Indirect Evidence for Release of Endothelium-Derived Relaxing Factor in Human Forearm Circulation In Vivo
Blunted Response in Essential Hypertension

Lilly Linder, MD, Wolfgang Kiowski, MD, Fritz R. Bühler, MD, and Thomas F. Lüscher, MD

In isolated blood vessels, acetylcholine releases endothelium-derived relaxing factor (EDRF). In vivo, the vasodilator action of acetylcholine may be mediated by EDRF, but prostacyclin or prejunctional inhibition of adrenergic neurotransmission may also be involved. Therefore, we investigated whether acetylcholine releases EDRF in humans in vivo and, if so, whether the response altered in essential hypertension. Acetylcholine was infused into the brachial artery, and forearm blood flow measured by venous occlusion plethysmography. In control subjects, acetylcholine (0.02–16 µg/min/100 ml tissue) increased flow from 2.4±5.0 to 20.6±5.2 ml/min/100 ml tissue (n=14; p<0.05) and decreased forearm vascular resistance from 42.0±4.1 to 6.0±1.4 units (p<0.03), a response comparable to that of sodium nitroprusside (0.6 µg/min/100 ml tissue). Acetylsalicylic acid (500 mg i.v.) given to block vascular prostacyclin production did not alter the response (n=14). α-Adrenoceptor blockade by phentolamine (12 µg/min/100 ml tissue) did not prevent the increase in flow evoked by acetylcholine. In hypertensive patients, the decrease in forearm vascular resistance induced by acetylcholine but not evoked by sodium nitroprusside was reduced as compared with controls (14.5±3.1 and 6.1±1.6 units, respectively; n=8; p<0.05). Thus, the vascular effects of acetylcholine in the human forearm circulation are independent of prostaglandins and adrenergic neurotransmission and therefore are most likely to be mediated by EDRF; the acetylcholine-induced release of EDRF is blunted in patients with essential hypertension. (Circulation 1990;81:1762–1767)

Acetylcholine is a vasodilator substance that is released from cholinergic nerves and possibly from some endothelial cells. Until recently, it was assumed that the muscarinic agonist causes vasodilatation via prejunctional inhibition of adrenergic neurotransmission. In 1980, Furchgott and Zawadzki demonstrated that endothelial cells release a potent and labile vasodilator substance (i.e., endothelium-derived relaxing factor [EDRF]) after stimulation with acetylcholine. In isolated arteries, the endothelium-dependent effects of acetylcholine fully account for its vasodilator action. In addition, acetylcholine can release prostacyclin from the blood vessel wall. Thus, three possible mechanisms could mediate the vascular action of acetylcholine in vivo: an inhibitory effect on noradrenaline release from sympathetic nerve endings, release of EDRF from endothelial cells, and stimulation of the production of prostacyclin from the blood vessel wall.

The present study was designed to investigate the possible mechanism involved in the vasodilator action of acetylcholine in the human forearm in vivo. In addition, the vascular effects of acetylcholine in normotensive subjects and in patients with uncomplicated essential hypertension were compared to delineate whether the response is altered in hypertension—as it is in isolated arteries of hypertensive animals.

Methods

The study sample consisted of 20 healthy volunteers (12 men and eight women; age, 20–73 years; mean, 34 years) with casual diastolic blood pressures of less than 90 mm Hg in the sitting position (Korotkoff phase V) and 14 outpatients (10 men and four women; age, 21–72 years; mean, 43 years) with

From the Department of Medicine, Division of Cardiology (Hypertension Service), and the Department of Research, University Hospital, Basel, Switzerland.

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Address for correspondence: Thomas F. Lüscher, MD; Division of Cardiology, University Hospital, CH-4031 Basel, Switzerland.

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uncomplicated essential hypertension (casual diastolic blood pressure, >95 mm Hg). Patients took no medication for at least 4 weeks before the study. All subjects had refrained from smoking and from drinking caffeine-containing beverages for 8 hours before the start of investigations. The study protocol was approved by the hospital ethical committee on the use of human subjects in clinical investigations, and informed consent was obtained from all participants.

Forearm Blood Flow Measurements

Forearm blood flow was measured bilaterally by venous occlusion plethysmography.\textsuperscript{18-22} A mercury-in-silastic strain gauge was placed at the upper third of the forearm, which rested comfortably on a support slightly above the level of the heart. The strain gauge was coupled to an electronically calibrated plethysmograph (model EC3, Hokanson).\textsuperscript{23} Venous occlusion was achieved by a blood pressure cuff applied proximal to the elbow and inflated to 40 mm Hg by a rapid cuff inflator (model EC10, Hokanson). The hand was excluded from the circulation by inflating a pediatric blood pressure cuff placed around the wrist to 50 mm Hg above systolic pressure 1 minute before and during the forearm blood flow measurement to eliminate the unpredictable influence of arteriovenous shunts in the hand. Experiments were done on the left (experimental) forearm while blood flow measurements on the right (control) arm served as continuous controls. Determinations of forearm blood flow were made by analyzing at least seven consecutive flow curve recordings, the mean value being taken for statistical evaluation. Forearm vascular resistance (FVR) was calculated by dividing mean arterial pressure by forearm blood flow. The electrocardiogram was monitored throughout the study.

Study Protocol

The study began at 8:00 AM and continued for approximately 4–5 hours. The subjects were supine in a quiet, air-conditioned room maintained at a constant temperature (20–22°C). The forearm volume was measured by water displacement using the Archimedes principle, and drug doses were adjusted accordingly. Under local anesthesia (lidocaine 1%), an 18-gauge catheter (Abbocath-T, Abbott, Sligo, Ireland) was inserted into the left brachial artery for regional infusion of acetylcholine, sodium nitroprusside, and phenolamine and for the recording of arterial pressure with a Statham P23 Pb pressure transducer. The subjects were allowed to rest for 30 minutes after completion of instrumentation. Basal forearm blood flow and intra-arterial blood pressure were recorded, and sodium nitroprusside was infused at a concentration of 0.6 μg/min/100 ml forearm tissue for 2 minutes. In previous studies, this dose of intra-arterial sodium nitroprusside was found to produce a maximal vasodilator response in the forearm without systemic hemodynamic effects.\textsuperscript{20-22} Approximately 30 minutes later, forearm blood flow returned to basal values. Subsequently, acetylcholine was infused in 11 increasing dosages (0.02–16 μg/min/100 ml forearm tissue) for 2–3 minutes with a constant-rate infusion pump (Sage Instruments Inc, New York). In eight normotensive subjects and six patients with essential hypertension, the infused volumes varied between 0.2 and 3.0 ml/min. For comparison of the eight normotensive and eight essential hypertensive, the volumes were held constant at 1 ml/min. After the dose-response curve to acetylcholine was completed, 500 mg acetylsalicylic acid (Aspégic) was infused intravenously during 20 minutes to inhibit the vascular production of prostacyclin.\textsuperscript{4,10,14,24} Approximately 40 minutes later, forearm blood flow returned to basal values, and the dose-response curve to acetylcholine was repeated. In a subgroup of normotensive subjects, acetylcholine was infused in four increasing dosages (0.3, 1, 4, and 16 μg/min/100 ml forearm tissue). After forearm blood flow had returned to basal values, the nonselective postjunctional α-adrenergic receptor antagonist phenolamine was infused at the constant rate of 12 μg/min/100 ml forearm tissue alone and with acetylcholine (dose range, 0.3–16 μg/min/100 ml forearm tissue).\textsuperscript{25,26} Results are expressed as mean ± SEM unless otherwise indicated. The \textit{t} test for paired or unpaired observations was used for statistical analysis. A two-tailed \textit{p} value of less than 0.05 was considered statistically significant.

Results

Vascular Effects of Acetylcholine

In normotensive subjects, stepwise increasing intra-arterial dosages of acetylcholine (from 0.02 to 16 μg/min/100 ml forearm tissue) augmented forearm blood flow from 2.4±0.5 to 20.6±5.2 ml/min/100 ml forearm tissue (\textit{n}=14; \textit{p}<0.05) (Figure 1). Intra-arterial blood pressure remained unchanged, and FVR decreased from 42.0±4.1 to 6.0±1.4 units
response curve to the muscarinic agonist was performed in four healthy volunteers before and after blockade of α-adrenergic receptors by phentolamine. Infusions of 0.3, 1, 4, and 16 μg/min/100 ml forearm tissue acetylcholine were applied alone and in combination with 12 μg/min/100 ml forearm tissue phentolamine in the same subjects. Phentolamine alone resulted in increase in forearm blood flow from 2.7±0.7 to 5.6±0.4 ml/min/100 ml forearm tissue (p<0.005). After intra-arterial infusion of acetylcholine alone, blood flow increased from 2.7±0.3 to 28.3±10.1 ml/min/100 ml forearm tissue (p<0.05). During simultaneous infusion of phentolamine and acetylcholine, forearm blood flow increased from 5.6±0.2 to 53.1±10.5 ml/min/100 ml forearm tissue (p<0.02). Thus, adrenergic blockade did not prevent but instead augmented the vasodilator response to the muscarinic agonist. Accordingly, the percent change of blood flow (expressed as percentage of the corresponding maximal forearm blood flow) and the percent change of resistance did not differ significantly between the two infusion series (Figure 4).

Vascular Effects of Acetylcholine in Hypertensive Subjects

Intra-arterial mean blood pressure averaged 83±3 mm Hg in normotensive subjects and 119±6 mm Hg in hypertensive patients and remained unchanged during the study (n=8, each group; p<0.001). Similarly, during the duration of the study, forearm blood flow did not change significantly in the control arm (data not shown). Intra-arterial infusions of increasing doses of acetylcholine (0.02–16 μg/min/100 ml forearm tissue) induced dose-dependent increases in forearm blood flow from 2.5±0.8 to 24.1±6.6 ml/min/100 ml forearm tissue (p<0.01) in the normotensive and from 3.1±0.3 to 13.7±3.8 ml/min/100 ml forearm tissue (p<0.02) in the hypertensive subjects. Accordingly, there was a dose-dependent

(n=14; p<0.03). The percent change of FVR (expressed as percentage of basal FVR) increased from 32.5±5.1 at the lowest dose of acetylcholine to 84.3±3.7 at the highest dose (p<0.001).

Acetylcholine and Vascular Prostacyclin Production

No significant difference in the forearm hemodynamic response to acetylcholine were found before and after intravenous application of 0.5 g acetylsalicylic acid (Aspéig) to inhibit the formation of vascular prostaglandins. Forearm blood flow increased from 3.2±0.6 to 23.5±5.7 ml/min/100 ml forearm tissue, similar to control conditions (n=14; p<0.05). FVR decreased from 34.4±4.0 to 6.1±1.4 units (n=14; p<0.02), and percent change of FVR increased from 20.2±3.8% to 83.2±3.2% (n=14; p<0.02) (Figure 2).

Acetylcholine and Sodium Nitroprusside

The increase in forearm blood flow and the decrease in vascular resistance evoked by a maximal dose of acetylcholine (16 μg/min/100 ml forearm tissue) were comparable to that induced by sodium nitroprusside (0.6 μg/min/100 ml forearm tissue) in the same subjects. Forearm blood flow after infusion of the maximal dose of acetylcholine and sodium nitroprusside was 20.6±5.2 and 13.2±1.8 ml/min/100 ml forearm tissue, (n=14; NS). Accordingly, FVR during infusion of the highest dose of acetylcholine (6.3±1.3 units) and during infusion of sodium nitroprusside (7.2±1.0 units) did not differ statistically (Figure 3).

Acetylcholine and α-Adrenergic Vasoconstriction

To evaluate a possible contribution of prejunctional inhibition of adrenergic neurotransmission to the vasodilator response of acetylcholine, a dose-
FIGURE 4. Bar graphs of changes in forearm blood flow in response to intra-arterially applied acetylcholine under control conditions (□) and after infusion of phentolamine (■) in the forearm circulation of normal subjects. Note the comparable percent increase in forearm blood flow in the presence and absence of phentolamine to block the vascular effects of the sympathetic nervous system. These experiments exclude a major contribution of prejunctional muscarinic inhibition of sympathetic neurotransmission to the vasodilator effects of acetylcholine in vivo.

decrease in vascular resistance from 45.3±6.6 to 6.1±1.6 units (p<0.001) in normotensive subjects and from 41.4±4.3 to 14.5±3.1 units (p<0.001) in hypertensive patients. The absolute change in forearm vascular resistance evoked by higher dosages of acetylcholine were less pronounced in hypertensive patients than in control subjects (n=8, each group; p<0.05) (Figure 5). In contrast, there was no significant difference between the two groups in response to sodium nitro-

FIGURE 5. Bar graph of changes in forearm vascular resistance evoked by the endothelium-dependent vasodilator acetylcholine and the endothelium-independent vasodilator sodium nitroprusside in normotensive subjects (□) and in patients with essential hypertension (■). Note there was an impaired response to acetylcholine but not to sodium nitroprusside in the hypertensive patients (*statistical significance, p<0.05).

FIGURE 6. Schematic of possible mechanisms of the vasodilator effects of acetylcholine in vivo. α1, α1-adrenergic receptor; Ach, acetylcholine; cAMP/cGMP, cyclic adenosine/guanosine monophosphate; EDRF, endothelium-derived relaxing factor; M, muscarinic receptor; NE, norepinephrine; PGI2, prostacyclin; −, inhibition of NE; \wedge \wedge, inhibition of production of PGI2.

Discussion

The present study provides indirect evidence for the release of EDRF in response to acetylcholine in resistance arteries of the human forearm circulation and demonstrates that this response is reduced in patients with uncomplicated essential hypertension.

That EDRF mediates the vasodilator actions of intra-arterially infused acetylcholine is based on the following observations (Figure 6).

First, intravenous infusion of acetylsalicylic acid to inhibit the production of vascular prostaglandins4,6–11,24 did not affect the increase in forearm blood flow observed during the infusion of the muscarinic agonist. Thus, a significant contribution of vascular prostaglandins to the vasodilator actions of acetylcholine in the human forearm in vivo can be excluded. Similar conclusions have been reached in isolated blood vessels of experimental animals and of humans.4–11

Second, an inhibition of adrenergic neurotransmission by acetylcholine-activating prejunctional muscarinic receptors has long been considered the main mechanism mediating the vascular effects of the
muscarinic agonist. To exclude a contribution of prejunctional effects of acetylcholine, we compared the vasodilator action of the cholinergic neurotransmitter in the presence and absence of phentolamine to block postjunctional α-adrenergic receptors. Phentolamine alone increased blood flow, indicating effective blockade of α-adrenergic receptors in the forearm circulation. Previous studies have shown that under these conditions, the effects of exogenous norepinephrine are inhibited. Thus, the effects of neurally released norepinephrine should be excluded. However, the vascular effects of acetylcholine were not prevented but instead augmented, leading to a comparable relative increase in forearm blood flow like under control conditions. Therefore, this suggests that the vascular effects of acetylcholine cannot be attributed to an inhibitory action on sympathetic neurons or to a stimulation of vascular prostacyclin production. Thus, it is likely that—in isolated human blood vessels obtained from the same vascular bed—intra-arterially applied acetylcholine activates muscarinic receptors on endothelial cells and evokes the release of EDRF. Indeed, in isolated mesenteric resistance arteries of the rat and in human subcutaneous resistance vessels, the relaxations evoked by acetylcholine are mediated by EDRF.

Finally, in line with this interpretation, the potency of the response to acetylcholine was comparable to that induced by sodium nitroprusside. Sodium nitroprusside, like EDRF, evokes vascular relaxation by increasing cyclic guanosine-3’5’-monophosphate (cGMP) in vascular smooth muscle. Thus, this study provides indirect evidence for the release of EDRF in resistance arteries of the human forearm circulation in vivo. Hence, EDRF may not only modulate vascular tone of large conduit arteries and veins in vivo but may also contribute to the regulation of resistance arteries where local blood flow is regulated. That inhibition of the L-arginine pathway increases blood pressure in experimental animals and decreases local blood flow in humans is consistent with that interpretation.

In experimental animals, chronic hypertension is associated with attenuated endothelium-dependent relaxations to acetylcholine. The impairment of endothelial function occurring in hypertension is closely correlated with the degree of hypertension and can be reversed by antihypertensive therapy. We have compared the vasodilator action of acetylcholine in normal subjects and in patients with uncomplicated essential hypertension. In the hypertensive group, the reduction in FVR in response to acetylcholine was significantly less pronounced than that of normotensive control subjects. In contrast, the maximal response to sodium nitroprusside did not differ between the two groups, suggesting that an altered endothelial release of EDRF rather than a decreased responsiveness of vascular smooth muscle cells to the factor is involved. As in experimental hypertension, the difference between normotensives and hypertensives was particularly pronounced at higher concentrations of acetylcholine.

That alterations in endothelial function occur in the forearm of patients with uncomplicated essential hypertension is particularly striking because this part of the circulation rarely develops hypertensive vascular disease. Thus, vascular beds such as the coronary, cerebral, renal, and limb circulation, which are known targets of hypertension, may exhibit much more pronounced changes in endothelial function. Indeed, in isolated human coronary arteries obtained from patients with coronary artery disease, the release of EDRF is severely reduced.

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L Linder, W Kiowski, F R Bühler and T F Lüscher

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