p = 0.047). Endothelin-1 may contribute to the reduction of venous compliance occurring in the early stages of essential hypertension and to the altered systemic hemodynamics in this condition.

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# Direct and Sympathetically Mediated Venoconstriction in Essential Hypertension

## Enhanced Responses to Endothelin-1

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## Abstract

**Endothelin-1 is a potent endothelium-derived vasoconstrictor peptide. Although circulating concentrations are not increased in essential hypertension, enhanced sensitivity to endothelin-1 has been observed in animal models of hypertension. We investigated dorsal hand vein responses to local infusion of endothelin-1 and norepinephrine in 12 patients with essential hypertension who had never received treatment and in 12 age and sex matched normotensive control subjects.**

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**Endothelin-1 may contribute to the reduction of venous compliance occurring in the early stages of essential hypertension and to the altered systemic hemodynamics in this condition. (*J. Clin. Invest.* 1994. 94:1359–1364.) Key words: vasoconstrictor peptide • blood pressure • endothelium • veins • sympathetic nervous system**

## Introduction

The endothelins are a family of peptides with extremely potent and characteristically sustained vasoconstrictor and vasopressor actions (1). Endothelin-1 is the predominant isoform in the vascular endothelium, where it is generated from its precursor, proendothelin-1 or 'big endothelin-1' (2). In addition to its direct vascular effects (3), endothelin-1 has inotropic (4) and mitogenic properties (5), influences salt and water homeostasis (6) and stimulates generation of renin, angiotensin II, aldosterone and epinephrine (6). Furthermore, centrally (7, 8) and peripherally (9, 10) administered endothelin-1 alters peripheral sympathetic activity. In view of these actions, there has been interest in the potential role that endothelin-1 may play in the pathophysiology of hypertension (11, 12).

Several investigators have invoked elevated concentrations of circulating immunoreactive endothelin-1 as evidence of increased production in essential hypertension (13, 14). However, endothelin-1 is cleared from the blood by the kidneys (14, 15) and the very high concentrations found in severe and accelerated phase hypertension are probably secondary to impaired renal clearance. Studies in hypertensive patients with normal renal function have shown similar concentrations of endothelin-1 to those in normotensives (16, 17). Indeed, in one study a negative correlation between blood pressure and plasma endothelin-1 was observed in the hypertensive group (16), making a global increase in generation of endothelin-1 unlikely as a cause of essential hypertension.

The results of studies examining vascular sensitivity to endothelin in hypertension are complex. In animal studies comparing WKY and SHR, both conduit (renal artery and aorta) and mesenteric resistance vessels from the hypertensive rat have been shown to be more sensitive to the effects of endothelin-1 by some investigators (18–21). Other investigators have reported decreased sensitivity to endothelin in the aorta and isolated mesenteric resistance arteries from SHR (22), DOCA-salt (23) and renovascular hypertensive rats (24). In vivo, endothelin-1 has been shown to have a greater pressor effect in SHR than WKY rats (25). In patients with essential hypertension, in vitro efficacy of endothelin in subcutaneous resistance arteries appears to be reduced (26). To date, in vivo responses to endothelin-1 have not been examined in patients with essential hypertension.

With continuing uncertainty regarding the vascular actions of endothelin-1 in hypertension, we have investigated whether venoconstriction to endothelin-1 is enhanced in patients with essential hypertension as compared with normotensive control subjects. We examined responses in veins for two reasons. First, the venous system is an important influence on cardiac output in its own right, and has been reported to be abnormal in early hypertension (27, 28). This raises the possibility that abnormal venous responses may contribute directly to the pathophysiology of essential hypertension. Second, studies of pressor responses or vasoconstriction in resistance beds in vivo may be confounded by the presence of vascular hypertrophy in resistance vessels (29), whereas this process does not appear to occur in veins (30).

We examined the effect of local intravenous infusion of endothelin-1 and norepinephrine on dorsal hand vein diameter,

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using norepinephrine as a control constrictor to detect any potential confounding effect produced by alterations in venous structure or function in hypertension. In view of the potential interaction between endothelin and activity of the sympathetic nervous system, and the known increase in sympathetic nerve activity in hypertension (31), we used the single deep breath response (32, 33) to assess the effect of endothelin-1 on sympathetically mediated venoconstriction in hypertensive and normotensive subjects. We chose the dorsal hand vein because responses to vasoactive drugs in these veins are very similar to those predicted from the pharmacological profile of action after systemic doses in vivo (34). In addition, there is clear evidence that cutaneous limb veins are under sympathetic venomotor control, whereas skeletal muscle veins do not participate in these reflexes (35, 36). Thus, responses in hand veins should reflect responses of the component of the venous system that is most important in physiological regulation of venous capacitance and cardiac preload. Finally, these studies do not require systemically active doses of drugs that may obscure any direct vascular action by direct effects on other organs, such as the heart and kidney, or activate reflex mechanisms due to changes in blood pressure.

## Methods

### *Subjects*

Consecutive patients with hypertension (BP > 160/100 mmHg) attending the Cardiovascular Risk Clinic at the Western General Hospital were considered for the study. Patients were only eligible for recruitment if there was no evidence of a secondary cause for hypertension; if mean daytime awake blood pressure was more than 140/90 mmHg on ambulatory monitoring (measurements every 30 min using Spacelabs 90207) (37); if there were no significant concurrent illnesses; and if they had never received antihypertensive therapy. Normotensive (BP < 140/90 mmHg) control subjects matched for age, sex, weight and height were recruited by advertisement. On the basis of power calculations (see *Data presentation and statistics*), we recruited 12 hypertensive and 12 normotensive control subjects. No subject received vasoactive or nonsteroidal anti-inflammatory drugs in the week before each phase of the study, and all abstained from alcohol for 24 h, and from food, caffeine containing drinks, and cigarettes for at least 3 h before any measurements were made. The studies were conducted with the approval of the Lothian Medicine and Clinical Oncology Ethics of Medical Research Sub-Committee and with the written, witnessed, informed consent of each subject.

### *Drugs*

A 23 SWG cannula (Abbott, Sligo, Republic of Ireland) was sited in a selected dorsal hand vein, without use of local anesthesia, in the direction of flow, for infusion of endothelin-1 (5 pmol/min; Clinalfa AG, Läufelfingen, Switzerland) and norepinephrine (6–768 pmol/min; Sterling-Winthrop, Guildford, United Kingdom). These doses, based on an estimated dorsal hand vein flow of 1 ml/min (34), would be expected to achieve local concentrations, within the infused hand vein, approximating to $10^{-8}$ M for endothelin-1 and to between $10^{-8}$ and $10^{-6}$ M for norepinephrine. Drugs were dissolved in saline. Ascorbic acid (Evans Medical, Horsham, UK) was added to norepinephrine solutions, at a final concentration of 10 μg/ml, to prevent degradation by oxidation (34). The total rate of infusion was maintained constant throughout all studies at 0.25 ml/min.

### *Measurements*

*Dorsal hand vein size.* The left hand was supported above the level of the heart by means of an arm rest. Internal diameter of the dorsal hand vein, distended by inflation of an upper arm cuff to 30 mmHg, was measured by the technique of Aellig (38). In brief, a magnetized lightweight rod rested on the summit of the infused vein ~ 1 cm downstream from the tip of the infusion cannula. This rod passed through the core of a linear variable differential transformer (LVDT) supported above the hand by a small tripod, the legs of which rested on areas of the dorsum of the hand free of veins. If venoconstriction occurs while this cuff is inflated, or if the cuff is deflated with consequent emptying of the vein, there is a downward displacement of the lightweight rod. This displacement causes a linear change in the voltage generated by the LVDT, and thus allows determination of the internal diameter of the vein, after calibration against standard displacements. Voltage output from the LVDT was transferred to a Macintosh personal computer file using a MacLab analogue-digital converter and Chart software (v. 3.2.8; both from AD Instruments, Castle Hill, NSW, Australia).

*Single deep breath venoconstrictor stimulus.* When vein size was stable, subjects were asked to breath out fully before breathing in as deeply as possible (32). They were asked to hold this inspiration for 10 s and avoid any tendency to breath out. The technique was practiced before the study to ensure that subjects did not perform a Valsalva maneuver. The deep breath stimulus usually causes a 5–20% venoconstriction in the 30 s after the maneuver (33).

*Blood pressure.* A well-validated semi-automated technique (Takeda UA 751 sphygmomanometer, Takeda Medical Inc., Tokyo, Japan) was used to measure blood pressure in duplicate in the non-infused arm (39).

*Endothelin assay.* Plasma immunoreactive endothelin was measured by radioimmunoassay (40). Immunoreactive endothelin was extracted from acidified plasma using SepPak C18 silica columns (Waters Associates, Milford, MA). Duplicate extracted samples and standards were incubated with rabbit polyclonal antibody raised against endothelin-1 (ITS Production B.V., Wijchen, The Netherlands; in 100 μl distilled water) and $^{125}$I-endothelin-1 (ITS; in 100 μl distilled water). After incubation for 18 h at 4°C, donkey anti-rabbit gamma globulin bound on solid phase (ITS; 100 μl) was added, and tubes were incubated for 30 min at room temperature. The amount of radioactivity in the antibody-bound fraction was determined by gamma counting for 3 min. The recovery of added endothelin-1 was 84%. Intra- and inter-assay coefficients of variation were 2.4% ($n$ = 6) and 4.2% ($n$ = 5), respectively. The sensitivity of this assay is 2 pg/ml endothelin. Cross reactivity of the assay with endothelin-1, endothelin-2, endothelin-3 and proendothelin-1 is 100, 52, 96, and 7%, respectively.

### *Study design*

Subjects rested recumbent during each phase, in a quiet room maintained at a constant temperature of between 22 and 25°C. An intravenous cannula was placed in the right antecubital vein under local anesthesia for blood sampling and the dorsal hand vein cannula and the LVDT sited. Saline was infused for 30 min during which vein size and blood pressure were measured every 5 min. Subjects were asked to take single deep breaths, to elicit sympathetically mediated venoconstriction, after 5 and 20 min of saline infusion. A venous blood sample was obtained from the non-infused arm for assay of circulating endothelin concentrations. Norepinephrine was then infused at incremental doubling doses of between 6 and 768 pmol/min for 10 min each to obtain a full dose response curve. Vein size was measured 5 and 10 min after starting infusion of each dose of norepinephrine. Blood pressure was measured 10 min after starting each dose of norepinephrine. Once a maximal response to norepinephrine was obtained saline was infused until vein size returned to basal values. Endothelin-1 was then infused at 5 pmol/min for 90 min with vein size measured every 5 min and blood pressure every 30 min. The sustained duration of action of endothelin-1 in human veins precluded randomizing the order of infusions of norepinephrine and endothelin-1 (41). Single deep breaths were taken at 28, 58, and 88 min. In a subset of subjects ($n$ = 8 from each group), a further blood sample was obtained from the non-infused arm at the end of the endothelin infusion for assay of circulating endothelin concentration.

### *Data presentation and statistics*

The power of the study to detect a 20% difference in endothelin-1–induced venoconstriction between 12 hypertensive and 12 normotensive

*Table I. Subject Characteristics*

| | Hypertensives | Normotensives |
|---|---|---|
| Number (n) | 12 | 12 |
| Age (yr) | 47 ± 3 | 48 ± 4 |
| Sex (M/F) | 8/4 | 8/4 |
| Supine systolic blood pressure (mmHg) | 159 ± 5* | 120 ± 3 |
| Supine diastolic blood pressure (mmHg) | 103 ± 2* | 75 ± 2 |
| Mean arterial blood pressure (mmHg) | 122 ± 3* | 90 ± 2 |
| Daytime ambulatory systolic pressure (mmHg) | 158 ± 6 | |
| Daytime ambulatory diastolic pressure (mmHg) | 103 ± 4 | |
| Creatinine (μmol/l) | 106 ± 5 | 90 ± 4 |
| Cholesterol (mmol/l) | 5.3 ± 0.2 | 5.1 ± 0.2 |
| Basal vein diameter (mm) | 0.8 ± 0.1 | 0.9 ± 0.2 |

* Indicates $P < 0.05$ for difference from normotensive subjects.

subjects was 90% at the 0.01 significance level. This calculation is based on the standard deviation of venoconstriction to endothelin-1 (10%) observed in a previous study (41). Basal vein size was calculated by taking the mean of the last three measurements before the start of the norepinephrine infusion, and is expressed in millimeters. Because basal vein size varies between subjects, responses to deep breath, norepinephrine and endothelin-1 are expressed as percentage change in vein size from basal in order to reduce the inter-subject variability. Venoconstriction with each dose of norepinephrine was calculated by averaging the two measurements for each dose. Individual norepinephrine dose–response curves were then analyzed using an iterative non-linear curve fitting program (Kaleidagraph, Abelbeck Software, CA) to obtain estimates of maximal responses ($E_{max}$) and norepinephrine dose producing a half-maximal response ($ED_{50}$). Individual $ED_{50}$ values were log transformed for statistical analysis and results shown as geometric means. Because serial measurements were made in each subject following infusion of endothelin-1, mean constriction to endothelin-1 over 90 min was calculated as a summary measure for each individual in order to avoid making multiple comparisons of data (42). The mean of the last two duplicate blood pressure measurements during saline infusion was used as baseline. All results are expressed as mean±standard error of the mean. Data were examined using Student's unpaired *t* test and by simple regression analysis. Statistical analyses were performed using StatView 512+ software for the Macintosh (Brainpower Inc., Calabasas, CA).

## Results

There was no significant difference between hypertensive and normotensive subjects in age, sex distribution, basal hand vein size, or renal function (Table I). Hypertensive and control subjects did not differ significantly in their venous sensitivity ($P = 0.46$ for $ED_{50}$) or responsiveness ($P = 0.30$ for $E_{max}$) to norepinephrine (Fig. 1). Vein diameter was no different from baseline following washout of the venoconstriction induced by norepinephrine before infusion of endothelin-1 in the hypertensive (0.8±0.1 mm; $P = 0.80$) and normotensive subjects (0.9±0.1 mm; $P = 0.69$).

Venoconstriction to endothelin-1 was substantially greater in hypertensive subjects (49±5%) than control subjects (27±2%; $P = 0.004$) (Fig. 2). Basal venoconstriction to a single deep breath was not different between hypertensive (17±3%) and control subjects (13±3%; $P = 0.37$). However, sympathetically mediated venoconstriction to deep breath was greater in hypertensive (67±7% at 90 min) but not normotensive subjects (11±3% at 90 min) after infusion of endothelin-1 ($P = 0.001$; Fig. 3). This was true even when venoconstriction

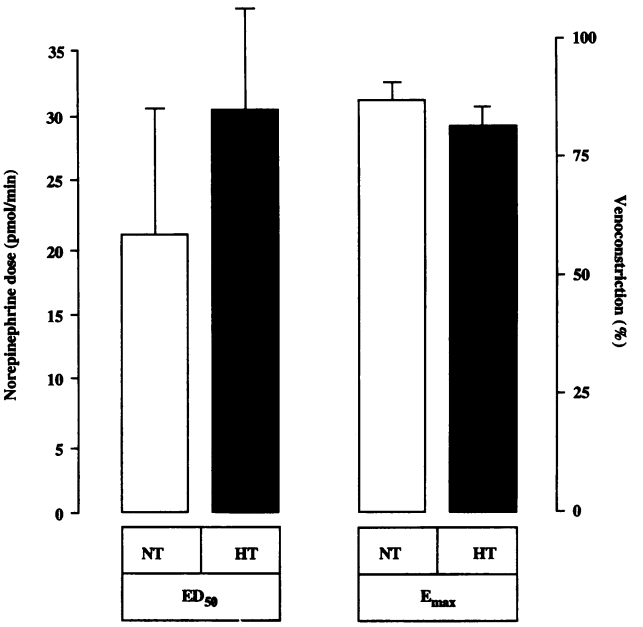


*Figure 1.* The norepinephrine dose producing a half-maximal response ($ED_{50}$; a measure inversely proportional to sensitivity) and the maximum responsiveness to norepinephrine ($E_{max}$). $ED_{50}$ is expressed as a geometric mean dose. There is no significant difference between normotensive (*NT*) and hypertensive (*HT*) subjects, either for $ED_{50}$ ($P = 0.46$) or $E_{max}$ ($P = 0.30$).

to deep breath was expressed as the absolute change in vein size rather than percentage change in order to account for the differences in underlying venoconstriction to endothelin-1 in hypertensive (0.14±0.03 mm at 90 min) and normotensive subjects (0.06±0.02 mm; $P = 0.03$).

Circulating plasma endothelin concentrations were similar in hypertensive (5.0±0.6 pg/ml) and control subjects (5.4±0.8 pg/ml; $P = 0.65$). Circulating endothelin concentrations did not change significantly from baseline following local infusion of endothelin in the hypertensives (baseline = 5.3±1.0; postendothelin = 6.2±1.1; $P = 0.32$; $n = 8$) or the normotensives (baseline = 5.5±1.2; postendothelin = 6.9±1.4; $P = 0.26$; $n = 8$). Blood pressure and heart rate did not alter significantly during infusion of norepinephrine or endothelin-1.

Venoconstriction to endothelin-1 was positively correlated on regression analysis with baseline systolic ($r = 0.85$; $P = 0.0004$), diastolic ($r = 0.69$; $P = 0.01$) and mean arterial pressure ($r = 0.82$; $P = 0.001$) in the hypertensive subjects. In contrast, in the normotensive subjects, venoconstriction to endothelin-1 was negatively correlated with baseline systolic ($r = -0.50$; $P = 0.10$), diastolic ($r = -0.51$; $P = 0.09$) and mean arterial pressure ($r = -0.58$; $P = 0.047$; Fig. 4). Venoconstriction to endothelin-1 did not correlate with basal vein size, $ED_{50}$ or $E_{max}$ to norepinephrine or plasma endothelin concentrations in either the hypertensive or normotensive subjects. There was no correlation, in either group, between blood pressure and plasma endothelin, or blood pressure and the $ED_{50}$ or $E_{max}$ to norepinephrine.

## Discussion

In these studies, we have shown that patients with essential hypertension have enhanced venoconstriction to endothelin-1

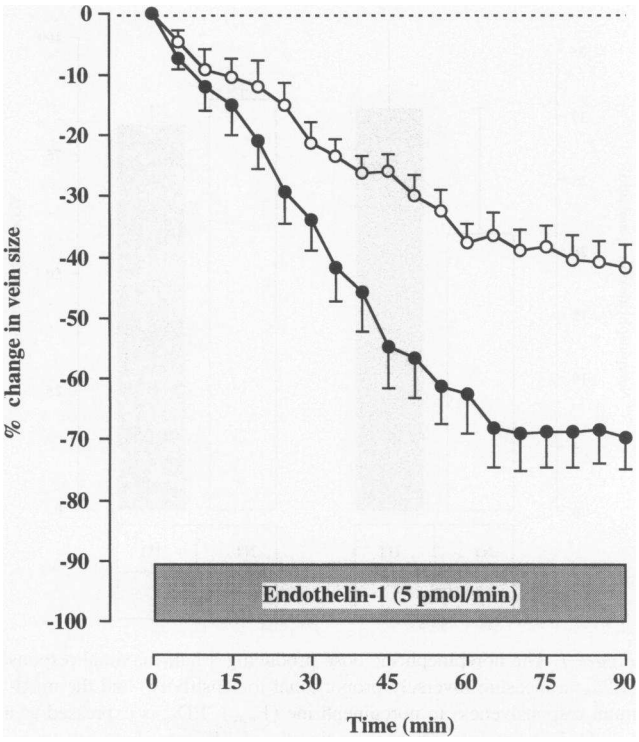


*Figure 2.* Venoconstriction to endothelin-1 in normotensive (○) and hypertensive (●) subjects. There is a significantly greater response in the hypertensive subjects ($P$ = 0.004 vs. normotensives).

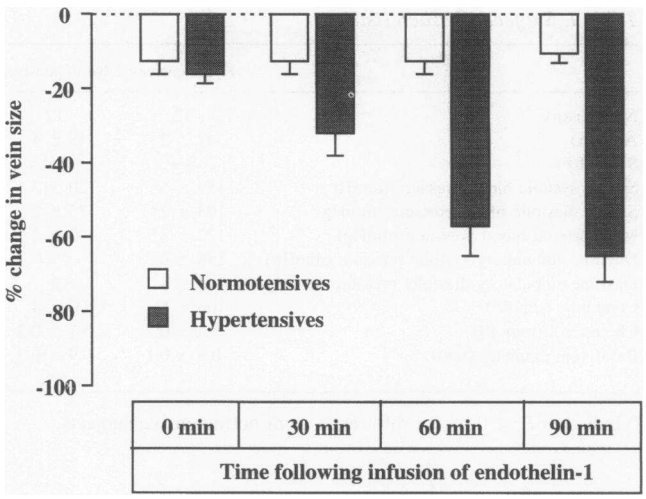


*Figure 3.* Sympathetically mediated venoconstriction induced by single deep breath before and during infusion of endothelin-1 in hypertensive and normotensive subjects. Endothelin-1 potentiates sympathetically mediated venoconstriction in the hypertensive subjects only ($P$ = 0.001 v. normotensives).

that is positively correlated with blood pressure. In addition, we have demonstrated that endothelin-1 substantially potentiates sympathetically mediated venoconstriction in hypertensive but not in normotensive subjects. We have also confirmed earlier work showing that essential hypertension is not associated with increased plasma endothelin concentrations (16) or with altered dorsal hand vein responses to norepinephrine (30).

We were only able to test responses to a single dose of endothelin-1 because the slow onset and long lasting action of endothelin-1 precludes the use of repeated doses in a single study to examine conventional dose–response relationships (41). Thus we cannot say whether the enhanced venous responsiveness to endothelin-1 in hypertension is due to increased sensitivity or responsiveness to the peptide. Different basal vein blood flow may have resulted in different concentrations of agonist reaching venous smooth muscle. However, total forearm blood flow is not decreased in essential hypertension (43), and there is no evidence for a selective redistribution of blood flow from the superficial to deep hand veins in hypertension. In addition, venous diameter was similar in the two groups, implying that any change in blood flow in the veins under consideration would have to be accounted for solely by an increase in velocity of blood flow. Furthermore, the almost identical responses to norepinephrine would suggest that delivery of agonist was similar in hypertensives and normotensives. The use of a constant rate of infusion helped to minimize the possibility that changes in flow through a vein might alter local release of endothelium derived mediators. In any case, such changes in flow are unlikely to have biased our results, as previous studies have shown that increasing the rate of drug infusion by up to 100%, but keeping the dose infused constant, does not alter dorsal hand vein responses to a number of agents (34, 38). The interval of at least 3 h between the last meal and first measurement minimized the possibility that high insulin levels may have altered responses. It is possible, however, that caffeine, which has a long half-life, may have been present in quantities sufficient to alter cardiovascular responses at the time of the study. It is very unlikely that the presence of vascular hypertrophy resulted in amplification of responses to endothelin because we examined responses in vessels which are not thought to undergo hypertrophy in hypertension and because responses to norepinephrine were not altered.

Our results are different from previous in vitro work that showed an apparent diminished efficacy of endothelin-1 in isolated small arteries of hypertensive patients after correction for media hypertrophy (26). This difference may be accounted for by methodological differences, such as the in vivo nature of the dorsal hand vein technique, the avoidance of local anesthesia, which might influence sympathetic responses, and the absence of vessel wall hypertrophy. Alternatively, the difference may be due to the type of vessel studied. However, in diseases associated with abnormalities in resistance or conduit vessels, such as essential hypertension and Raynaud's disease, similar abnormalities are found in hand veins (44, 45).

There are several potential mechanisms for enhanced venous responsiveness to endothelin-1 in essential hypertension. First, there may be decreased local venous endothelin-1 generation. Plasma concentrations of immunoreactive endothelin, which are thought to reflect local concentrations at the interface between endothelial and vascular smooth muscle cells (2), were no different between hypertensive and control subjects, suggesting that there was no global increase in endothelin generation in the hypertensive subjects. However, because endothelial generation of endothelin-1 is directed mainly abluminally (2), it is conceivable that local endothelin generation differs between hypertensive and normotensive subjects. Thus, it is possible, though perhaps unlikely, that our findings may have been due to increased endothelin receptor number or affinity caused by decreased vascular generation of endothelin.

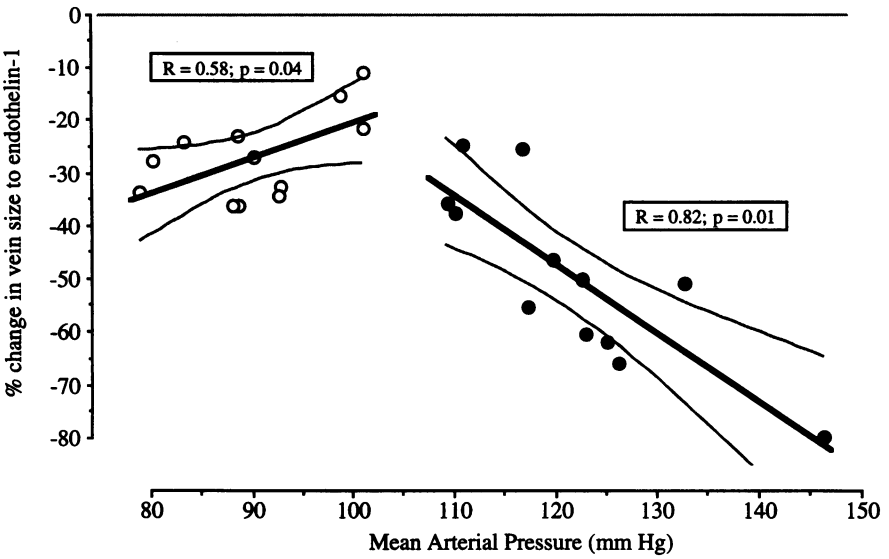


*Figure 4.* Regression curves (with 95% confidence intervals) for the correlation between blood pressure and change in vein size to endothelin-1 in normotensive (○) and hypertensive (●) subjects. There is a negative correlation in normotensive subjects but a positive correlation in hypertensive subjects.

Second, the modulating influence of endothelium-derived vasodilator substances on endothelin-1 induced venoconstriction may be abnormal in essential hypertension (46), resulting in enhanced responses without changes in generation or sensitivity. This would presuppose that impaired endothelial function in hypertension is not the result of elevated pressure per se; recent studies in resistance vessels suggest that impaired endothelial function is not normalized with lowering of blood pressure by anti-hypertensive treatment (47). There is no published work examining endothelium-dependent responses in veins of hypertensive subjects, although insulin-mediated venodilatation, which may be endothelium dependent, is clearly abnormal in mild hypertension (44). However, the unaltered response to norepinephrine in hypertensives, which has endothelial effects, would suggest that endothelial abnormalities are unlikely to fully account for our findings.

Third, it is possible that these findings represent an abnormality of venous smooth muscle in hypertension. The underlying abnormality may be a change in receptor sub-type expression, receptor number or receptor affinity. Alternatively, there may be an abnormality in second messenger systems resulting in a specific amplification of responses to endothelin-1, for example in G-proteins.

Finally, our results may be due to enhanced facilitation of sympathetic vasoconstriction by endothelin in hypertension. It has been suggested from in vitro studies that endothelin may increase peripheral sympathetic activity through postsynaptic potentiation of the effects of norepinephrine (10). However, these findings have not been confirmed in vivo in the forearm resistance bed of healthy subjects (48). The potentiation of sympathetically induced venoconstriction by endothelin-1 only in hypertensive subjects would be consistent with this explanation for our findings. However, at least in normal subjects, dorsal hand veins have no underlying sympathetic tone, so facilitation of sympathetic activity is unlikely to be the sole or initiating mechanism involved.

Although enhanced venoconstriction to endothelin-1 in hypertension may be an epiphenomenon, not causally related to the elevation of blood pressure, this appears unlikely given the positive correlation with blood pressure in hypertensive subjects (Fig. 4). Even so, enhanced venoconstriction to endothelin-1 may occur only secondarily to the increase in blood pressure. However, if this was the case one might also expect a positive correlation between blood pressure and endothelin-induced venoconstriction in normotensive subjects. That there is a negative correlation in these subjects suggests that enhanced venoconstriction to endothelin-1 may be a causative factor in patients with essential hypertension.

These findings in capacitance vessels suggest that exaggerated responsiveness to endothelin-1 may contribute to reduced venous compliance in hypertension. This may, in turn, contribute to the raised cardiac preload and cardiac output observed in the early stages of essential hypertension (27, 28). Further in vivo studies in resistance vessels will help to show whether there is a global abnormality in endothelin responsiveness in essential hypertension, although such studies will need to take account of structural changes in these vessels. The role of endothelin in hypertension may be further clarified with the use of endothelin receptor antagonists, which are currently entering clinical investigation (49).

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