Endothelium-Derived Hyperpolarizing Factor Mediates Bradykinin-Stimulated Tissue Plasminogen Activator Release in Humans

Ayaz M. Rahman, *Emory University*
Jonathan Murrow, *Emory University*
Muhiddin A. Ozkor, *Emory University*
Nino Kavtaradze, *Emory University*
Ji Lin, *Emory University*
Christine De Staercke, *Ctr Dis Control & Prevent*
W. Craig Hooper, *Ctr Dis Control & Prevent*
Amita Manatunga, *Emory University*
Salim Hayek, *Emory University*
Arshed Quyyumi, *Emory University*

Journal Title: Journal of Vascular Research

Volume: Volume 51, Number 3

Publisher: Karger Publishers | 2014-01-01, Pages 200-208

Type of Work: Article | Post-print: After Peer Review

Publisher DOI: 10.1159/000362666

Permanent URL: https://pid.emory.edu/ark:/25593/txcxj

Final published version: http://dx.doi.org/10.1159/000362666

HHS Public Access

Author manuscript

J Vasc Res. Author manuscript; available in PMC 2015 June 04.

Published in final edited form as:

J Vasc Res. 2014 ; 51(3): 200–208. doi:10.1159/000362666.

Endothelium-Derived Hyperpolarizing Factor Mediates Bradykinin Stimulated Tissue Plasminogen Activator Release In Humans

Ayaz M. Rahman, **Jonathan R. Murrow**, **Muhiddin A. Ozkor**, **Nino Kavtaradze**, **Ji Lin**[*], **Christine De Staercke**[†], **W. Craig Hooper**[†], **Amita Manatunga**[*], **Salim Hayek**, and **Arshed A. Quyyumi**

Division of Cardiology, Department of Medicine, Emory University School of Medicine, Atlanta, GA

[*]Rollins School of Public Health, Emory University, Atlanta, GA

[†]Centers for Disease Control and Prevention, National Center on Birth Defects and Developmental Disabilities, Division of Blood Disorders, Atlanta, GA.

Abstract

Aims—Bradykinin stimulates tissue plasminogen activator (t-PA) release from human endothelium. Although bradykinin stimulates both nitric oxide and endothelium-derived hyperpolarizing factor (EDHF) release, the role of EDHF in t-PA release remains unexplored. This study sought to determine the mechanisms of bradykinin-stimulated t-PA release in the forearm vasculature of healthy human subjects.

Methods—In 33 healthy subjects (age 40.3±1.9 years) forearm blood flow (FBF) and t-PA release were measured at rest, and after intra-arterial infusions of bradykinin (400ng/min) and sodium nitroprusside (3.2 mg/min). Measurements were repeated after intra-arterial infusion of TEA (1 μmol/min), fluconazole (0.4 $\mu mol \cdot min^{-1} \cdot L^{-1}$), and N^G-monomethyl-L-arginine (L-NMMA, 8 μmol/min) to block nitric oxide, and their combination in separate studies.

Results—Bradykinin significantly increased net t-PA release across the forearm (P<0.0001). Fluconazole attenuated both bradykinin-mediated vasodilation (-23.3±2.7% FBF, P<0.0001) and t-PA release (from 50.9±9.0 to 21.3±8.9 ng/min/100ml, P=0.02). TEA attenuated FBF (-14.7±3.2%, P=0.002) and abolished bradykinin-stimulated t-PA release (from 22.9+5.7 to - 0.8±3.6 ng/min/100ml, P=0.0002). L-NMMA attenuated FBF (P<0.0001), but did not inhibit bradykinin-induced t-PA release (P=NS).

Corresponding Author: Arshed A. Quyyumi, MD, FRCP Professor of Medicine Emory University School of Medicine 1462 Clifton Rd. NE, Suite F506 Atlanta, GA 30322 Tel: (404) 727-3655 Fax: (404) 712-8785 aquyyum@emory.edu.

Disclosures: none

Clinical Trial Registration:
http://clinicaltrials.gov/ct2/show/NCT00166166?term=quyyumi&rank=6
ClincalTrials.gov Identifier: NCT00166166

Conclusion—Bradykinin-stimulated t-PA release is partly due to cytochrome P450-derived epoxides, and is inhibited by K^+_{ca} channel blockade. Thus, bradykinin stimulates both EDHF-dependent vasodilation and t-PA release.

Keywords

endothelium; endothelium-derived hyperpolarizing factors; fibrinolysis; tissue plasminogen activator; bradykinin

INTRODUCTION

The endogenous fibrinolytic system is a crucial component of blood flow regulation and protects against intravascular thrombosis. Impairment of endogenous fibrinolysis characterized by inhibition of tissue plasminogen activator (tPA) release is thought to be an underlying factor in endothelial dysfunction and atherothrombosis.[1,2] The endothelium regulates vasodilator tone and fibrinolytic capacity by releasing vasoactive substances including nitric oxide (NO), prostacyclin, and endothelium-derived hyperpolarizing factor (EDHF).[3, 4] Bradykinin is an endogenous cardioprotective vasoactive polypeptide that stimulates the release of these molecules and t-PA from the endothelium.[5-7] t-PA is synthesized in the endothelial cell, stored in small dense granules and released in a dose-dependent manner in response to bradykinin.[6, 8] t- PA converts plasminogen to a proteolytic enzyme, plasmin, which digests fibrin-dependent thrombi.[9] Although it is known that bradykinin causes vasodilation via activation of the B_2 receptor and subsequent release of NO, prostacyclin, EDHF, and t-PA, the downstream mechanisms governing t-PA release have not been fully elucidated.[5, 10, 11]

There is mounting pre-clinical evidence to suggest that t-PA release is mediated by EDHF.[12, 13] Persistent vasodilation after inhibition of NO and cyclooxygenase can be largely attributed to hyperpolarization by EDHF.[3, 14] The hallmark of hyperpolarization is activation of endothelial and/or smooth muscle cell calcium-activated potassium (K^+_{ca}) channels and their inhibition by apamin and charybdotoxin in pre-clinical studies.[3, 15] In human studies, tetraethylammonium chloride (TEA) antagonizes K^+_{ca} channels, thus facilitating characterization of EDHF-dependent responses.[16, 17] Endothelium-dependent hyperpolarization may also be partly due to the release of epoxyeicosatrienoic acids (EETs) from cytochrome P450-dependent metabolism of arachidonic acid and subsequent stimulation of K^+_{ca} channels on endothelial cells.[18] The role of EETs as potential EDHFs has been investigated in humans using azole compounds such as fluconazole that selectively inhibit epoxidation (EET generation) of arachidonic acid.[14, 18, 19]

Since bradykinin-mediated t-PA release may be crucial in maintaining endogenous thrombolytic potential, it is important to determine its underlying mechanisms. Our overall goal was to investigate whether EDHF release stimulates t-PA, with the hypothesis that bradykinin stimulates EDHF-mediated t-PA release in the human circulation. We investigated whether activation of the K^+_{ca} channels and/or cytochrome P450 derived EETs mediates t-PA release.

METHODS

Subjects

Thirty-three healthy subjects aged 21 to 60 years and free of smoking, hypercholesterolemia, hypertension, diabetes, cardiovascular disease, medication use, or any other systemic disorder participated in the study (ClincalTrials.gov Identifier: NCT00166166) (Table 1). Written informed consent was obtained and the study was approved by the Emory University Institutional Review Board and the Food and Drug Administration.

Experimental Protocols

Subjects were enrolled into 3 separate protocols described below, performed sequentially. Measurements were performed after an overnight fast in a quiet, temperature-controlled (22 to 24°C) room. Subjects received 975mg of oral aspirin 90 minutes prior to initiation of the study to inhibit prostanoid release.[20, 21] An intravenous catheter was placed into a deep antecubital vein for venous sampling and an arterial cannula into the brachial artery for arterial pressure monitoring, drug delivery and blood sampling.

Forearm Blood Flow Measurements

Simultaneous measurements of FBF were obtained in both arms with the use of a dual-channel venous occlusion strain gauge plethysmograph (model EC6, DE Hokanson, Bellevue, WA) as previously described.[14] The mercury-filled silastic strain gauge was placed around the forearm and connected to a plethysmograph calibrated to measure the percent change in volume.[14] An upper arm cuff was inflated to 40 mmHg to occlude venous outflow and FBF measurements were recorded every 15 seconds up to eight times, and a mean FBF value in $mL \cdot min^{-1} \cdot 100\ mL^{-1}$was computed. Forearm vascular resistance was calculated as the mean arterial pressure ÷ FBF and expressed as mmHg/ $mL \cdot min^{-1} \cdot 100\ mL^{-1}$.

Protocol 1: Effect of K^{+}_{ca} Channel Activation on BK-Stimulated t-PA Release

FBF was measured after a 30-minute rest during saline infusion (total infusion rate 2.5ml·min-1) and after intra-arterial infusions of 100, 200, and 400 ng/min of bradykinin (Clinalfa, Switzerland) given for 8 minutes each (n=18). After 30 minutes, intra-arterial TEA (Sigma-Aldrich, Allentown, PA, sterilized and tested for pyrogenicity by the Emory Investigational Drug Pharmacy) was infused at 1 μmol/min for 8 minutes. When given at 0.25 to 1 mg/min, TEA selectively inhibits K^{+}_{Ca} channels, but reduces FBF with bradykinin only at 1 mg/min (<0.6μmol/min).[16, 22, 23, 24] This dose and timing was effective in attenuating FBF and agonist-stimulated vasodilation in previous studies.[4, 16, 25] While continuing the infusion of TEA, bradykinin infusions were re-administered. Arterial blood pressure and FBF measurements were repeated in the last 2 minutes of each infusion. Arterial and venous blood sampling were performed at baseline prior to the infusion of drug and at peak doses of bradykinin.

Protocol 2: Effect of Cytochrome P450 Metabolites to BK-Stimulated t-PA Release

FBF was measured at rest during saline infusion and after intra-arterial infusion of escalating doses of bradykinin (n=11). Thirty minutes later, fluconazole (Pfizer, New York, NY) was infused intra-arterially at 0.4 μmol·min^{-1}·L^{-1} for 5 minutes. This dose has been effective in attenuating vasodilation during sustained flow conditions.[4, 17] While continuing the infusion of fluconazole, escalating doses of bradykinin were administered. Finally, combined intra-arterial infusions of fluconazole and TEA were given for 8 minutes and bradykinin infusions repeated. This protocol allowed assessment of the contribution of cytochrome P450 metabolites alone and in combination with K^+_{ca} channel activation to BK-stimulated t-PA release.

Protocol 3: Effect of NO to BK-Stimulated t-PA release

The contribution of NO to bradykinin stimulated t-PA release was also determined in the presence of K^+_{ca} channel activation (n=13). FBF was measured at rest and after intra-arterial infusion of bradykinin. Thirty minutes later, TEA (1 μmol/min) was infused for 8 minutes, and followed by repeat infusions of bradykinin. After 30 minutes, TEA and N^G-monomethyl-L-arginine (L-NMMA) (Clinalfa, Switzerland) at 8 μmol/min were infused together for 8 minutes to inhibit EDHF and NO synthesis, followed by repeat administration of bradykinin in escalating doses while continuing infusions of the two antagonists. This dose and timing of L-NMMA was effective in attenuating FBF and agonist-stimulated vasodilation in previous studies.[16, 25]

Effect of sodium nitroprusside on t-PA release

Finally, in 30 subjects, the comparative contribution of the endothelium-independent vasodilator, sodium nitroprusside (SNP) and bradykinin was investigated. FBF was measured during rest and after intra-arterial infusion of escalating doses of bradykinin. Thirty minutes later, SNP (Hospira, Lakeforest, IL) was infused intra-arterially at 1.6, and 3.2 μg/min.

Blood Sampling and Biochemical Assays

Simultaneous arterial and venous blood samples were obtained from the infused arm before and after peak doses of vasodilator drugs, cooled, centrifuged, and stored in tubes containing 0.5 M citrate buffer (Diapharma, West Chester, OH) at -70°C. t-PA antigen levels were determined in duplicate using a two-site enzyme linked immunosorbent assay (Diapharma, West Chester, OH) as described previously.[26] Net release or uptake rates at each time point were calculated: net release = $(C_V\text{-}C_A)\ X^1$, where C_V and C_A represent the concentration of t-PA in the brachial vein and artery, respectively.

Statistical Analysis

To determine the effects of the interventions on FBF and forearm vascular resistance, changes in the flow and resistance responses between the control, single blockade, and combined blockade were compared using repeated measures ANOVA. To determine the effects of treatments on net t-PA release, changes in net t-PA release of control, single blockade, and combined blockade were compared using one-way ANOVA with the Tukey

post hoc test. Changes of the response were converted to percentage changes of least squares means when presented in the text. Data are presented as mean ± SEM in the text. The least squares means and standard errors are presented in the figures. A P value less than 0.05 was considered statistically significant.

RESULTS

Baseline Subject Characteristics

Thirty-three healthy (age 40.3±1.9 years, 64% male) normotensive, non-diabetic, non-obese, nonsmoking subjects with normal serum cholesterol were studied (Table 1). During intrabrachial drug infusions, no changes in blood pressure or heart rate were observed.

Effect of K^+_{ca} Channel Activation on FBF and BK-Stimulated t-PA Release

Bradykinin produced dose-dependent increase in FBF (P=0.002) and decrease in vascular resistance (P=0.03, Figure 1A, 1B). Bradykinin significantly increased the arterio-venous t-PA concentration gradient and net t-PA release across the forearm (from -0.3±0.5 to 36.9±5.5 ng/min/100ml, P=0.002, Table 2, Figure 1C). There was no association between net t-PA release and age, sex, BMI, total cholesterol, and LDL levels.

Infusion of TEA decreased resting FBF by 14%, P=0.0001 and increased vascular resistance by 18%, P=0.03. Co-administration of TEA with bradykinin attenuated the overall response with a 15%, P=0.002 lower FBF and a 21%, P=0.03 higher vascular resistance (Figure 1A, 1B). TEA infusion effectively abolished t-PA release with bradykinin (from 22.9±5.7 to -0.8±3.6 ng/min/100ml, P=0.0002) and the arterio-venous t-PA concentration gradient (Table 2), indicating that the effect of bradykinin on endothelial t-PA release is mediated entirely through K^+_{ca} channel activation (Figure 1C).

Effect of Cytochrome P450 Metabolites and K^+_{ca} Channel Activation on FBF and BK-Stimulated t-PA Release

Infusion of fluconazole decreased resting FBF by 19%, P=0.001 and increased vascular resistance by 27%, P=0.001. Fluconazole also blunted the overall vasodilator response to bradykinin with a 23%, P<0.0001 reduction in FBF and a 35%, P<0.0001 higher vascular resistance (Figure 2A, 2B). Fluconazole infusion attenuated the t-PA release with BK (from 50.9+9 to 21.3+8.9 ng/min/100ml, P=0.02) and the arterio-venous t-PA concentration gradient (Table 2), indicating significant contribution of cytochrome P450 metabolites to BK-stimulated t-PA release (Figure 2C). Infusion of TEA after fluconazole reduced FBF by an additional 20% (P<0.0001, n=10) and increased vascular resistance by a further 24%, P<0.0001 (Figure 2A, 2B). In the presence of fluconazole, infusion of TEA trended to attenuate the arterio-venous t-PA gradient (Table 2), but the further reduction in net t-PA release did not reach statistical significance (Figure 2C). However, TEA infusion attenuated the t-PA release with bradykinin to a greater extent than fluconazole (85±4% versus 61±11% reduction, respectively, P=0.05), indicating that of the potential hyperpolarization mechanisms, K^+_{ca} channel activation is the predominant EDHF signaling pathway mediating bradykinin-stimulated t-PA release.

Effect of NO and K^+_{ca} Channel Activation on FBF and BK-Stimulated t-PA release

To assess the comparative contribution of K^+_{ca} channel activation and NO to bradykinin-stimulated t-PA release, individual and combined blockade with TEA and L-NMMA were studied. As seen previously, infusion of TEA attenuated the vasodilator response to bradykinin with a 17%, P<0.0001 reduction in FBF (Figure 3A). Similarly, t-PA release with bradykinin was reduced from 20.4±6 to 3.5±3 ng/min/100ml, P=0.02, indicating a significant contribution of K^+_{ca} channel activation to bradykinin-stimulated t-PA release (Figure 3C). Infusion of LNMMA after TEA reduced FBF by an additional 21%, P<0.0001 and increased vascular resistance by a further 42%, P=0.04 (Figure 3A, 3B). In the presence of TEA, infusion of LNMMA did not further affect net t-PA release (P=0.25, Figure 3C, Table 2), indicating that bradykinin-stimulated t-PA release is through a K^+_{ca} channel-dependent and NO-synthase-independent pathway.

Effect of sodium nitroprusside on t-PA release

Infusions of bradykinin and SNP resulted in similar increases in FBF (P=0.7) and decreases in FVR (Figure 4A). However, in contrast to bradykinin, the t-PA response (-1.8±2 versus -0.6±0.4 ng/min/100ml, p=NS) and the arterial-venous t-PA concentration gradient (-0.4±0.3 versus -0.2±0.3, p=0.5) remained unchanged with SNP, indicating that SNP does not stimulate endothelial t-PA release Figure 4B).

DISCUSSION

Herein, we demonstrate that although both NO and EDHF contribute to bradykinin-induced vasodilation, t-PA release is solely mediated by EDHF signaling pathways. We confirmed that cytochrome P450-derived EETs partly contribute to bradykinin-stimulated t-PA release, and now show that it is completely abolished by inhibition of K^+_{ca} channels, the final site of action for several putative EDHFs. [27] Finally, we demonstrate a lack of contribution of NO to bradykinin-stimulated t-PA release.[11]

Endothelial fibrinolytic activity is an endogenous protective mechanism against the development and propagation of arterial thrombi.[28] In the fibrinolytic system, plasminogen is activated to plasmin that degrades fibrin into soluble fibrin degradation products.[29] The endothelium releases t-PA through a calcium and G protein-dependent pathway in response to a variety of endogenous mediators including catecholamines, thrombin, and bradykinin.[30, 31] Endogenous bradykinin is produced by activation of the plasma kallikrein-kinin system on endothelial cells and bradykinin B_2 receptor blockade inhibits t-PA release.[10, 11]

In the intact human vasculature, endothelium-dependent hyperpolarization can be mediated by agonists such as bradykinin and by physical stimuli including increases in shear stress that all increase intracellular calcium, causing opening of endothelial K^+_{ca} channels and initiating downstream processes that explain the EDHF phenomena.[3, 4] Candidate EDHFs include EETs synthesized from the cytochrome P450-dependent metabolism of arachidonic acid, and hydrogen peroxide generated from the degradation of superoxides through various endothelial oxidases such as NADPH-oxidase (NOX) or through superoxide dismutase-

dependent dismutation.[32, 33] The availability of TEA, a specific K^+_{ca} channel antagonist, for use in humans has enabled investigations of EDHF activity in the forearm microcirculation.[16, 17, 22] We now demonstrate that in addition to stimulating vasodilation, bradykinin stimulates t-PA release that is completely abolished by K^+_{ca} channel inhibition.[6, 34]

Endothelium-derived cytochrome P450 metabolites of arachidonic acid can hyperpolarize membranes primarily by activating the K^+_{ca} channels, although the identity of the specific EETs remains controversial.[35-37] Herein we demonstrate that fluconazole, an inhibitor of cytochrome 2C9, attenuated bradykinin-mediated t-PA release, suggesting cytochrome P450 metabolites contribute significantly to t-PA release,[19] observations that have been confirmed in both pre-clinical,[14, 38] and clinical studies in both healthy and hypertensive subjects.[27]

Because inhibition of EETs had a lesser effect than inhibition of K^+_{ca} channels on bradykinin-stimulated t-PA release, it appears that other factors that stimulate K^+_{ca} channels also contribute to EDHF activity, which in pre-clinical studies has been attributed to hydrogen peroxide, gap junctions or elevations in K^+ release from endothelial cells.[3, 18, 39-41] Hydrogen peroxide predominantly contributes to bradykinin-induced human arteriolar vasodilation[42] and to release of t-PA from rat hearts,[43] but its role in the human circulation *in vivo* remains to be established. Thus, although hydrogen peroxide activates K^+_{ca} channels and can function as an EDHF, its contribution to vascular homeostasis is complex.[44] It is not only a hyperpolarizing factor, but depending on the species, blood vessel, and concentration, hydrogen peroxide can be a signaling molecule and may also cause direct smooth muscle relaxation or depolarization of the smooth muscle and vasoconstriction.[3]

It may be argued that the bradykinin-mediated t-PA release is at least partly flow-mediated. However, with similar increases in blood flow with SNP, we and previous investigators found no increase in t-PA.[6, 11, 27, 45, 46] Moreover, infusion of L-NMMA selectively attenuated FBF without affecting t-PA response to bradykinin.

Limitations

L-NMMA, fluconazole, and TEA are competitive inhibitors and may not completely inhibit NO, cytochrome P450 pathways, and K^+_{ca} channels, respectively, and thus likely underestimate the physiologic importance of these mediators *in vivo*.[47, 48] TEA also acts as a competitive inhibitor of the nicotinic receptor and reduces the water permeability of human AQP1 channels; however, in the doses administered, it has been shown to selectively inhibit K^+_{ca} channels.[49, 50] It is assumed that TEA effects are reflecting endothelium-dependent hyperpolarization as we cannot measure membrane potentials in this in vivo study. Fluconazole is not a specific inhibitor of cytochrome 2C9; however, we have shown significant inhibition of resting and bradykinin-mediated vasodilation with this inhibitor.[4] Previous studies with a specific 2C9 inhibitor, sulphaphenazole, or with miconazole have yielded contradictory data.[19, 51-53] We did not find any ethnic differences in bradykinin-mediated t-PA release even though ethnicity affects the vasodilator response to bradykinin; however, our study was not powered to explore this issue.[54] Our experiments were conducted in the setting of cyclooxygenase inhibition and therefore are unable to explore

possible interactions with prostaglandins. Nevertheless, previous studies demonstrated no alteration in bradykinin-mediated t-PA release with cyclooxygenase inhibition.[11, 55, 56]

CONCLUSIONS

We demonstrate that bradykinin-stimulated t-PA release is via endothelium-dependent hyperpolarization, partly through cytochrome P450-derived epoxides and ultimately via activation of K^+_{ca} channels. Although bradykinin stimulates both NO and EDHF-dependent vasodilation, bradykinin-mediated t-PA release is purely EDHF-dependent. Both endothelium-dependent vasodilation and endothelial fibrinolytic capacity, measured as t-PA release, appear to predict the risk of future cardiovascular events.[57, 58] Since abnormalities in the EDHF signaling pathway contribute to both abnormal vasodilation and increased thrombotic risk, agonists that enhance EDHF activity, such as epoxide hydrolase inhibitors, need to be investigated for their thrombolytic actions

Acknowledgments

Funding sources:

National Institutes of Health Research Grant RO1 HL79115, and in part by PHS Grant UL1 RR025008 from the Clinical and Translational Science Award Program, and PHS Grant M01 RR00039 from the General Clinical Research Center program, National Institutes of Health, National Center for Research Resources, the British Cardiovascular Society Research Fellowship, National Blood Foundation, NIH NRSA T32 Training Grant, American College of Cardiology Foundation Keating Fellowship, and the American Heart Association Beginning Grant-in-Aid

Abbreviations

ASA	Acetylsalicylic acid
BK	Bradykinin
COX	Cyclooxygenase
EDHF	Endothelium-Derived Hyperpolarizing Factor
EETS	Epoxyeicosatrienoic acid
eNOS	Endothelial Nitric Oxide Synthase
FBF	Forearm Blood Flow
FLU	Fluconazole
H_2O_2	Hydrogen peroxide
K^+_{Ca}	Calcium-activated potassium channels
L-NMMA	N^G-monomethyl-L-arginine
NO	Nitric Oxide
PGI_2	Prostacyclin
SNP	Sodium Nitroprusside
TEA	Tetraethylammonium chloride

tPA Tissue Plasminogen Activator

REFERENCES

1. Newby DE, Wright RA, Labinjoh C, Ludlam CA, Fox KA, Boon NA, Webb DJ. Endothelial dysfunction, impaired endogenous fibrinolysis, and cigarette smoking: A mechanism for arterial thrombosis and myocardial infarction. Circulation. 1999; 99:1411–1415. [PubMed: 10086962]
2. Newby DE, McLeod AL, Uren NG, Flint L, Ludlam CA, Webb DJ, Fox KA, Boon NA. Impaired coronary tissue plasminogen activator release is associated with coronary atherosclerosis and cigarette smoking: Direct link between endothelial dysfunction and atherothrombosis. Circulation. 2001; 103:1936–1941. [PubMed: 11306520]
3. Feletou M, Vanhoutte PM. Endothelium-dependent hyperpolarizations: Past beliefs and present facts. Ann Med. 2007; 39:495–516. [PubMed: 17852039]
4. Ozkor MA, Murrow JR, Rahman AM, Kavtaradze N, Lin J, Manatunga A, Quyyumi AA. Endothelium-derived hyperpolarizing factor determines resting and stimulated forearm vasodilator tone in health and in disease. Circulation. 2011; 123:2244–2253. [PubMed: 21555712]
5. Vanhoutte PM. Endothelium and control of vascular function. State of the art lecture. Hypertension. 1989; 13:658–667. [PubMed: 2661425]
6. Brown NJ, Gainer JV, Stein CM, Vaughan DE. Bradykinin stimulates tissue plasminogen activator release in human vasculature. Hypertension. 1999; 33:1431–1435. [PubMed: 10373228]
7. Linz W, Wiemer G, Scholkens BA. Beneficial effects of bradykinin on myocardial energy metabolism and infarct size. Am J Cardiol. 1997; 80:118A–123A.
8. Emeis JJ, van den Eijnden-Schrauwen Y, van den Hoogen CM, de Priester W, Westmuckett A, Lupu F. An endothelial storage granule for tissue-type plasminogen activator. J Cell Biol. 1997; 139:245–256. [PubMed: 9314543]
9. Fox KA, Robison AK, Knabb RM, Rosamond TL, Sobel BE, Bergmann SR. Prevention of coronary thrombosis with subthrombolytic doses of tissue-type plasminogen activator. Circulation. 1985; 72:1346–1354. [PubMed: 3933849]
10. Schmaier AH. The plasma kallikrein-kinin system counterbalances the renin-angiotensin system. J Clin Invest. 2002; 109:1007–1009. [PubMed: 11956236]
11. Brown NJ, Gainer JV, Murphey LJ, Vaughan DE. Bradykinin stimulates tissue plasminogen activator release from human forearm vasculature through b-2 receptor-dependent, no synthase-independent, and cyclooxygenase-independent pathway. Circulation. 2000; 102:2190–2196. [PubMed: 11056091]
12. Node K, Ruan XL, Dai J, Yang SX, Graham L, Zeldin DC, Liao JK. Activation of galpha s mediates induction of tissue-type plasminogen activator gene transcription by epoxyeicosatrienoic acids. J Biol Chem. 2001; 276:15983–15989. [PubMed: 11279071]
13. Muldowney JA 3rd, Painter CA, Sanders-Bush E, Brown NJ, Vaughan DE. Acute tissue-type plasminogen activator release in human microvascular endothelial cells: The roles of galphaq, plc-beta, ip3 and 5,6-epoxyeicosatrienoic acid. Thromb Haemost. 2007; 97:263–271. [PubMed: 17264956]
14. Halcox JP, Narayanan S, Cramer-Joyce L, Mincemoyer R, Quyyumi AA. Characterization of endothelium-derived hyperpolarizing factor in the human forearm microcirculation. Am J Physiol Heart Circ Physiol. 2001; 280:H2470–2477. [PubMed: 11356600]
15. Scotland RS, Madhani M, Chauhan S, Moncada S, Andresen J, Nilsson H, Hobbs AJ, Ahluwalia A. Investigation of vascular responses in endothelial nitric oxide synthase/cyclooxygenase-1 double-knockout mice: Key role for endothelium-derived hyperpolarizing factor in the regulation of blood pressure in vivo. Circulation. 2005; 111:796–803. [PubMed: 15699263]
16. Inokuchi K, Hirooka Y, Shimokawa H, Sakai K, Kishi T, Ito K, Kimura Y, Takeshita A. Role of endothelium-derived hyperpolarizing factor in human forearm circulation. Hypertension. 2003; 42:919–924. [PubMed: 14557280]

17. Bellien J, Iacob M, Gutierrez L, Isabelle M, Lahary A, Thuillez C, Joannides R. Crucial role of no and endothelium-derived hyperpolarizing factor in human sustained conduit artery flow-mediated dilatation. Hypertension. 2006; 48:1088–1094. [PubMed: 17075035]
18. Miura H, Wachtel RE, Liu Y, Loberiza FR Jr. Saito T, Miura M, Gutterman DD. Flow-induced dilation of human coronary arterioles: Important role of ca(2+)-activated k(+) channels. Circulation. 2001; 103:1992–1998. [PubMed: 11306529]
19. Bellien J, Joannides R, Iacob M, Arnaud P, Thuillez C. Evidence for a basal release of a cytochrome-related endothelium-derived hyperpolarizing factor in the radial artery in humans. Am J Physiol Heart Circ Physiol. 2006; 290:H1347–1352. [PubMed: 16339823]
20. Hong SL. Effect of bradykinin and thrombin on prostacyclin synthesis in endothelial cells from calf and pig aorta and human umbilical cord vein. Thromb Res. 1980; 18:787–795. [PubMed: 6774443]
21. Duffy SJ, Tran BT, New G, Tudball RN, Esler MD, Harper RW, Meredith IT. Continuous release of vasodilator prostanoids contributes to regulation of resting forearm blood flow in humans. Am J Physiol. 1998; 274:H1174–1183. [PubMed: 9575920]
22. Honing ML, Smits P, Morrison PJ, Rabelink TJ. Bradykinin-induced vasodilation of human forearm resistance vessels is primarily mediated by endothelium-dependent hyperpolarization. Hypertension. 2000; 35:1314–1318. [PubMed: 10856283]
23. Stanfield PR. Tetraethylammonium ions and the potassium permeability of excitable cells. Rev Physiol Biochem Pharmacol. 1983; 97:1–67. [PubMed: 6306751]
24. Langton PD, Nelson MT, Huang Y, Standen NB. Block of calcium-activated potassium channels in mammalian arterial myocytes by tetraethylammonium ions. Am J Physiol. 1991; 260:H927–934. [PubMed: 1900393]
25. Gilligan DM, Guetta V, Panza JA, Garcia CE, Quyyumi AA, Cannon RO 3rd. Selective loss of microvascular endothelial function in human hypercholesterolemia. Circulation. 1994; 90:35–41. [PubMed: 8026018]
26. Ridker PM, Vaughan DE, Stampfer MJ, Manson JE, Shen C, Newcomer LM, Goldhaber SZ, Hennekens CH. Baseline fibrinolytic state and the risk of future venous thrombosis. A prospective study of endogenous tissue-type plasminogen activator and plasminogen activator inhibitor. Circulation. 1992; 85:1822–1827. [PubMed: 1572039]
27. Giannarelli C, Virdis A, De Negri F, Magagna A, Duranti E, Salvetti A, Taddei S. Effect of sulfaphenazole on tissue plasminogen activator release in normotensive subjects and hypertensive patients. Circulation. 2009; 119:1625–1633. [PubMed: 19289643]
28. Gorog DA. Prognostic value of plasma fibrinolysis activation markers in cardiovascular disease. J Am Coll Cardiol. 2010; 55:2701–2709. [PubMed: 20538163]
29. Rijken DC, Lijnen HR. New insights into the molecular mechanisms of the fibrinolytic system. J Thromb Haemost. 2009; 7:4–13. [PubMed: 19017261]
30. Stein CM, Brown N, Vaughan DE, Lang CC, Wood AJ. Regulation of local tissue-type plasminogen activator release by endothelium-dependent and endothelium-independent agonists in human vasculature. J Am Coll Cardiol. 1998; 32:117–122. [PubMed: 9669258]
31. van den Eijnden-Schrauwen Y, Atsma DE, Lupu F, de Vries RE, Kooistra T, Emeis JJ. Involvement of calcium and g proteins in the acute release of tissue-type plasminogen activator and von willebrand factor from cultured human endothelial cells. Arterioscler Thromb Vasc Biol. 1997; 17:2177–2187. [PubMed: 9351387]
32. Fleming I, Michaelis UR, Bredenkotter D, Fisslthaler B, Dehghani F, Brandes RP, Busse R. Endothelium-derived hyperpolarizing factor synthase (cytochrome p450 2c9) is a functionally significant source of reactive oxygen species in coronary arteries. Circ Res. 2001; 88:44–51. [PubMed: 11139472]
33. Lambeth JD. Nox enzymes, ros, and chronic disease: An example of antagonistic pleiotropy. Free radical biology & medicine. 2007; 43:332–347. [PubMed: 17602948]
34. Witherow FN, Dawson P, Ludlam CA, Webb DJ, Fox KA, Newby DE. Bradykinin receptor antagonism and endothelial tissue plasminogen activator release in humans. Arterioscler Thromb Vasc Biol. 2003; 23:1667–1670. [PubMed: 12869356]

35. Fisslthaler B, Popp R, Kiss L, Potente M, Harder DR, Fleming I, Busse R. Cytochrome p450 2c is an edhf synthase in coronary arteries. Nature. 1999; 401:493–497. [PubMed: 10519554]
36. Campbell WB, Gebremedhin D, Pratt PF, Harder DR. Identification of epoxyeicosatrienoic acids as endothelium-derived hyperpolarizing factors. Circ Res. 1996; 78:415–423. [PubMed: 8593700]
37. Weston AH, Feletou M, Vanhoutte PM, Falck JR, Campbell WB, Edwards G. Bradykinin-induced, endothelium-dependent responses in porcine coronary arteries: Involvement of potassium channel activation and epoxyeicosatrienoic acids. Br J Pharmacol. 2005; 145:775–784. [PubMed: 15895105]
38. Gauthier KM, Edwards EM, Falck JR, Reddy DS, Campbell WB. 14,15-epoxyeicosatrienoic acid represents a transferable endothelium-dependent relaxing factor in bovine coronary arteries. Hypertension. 2005; 45:666–671. [PubMed: 15699460]
39. Fleming I. Cytochrome p450 and vascular homeostasis. Circ Res. 2001; 89:753–762. [PubMed: 11679404]
40. Fleming I, Busse R. Endothelium-derived epoxyeicosatrienoic acids and vascular function. Hypertension. 2006; 47:629–633. [PubMed: 16490839]
41. Feletou M, Vanhoutte PM. Endothelium-derived hyperpolarizing factor: Where are we now? Arterioscler Thromb Vasc Biol. 2006; 26:1215–1225. [PubMed: 16543495]
42. Larsen BT, Gutterman DD, Sato A, Toyama K, Campbell WB, Zeldin DC, Manthati VL, Falck JR, Miura H. Hydrogen peroxide inhibits cytochrome p450 epoxygenases: Interaction between two endothelium-derived hyperpolarizing factors. Circ Res. 2008; 102:59–67. [PubMed: 17975109]
43. Winnerkvist A, Wiman B, Valen G, Vaage J. Oxidative stress and release of tissue plasminogen activator in isolated rat hearts. Thromb Res. 1997; 85:245–257. [PubMed: 9058499]
44. Matoba T, Shimokawa H, Kubota H, Morikawa K, Fujiki T, Kunihiro I, Mukai Y, Hirakawa Y, Takeshita A. Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in human mesenteric arteries. Biochemical and biophysical research communications. 2002; 290:909–913. [PubMed: 11798159]
45. Newby DE, Wright RA, Ludlam CA, Fox KAA, Boon NA, Webb DJ. An in vivo model for the assessment of acute fibrinolytic capacity of the endothelium. Thromb Haemostasis. 1997; 78:1242–1248. [PubMed: 9364992]
46. Pretorius M, Brown NJ. Endogenous nitric oxide contributes to bradykinin-stimulated glucose uptake but attenuates vascular tissue-type plasminogen activator release. J Pharmacol Exp Ther. 2010; 332:291–297. [PubMed: 19841473]
47. Rees DD, Palmer RM, Hodson HF, Moncada S. A specific inhibitor of nitric oxide formation from l-arginine attenuates endothelium-dependent relaxation. Br J Pharmacol. 1989; 96:418–424. [PubMed: 2924084]
48. Coleman HA, Tare M, Parkington HC. Endothelial potassium channels, endothelium-dependent hyperpolarization and the regulation of vascular tone in health and disease. Clin Exp Pharmacol Physiol. 2004; 31:641–649. [PubMed: 15479173]
49. Akk G, Steinbach JH. Activation and block of mouse muscle-type nicotinic receptors by tetraethylammonium. The Journal of physiology. 2003; 551:155–168. [PubMed: 12824448]
50. Brooks HL, Regan JW, Yool AJ. Inhibition of aquaporin-1 water permeability by tetraethylammonium: Involvement of the loop e pore region. Molecular pharmacology. 2000; 57:1021–1026. [PubMed: 10779387]
51. Halcox JPJ, Narayanan S, Cramer-Joyce L, Mincemoyer R, Quyyumi AA. Characterization of endothelium-derived hyperpolarizing factor in the human forearm microcirculation. Am J Physiol Heart Circ Physiol. 2001; 280:H2470–2477. [PubMed: 11356600]
52. Fichtlscherer S, Dimmeler S, Breuer S, Busse R, Zeiher AM, Fleming I. Inhibition of cytochrome p450 2c9 improves endothelium-dependent, nitric oxide-mediated vasodilatation in patients with coronary artery disease. Circulation. 2004; 109:178–183. [PubMed: 14662709]
53. Passauer J, Bussemaker E, Lassig G, Pistrosch F, Fauler J, Gross P, Fleming I. Baseline blood flow and bradykinin-induced vasodilator responses in the human forearm are insensitive to the cytochrome p450 2c9 (cyp2c9) inhibitor sulphaphenazole. Clin Sci (Lond). 2003; 105:513–518. [PubMed: 12826020]

54. Rosenbaum DA, Pretorius M, Gainer JV, Byrne D, Murphey LJ, Painter CA, Vaughan DE, Brown NJ. Ethnicity affects vasodilation, but not endothelial tissue plasminogen activator release, in response to bradykinin. Arterioscler Thromb Vasc Biol. 2002; 22:1023–1028. [PubMed: 12067915]

55. Ohlmann P, Martinez MC, Schneider F, Stoclet JC, Andriantsitohaina R. Characterization of endothelium-derived relaxing factors released by bradykinin in human resistance arteries. Br J Pharmacol. 1997; 121:657–664. [PubMed: 9208131]

56. Nakashima M, Mombouli JV, Taylor AA, Vanhoutte PM. Endothelium-dependent hyperpolarization caused by bradykinin in human coronary arteries. J Clin Invest. 1993; 92:2867–2871. [PubMed: 8254041]

57. Halcox JP, Schenke WH, Zalos G, Mincemoyer R, Prasad A, Waclawiw MA, Nour KR, Quyyumi AA. Prognostic value of coronary vascular endothelial dysfunction. Circulation. 2002; 106:653–658. [PubMed: 12163423]

58. Ridker PM, Vaughan DE, Stampfer MJ, Manson JE, Hennekens CH. Endogenous tissue-type plasminogen activator and risk of myocardial infarction. Lancet. 1993; 341:1165–1168. [PubMed: 8098074]

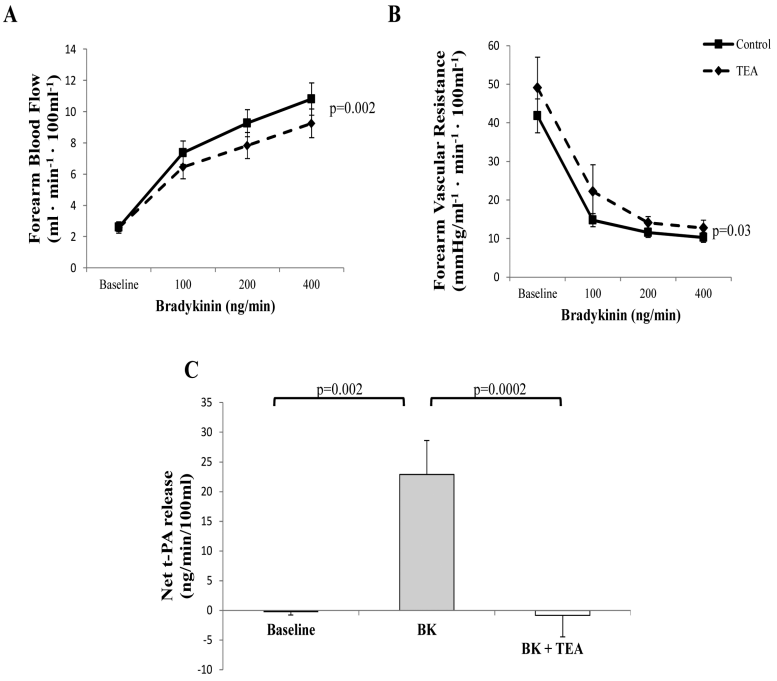

Figure 1. Contribution of K^+_{ca} channel activation to bradykinin-stimulated vasodilation, and t-PA release

Forearm blood flow (A) and forearm vascular resistance (B) changes in response to bradykinin (100, 200, and 400 ng/min) alone, and after infusion of TEA (n=18). Net t-PA release (C) in response to peak dose of bradykinin (400 ng/min) and after infusion of TEA. Data shown as mean ± SEM.

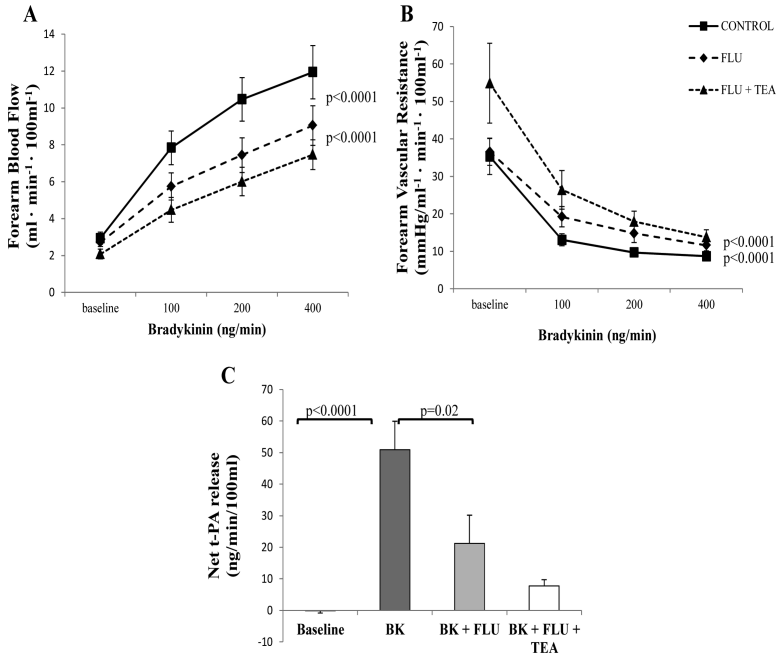

Figure 2. Contribution of cytochrome P450 metabolites and K^+_{Ca} channel activation to bradykinin-stimulated vasodilation and t-PA release

Forearm blood flow (A) and forearm vascular resistance (B) changes in response to bradykinin (100, 200, and 400 ng/min) alone, after infusion of fluconazole, and combined infusions of fluconazole and TEA (n=11). Net t-PA release (C) in response to peak dose of bradykinin (400 ng/min) and after initial infusion of fluconazole, followed by combined fluconazole and TEA infusions. Data shown as mean ± SEM.

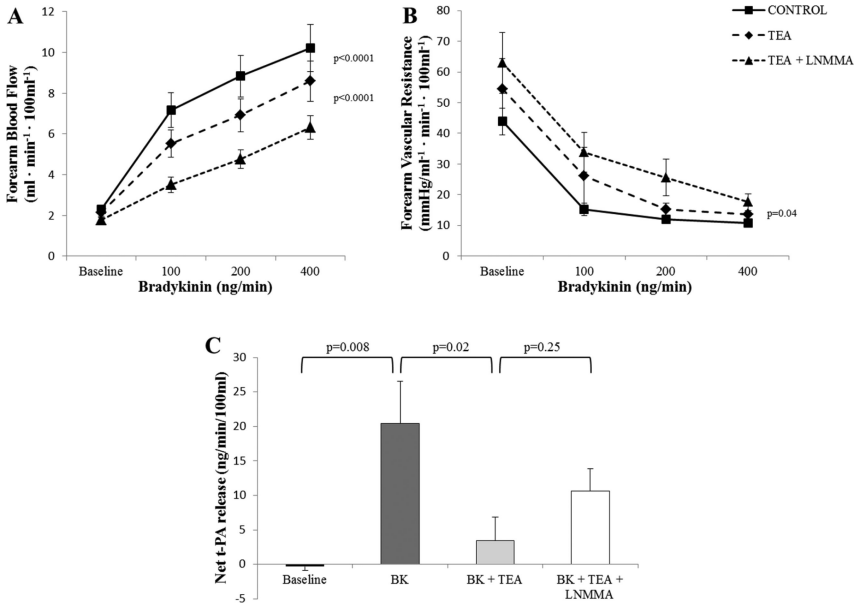

Figure 3. Contribution of NO and K^{+}_{ca} channel activation to bradykinin-stimulated vasodilation and t-PA release

Forearm blood flow (A) and forearm vascular resistance (B) in response to bradykinin (100, 200, and 400 ng/min) alone, after initial infusion of TEA, and combined infusion of TEA and L-NMMA in healthy (n=13) subjects. Data shown as mean ± SEM. Box plot of net t-PA release (C) in response to peak dose of bradykinin (400 ng/min) and after infusion of TEA, followed by combined TEA and L-NMMA infusions. Minimum and maximum values depicted by whiskers, the box signifies lower and upper quartiles, the median is represented by the short black line within the box, and the mean by the symbol marker.

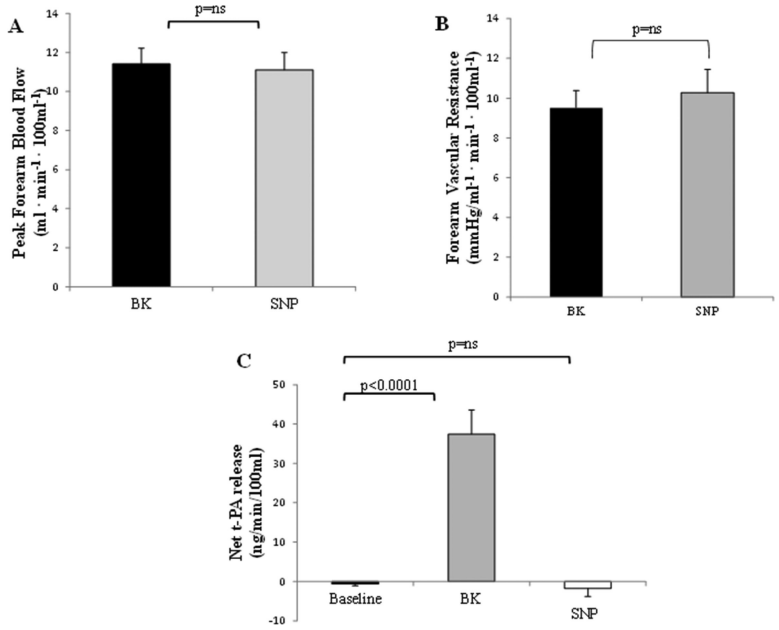

Figure 4. Contribution of bradykinin and sodium nitroprusside to vasodilation and t-PA release

Forearm blood flow (A), vascular resistance (B), and net t-PA release (C) in response to peak doses of bradykinin (400 ng/min) and sodium nitroprusside (3.2 μg/min). Data shown as mean ± SEM.

Table 1

Subject characteristics

Male, n (%)	21 (63.6)
Age, years	40.3±1.9
Ethnicity, n (%)	
White	8 (24.2)
Black	21 (64.3)
Hispanic	4 (12.1)
Systolic BP, mmHg	121.6±2
Diastolic BP, mmHg	73±1.4
Heart rate, bpm	68±1.5
Body mass index (kg/m^2)	28±1
Fasting blood glucose (mg/dL)	86.4±1.4
Triglycerides (mg/dL)	102.4±9.5
Total Cholesterol (mg/dL)	170±6.4
High Density Lipoprotein Cholesterol (mg/dL)	54.3±2.3
Low Density Lipoprotein (mg/dL)	95.3±5.7
Tobacco smoking (%)	0
Hematocrit (%)	39.4±0.6

Table 2

Effect of interventions on arterial-venous (AV) gradient

Venous-arterial t-PA difference (ng/ml)	Control (n=33)	+TEA (n=18)	+Fluconazole (n=11)	+Fluconazole +TEA (n=10)	+TEA +L-NMMA (n=13)
Baseline	−0.2 ± 0.3	−0.3 ± 0.3	−0.2 ± 0.8	−0.2 ± 0.8	−0.2 ± 0.4
Bradykinin (400 ng/min)	5.6 ± 0.8*	0.03 ± 0.7†	4.4 ± 1.4†	1.6 ± 0.4	2.6 ± 0.9

*P < 0.05 versus baseline

†P < 0.01 versus control