The human coronary vasodilatory response to acute mental stress is mediated by neuronal nitric oxide synthase

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ACUTE MENTAL STRESS can impact on cardiovascular morbidity and mortality, triggering myocardial infarction, ventricular arrhythmia, and left ventricular failure (11). Analogous to exercise-induced stress, mental stress can exert detrimental effects due to suboptimal coupling between myocardial oxygen demand and blood flow (31). The physiological cardiovascular response to mental stress mirrors that to other sympathetic stimuli and includes a catecholamine-driven increase in heart rate, blood pressure, and cardiac contractility (17). Coronary blood flow increases in parallel to the higher myocardial O2 demand, mediated mainly by reduction in coronary vascular resistance. Factors identified to mediate coronary vasodilator reserve include Ca2+-activated K+ channels (27), adenosine, and nitric oxide (NO) (45). The vasomotor response to mental stress can be attenuated or even reversed in the presence of coronary artery disease, a condition that features endothelial dysfunction and reduced NO availability (9, 41, 43).

Until relatively recently, it was generally assumed that the NO responsible for mediating local increases in blood flow was generated by endothelial NO synthase (eNOS) expressed in endothelial cells (10, 16). However, studies using intra-arterial infusion of a selective neuronal NO synthase (nNOS) inhibitor show that local nNOS-derived NO is a major contributor to the basal regulation of microvascular tone and blood flow in the human forearm and coronary circulations (32, 33). It was also found that local nNOS is involved in mental stress-induced forearm vasodilatation in healthy humans. In the present study, we investigated the role of nNOS in the changes in coronary blood flow during acute mental stress in humans.

METHODS

The study was conducted in accordance with the Declaration of Helsinki and received ethical approval from the local Research and Ethics Committee. All participants provided written informed consent. Eleven patients (6 men and 5 women, mean age: 58 ± 14 yr) undergoing elective diagnostic coronary angiography at King’s College Hospital (London, UK) were included in the study. Patients had to have at least one angiographically unobstructed coronary artery as the study vessel. Those with a history of coronary disease, myocardial infarction, left ventricular impairment, dysrythmia, or renal impairment were excluded. Participants abstained from food for at least 6 h before cardiac catheterization and from all medication (except aspirin) on the day of the procedure.

Standard diagnostic coronary angiography was performed in a quiet, temperature-controlled cardiac catheterization laboratory with digital cineangiography. After completion of the diagnostic procedure, a 6-F guide catheter was positioned in the study artery, and a 0.014-in. intracoronary Doppler wire (FloWire, Volcano Therapeutics, Rancho Cordova, CA) was advanced into a proximal segment that was free from side branches. The Doppler wire was interfaced
with a real-time spectral analysis system (ComboMap Pressure and Flow system, Volcano Therapeutics) to record the average peak velocity (APV) of blood flow. APV recordings were taken before coronary angiography at each stage of the protocol, which was performed using non-ionic contrast medium (Omnipaque, GE Healthcare) and without altering the angle of projection during the study. Offline analysis was performed using an automated quantitative coronary edge detection system (Philips) to measure changes in epicardial coronary artery diameter in a 2.5- to 5-mm length segment of vessel, ~2.5 mm distal to the tip of the Doppler wire. Coronary flow was derived from the APV and arterial diameter (12).

All drugs were infused via the guide catheter into the study artery at a rate of 2 ml/min. The selective nNOS inhibitor S-methyl-L-thiocitrulline (SMTC) was obtained from Merck Millipore and was prepared to good medical practice standards for human use in a nationally accredited pharmaceutical manufacturing facility. Substance P was obtained from Bachem (Bubendorf, Switzerland), and isosorbide dinitrate (ISDN) was obtained from Schwarz Pharma (Watford, UK).

A schematic representation of the protocol is shown in Fig. 1. Measurements began once a steady baseline was achieved during intracoronary infusion of 0.9% saline. Substance P was then infused for 2 min at 20 pmol/min, a dose that elicits eNOS-mediated, endothelium-dependent vasodilation without inducing systemic effects (24). After a washout period with normal saline, a 1-mg ISDN bolus was administered to assess endothelium-independent vasodilatation (32). After a further saline washout, the Stroop color-word test was performed to elicit mental stress (15). This was followed by a recovery period after which SMTC (0.625 µmol/min) was infused for 7 min. This dose of SMTC has previously been shown to provide selective inhibition of nNOS in the coronary circulation in a similar patient population (32). SMTC infusion was continued while the Stroop test was repeated. In two patients, a second Stroop test was performed during saline instead of SMTC infusion to confirm that it evoked a reproducible increase in blood flow. Aortic pressure, heart rate, and APV were recorded at baseline and after each phase of the protocol, and coronary angiography was performed to quantify coronary diameter. The ECG was continuously monitored.

**Statistical analyses.** Subject characteristics are summarized as means ± SD; other results are presented as means ± SE. Vasodilator/constrictor responses were analyzed both as absolute change in coronary flow and as the percent change from the immediately preceding baseline. Responses were compared by ANOVA for repeated measures with adjustment for baseline values of coronary blood flow where appropriate [ANOVA/analysis of covariance (ANCOVA)]. Bonferroni correction for multiple comparisons was applied. All tests were two-tailed, and differences were considered significant at \( P < 0.05 \).

### RESULTS

Baseline subject characteristics are shown in Table 1. The indications for coronary angiography included chest pain (7 subjects), dyspnea (2 subjects), nonsustained ventricular tachycardia on Holter monitoring (1 subject), and preoperative assessment in valvular disease (1 subject). Four subjects underwent functional testing for ischemia (either stress echocardiography, myocardial perfusion scintigraphy, or exercise treadmill testing), which was positive in three cases. Ten subjects had all three coronary arteries smooth and unobstructed; the 11th subject had minor irregularities (<10% stenosis) in all arteries. None of the subjects developed adverse reactions, symptoms, or ECG changes of ischemia during the infusions or mental stress.

**Effect of mental stress and SMTC on coronary flow.** The hemodynamic responses and changes in coronary blood flow and coronary conductance after mental stress are shown in Table 2. The changes in heart rate and blood pressure during mental stress were minimal and similar with or without SMTC.

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**Table 1. Baseline characteristics of patients**

| Characteristic            | Value            |
|---------------------------|------------------|
| \( N \)                   | 11               |
| Age, yr                   | 58 ± 14          |
| Sex, men/women            | 6/5              |
| Hypertension, \( n \)     | 7                |
| Diabetes mellitus, \( n \) | 2               |
| Hypercholesterolemia, \( n \) | 8            |
| Smoker, \( n \)           | 0                |
| Body mass index, kg/m²    | 28.2 ± 3.2       |
| Medication, \( n \) (%)   |                  |
| Angiotensin-converting enzyme inhibitor/angiotensin receptor blocker | 4 (36)         |
| \( β \)-Blockers or Ca²⁺ channel blocker | 5 (45)        |
| Statins                   | 2 (18)           |
| Study artery, \( n \)     |                  |
| Left anterior descending coronary artery | 7               |
| Circumflex artery         | 3                |
| Right coronary artery     | 1                |

Values are means ± SD; \( n \), no. of subjects.

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**Table 2. Hemodynamic responses during mental stress with or without SMTC**

|                     | Saline | Stress | SMTC |
|---------------------|--------|--------|------|
| Heart rate, beats/min | 59 ± 3.7 | 60 ± 3.9 | 55 ± 3.0 |
| Systolic BP, mmHg    | 117 ± 3.7 | 120 ± 6.0 | 114 ± 4.0 |
| Mean BP, mmHg        | 84 ± 1.3 | 83 ± 2.7 | 81 ± 3.0 |
| Diastolic BP, mmHg   | 68 ± 2.3 | 64 ± 2.5 | 65 ± 3.9 |
| Coronary blood flow, ml/min | 38 ± 3.9 | 52 ± 5.4† | 31 ± 4.0 |
| Coronary conductance, units | 0.49 ± 0.04 | 0.61 ± 0.08* | 0.42 ± 0.06 |

Values are means ± SE. SMTC, S-methyl-L-thiocitrulline; BP, blood pressure. *\( P < 0.05 \) and †\( P < 0.01 \) vs. the preceding baseline. ‡Significant interaction between groups.
Mental stress increased coronary flow by 34 ± 7.0% (P < 0.01; Fig. 2A). SMTC reduced basal coronary flow by 20 ± 4.7% (P = 0.01), consistent with previous work (32, 33). ISDN increased diameter by 3.9 ± 2.0% (P = 0.01), consistent with previous work (32, 33).

Effect of mental stress and SMTC on epicardial coronary diameter. Mental stress increased coronary artery diameter by 6.9 ± 3.7% (P = 0.02; Fig. 3). SMTC reduced coronary artery diameter by 5.1 ± 1.6% (P < 0.01) and abolished the mental stress-induced increase to 0.5 ± 2.8% (P = 0.98 compared with the preceding baseline). ISDN increased diameter by 3.9 ± 2.0% (P = 0.01), and substance P increased it by 3.1 ± 1.6% (P = 0.07). There was no significant correlation between the percent change in coronary diameter and percent change in coronary blood flow in response to acute mental stress (r² = 0.19, P = 0.15).

DISCUSSION

NO plays an important role in the regulation of vasomotor tone in the human coronary circulation, both during resting conditions and under situations of increased metabolic demand. At rest, it maintains a state of tonic vasodilation in resistance vessels (21) and conduit epicardial arteries (22). After stimuli such as mental stress and cold pressors (3, 9, 13, 26), NO mediates dynamic changes in vascular tone that antagonize catecholamine-mediated vasoconstriction.

Until relatively recently, investigations of the role of NO in regulating coronary vascular tone in humans relied mainly on the use of the nonselective NOS inhibitor Nω-nitro-L-arginine (L-NMMA) or of endothelium-dependent agonists, such as ACh, substance P, and fluid shear stress (14, 21, 36–38). Based on such studies, eNOS expressed in the endothelium was assumed to be the main source of local NO that regulated vascular tone. However, experimental animal studies (25) have indicated that nNOS expressed in perivascular nerves or the vascular smooth muscle might also regulate local vessel tone. In line with this, nNOS is reported to be expressed in human coronary artery smooth muscle cells (18) and coronary perivascular nerves in rats and dogs (34, 44).

In first-in-human studies with the nNOS-selective inhibitor SMTC, our laboratory previously demonstrated that basal blood flow in the human coronary circulation in vivo is under tonic regulation by local nNOS-derived NO (32). In these experiments, we found that SMTC had no effect on endothelium-dependent vasodilation induced by substance P, which was, however, inhibited by L-NMMA. Similarly, local SMTC infusion into the forearm circulation of healthy human subjects reduced basal blood flow in an L-arginine-dependent manner but without affecting the vasodilator response to ACh (33). Furthermore, we found that mental stress-induced increases in forearm blood flow in healthy men were significantly blunted...
by local intra-arterial infusion of SMTC, suggesting that local nNOS was involved in this setting. Previous work in animals has shown that nNOS-dependent effects on vessel tone vary significantly by vascular bed (10, 19, 23), and so it was important to establish whether nNOS is involved in mental stress-induced vasodilatation in the human coronary circulation.

The novel finding of this study is that nNOS is responsible for mediating mental stress-induced vasodilation in the resistance vasculature of the human coronary circulation. The changes in coronary flow and conductance were not related to significant changes in systemic hemodynamics, suggesting that they represented a direct vasodilator action of nNOS-derived NO and not effects secondary to altered myocardial O2 demand. This role of nNOS contrasts to pacing-induced increases in coronary blood flow, which we recently reported were mediated by eNOS-derived NO (35). The activation of eNOS during pacing is likely to involve increased endothelial shear stress, whereas nNOS activation during mental stress may involve perivascular nitricergic nerves in the coronary vessel wall and their autonomic activation (2, 5).

The baseline response to mental stress in the present study was variable, ranging from a 1–77% increase in blood flow, similar to our laboratory’s previous observations in the forearm. Previous studies on mental stress-induced vasodilation in humans have reported average increases ranging from a 55% increase in blood flow (9) to a 10% increase in flow (43), which may be related to the differing characteristics of study participants. Specific factors that could have influenced the vascular response to stress in our study include vasoactive medications, body mass index, and diabetes. Local characteristics of the vessel studied could also have influenced the response, although we selected vessels that were angiographically free of stenosis. The use of different techniques to elicit mental stress may also contribute to variation in responses. In the present study, SMTC reduced basal coronary flow, as previously documented (32, 33), and substantially attenuated the mental stress-induced increase in blood flow. The degree of attenuation in stress-induced vasodilation is similar to that previously observed in the forearm (20), although it should be noted that subject characteristics were significantly different between these studies. The present study by necessity was undertaken in patients undergoing diagnostic coronary angiography and, therefore, included older patients with multiple risk factors for coronary disease and on oral medications.

Impaired mental stress-induced increases in flow have been reported in coronary artery disease (9, 31), metabolic syndrome (8), and non-flow-limiting atherosclerosis (43). Interestingly, we found little correlation between the response to substance P and the response to mental stress, suggesting that eNOS-mediated vasodilator function may not be the major determinant of the mental stress response. It is possible, therefore, that the variations in the mental stress response could, in part, be explained by the presence of nNOS dysfunction in these conditions (7, 30, 39). Potential underlying mechanisms include nNOS uncoupling, which has been documented in conditions of oxidative stress, such as atherosclerosis, and increased levels of the endogenous NO synthase inhibitor asymmetric dimethylarginine, which has been observed in conditions such as diabetes and hypertension (29).

Numerous studies have been performed to explore the impact of stress on patients with coronary artery disease, and a recent meta-analysis found that mental stress-induced ischemia is associated with an approximately doubled risk of cardiac events (myocardial infarction, revascularization, and unstable angina) and total mortality (42). The finding of reduced coronary blood flow during mental stress has also been shown to be a predictor of daily ischemia in patients with coronary artery disease, independent of exercise-induced ischemia (4).

Little is known about the long-term outcomes associated with impaired mental stress-induced vasodilation in people who have been diagnosed with “normal coronary arteries.” There are some important considerations to bear in mind. First, it is now established that coronary angiography can underestimate the degree of plaque burden compared with postmortem histology (40). Second, a degree of “visual-functional” mismatch occurs on coronary angiography, which can underestimate a plaque’s functional severity in up to 40% of cases (28). Third, it is increasingly understood that the majority of acute coronary syndromes arise from plaques that are not necessarily flow limiting in size but share vulnerable characteristics that ultimately lead to their rupture (6). Indeed, Yeung et al. (43) found that mental stress triggers vasoconstriction at the site of both obstructive and nonobstructive plaques in epicardial arteries, with an accompanying decrease in myocardial blood flow. Additionally, Arrighi et al. (1) found that mental stress caused increased coronary resistance and impaired myocardial blood flow in regions subtended by vessels without significant stenosis. In the present study, we observed nNOS-dependent changes in epicardial coronary artery diameter, as well as coronary blood flow, in response to acute mental stress. These effects were not correlated with each other, suggesting that they may both reflect direct effects of nNOS, but we cannot exclude the possibility that the changes in epicardial diameter might be secondary to the changes in blood flow. Given the identification of nNOS as a mediator of stress-induced coronary flow in individuals with angiographically normal arteries, it would be of interest to examine its function in patients with established coronary disease.

In conclusion, this study identified nNOS as the major NO synthase isoform responsible for mediating the vasodilator response to mental stress in the human coronary circulation. Due to the complex interplay that exists between mental stress and vasodilator function in the coronary circulation, dysfunctional blood flow responses to mental stress may contribute to the development of stress-induced myocardial ischemia, irrespective of the presence of angiographically significant coronary artery disease.

Study limitations. We estimated coronary blood flow from flow velocity rather than using thermodilution, but flow velocity has been widely used in studies of coronary vascular function. All of the participants in our study either had risk factors for vascular endothelial dysfunction and/or were on vasoactive medications, so that the current results do not necessarily reflect the role of nNOS in completely healthy humans. To minimize potential confounding effects of these factors, we studied arteries that appeared angiographically smooth and unobstructed, and we omitted medications on the day of the study. In addition, the impact of coexisting atherosclerosis risk factors in this study was not sufficient to impair endothelium-dependent vasodilatation to substance P.
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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

S.G.K., N.M., H.S., A.R.C., K.M., F.K., and A.M.S. performed experiments; S.G.K., N.M., H.S., and K.O. analyzed data; S.G.K., N.M., P.C., and A.M.S. interpreted results of experiments; S.G.K. prepared figures; S.G.K. drafted manuscript; S.G.K., N.M., P.C., and A.M.S. edited and revised manuscript; A.M.S. conceived and designed research; all authors approved final version of manuscript.

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