Bradykinin-Induced Vasodilation of Human Forearm Resistance Vessels Is Primarily Mediated by Endothelium-Dependent Hyperpolarization

Marina L.H. Honing, Paul Smits, Paul J. Morrison, Ton J. Rabelink

Abstract—Bradykinin (BK) stimulates endothelial cells to release a number of relaxing factors, such as NO, prostaglandins (PGs), and an endothelium-derived hyperpolarizing factor (EDHF). However, the contributions of NO, PG, and EDHF in the vascular relaxation to BK vary with species and anatomic origin of blood vessels used. Therefore, the present study was designed to investigate the contributions of NO, PG, and EDHF in vasodilation caused by BK in human forearm resistance vessels. Forearm blood flow (FBF) was recorded with venous occlusion plethysmography in healthy nonsmoking subjects. At first, studies were performed to validate the NO clamp technique for its ability to inhibit endogenous NO generation. Brachial artery infusion of serotonin (0.6, 1.8, and 6 ng · 100 mL forearm volume [FAV]⁻¹ · min⁻¹) caused significant forearm vasodilation (2.6 to 4.6 mL · 100 mL FAV⁻¹ · min⁻¹), which is known to be NO mediated. Indeed, during the NO clamp, cumulative doses of serotonin caused no vasodilation (2.4 to 2.6 mL · 100 mL FAV⁻¹ · min⁻¹), indicating that the generation of endogenous NO was completely blocked. Thereafter, the vasodilative actions of BK were investigated. Brachial artery infusion of BK (50, 100, and 200 ng · 100 mL FAV⁻¹ · min⁻¹) caused significant forearm vasodilation in all studies (from 3.1 to 20.4 mL · 100 mL FAV⁻¹ · min⁻¹). After the inhibition of cyclooxygenase and NO synthase activity through the use of aspirin and the NO-clamp technique, BK increased FBF in a similar manner (3.9 to 18.9 mL · 100 mL FAV⁻¹ · min⁻¹), indicating that the vasodilative actions of BK are independent of NO and PG generation. However, vasodilation caused by the 2 lower doses of BK were significantly attenuated after K⁺ channel activity was blocked with tetraethylammonium chloride (0.1 mg · 100 mL FAV⁻¹ · min⁻¹), suggesting that in the lower dose range, BK mediates vasodilation through the opening of vascular potassium channels. In conclusion, BK is a potent vasodilator peptide in human forearm resistance vessels, causing vasodilation through hyperpolarization of the vascular wall independent of NO and PG production. In addition, the NO-clamp technique is a valid instrument to investigate the contribution of NO in the vasodilative response to different agents. (Hypertension. 2000;35:1314-1318.)

Key Words: bradykinin ■ hyperpolarization ■ tetraethylammonium chloride ■ nitric oxide ■ prostaglandins

Bradykinin (BK) is one of the most potent vasodilator substances known. Several studies have demonstrated with the use of animal or human arteries that BK stimulates endothelial cells to release a number of relaxing factors, such as NO, prostaglandins (PGs), and an endothelium-derived hyperpolarizing factor (EDHF). However, the contributions of NO, PG, and EDHF to the vascular relaxation to BK vary with species and anatomic origin of the blood vessels used.

In vitro studies performed on isolated blood vessels demonstrate that the stimulation of NO release by acetylcholine is more prominent in larger arteries. In contrast, the contribution of EDHF to endothelium-dependent relaxation by BK is significant larger in microvessels. These findings become relevant when interpreting the results of in vivo studies that measure hemodynamic parameters such as blood pressure and vascular resistance. For example, M ü gge et al investigated in vivo the response to acetylcholine in the perfused rabbit hindlimb, demonstrating that the inhibition of NO synthesis had no effect on acetylcholine-induced decrease in vascular resistance. Therefore, the rabbits were killed, and the femoral artery was cut into rings and suspended in organ bath chambers for isometric tension recordings. Interestingly, in vitro, the femoral artery of the same rabbit showed a reduced vasodilation in response to acetylcholine after the production of NO was blocked. These results indicate that acetylcholine stimulates the release of NO in larger arteries but that the clinically significant decrease in vascular resistance, due to dilation of resistance arteries, is caused by a different potent relaxing factor.
When BK is injected intravenously into mammals, it causes a rapid fall of blood pressure due to arteriolar vasodilation. These hemodynamic effects are currently attributed to an enhanced production of NO and possibly some release of PG and EDHF. However, the importance and contribution of EDHF in the hemodynamic changes caused by BK still remain unclear. For example, Kato et al demonstrated a less important role for NO in the vasodilation of coronary resistance arteries compared with epicardial vessels. Indeed, Nakashima et al demonstrated with electrophysiological data that in the human coronary artery, EDHF is an important contributor to the endothelium-dependent relaxations in response to BK. Therefore, the present study was designed to investigate the contributions of NO, PG, and EDHF in vasodilation caused by BK in human forearm resistance vessels.

Methods

Thirty-two healthy, nonsmoking subjects ranging in age from 18 to 45 years participated in 3 studies. The local ethics committee approved all studies, and written informed consent was obtained from the volunteers before the start of any investigation. None of the subjects received vasoactive or nonsteroidal anti-inflammatory drugs in the week before start of the study. Alcohol and all caffeine-containing beverages were withheld for 12 hours before the study. All studies were performed in a quiet room kept at a constant temperature between 22°C and 24°C. The subjects were supine with both forearms resting slightly above heart level.

The brachial artery of the nondominant arm was cannulated with a 20-gauge catheter after local anesthesia of the skin with 2% lidocaine (Astra Pharmaceuticals Ltd). Forearm blood flow (FBF) was measured simultaneously in both arms with venous occlusion plethysmography with the use of calibrated mercury-in-Silastic strain gauges applied to the forearm (Hokanson EC-4). A microcomputer-based R wave–triggered system for online, semicontinuous monitoring was used. During the experiments, upper arm cuffs were intermittently inflated to 40 mm Hg for 4 heartbeats every 15 seconds to prevent venous outflow from the forearm. Wrist cuffs were inflated 40 mm Hg above the actual systolic pressure to exclude the hands from the circulation. FBF measurements were recorded during a 2-minute period at 5-minute intervals. Intra-arterial blood pressure was continuously monitored. Saline (0.9%; Baxter Healthcare Ltd) was infused for ≥30 minutes, until FBF had stabilized, before the administration of drugs. Drugs and peptides, with the exception of aspirin, were dissolved in physiological saline and infused intra-arterially at locally active doses. The infusion rate was kept constant at 90 mL/h. All solutions were prepared aseptically from sterile stock solutions or ampules on the day of the experiment and stored at 4°C until use. On the day of use, tetraethylammonium chloride (TEA) was reconstituted from a sterile stock powder, diluted in 0.9% NaCl to a concentration of 1 mg/mL, and passed through a 0.22-μm Millipore filter.

Study 1: Effects of Inhibition of Endogenous NO Generation, With the NO Clamp Technique, on Endothelium-Dependent and -Independent Vasodilation

To confirm that the NO clamp technique is a valid instrument to investigate the contribution of NO to the vasodilator response to BK (ie, that the technique effectively inhibits generation of endogenous NO), we performed 2 separate experiments in a total of 16 healthy volunteers.

In the first group, venous occlusion plethysmography of the forearm was performed in 8 subjects. Serotonin (5-hydroxytryptamine [5-HT]; 0.6, 1.8, and 6.0 ng · 100 mL forearm volume [FAV]−1 · min−1; Sigma Chemical Co) was infused into the brachial artery under 2 different conditions: with an intact NO system and with a clamped NO system. The NO clamp involves the stimulation of normal basal NO activity during continuous inhibition of endogenous NO synthesis. This technique can be used to investigate the vasoactive mechanisms of different agents and to demonstrate whether vasodilation to a particular agent is NO mediated. The NO clamp is achieved by infusing Nω-mono-L-arginine (L-NMMA; 200 μg · 100 mL FAV−1 · min−1; Institut für Pharmazie, Universität Leipzig, Germany), a competitive inhibitor of NO synthase, throughout the experiment. After 10 minutes of L-NMMA infusion, the vasoconstriction by L-NMMA was subsequently counteracted by concurrent infusion of ascending doses of sodium nitroprusside (SNP; 30 to 180 ng · 100 mL FAV−1 · min−1) until blood flow had returned to baseline values. Return to baseline is a very important aspect because only when FBF is completely returned to baseline can it be compared with the situation before initiation of the NO clamp. Thereafter, L-NMMA and SNP are infused at constant rates for the remainder of the study.

In a second group of 8 healthy subjects, SNP (6, 60, 180, and 600 ng · 100 mL FAV−1 · min−1; Menek) was infused into the brachial artery, again first in an intact NO system and second in a clamped NO system. This experiment allowed us to investigate a possible interaction of the NO clamp with endothelium-independent vasodilation.

In a previous study, we demonstrated that over time, the NO clamp was stable, keeping baseline FBF constant during the experiment.

Study 2: Role of NO and PGs in BK-Mediated Vasodilation

Venous occlusion plethysmography of the forearm was performed in 8 subjects. BK was infused into the brachial artery in increasing dosages of 50, 100, and 200 ng · 100 mL FAV−1 · min−1 (Clinalfa). Subsequently, BK infusion was repeated during inhibition of the endogenous NO system in the forearm by use of the NO clamp, as described previously.

To block the generation of vasoactive prostaglandins and thromboxanes, 600 mg carbasa late calcium (Dagra Pharma BV) was administered orally 30 minutes before the start of the measurements. Previously, 600 mg carbasa late calcium has been shown to completely block cyclooxygenase activity by at least 85%, with recovery occurring during the next 6 hours.

Study 3: Role of Hyperpolarization in Vasodilation Caused by BK

Venous occlusion plethysmography of the forearm was performed in a total of 8 subjects. The dose-response curves to BK (50, 100, and 200 ng · 100 mL FAV−1 · min−1) were measured alone and after inhibition of large conductance Ca2+-dependent potassium channels with TEA (0.1 mg · 100 mL FAV−1 · min−1). Because charybdotoxin and iberiotoxin, the 2 most selective blockers of the calcium-dependent potassium channels, are too toxic for human application, we chose TEA to investigate the role of KCa channel activation on the vascular effects of BK. TEA antagonizes different types of potassium channels with varying degrees of potency. However, TEA has been shown to selectively block single KCa channels in arterial smooth muscle cells at concentrations of <1 mmol/L. We administered TEA intra-arterially at an infusion rate of 0.1 mg · 100 mL FAV−1 · min−1, which correlates with a calculated local plasma concentration of 0.5 mmol/L.

Before BK infusion, TEA was infused for 30 minutes into the brachial artery to investigate whether inhibition of hyperpolarization with TEA influenced basal FBF. Subsequently, cumulative doses of BK were coinfused with TEA.

Statistical Analysis

FBF is expressed as mL · 100 mL FAV−1 · min−1. The final 6 blood flow recordings of each infusion step from both measurement and control arms were used to calculate the mean FBF. Recordings made in the first 30 seconds after wrist-cuff inflation were not used for
The vasoconstriction that was counteracted with incremental doses of SNP until baseline FBF was restored (without NO clamp 3.1±0.4, with NO clamp 3.9±0.9). Baseline FBF was kept constant for ≥20 minutes until infusion of BK was started. After the inhibition of cyclooxygenase and NO synthase activity, BK increased FBF in a similar manner (from 3.9±0.9 to 18.9±2.0), indicating that the increase in blood flow is not dependent on NO and PG production (Figure 3). After infusion of the highest dosage of BK (200 ng · 100 mL FAV⁻¹ · min⁻¹) was stopped, the infusion of L-NMMA and SNP (NO clamp) was continued until baseline FBF was restored (from 3.9±0.9 to 18.9±2.0 to 2.7±0.3), demonstrating that the NO clamp remained stable during BK infusion and did not influence or augment vasodilation to BK.

Study 3: Effects of Potassium Channel Inhibition on Vasodilation Caused by BK
Under normal basal conditions, the infusion of incremental doses of BK caused a significant increase in FBF (from 3.8±0.6 to 20.2±2.0). Baseline FBF was not significantly affected after a 30-minute infusion of TEA (from 3.2±0.5 to 2.6±0.6). However, the 2 lowest dosages of BK (50 and 100 ng · 100 mL FAV⁻¹ · min⁻¹) were significantly attenuated in...
the presence of TEA, suggesting that vasodilation caused by low-dose BK is mediated by the opening of vascular potassium channels (50 ng · 100 mL FAV⁻¹ · min⁻¹; without TEA 17.5 ± 2.6; with TEA 6.5 ± 1.9; and 100 ng · 100 mL FAV⁻¹ · min⁻¹; without TEA 19.7 ± 1.8, with TEA 13.2 ± 2.8; both P < 0.05). The highest dosage of BK (200 ng · 100 mL FAV⁻¹ · min⁻¹) was not affected by TEA (without TEA 20.2 ± 2.0, with TEA 19.7 ± 3.0; Figure 4).

In control experiments, we found that vasodilation induced by SNP was not inhibited by TEA (Paul Smits, unpublished data, 1999), indicating that TEA has no inhibitory effect on endothelium-independent vasodilation.

Discussion

We demonstrated that BK caused a significant vasodilation in resistance vessels of the human forearm. These vasodilator actions of BK seem to be primarily mediated by opening of calcium-dependent potassium channels and are largely independent of endothelium-derived NO and PG.

Our findings are in agreement with previous in vivo studies that demonstrate endothelium-derived relaxing factors other than NO may mediate BK-induced vasodilation in certain vascular resistance beds, such as the coronary circulation. Our data do not exclude that BK stimulates the release of NO from the forearm microvascular bed is representative of the effects of BK on coronary microcirculation.

However, our findings are in contrast with 2 in vivo studies that demonstrate BK does indeed stimulate the release of NO in the forearm resistance vessels. However, this argument is unlikely because to obtain the NO clamp, the infusion of L-NMMA caused a significant vasoconstriction in the forearm resistance vessels, providing evidence that the tonic release of NO from the forearm vasculature to maintain resting basal vascular tone is inhibited. Furthermore, previous studies have demonstrated that 5-HT–induced vasodilation is entirely NO mediated and coinfusion of 5-HT during the NO clamp in the present study completely abolished the 5-HT–induced vasodilation, suggesting optimal blockade of stimulated NO generation.

In addition, our findings are in contrast with the results of many in vitro organ chamber experiments that demonstrate BK is indeed able to stimulate the release of NO from endothelial cells. A possible explanation could be that in vitro organ chamber experiments cannot be extrapolated to in vivo hemodynamics, as demonstrated by Mügge et al. Organ chamber experiments record changes in isometric tension of small rings of isolated blood vessels. In contrast, venous occlusion plethysmography measures changes in blood flow caused by a reduction in peripheral resistance in the smaller arterioles of the forearm vessels. Therefore, our data do not exclude that BK stimulates the release of NO in the larger arteries of the forearm.

We were able to inhibit the increase in blood flow to respond to lower doses of BK with the calcium-dependent potassium channel inhibitor TEA, indicating that the initial increase in FBF is caused by hyperpolarization of the vascular wall. Our findings are in agreement with studies performed by Urakami-Harasawa et al. and Nagao et al. that demonstrated smaller peripheral arteries are more dependent on hyperpolarization than on NO. In contrast, vasodilation caused by the highest dose of BK could not be abolished after the production of NO and PG was blocked and KCa channel activation was inhibited, indicating that other relaxing factors are released by high doses of BK. Indeed, studies have demonstrated that BK stimulates the release of many mediators, such as histamine, leukotrienes, metabolites of arachidonic acid, and epoxyeicosanoids. The release of these mediators could be the result of direct receptor-induced stimulation of mast cells by BK, which are present in the adventitial layer of the vessel wall, or indirect non–receptor-mediated stimulation of the vessel wall and surrounding tissues. In addition, direct vascular smooth muscle cell stimulation by BK should be considered because studies have demonstrated the presence of BK receptors on smooth muscle cells. However, organ chamber experiments have demonstrated that the removal of the endothelial layer from arteries resulted in a total abolishment of vasorelaxation to BK, indicating that the contribution of BK receptors on vascular smooth muscle cells in the total amount of vasodilation caused by BK can be neglected. Also, we used TEA, which is an inhibitor of large conductance calcium-dependent potassium channels, leaving other known potassium channels unaffected to interact with other possible mediators.

The clinical relevance of our presented data pertains to the actions of ACE inhibitors. At the present, it is accepted that the hypotensive and antiproliferative actions of ACE inhibitors are due not only to decreased production of angiotensin II but also to decreased degradation of BK with consequent enhanced production of NO. In the present study, we demonstrated that the vasodilative effects of BK in human
resistance vessels are less dependent on NO and are caused by hyperpolarization of the vessel wall.

References
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