Transvascular Albumin Leakage and Forearm Vasodilatation to Acetylcholine in Essential Hypertension

Roberto Pedrinelli, Giulia Dell’Omo, Simona Bandinelli, Giuseppe Penno, and Mario Mariani

The impact of hypertension on microvascular permeability and nitric oxide-mediated endothelial vasomotion in humans has been studied by measuring either the transcapillary albumin escape rate (TERalb, a measure of permeability through systemic capillary endothelium where most of the albumin permeation takes place) and forearm vasodilatation to locally infused acetylcholine (used as a probe for the nitric oxide-releasing potential of arteriolar endothelial cells). It is unknown, however, how the two parameters relate to each other in the same hypertensive subject. This piece of evidence may enhance our understanding about the relative effect of hypertension on two biological functions (ie, permeability and nitric oxide-mediated vasomotion), both dependent on vascular endothelium, and also may allow to appreciate in greater detail the profile of parameters frequently used as markers of microvascular dysfunction in human hypertension. For these reasons, TERalb (the 1-h decline rate of intravenous $^{125}$I-albumin) and forearm vasodilatation (strain gauge venous plethysmography) to graded intraarterial acetylcholine infusion were measured in 44 never-treated men with uncomplicated essential hypertension, and 15 male normotensive controls with comparable age, lipids, and proportion of current smokers. TERalb was increased in patients, whereas acetylcholine-mediated vasodilatation did not differ significantly between the two groups, indicating a heterogeneous impact of elevated blood pressure on capillary permeability and endothelial vasomotion in still uncomplicated mild to moderate essential hypertensive patients. The dissociation between TERalb and forearm responsiveness to acetylcholine also demonstrates that different endothelial-dependent biologic parameters do not behave uniformly in human hypertension. Am J Hypertens 2000;13:256–261 © 2000 American Journal of Hypertension, Ltd.

**KEY WORDS:** Hypertension, capillary permeability, endothelial function, acetylcholine.
Forty-four never-treated uncomplicated patients with stage 1 to 3 essential hypertension seen at our Outpatient Centre for diagnostic screening. Hypertension was identified on the basis of repeated casual blood pressure (BP) determinations greater than 140/90 mm Hg (Table 1) in absence of antihypertensive or hypolipidemic drugs. Fasting blood glucose was less than 6.0 mmol/L (120 mg/dL), HbA1c less than 6% and oral glucose tolerance was normal (2-h after oral glucose load <7.8 mmol/L [140 mg/dL]). Serum creatinine was less than 110 μmol/L (1.2 mg/dL), total serum cholesterol less than 7.8 mmol/L (300 mg/dL), normal urinary sediment, no urinary tract infection, body mass index (BMI) less than 30 kg/m², and no evidence (ejection fraction ≥50%) or history of congestive heart failure, peripheral arterial vascular disease, chronic obstructive pulmonary disease. Renal ultrasound scan showed normal-sized kidneys, and routine clinical and hematologic examinations excluded other secondary forms of hypertension. Normal subjects were chosen on the basis of normal physical examination, routine blood and urinary tests, BP, electrocardiogram, abdominal echograms, and ankle/brachial index. Experimental evaluations were completed in a 2-week period.

According to institutional guidelines, subjects were aware of the investigational nature of the study and agreed to participate. The study was carried out in accordance with the Declaration of Helsinki and the protocol was approved by the local ethical committee.

**Experimental Procedures** TERalb TERalb determination was carried out as described previously. In short, 125I-labeled human serum albumin (6 to 8 μCi, 222 to 296 kBq, SARI-125 A-2; SORIN Biomedica, Saluggia, Italy) was injected as a bolus after a 30-min rest in the sitting position and blood was withdrawn from the contralateral arm every 5 min during the hour after the injection. Radioactivity was measured (Cobra 5000 γ-counter; Packard, Downers Grove, Illinois) in duplicate in whole blood samples centrifuged for 10 min at 3000 g for 40 min. Hematocrit (Coulter Counter 55; Coulter Electronics, Bedfordshire, U.K.) was determined in each sample. Serum albumin was measured by immunonephelometry (Behring Laser nephelometer System, Behring, Marburg, Germany, interassay variation coefficient: 5.2%). 125I labeling was obtained by electrolytic technique, a procedure that does not alter the biological behavior of albumin in vivo. Free 125I was eluted by passage in a Sephadex G-25-M column (Column PD-10; Pharmacia, Uppsala, Sweden), a purification step that reduced free 125I content in the injected dose to less than 1%. Studies were run between 2:00 and 4:00 pm after a 4-h fast and no tea, coffee, alcohol, or tobacco from the early morning.

BP was measured 12 to 15 times by an automated oscillometric device (SpaceLabs 90207, SpaceLabs, Redmond, Washington, USA) during the TERalb determination.

**Forearm infusion protocol** Forearm studies were performed in a quiet, air-conditioned room. Subjects fasted and were instructed to refrain from heavy exercise and avoid smoking and emotional excitement from the day before the experiment. A 22-gauge polyethylene catheter (Angiocath, Becton Dickinson, St.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Controls (n = 15)</th>
<th>EH (n = 44)</th>
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<tbody>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>126 ± 7</td>
<td>151 ± 12†</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>78 ± 6</td>
<td>96 ± 8‡</td>
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<tr>
<td>Mean BP (mm Hg)</td>
<td>94 ± 6</td>
<td>115 ± 8‡</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>51 ± 18</td>
<td>49 ± 11</td>
</tr>
<tr>
<td>Current smokers</td>
<td>4/15 (27%)</td>
<td>10/44 (22%)</td>
</tr>
<tr>
<td>FBF (mL/min/dL)</td>
<td>4 ± 1</td>
<td>3.8 ± 1.3</td>
</tr>
<tr>
<td>FVR (units)</td>
<td>24 ± 6</td>
<td>33 ± 9‡</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.1 ± 1</td>
<td>5.3 ± 1</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.2 ± 0.9</td>
<td>3.6 ± 1.2</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.1 ± 0.2</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>Triglycerides (mmol/L, medians &amp; range)</td>
<td>1.1 (0.6–3.9)</td>
<td>1.4 (0.6–4.6)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25 ± 2.5</td>
<td>26.3 ± 2.3*</td>
</tr>
<tr>
<td>Plasma volume (mL/1.73 m²)</td>
<td>2745 ± 666</td>
<td>2813 ± 593</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>35 ± 7.3</td>
<td>36 ± 4.1</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>45 ± 3.1</td>
<td>45 ± 3.2</td>
</tr>
</tbody>
</table>

Means ± SD unless otherwise specified.
* P < .01, † P < .001 v controls.
Louis, MO, USA) was inserted into the right brachial artery, the arterial line connected to an infusion pump (Perfusor, Secura FT, Braun, Melsungen, Germany) and subjects were then allowed to rest for approximately 30 min. Total forearm blood flow (FBF) was measured by venous plethysmography with a strain-gauge apparatus (Hokanson, EC 5R Plethysmograph, Hokanson, Issaquah, WA, USA). In our laboratory, strands are made of Silastic tubing of 0.4 mm inner diameter and 0.8 mm outer diameter filled with mercury. The gauge was applied on the arm, 5 to 6 cm distal to the elbow at a tension sufficient to keep the gauge in the same position throughout the experiment. Patient forearm was kept on a table, slightly flexed and inclined at about 45° to the horizontal plane with the wrist and hand supported by sand bags. One minute before FBF determination, a pneumatic pediatric cuff was placed around the wrist and inflated to suprasystolic arterial blood pressure to exclude the hand vascular region. A second cuff was placed proximal to the plethysmograph and automatically inflated to a pressure of 40 mm Hg to allow FBF measurement according to the venous occlusion method. Preliminary experiments showed that brachial artery cannulation caused no specific FBF changes on the infused side. All the tracings were read by a single observer (G. D.), with a within-observer variability of 3.5% (variation coefficient, n = 9 blind readings of the same tracing). In our experimental conditions, repeated FBF measurements (during a 1-h period) in a separate group of subjects (n = 7) gave a 15 ± 6% (SD) variation coefficient in the absence of any intervention. Forearm volume was measured according to the water displacement method; BP was measured every 5 min throughout the study on the contralateral arm by an automated device (NIBP KO 7267.004, Kontron Instruments, Watford Herts, UK).

Fresh solutions of ACh HCl (Miovisin, Farmiga, Pisa, Italy 7.5, 15 and 30 μg/min, 5 min each) and sodium nitroprusside (SNP, Nipride, Malesci, Firenze, Italy), used as an internal control for NO-independent sodium nitroprusside (SNP, Nipride, Malesci, Firenze, Italy), used as an internal control for NO-independent.

**Data Processing**

Plasma $^{125}$I-albumin concentration (cpm/mL) was plotted on a semilogarithmic scale, and the transcapping escape rate (%/h) was calculated from the monoexponential disappearance rate constant of the $^{125}$I curve from 10 to 60 min. Plasma volume (mL/1.73 m$^2$) was determined by retropolation to zero time of the disappearance curve corrected for the injected dose of tracer obtained by weighing the syringes before and after the injection. FBF (mL/min/dL of forearm volume) was the mean of four to five determinations obtained over the last 2 min of each experimental period. Because BP did not change during the infusion, drug-induced effects were evaluated in terms of FBF changes, analyzed either as percentages from baseline or area under curve (AUC) values (trapezoidal rule). BP values were the average of the multiple recordings taken during the TERalb and the plethysmographic studies. Mean BP (diastolic +1/3 pulse pressure), forearm vascular resistance (FVR, MBP/FBF), BMI (body weight/squared surface area), LDL cholesterol [CHOLTOT - (CHOLHDL + triglyceride/5)] were derived from standard formulae.

**Statistics**

Statistical significance of the differences was tested by one-way analysis of variance for continuous variables and by $\chi^2$ statistics for categorical variables. Associations were evaluated by Pearson’s correlation coefficients. Statistical significance was set at a $P < .05$. Descriptive statistics were means ± standard deviation unless otherwise specified. Calculations were performed by using Statgraphics Plus (Manugistic Inc, Release 1997, Rockville, MD, USA).

**RESULTS**

Age, lipids, proportion of current smokers, and FBF did not differ between patients and controls, whereas FVR and BMI were higher in hypertensives (Table 1).

**TERalb**

TERalb was higher (9.2 ± 2.7 vs 7.2 ± 1%/h, $P < .008$, Figure 1, left) in patients. Plasma volume, serum albumin concentration, and hematocrit did not differ from controls (Table 1). No statistically significant association was found between TERalb and any of the continuous variables reported in Table 1, except systolic BP, both in the overall group ($r = 0.42, P < .0008, n = 59$, Figure 1, right) and in the hypertensive subgroup ($r = 0.32, P < .03, n = 44$).

**Intraarterial Infusions**

FBF increased dose dependently during ACh and SNP (Figure 2, top). The AUC of the corresponding time FBF profile (AUC$_{ACH}^{125}$: 233 ± 93 v 264 ± 106 mL/dL × 15 min$^{-1}$; AUC$_{SNP}^{125}$: 191 ± 39 v 216 ± 49 mL/dL × 15 min$^{-1}$) and the
percent FBF changes during ACh and SNP (Figure 2, bottom) did not differ between patients and controls. Forearm volumes (controls: 972 ± 45 vs hypertensives: 1021 ± 50 mL) were similar.

Relationship Between TERalb and ACh-mediated Local Vasodilatation

Peak responses to ACh and TERalb values were unrelated (r = 0.03, n = 59) (Figure 3).

DISCUSSION

The coexistence of abnormal TERalb values with preserved ACh-mediated arteriolar responsiveness indicates a trend toward a heterogeneous impact of elevated BP on capillary permeability and endothelial

vasomotion in mild to moderate, still uncomplicated essential hypertensive patients. The dissociation between TERalb and forearm ACh responsiveness also demonstrates that, despite common dependence on the function of endothelial cells, different indices of microvascular damage do not behave uniformly in hypertensive patients. Although NO contributes to modulate both permeability and vasomotion, its involvement in the two biologic processes differs, as the transport of macromolecules across the endothelium uses several other mechanisms involving the matrix as well (see Ref. for review). On the other hand, ACh responses reflect mainly NO bioavailability, although shortcomings of ACh as a probe include activation of NO-independent mechanisms, poor correspondence with the underlying tonic activity of the L-arginine-NO system, and unpredictable dependence on endogenous muscarinic receptor reserve and acetylcholine-esterase activity among individuals. In addition, more speculative reasons may contribute to the observed dissociation between TERalb and ACh-mediated forearm vasodilatation. For example, the vascular functions explored by the two parameters may respond with a different time course to the noxious hypertensive stimulus; actually, one might infer from our data that capillary permeability is impaired before the development of a dysfunctional NO-mediated vasomotion, although this possibility cannot be discerned from a cross-sectional study such as ours. Different sensitivity is a next possibility, in that defects in ACh-mediated vasodilatation identify more advanced endothelial dysfunctions, whereas TERalb could be sensitive to milder forms. This latter hypothesis is consistent with in vitro models predicting marked increases in macromolecular transport even when endothelial surface is only minimally altered. The relevance of this model to the human situation is unknown.
Abnormal TERalb in Essential Hypertensives  A result in itself worthy of some additional comment was the confirmation of previous reports3–7 showing a faster transvascular albumin escape rate in never-treated, glucose-tolerant, uncomplicated essential hypertensives. This behavior is probably a consequence of an abnormal permeation through capillaries of the extrarenal organs,8 although the relative contribution of various organs and tissues with different permeability cannot be identified precisely on the basis of these data. Moreover, the albumin escape rate is a complex measurement that depends not only on the intrinsic permeability but also on the pressures and flow rates in the blood vessels, the total cross-sectional area, and likely also on the capacity of the lymphatics to clear interstitial fluid and dissipate interstitial hydraulic pressure through passive flow and active pumping. Therefore, mechanisms potentially responsible for the abnormal microvascular passage of albumin in our patients include capillary hyperfiltration due to unbalanced pre- or postcapillary pressures.25 Because two main determinants of postcapillary pressure such as cardiac function and plasma volume were normal in our patients, one should hypothesize elevated precapillary pressure driven by arterial hypertension, in agreement with the positive correlation with systolic BP levels. However, elevated perfusion pressure explained only a small portion of the overall TERalb variability, and, furthermore, transvascular albumin leakage can be abnormal even in normotensive nondiabetic atherosclerotic patients.26 Increased microcirculatory area due to opening of nonperfused capillaries could theoretically contribute to increase TERalb8 but the evidence of capillary rarefaction in the crocirculatory area due to opening of nonperfused arterioles, difficult to reconcile with the view of essential hypertension as a clinical condition invariably characterized by impaired endothelial-mediated vasodilatation.13 Perhaps, reduced muscarinic responsiveness may develop at more complicated stages or with longer duration of disease,36 whereas the hypertension process in our group of never-treated, mild hypertensive patients was likely of short duration. Subgroups whose genetic characteristics12 may, by chance, have prevailed in some but not other series of patients should probably considered as well. We have no arguments in favor or against these possibilities, and, on the other hand, our study did not aim to address these specific issues.

In conclusion, the data show a dissociation between TERalb and forearm responsiveness to ACh, indicating that indices dependent on different endothelium-dependent microvascular functions do not behave uniformly in mild to moderate uncomplicated essential hypertensive patients.

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