Endothelial cells play a key role in the local regulation of vascular tone by the release of vasodilator and vasoconstrictor substances, including endothelium-derived relaxing factor (now identified as nitric oxide) and endothelium-derived contracting factors (constrictor prostanoids and possibly endothelin), respectively.1

Confirming studies in animals,2–9 data from patients with essential hypertension have shown that the vasodilation to acetylcholine, an endothelium-dependent vasodilator,10 is reduced compared with normotensive control subjects.11,12 This suggests that endothelium-dependent responses are curtailed also in human genetic hypertension. However, no data seem to be available regarding responses to endothelium-dependent vasodilators in secondary forms of human hypertension.

The present study was designed to determine whether or not endothelium-dependent vasodilatation to acetylcholine is reduced in secondary forms of human hypertension (primary aldosteronism and renovascular hypertension) and, if so, whether or not a cyclooxygenase-dependent vasoconstrictor mechanism may be involved.

Methods

Subjects

Forty patients (Table 1) participated in the present study. Office arterial blood pressure was measured by sphygmomanometer once a week for 3 weeks before inclusion. Patients with primary or secondary forms of hypertension had already been screened during a previous admission to the unit and were recruited again together with healthy volunteers to perform the study. Patients and subjects were divided into four groups: 1) normotensive subjects (n = 12), with no family history of essential hypertension and arterial pressures of <140/90 mm Hg; 2) essential hypertensive patients (n = 12), with arterial blood pressures >160/95 mm Hg; 3) hypertensive patients with primary aldosteronism hypertensive patients (n = 8), defined by an arterial blood pressure >160/95 mm Hg, by the combined presence of hypokalemia plus an increased production of aldosterone despite low levels of plasma renin activity, and by the existence of an adrenal adenoma (determined by computed tomographic scan). Five patients of this group underwent surgery, and the presence of an aldosterone-producing adenoma was confirmed by histological examination. In these five patients, the study was repeated no longer than 1 month after surgery, when blood pressure (before surgery: 166.3±8.4/98.6±5.3 mm Hg; after: 123.8±6.4/83.1±4.8 mm Hg; p<0.001) and plasma potassium (before surgery: 3.3±0.2 mmol/L; after: 4.0±0.3 mmol/L; p<0.001) were normalized. 4) The last group included renovascular hypertensive patients (n = 8), defined by an arterial blood pressure >140/95 mm Hg and the arteriographic demonstration of a significant renal artery stenosis combined with the suppression of renin secretion in the nonstenotic kidney.

The normotensive subjects and the patients with essential and renovascular hypertension were matched...
for age, sex, body weight, and other clinical variables (Table 1). Because of the difficulty of recruitment, patients with primary hyperaldosteronism could not be perfectly matched with the other groups in terms of age, sex, body weight, forearm volume, and forearm blood flow.

The protocol was approved by the local institutional review committee; patients were aware of the investigational nature of the study and consented to it. Essential and renovascular hypertensive patients discontinued antihypertensive treatment 2 weeks before the study; patients with hyperaldosteronism discontinued treatment with spironolactone 4 weeks before the study. No patient was on treatment other than antihypertensive medications.

**Experimental Procedure**

All studies were performed at 8 AM after overnight fasting with individuals lying supine in a quiet air-conditioned room (22-24°C). As previously described, a polyethylene cannula (21 gauge, Abbot, Sligo, Ireland) was inserted into the brachial artery with patients under local anesthesia (2% lidocaine). The cannula was connected through stopcocks to a pressure transducer (model MS20, Electromedics, Englewood, Colo.) for the determination of systemic mean arterial blood pressure (systolic pressure plus diastolic pressure) and heart rate (model VSM1, Physiocontrol, Redmond, Wash.) and for intra-arterial infusions. Forearm blood flow was measured in both forearms (experimental and contralateral) by strain-gauge venous plethysmography (LOOSCO, GL LOOS, Amsterdam, The Netherlands). The circulation to the hand was occluded 1 minute before each measurement of forearm blood flow by inflating a pediatric cuff around the wrist at suprasystolic blood pressure.

**Experimental Design**

Endothelium-dependent vasodilatations were estimated by performing a dose-response curve to intra-arterial acetylcholine (cumulative increase of the infusion rates: 0.15, 0.45, 1.5, 4.5, 15 µg/100 mL forearm tissue per minute for 5 minutes at each dose). To estimate whether or not endothelium-dependent cyclooxygenase-dependent constrictor substances may be released by acetylcholine, we repeated the experiment during an infusion of indomethacin (50 µg/100 mL forearm tissue per minute; an inhibitor of cyclooxygenase) started 30 minutes before the administration of acetylcholine. This dose was selected to obtain a plasma concentration of indomethacin in the range of 10⁻⁹ M. Endothelial-independent vasodilations were assessed with a dose-response curve to intra-arterial sodium nitroprusside (cumulative increase by 0.1, 0.3, and 1 µg/100 mL forearm tissue per minute for 3 minutes each). These rates were selected to induce vasodilations comparable to those obtained with acetylcholine. The infusion sequence of the two drugs was randomized, and 45 minutes of recovery was allowed between the two experimental steps.

**Data Analysis**

Data were analyzed in terms of changes in forearm blood flow and forearm vascular resistance (calculated as the ratio between mean intra-arterial blood pressure and forearm blood flow). Because mean arterial blood pressure did not significantly change during the study, increments in forearm blood flow were taken as evidence of local vasodilatation. Results are expressed as mean±SEM. Data were analyzed statistically by t test for paired or unpaired observations and by analysis of variance for intergroup comparisons. Wilcoxon's test was used to check the statistical significance of the difference between nonparametric values. Differences were considered statistically significant at a value of *p* < 0.05.

**Drugs**

Acetylcholine HC1 (Farmigea S.p.A., Pisa, Italy), indomethacin (Limometacen; Chiesi Farmaceutici S.p.A., Parma, Italy), and sodium nitroprusside (Malesci, Milan, Italy) were obtained from commercially available sources. Acetylcholine and indomethacin were diluted freshly to the desired concentration by adding normal saline. Sodium nitroprusside was dissolved in glucosate solution and protected from light by aluminum foil.

**Results**

Essential and secondary hypertensive patients had comparable values of blood pressure, and plasma total
Acetylcholine

The vasodilation of the forearm induced by acetylcholine was significantly reduced \( (p<0.01) \) in essential hypertensive patients (percent increments from basal: 8.9±3.4%, 22.1±4.5%, 73.7±32.8%, 192.6±49.3%, and 324.2±67.3%) compared with normotensive control subjects (percent increments from basal: 10.3±2.4%, 28.2±5.8%, 192.3±44.3%, 558.9±77.5%, and 689.7±92.3%) (Figure 1). The response to acetylcholine was not significantly altered by indomethacin in normotensive subjects (percent increments from basal: 7.9±1.9%, 31.6±6.1%, 184.2±41.6%, 534.2±71.7%, and 671.8±88.1%), whereas the cyclooxygenase inhibitor significantly \( (p<0.01) \) increased the response to acetylcholine in essential hypertensive patients (percent increments from basal: 7.1±2.3%, 21.3±4.7%, 79.2±22.2%, 299.2±52.7%, and 602.2±78.6%) (Figure 1). After indomethacin, the vasodilator effect of the highest dose of acetylcholine was not statistically different between essential hypertensive patients and normotensive subjects. In normotensive subjects and essential hypertensive patients, indomethacin did not change basal forearm blood flow (data not shown).

In patients with primary aldosteronism, the vasodilator effect of acetylcholine was reduced \( (p<0.01) \) compared with normotensive subjects (percent increments from basal: 11.5±4.5%, 48.9±7.8%, 93.6±24.2%, 353.2±48.2%, and 482.9±67.3%) (Figure 2). The response to acetylcholine was not modified by indomethacin (percent increments from basal: 28.3±2.5%, 53.7±6.8%, 93.5±11.4%, 348.3±43.8%, and 464.1±58.8%). When the dose–response curve to acetylcholine was repeated within 1 month after surgery as soon as arterial blood pressure and plasma potassium were normalized \( (n=5) \), the vasodilator response to the drug was increased \( (p<0.01) \) (percent increments from basal: 27.9±4.1%, 58.2±6.4%, 166.3±22.7%, 690.2±76.4%, and 827.9±98.3%) and was no longer different from normotensive control subjects (Figure 2).

In patients with renovascular hypertension, the vasodilation induced by acetylcholine was significantly reduced \( (p<0.01) \) compared with normotensive subjects (percent increments from basal: 12.8±4.5%, 42.8±6.2%, 154.3±24.7%, 293.3±37.5%, and 420.4±69.6%) (Figure 2) and was not significantly modified by indomethacin (percent increments from basal: 20.6±2.4%, 32.3±4.9%, 150.3±8.5%, 233.4±28.9%, and 447.2±57.8%). Also, in hyperaldosteronism and renovascular hypertensive patients, indomethacin did not affect basal forearm blood flow (data not shown).

Sodium Nitroprusside

In all subjects, sodium nitroprusside caused a dose-dependent vasodilation that was not statistically different among the four groups (percent increments from basal: normotensive subjects: 191.8±32.7%, 353.6±53.2%, 514.6±56.9%; essential hypertensive patients: 182.6±38.3%, 326.2±49.5%, 471.7±53.9%; primary aldosteronism hypertensive patients: 258.2±54.1%, 432.6±74.3%, 543.6±88.6%; after surgery: 233.6±54.3%, 411.4±56.3%, 506.1±63.1%; renovascular hypertensive patients: 235.6±63.6%, 413.7±67.4%, 554.2±79.2%).

Discussion

In the present study, we used acetylcholine to induce endothelium-dependent vasodilation. The validity of this approach has already been demonstrated in previous in vivo and in vitro studies using animal and human blood vessels.\(^1\)\(^-\)\(^12\)\(^-\)\(^17\)\(^-\)\(^18\) The results demonstrate a re-
duced forearm vasodilation to acetylcholine in patients with secondary forms of human hypertension such as primary aldosteronism and renovascular hypertension. Moreover, in essential hypertensive patients, but not in patients with secondary hypertension, treatment with an inhibitor of cyclooxygenase can partially restore the responsiveness to acetylcholine.

The present finding of a reduced acetylcholine-mediated vasodilation in essential hypertensive patients (compared with normotensive subjects) confirms earlier observations in animals and humans. The observation that there was no difference in the vasodilator effect of sodium nitroprusside between the two groups tends to exclude a nonselective defect in responsiveness of vascular smooth muscle cells to vasodilators in essential hypertension. In particular, because sodium nitroprusside acts on the same cellular mechanism (soluble guanylate cyclase stimulation) as endothelium-derived relaxing factor (nitric oxide), the reduced responsiveness to acetylcholine in essential hypertensive patients does not appear to be a consequence of reduced responsiveness of smooth muscle cells to normal levels of relaxing factors.

In primary aldosteronism, a volume-dependent form of hypertension, vasodilatation to acetylcholine was reduced compared with normotensive subjects despite a similar vasodilation to sodium nitroprusside. Thus, these data suggest that endothelium-dependent vasodilation to acetylcholine is reduced in hypertensive patients with primary aldosteronism. This interpretation is supported by the observation that in patients with primary aldosteronism studied after surgery, when arterial blood pressure and plasma potassium had returned to normal levels, the vasodilation to acetylcholine was increased and was no longer different from that in normotensive subjects. The normalization of the response to acetylcholine cannot be attributed to structural adjustments, because the vasodilatation to sodium nitroprusside did not differ from that obtained before surgery. Taken together, these data indicate that primary aldosteronism causes a reduced endothelium-dependent vasodilation and that this endothelium defect can be corrected by an appropriate treatment. These results are in agreement with animal experiments showing a reduced endothelium-dependent relaxation to acetylcholine in the aorta of the hypertensive salt-sensitive Dahl rat, an animal model of volume-induced hypertension that is similar in some ways to that induced by primary aldosteronism in humans. In that animal model, the endothelial responsiveness was restored by antihypertensive treatment, potassium supplementation, or both.

Also, in patients with renovascular hypertension, the vasodilation to acetylcholine was reduced compared with that in normotensive subjects in the absence of a difference in the direct vasodilator effect of sodium nitroprusside. Thus, these data are in agreement with previous results obtained in mesenteric resistance arteries of two-kidney, one clip renovascular hypertensive rats. The available evidence thus suggests the existence of reduced endothelium-dependent vasodilatation in the forearm of renovascular hypertensive patients.

Another aim of the present study was to investigate whether or not the cyclooxygenase-dependent endothelium-derived contracting factor may be involved in the impairment of endothelium-dependent responsiveness in human hypertension. This possibility was based on the observation that endothelium-dependent relaxations to acetylcholine are depressed in isolated large conduit and small resistance arteries obtained from spontaneously hypertensive rats and stroke-prone spontaneously hypertensive rats, respectively, because of the concomitant release by the endothelial cells of vasoconstrictor products of cyclooxygenase, most likely prostaglandin endoperoxides. The present data show that in essential hypertensive patients, the vasodilator effect of acetylcholine was increased by treatment with indomethacin, and at the highest concentration of the cholinergic transmitter, the vasodilatation induced was not different from that observed in normotensive control subjects. These results are consistent with those obtained in the aorta from spontaneously hypertensive rats, in which cyclooxygenase inhibition restored the endothelial responsiveness at the higher concentrations of acetylcholine. By contrast, treatment with indomethacin did not affect the vasodilator action of acetylcholine either in secondary hypertensive patients or in normotensive subjects. Hence, and in agreement with previous reports in hypertensive animals, the present data suggest that the release of a cyclooxygenase-derived endothelium-derived contracting factor blunts endothelium-dependent vasodilation in human essential hypertension. However, this is not the case in secondary forms of hypertension or in normotensive subjects, a conclusion that is also in line with earlier work obtained in animal models.

In conclusion, human hypertension, whether essential or secondary to primary aldosteronism or renovascular disease, is accompanied by reduced endothelium-dependent vasodilations to acetylcholine. Thus, whatever the etiology of hypertension, a defect of the endothelium may contribute to the increase in peripheral resistance that is characteristic of the disease. In addition, a cyclooxygenase-dependent vasoconstrictor substance, possibly endothelium-derived contracting factor, contributes to the reduced endothelium-dependent responsiveness in essential hypertension but not in primary aldosteronism or renovascular hypertension. The fact that this endothelium-derived contracting factor is detectable only in essential hypertensive patients is particularly striking, because this might mean that this substance might be involved in the etiopathogenesis of essential hypertension itself.

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