Effect of Endothelin Blockade on Basal and Stimulated Forearm Blood Flow in Patients With Essential Hypertension

Paul Martin, Daniel Ninio, Henry Krum

Abstract—Endothelin receptor antagonism lowers blood pressure in patients with essential hypertension, but the contribution of the endothelin system to increased vascular tone in these patients remains controversial. We used strain-gauge venous plethysmography to measure changes in basal and metabolically stimulated (peak reactive hyperemia [PRH]) forearm blood flow (FBF) induced by continuous intrabrachial infusion of SB 209670, a dual endothelin type A/endothelin type B receptor antagonist (ETRA) in 11 patients with hypertension and 12 healthy age-matched control subjects. In both groups, ETRA caused significant vasodilation (increase in FBF compared with baseline over the last 30 minutes of ETRA infusion: 75±12% in control subjects, 40±13% in hypertension patients; P<0.01 for both groups). By repeated-measures ANOVA, there was no greater increase in FBF after ETRA in hypertension patients compared with control subjects. In addition, FBF responses to PRH, expressed as percent change from baseline (prePRH), were similar in both groups (control subjects: preETRA, 1381±222%; during ETRA, 921±178%; and hypertension patients: preETRA, 1232±221%; during ETRA, 865±285%). We conclude that basal and stimulated vasodilation induced by short-term ETRA infusion in the forearm vasculature of hypertension patients is not increased compared with that of control subjects. An enhanced contribution of the endothelin system to vascular tone in hypertension patients could not be demonstrated using this experimental approach. (Hypertension. 2002;39:821-824.)

Key Words: endothelin ■ hypertension, essential ■ plethysmography ■ blood flow ■ SB 209670

Endothelin-1 (ET-1) is a 21-amino-acid peptide synthesized primarily by endothelial cells. Approximately 80% of secreted ET-1 is directed abluminally, mediating contraction of the adjoining vascular smooth muscle cell via endothelin type A (ET_{A}) and type B (ET_{B}) receptors. ET-1 can also act in a paracrine and autocrine manner on endothelial cell ET_{A} receptors to stimulate NO and prostacyclin release, which in turn mediates vascular smooth muscle cell relaxation. The net effect of ET-1 release is vasoconstriction in normal subjects.

There has, however, been some uncertainty regarding the contribution of ET-1 to the pathogenesis and/or progression of essential hypertension in man. The development of specific endothelin receptor antagonists (ETRAs) has enabled clinical investigators to further assess this contribution.

Chronic (4-week) administration of an ETRA (bosentan) lowers blood pressure in patients with essential hypertension, but whether the endothelin system is specifically involved in the increased vascular tone of these patients remains controversial. Local intraarterial infusion of an ETRA into a vascular bed, with measurement of effects on resting blood flow, has been used in an attempt to address this specific question. In one such study, infusion of a selective ET_{A} receptor antagonist into the forearm vasculature of patients with hypertension caused significant vasodilation; this was greater than that observed in normal subjects. However, this difference was largely accounted for by the absence of significant vasodilation in the normal subjects, a finding at odds with a number of previous studies, which have consistently observed substantial vasodilation to an ET_{A} antagonist in these subjects. In addition, the contribution of ET-1 to the vasodilator response to metabolic stimuli such as postischemic reactive hyperemia has not been previously determined in patients with hypertension.

In an attempt to clarify the above issues, the present study sought to compare the effect of a potent dual ET_{A}/ET_{B} antagonist on vascular tone in patients with essential hypertension and normal subjects, both at rest and during metabolic vasodilation.

Methods

Study Subjects

Hypertensive Patients

Hypertensive patients had to be between ages 40 to 70 years, with documented mild essential hypertension, and had to be receiving ≥1 antihypertensive medication or be newly diagnosed with blood pressure >140/90 mm Hg. Elevated blood pressure levels were confirmed after withdrawal of regular antihypertensive therapy over...
a 2- to 4-week placebo run-in period. Patients could be on no medications that might alter cardiovascular function and could have no concomitant cardiovascular diseases that might alter endothelial function. These diseases included diabetes mellitus, hypercholesterolemia (or being on lipid-lowering medication), and chronic heart failure.

Normal Subjects

Age- and gender-matched healthy control subjects had to be free of concomitant cardiovascular diseases or other diseases affecting forearm blood flow (FBF; as for hypertensive patients) and could be on no regular medication that might affect blood pressure control or cardiovascular homeostasis.

The study was approved by the Alfred Group of Hospitals Ethics Committee, and written informed consent was obtained from all participants.

Study Design

Subjects were required to rest in a recumbent state throughout the studies, which were performed in a quiet room maintained at a controlled temperature of between 22°C and 25°C.

No study subjects could receive vasoactive medication, including aspirin or nonsteroidal antiinflammatory drugs in the week before the study. All subjects were required to abstain from alcohol and caffeine-containing drinks for 24 hours and from food for at least 5 hours before any measurements were made.

A 24-gauge cannula was inserted into the subject’s nondominant brachial artery under sterile conditions. All participants then rested for at least 30 minutes to establish stable blood pressure and FBF values. Baseline resting FBF and blood pressure measures were then recorded every 5 minutes over a 30-minute period of vehicle infusion before commencement of the ETRA infusion. Baseline peak reactive hyperemia (PRH) measurements were performed at the end of this period. ETRA infusion was then commenced with FBF and blood pressure measured every 5 minutes during the infusion. The infusion continued for at least 75 minutes in all patients to achieve steady state. PRH was remeasured at the end of this period just before cessation of the ETRA infusion.

FBF Measurement

FBF was measured in the nondominant arm by venous occlusion plethysmography with a mercury-in-silastic strain gauge (D.E. Hokanson, Bellevue, Wash) that had been electrically calibrated, using methods previously described. The pressure of the collecting cuff was increased to 20 mm Hg higher than the systolic pressure immediately before FBF measurement to isolate the hand circulation.

Recordings of FBF were made over 2.5-minute periods at 5-minute intervals for the duration of the study. Voltage output was transferred to a Macintosh personal computer (MAC LC200, Apple Computer) using a MacLab analog-digital converter and Chart software (Version 3.6; AD Instruments). The mean of the final 5 measurements of each recording period was used for analysis and was expressed in milliliters per 100 mL of forearm tissue per minute, as previously described.

Postischemic Reactive Hyperemia Assessment

Hyperemia was produced by application of an occluding limb cuff to the upper arm for 4 minutes. On release of the cuff, a significant increase in FBF occurs, with return to resting FBF over the next few minutes. We assessed PRH by measuring the peak FBF immediately after release of the cuff. Data are expressed as percent change from the FBF measurements made before release of the cuff.

Blood Pressure and Heart Rate Measurement

Mean arterial blood pressure and heart rate were measured at 5-minute intervals using a Dinamap 1846 SX semi-automated blood pressure monitor (Critikon, Inc) from a cuff placed on the dominant arm.

Clinical Characteristics of the Study Population

<table>
<thead>
<tr>
<th>Demographic Parameters</th>
<th>Hypertensive Patients (n=11)</th>
<th>Normal Subjects (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>111 ± 2*</td>
<td>89 ± 2</td>
</tr>
<tr>
<td>Age, y</td>
<td>54 ± 2</td>
<td>54 ± 3</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>88 ± 6</td>
<td>77 ± 4</td>
</tr>
<tr>
<td>Height, cm</td>
<td>171 ± 3</td>
<td>169 ± 2</td>
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<tr>
<td>Gender, male/female</td>
<td>9/2</td>
<td>8/4</td>
</tr>
<tr>
<td>Total plasma cholesterol, mmol/L</td>
<td>5.2 ± 0.5</td>
<td>5.4 ± 0.3</td>
</tr>
<tr>
<td>Serum creatinine, mmol/L</td>
<td>0.09 ± 0.004</td>
<td>0.09 ± 0.003</td>
</tr>
<tr>
<td>Plasma glucose, mmol/L</td>
<td>5.2 ± 0.1</td>
<td>4.8 ± 0.2</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. *P < 0.001 normal subjects vs hypertensive patients.

Drug Administration

The ETRA infused was SB 209670 (Smith Kline Beecham Pharmaceutical), a dual (ET_A/ET_B) receptor antagonist (ETRA). This agent was chosen as it potently inhibits both endothelin receptor subtypes (K_i:ET_A=0.2 nmol/L, ET_B=12 nmol/L), including the endothelial cell ET_A receptor (Dr E Ohlstein, personal communication, 2000). The agent was infused at an initial dose of 0.5 μg/kg per min for 10 minutes (first dose), then 5 μg/kg per min for 5 minutes (loading dose), and subsequently 0.5 μg/kg per min thereafter (maintenance dose). Doses were chosen to achieve a concentration in plasma that was locally active but subsystemic. All solutions were prepared aseptically from sterile stock solutions on the day of the study. The drug was dissolved in dextrose 5% (Baxter Healthcare Ltd) and infused at a rate of 1.0 mL/min using a constant-rate infusion pump (Braun) through a bacterial filter.

Statistical Analysis

Data are presented as mean ± SEM. Normal subjects and patients with hypertension were matched as far as possible for baseline resting FBF to allow for subsequent responses to be compared as percent change from baseline. SPSS version 10 was used for statistical analysis. Within-group analyses were performed by Student’s paired t test. Between-group comparisons were performed using unpaired Student’s t test and repeated-measures ANOVA when appropriate. When a statistical difference was detected by ANOVA, the Bonferroni multiple-comparison procedure was used to define differences between the results. Statistical significance was accepted when P < 0.05.

Results

Subject Baseline Demographic Parameters

Study population characteristics are summarized in the Table. Normal subjects and patients with hypertension were well matched, with the exception of mean arterial pressure, which was significantly higher, as expected, in patients with hypertension.

Blood Flow Measurements at Baseline

Following 30 minutes of infusion of vehicle to establish a steady state, resting FBF measurements were similar between normal subjects (3.7 ± 0.5 mL/min per 100 mL forearm volume) and patients with hypertension (5.2 ± 0.6 mL/min per 100 mL forearm volume), with P = NS for differences between the 2 groups.

Baseline PRH as a percentage of resting FBF was also similar between the 2 groups (1381 ± 222% in normal sub-
Blood Flow Measurements During Endothelin Blockade

Changes in resting FBF in the 2 groups during infusion of the ET receptor antagonist, such as occurs in the Figure. ET receptor antagonist (ETRA) caused progressive vasodilation in both normal subjects and patients with hypertension. By repeated-measures ANOVA, this was significantly different from baseline in both groups (P<0.01). However, there were no significant differences in FBF responses between groups. As assessed over the last 30 minutes of ETRA infusion, the percent increase in FBF was 75±12% in normal subjects and 40±13% in patients with hypertension (P=NS between groups). Similarly, there were no differences between groups when absolute FBF responses to ETRA were assessed over the last 30 minutes of ETRA infusion (6.1±1.0 mL/min per 100 mL forearm volume in normal subjects and 6.8±0.8 mL/min per 100 mL forearm volume in patients with hypertension; P=NS between groups).

PRH as a percentage of baseline FBF decreased in both groups during ETRA infusion, because of a higher resting baseline resulting from ETRA-mediated vasodilation in both groups. In normal subjects, PRH increased to 921±78% above baseline during ETRA infusion, whereas in hypertensive patients this increase was 865±285%. The difference between the 2 groups was not significant.

There were no significant hemodynamic differences during the course of the ETRA infusion in either group. The mean recorded fall in mean arterial blood pressure during ETRA infusion was 3±1 mm Hg, observed in both groups. Heart rate did not change throughout in either group.

Discussion

The present study demonstrates that blockade of the endothelin system results in a substantial and significant vasodilator effect in both normal subjects and patients with essential hypertension. However, vasodilation in response to endothelin blockade, both in the resting state and in response to metabolic stimulation, was not increased in patients with hypertension compared with normal subjects.

Our observation of vasodilatation by ETRA in normal subjects is consistent with ET-1 being one of the most potent vasoconstrictors described. This observation has been previously reported by many groups, mainly using the selective ET A receptor antagonist BQ-123. In contrast, Cardillo et al demonstrated that both BQ-123 and combined ET A and ET B receptor blockade resulted in no significant effect on vascular tone in normotensive subjects. The discrepancy between these findings and those of previous studies cannot be accounted for by differences in the study protocols, because both the dose and the duration of infusion of BQ-123 were identical to those used in many previous studies. It is also noteworthy that a recent study has reported not only local, but also systemic, effects of BQ-123 at the doses used by many of these investigators, making interpretation of these data even more difficult.

The key finding of the present study was that vasodilatation to ETRA in patients with essential hypertension was not increased compared with that in normal subjects. This is in contrast to previous studies, in which an enhanced vasodilator effect of ET blockade in patients with essential hypertension was observed. As mentioned, this relative increase in vasodilation in hypertensive patients appears to arise, at least in part, from the limited vasodilation observed in normal subjects.

Our observations are consistent with data from several previous ex vivo and in vitro human studies that demonstrated an absent or attenuated vascular responsiveness to exogenous ET-1 in many cardiovascular diseases. Blunted vasoconstrictor effects of endogenous endothelin-1 have been observed in many (but not all) studies of patients with essential hypertension, chronic heart failure, diabetes mellitus, and chronic renal failure. Such changes may be related to receptor downregulation or postreceptor translational changes in the setting of local activation of the agonist. Local activation of the endothelin system has been well demonstrated in a number of animal and human studies. Thus, short-term blockade of the receptor may similarly demonstrate an attenuated vascular response, as occurred in the present study. This possibility is supported by the recent observation of a decreased vasodilator response to ETRA in patients with congestive heart failure, a condition in which the endothelin system is clearly activated, both locally and systemically.

These observations are also similar to those reported for other vasoconstrictor peptides, eg, angiotensin II. Vascular responsiveness to peptide antagonism is not enhanced in patients with hypertension compared with control subjects. However, these observations do not diminish the importance of these neurohormonal systems in the pathogenesis and progression of disease or the therapeutic benefit of blockade of these systems. Thus, assessment of vascular responsiveness to short-term receptor blockade may not be the most appropriate method by which to assess the functional activity of an agonist acting on the vasculature.

In addition to assessing the effect of endothelin blockade on resting vascular tone in patients with hypertension and normal subjects, the present study also sought to assess the relative contribution of ET-1 to vasodilation that occurs in response to metabolic stimuli such as postischemic hyperemia. We found no differences in PRH responses between hypertensive and normal subjects in either the absence or presence of ETRA. This finding is consistent with the responses observed to ETRA on resting vascular tone between the 2 groups in the present study. As with the resting studies, these
findings do not exclude the possibility of an enhanced contribution of ET-1 to limit PRH in patients with hypertension.

Study Limitations

We did not perform a placebo infusion as the primary focus of the study was to compare responses to the same dose of ETRA in normal subjects and patients with the disease (essential hypertension). In addition, we did not include a control dilator that acts through direct mechanisms on vascular smooth muscle, such as hydralazine, which may have provided additional important mechanistic information. We did not infuse ET-1, ET-3, or sarafotoxin 6c to study the effect of selective agonism on different endothelin receptors. In addition, logistic considerations prevented us from delineating a dose-response to the antagonist. We infused the ETRA for a prolonged period of time based on the known kinetics of the drug. Whether a more prolonged infusion period would have yielded different results is unknown. However, it appears that steady-state pharmacodynamics were reached in the study, based on the relatively stable FBF values in both groups over the last 30 minutes of the study.

Conclusions

The present study demonstrates that infusion of the dual ETRA SB 209670 into the forearm vasculature of normal subjects resulted in significant vasodilatation at rest. Forearm vasodilatation in response to ETRA was also observed in patients with essential hypertension; this was not increased in comparison to normal subjects. Endothelin receptor antagonism modified postischemic vasodilatation in a similar manner in both groups. Therefore, ET-1 appears to contribute to the regulation of both resting and hyperemic vascular tone in normal subjects; an enhanced contribution in patients with essential hypertension could not be demonstrated using this experimental approach. Specifically, this study involved acute inhibition of an endothelin system that may be locally activated with an endothelin receptor downregulation; an attenuated response to the antagonist could therefore arise in this setting. Therefore, the present data do not preclude an important role for ET-1 in the pathogenesis and progression of essential hypertension nor the possibility that blockade of the endothelin system may be of therapeutic benefit in this condition.

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References


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