Mental Stress Induces Prolonged Endothelial Dysfunction via Endothelin-A Receptors

Lukas E. Spieker, MD; David Hürlimann, MD; Frank Ruschitzka, MD; Roberto Corti, MD; Frank Enseleit, MD; Sidney Shaw, PhD; Daniel Hayoz, MD; John E. Deanfield, MD; Thomas F. Lüscher, MD; Georg Noll, MD

Background—Mental stress is a risk factor for atherosclerosis and may precipitate myocardial ischemia and infarction. Because endothelial dysfunction is an early manifestation of atherosclerosis, we investigated the impact of mental stress on endothelial function.

Methods and Results—The effects of a 3-minute mental stress task on endothelium-dependent vasodilation were studied in healthy subjects without cardiovascular risk factors. Flow-mediated (FMD) and nitroglycerin (0.4 mg sublingual)-induced vasodilation were studied before and after mental stress by high-resolution ultrasound of the radial artery. Additionally, FMD was assessed before and 10 to 45 minutes after mental stress during intraarterial infusion of a selective endothelin A receptor antagonist (BQ-123, 1 nmol/min) or saline, respectively. Endothelium-dependent vasodilation was reduced by half for about 45 minutes (8.0±1.1% versus 4.1±1.0%; P<0.002), whereas endothelium-independent vasodilation to nitroglycerin remained unaffected (15.6±1.6 versus 14.3±1.3%; NS). Intraarterial infusion of BQ-123, a selective endothelin-A receptor antagonist, but not saline prevented the impairment of endothelium-dependent vasodilation (8.6±1.2 versus 9.4±1.3%; NS). In contrast, intraarterial infusion of norepinephrine of similar duration as mental stress did not inhibit FMD.

Conclusions—Mental stress induces prolonged endothelial dysfunction, which is prevented by selective endothelin-A receptor antagonism. This represents a novel and important link between mental stress and atherosclerotic vascular disease. (Circulation. 2002;105:2817-2820.)

Key Words: atherosclerosis • endothelin • nitric oxide • reactive hyperemia

Mental stress triggers myocardial ischemia in patients with coronary artery disease.1,2 In fact, sudden life stressors, such as anger, bereavement, earthquakes, or war can even precipitate cardiac events such as acute myocardial infarction or sudden cardiac death.3,4 However, the mechanisms by which stress translates into vascular injury still remain elusive.

Cardiovascular risk factors, eg, hypercholesterolemia and hypertension, impair endothelial function as they blunt bioavailability of NO and enhance ET-1 formation in the vascular wall. Hence, the aim of this study was to investigate the effects of mental stress on endothelial function in humans and to delineate the mechanisms involved.

Methods

Subjects

Twenty-three healthy subjects (aged 20 to 31 years; body mass index, 21.6±1.7 kg/m2; arterial blood pressure, 113±8/67±7 mm Hg; total cholesterol 4.5±0.3 mmol/L) without any cardiovascular risk factor participated in the study. All subjects gave written informed consent and the study protocol was approved by the local research ethics committee of the University Hospital Zürich, Switzerland.

Study Protocol

Flow-mediated dilation (FMD) and nitroglycerin (0.4 mg, Nitrolingual Spray, Pohl-Boskamp, Germany)-induced vasodilation of the radial artery were assessed before and 10 minutes after a 3-minute mental stress test with a high-resolution A-mode ultrasonic echotracking device (Nius, Asulab, Switzerland) as described previously.5,6 FMD of the radial artery was induced by release of a wrist cuff inflated to suprasystolic pressure for 5 minutes. The intraobserver reproducibility in our laboratory for radial artery diameter determinations is 2.900±0.085 versus 2.939±0.087 (r=0.99, P<0.0001), and the variability 0.027±0.005 (ie, 0.9±0.2%). During the highly reproducible and observer-independent mental stress task, the volunteers had to respond as fast as possible to color lights flashing in random order by pressing a push-button of the corresponding color.7,8 Blood pressure and heart rate were measured at the finger of the left arm (Finapres, Ohmeda, USA). The volunteers were allocated to different protocol arms: 7 subjects underwent measurements

Received March 28, 2002; revision received April 29, 2002; accepted April 29, 2002.
From Cardiology (L.E.S., D.H., F.R., R.C., F.E., T.L., G.N.), University Hospital, Zürich; the Department of Clinical Research (S.S.), Inselspital, Bern; Division of Hypertension and Vascular Medicine (D.H.), Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland; and Cardiology (J.E.D.), Greater Ormond Street Hospital for Children, London, UK.
Guest Editor for this article was Kathryn G. Lamping, PhD, VA Medical Center, Iowa City, Iowa.
Correspondence to Georg Noll, MD, FESC, Cardiology, University Hospital, CH-8091 Zürich, Switzerland. E-mail karnog@usz.unizh.ch
© 2002 American Heart Association, Inc.

Circulation is available at http://www.circulationaha.org

DOI: 10.1161/01.CIR.0000021598.15895.34

2817
before and after stress testing; in 5 subjects, FMD was repeatedly measured before and 10, 30, and 45 minutes after mental stress; and in 6 subjects, FMD was measured before and 10 minutes after completing the mental stress task during continuous intraarterial infusion of BQ-123 (1 nmol/min; Clinalfa, Switzerland), a selective endothelin-A receptor (ET_A) receptor antagonist, started 15 minutes before the mental stress test. The 10-minute measuring point was selected because the acute hemodynamic effects of mental stress had disappeared at that time. In control experiments (n=11), saline (0.9% NaCl) was infused intraarterially. Drugs were infused with a constant rate infusion pump (1 mL/min; Perfusor, Braun, Germany) through an indwelling catheter (20G; Ohmeda, Swindon, UK) inserted under local anesthesia into the brachial artery of the nondominant arm. In contrast to pilot experiments and previous studies with higher dosages (10 and 100 nmol/min), in the present study a 10-times lower dosage of BQ-123 was chosen to exclude any hemodynamic effect. In further experiments (n=6), radial artery blood flow was measured before and after mental stress (PW-Doppler; angle of insonation, 60°; range gate in the center of the artery). Venous blood samples were drawn before and immediately after the mental stress test in additional experiments (n=8). Circulating ET-1 and norepinephrine levels were measured by radioimmunoassay. In separate experiments, FMD was measured before and after mental stress by terminating a brief (3 minutes) intraarterial infusion of norepinephrine (30 ng/min) to simulate an activation of the sympathetic nervous system. Baseline hemodynamic conditions were reestablished 10 minutes after completing the mental stress task (mean arterial blood pressure, 82±2 mm Hg; heart rate, 64±6 beats per minute; radial artery blood flow, 66±11 versus 75±10 mL/min; radial artery diameter, 2.82±0.13 versus 2.86±0.13 mm; all NS). However, FMD was reduced by half to 4.1±1.0% (P<0.002 versus baseline; Figure 1A), whereas nitroglycerin-induced vasodilation remained unaltered (14.3±1.3%; NS; Figure 1A). FMD progressively returned to baseline levels over a period of approximately 45 minutes (Figure 2; P=0.0002 for trend). The impairment in FMD of the radial artery after mental stress testing; in 5 subjects, FMD was repeatedly measured before and 10, 30, and 45 minutes after mental stress; and in 6 subjects, FMD was measured before and 10 minutes after completing the mental stress task during continuous intraarterial infusion of BQ-123 (1 nmol/min; Clinalfa, Switzerland), a selective endothelin-A receptor (ET_A) receptor antagonist, started 15 minutes before the mental stress test. The 10-minute measuring point was selected because the acute hemodynamic effects of mental stress had disappeared at that time. In control experiments (n=11), saline (0.9% NaCl) was infused intraarterially. Drugs were infused with a constant rate infusion pump (1 mL/min; Perfusor, Braun, Germany) through an indwelling catheter (20G; Ohmeda, Swindon, UK) inserted under local anesthesia into the brachial artery of the nondominant arm. In contrast to pilot experiments and previous studies with higher dosages (10 and 100 nmol/min), in the present study a 10-times lower dosage of BQ-123 was chosen to exclude any hemodynamic effect. In further experiments (n=6), radial artery blood flow was measured before and after mental stress (PW-Doppler; angle of insonation, 60°; range gate in the center of the artery). Venous blood samples were drawn before and immediately after the mental stress test in additional experiments (n=8). Circulating ET-1 and norepinephrine levels were measured by radioimmunoassay. In separate experiments, FMD was measured before and after mental stress by terminating a brief (3 minutes) intraarterial infusion of norepinephrine (30 ng/min) to simulate an activation of the sympathetic nervous system. Baseline hemodynamic conditions were reestablished 10 minutes after completing the mental stress task (mean arterial blood pressure, 82±2 mm Hg; heart rate, 64±6 beats per minute; radial artery blood flow, 66±11 versus 75±10 mL/min; radial artery diameter, 2.82±0.13 versus 2.86±0.13 mm; all NS). However, FMD was reduced by half to 4.1±1.0% (P<0.002 versus baseline; Figure 1A), whereas nitroglycerin-induced vasodilation remained unaltered (14.3±1.3%; NS; Figure 1A). FMD progressively returned to baseline levels over a period of approximately 45 minutes (Figure 2; P=0.0002 for trend). The impairment in FMD of the radial artery after
mental stress was not correlated to changes in blood pressure and heart rate during mental stress. Intraarterial infusion of BQ-123 completely prevented the decrease in FMD of the radial artery induced by mental stress (8.6±1.3% before versus 9.4±1.3% 10 minutes after completing the stress task, NS; Figure 1B). Hemodynamic conditions before and 10 minutes after completing the stress task were equal (mean arterial blood pressure, 79±3 versus 73±3 mm Hg; heart rate, 56±2 versus 56±2 bpm; radial artery diameter, 2.87±0.16 versus 2.90±0.16 mm; all NS). In contrast, intraarterial saline infusion did not affect the reduction in FMD induced by mental stress (9.4±1.4% versus 4.4±0.8%, P=0.02; between group, P=0.039). There were no significant differences between the study groups receiving BQ-123 or saline, respectively.

An intraarterial norepinephrine infusion of similar duration as mental stress (3 minutes) did not change FMD (7.0±0.5% before versus 7.4±0.6% ten minutes after terminating the infusion; NS), although local circulating norepinephrine levels increased considerably (from 278±47 to 1065±172 pmol/L, shortly after the infusion; P=0.01). Basal vessel diameter (2.62±0.15 versus 2.59±0.15 mm; NS) and blood flow (59±8 versus 63±3 mL/min; NS) were equal before and 10 minutes after completing the infusion.

**Discussion**

This study provides the first evidence that sudden mental stress induces prolonged endothelial dysfunction via activation of ET₄ receptors. Mental stress reduced the endothelium-dependent, NO-mediated vasodilation by half, whereas that to the endothelium-independent vasodilator nitroglycerin remained unaffected, consistent with impaired endothelial function. Interestingly, the response remained reduced for almost an hour and was prevented by the selective ET₄ antagonist BQ-123 identifying ET₄ activation as the responsible mechanism.

Flow-dependent vasodilation of large conduit arteries is a physiologically important adaptive response of the cardiovascular system during stress mediated by NO.⁶,¹¹ Impaired flow-dependent vasodilation after mental stress reflects an acute imbalance of endothelium-derived NO and ET-1. Indeed, ET-1 reduces the vasodilator effects of NO.¹² In line with this, the impairment of endothelial function induced by mental stress was prevented by ET₄ receptor blockade.

We have previously described a small increase of plasma ET-1 levels after mental arithmetic in offspring of hypertensive but not normotensive parents.¹³ As most of ET-1 produced by endothelial cells is secreted abuminally where it exerts its actions in a paracrine rather than endocrine fashion, circulating ET-1 levels thus do not reflect the local activity of ET-1 in vascular tissue.¹⁴ This is particularly interesting because vascular tissue and circulating levels of ET-1 are elevated in patients with atherosclerosis,¹⁵,¹⁶ In patients with coronary artery disease, vascular tissue levels increase as the process becomes unstable and particularly after infarction.¹⁷,¹⁸

A contribution of the sympathetic nervous system to the derangement of vascular function after mental stress could be excluded. An intraarterial infusion of norepinephrine of similar duration as mental stress resulting in a 4-fold increase of plasma norepinephrine only caused a short transient vasoconstriction for about 5 minutes but did not affect endothelial function 10 minutes later when baseline conditions were reached.

The findings of the present study are in line with the concept of an atherogenic effect of mental stress.¹⁹ Endothelial dysfunction induced by mental stress offers a novel and unique link between psychological factors and the pathogenesis of atherosclerosis similarly to other risk factors that impair endothelial function, eg, hypercholesterolemia and hypertension. Because the underlying mechanism is ET₄ receptor activation, ET₄ receptor antagonism may represent a new therapeutic strategy in the prevention of atherosclerotic vascular disease and its complications.

**Acknowledgments**

This study was supported by the Swiss National Research Foundation (grants 32-51069.97/1 and 32-52690.97), the Swiss Heart Foundation, and the Stanley Thomas Johnson Foundation. The authors thank Manuela Zahno and Rosy Hug for expert technical assistance.

**References**


10. Deleted in proof.


Mental Stress Induces Prolonged Endothelial Dysfunction via Endothelin-A Receptors
Lukas E. Spieker, David Hürlimann, Frank Ruschitzka, Roberto Corti, Frank Enseleit, Sidney Shaw, Daniel Hayoz, John E. Deanfield, Thomas F. Lüscher and Georg Noll

Circulation. 2002;105:2817-2820; originally published online May 20, 2002; doi: 10.1161/01.CIR.0000021598.15895.34
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/105/24/2817

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/