In vivo measurement of flow-mediated vasodilation in living rats using high-resolution ultrasound

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Submitted 2 July 2007; accepted in final form 30 November 2007

The measurement of endothelial vasodilator function as flow-mediated dilation (FMD) has emerged as a useful tool for atherosclerosis research and is used as a barometer for cardiovascular health in humans (27, 28). The severity of endothelial dysfunction correlates with a patient’s risk for experiencing an initial or recurrent cardiovascular event (28). In the presence of risk factors for cardiovascular disease, such as age, diabetes, hypertension, smoking, and dyslipidemia (8–10, 14), the endothelium loses its normal regulatory control of vessel wall homeostasis (10). Endothelial dysfunction is characterized by a loss of endothelial control over vascular tone, thrombosis, and vessel wall remodeling. A growing number of therapeutic interventions known to decrease cardiovascular risk, including exercise, lipid lowering, dietary interventions, smoking cessation, weight reduction, or medication with angiotensin-converting enzyme inhibitors and statins, have been shown to improve endothelial function (28). These observations underscore the usefulness of measuring endothelial function as a diagnostic indicator of cardiovascular health. However, many aspects of the underlying mechanisms are not fully understood due to limitations associated with human subjects as well as the lack of a validated living animal model of conduit artery flow-mediated vasodilation.

In humans, endothelial vasodilator function can be quantified by monitoring FMD, the dilation of conduit arteries in response to physiologically relevant increases in wall shear stress (WSS) induced by ischemic dilation of the downstream microvasculature (2, 13, 20). Increases in WSS, i.e., the tangential force exerted by the flow of blood over the surface of the endothelium, lead to a rapid activation of endothelial nitric oxide (NO) synthase (NOS) with consequent increases in NO formation (21). Accordingly, FMD is believed to be largely abolished following NOS inhibition and therefore has been used to provide a “read out” of local vascular NO availability. Newer literature points toward NO-independent FMD mechanisms and their potential connection to the development of vascular disease and the occurrence of cardiovascular events (20). Under conditions of decreased NO activity, other factors such as endothelium-derived hyperpolarizing factor (EDHF) and cyclooxygenase (COX) products appear to play a more dominant role in modulating vascular tone (5). The different processes implicated in vascular reactivity are not well understood but are the subject of increasing interest.

Despite the need for a better understanding of these mechanisms, there is currently no FMD-related integrative physiological model available that is concordant with human FMD and allows repeated studies in living rodents. Such a model could greatly facilitate cardiovascular research as a useful tool in cases where human clinical research is impractical or prohibited due to ethical issues associated with studies of experimental drug (7), gene, and cell therapies (24, 30) and when tissue sampling is required. Here we show the feasibility of an FMD-related approach to study both NOS-dependent and independent vasodilation of the femoral artery (FA) in anesthetized rats during reactive hyperemia following ischemia using high-resolution ultrasound.
MATERIALS AND METHODS

Animals and Ultrasound Measurements

All animal procedures were approved by the University of California, San Francisco (UCSF) Institutional Animal Care and Use Committee, and rats were housed in the UCSF animal facility and fed a standard diet ad libitum (Purina, Richmond, IA). Male Sprague-Dawley rats (Charles River, Portage, MI) were anesthetized with isoflurane (4% induction and 2.5% maintenance), and body core temperature was closely monitored by a rectal probe and kept stable by performing the examination on a heated examination table with warming lamps directed at the animal. All infusions were prepared with warm (37°C) saline as vehicle. If not stated otherwise, experiments were performed in 9- to 10-wk-old rats. In the last set of experiments, 9- to 10-wk-old (200–300 g) and 20–24-wk-old (500–600 g) rats were compared. The FA (Fig. 1) was visualized with a 35-MHz transducer (axial resolution of 50 μm; VisualSonics, Toronto, Canada). After the identification of the FA by its characteristic flow pattern, the position of the probe was optimized to show clear vessel wall/lumen interfaces, allowing automated recognition by the analysis software. When the clearest image of the vessel walls was obtained (Fig. 1), the settings were optimized, and the probe was fixed in a stand and not changed throughout the investigations. Experiments were started after a 15-min equilibration period and when a stable-body core temperature was achieved. The image and flow analyses were performed off-line from recorded loops using an automated system (Brachial Analyzer 5, Medical Imaging Applications, Coralville, IA). All vasodilation results are presented as Δ% change: (Diameterpostischemia − Diameterbaseline)/Diameterbaseline × 100. Flow was calculated as π × (diameter/2)² × mean flow velocity (V). V was calculated as velocity time integral × duration of heart cycle. WSS was calculated as 8 × μ × V/diameter, where blood viscosity (μ) was assumed to be constant at 0.035 dyn·s⁻¹·cm⁻². See Fig. 1 for representative readings over four heart cycles. All diameter readings were taken at diastole, and flow velocity represents the mean angle-corrected Doppler-flow velocity.

Pharmacological and Flow-Mediated Induction of Vasodilation

Nitroglycerin. After obtaining baseline readings for diameter, blood pressure, and heart rate, five increasing bolus doses of nitroglycerin (NTG; 8.8 × 10⁻⁴–10⁻⁸ M, 0.1–100 μg in 200 μl vehicle followed by 300 μl to flush the infusion system, which had a dead volume of ~200 μl, n = 4; Hospira, Lake Forest, IL) were administered via tail-vein catheter (21G Butterfly, Abbott, North Chicago, NC). Repeated readings were taken immediately after injection (0 min) and 1, 2, 3, and 4 min after each injection with 10-min breaks between doses. The blood pressure and heart rate were measured noninvasively with a computerized tail-cuff method (NIBP-8; Columbus Instruments, Columbus, OH) (12). Vehicle controls were performed in parallel groups of animals.

Acetylcholine. Because of the short half life and potentially severe hemodynamic systemic side effects, acetylcholine was injected locally into the hindlimb circulation. We inserted a saline-filled polyethylene (PE)-50 catheter with a pressure transducer (Powerlab Adinstruments/8sp, Milford, MA) into the left common carotid artery and advanced it to the iliac bifurcation (n = 6). After baseline readings, six increasing bolus doses of acetylcholine (Sigma-Aldrich, St. Louis, MO) were administered via the intra-aortic catheter (10⁻⁹–10⁻⁴ mol/l in 200 μl vehicle followed by 300 μl vehicle). Repeated readings were taken immediately after injection (0 min), and 0.5, 1, 1.5, 2, 2.5, and 3 min after injection with 10-min breaks between injections. Blood pressure and heart rate were measured via the fluid-filled intra-aortic catheter. Acetylcholine stock solutions were prepared freshly with vehicle on each day. Vehicle control injections were performed in parallel groups of animals.

Adenosine. To pharmacologically mimic the blood flow increase during reactive hyperemia, we administered a bolus dose of adenosine (100 μl of 10⁻⁵ mol/l) as detailed for acetylcholine (Hospira). Blood pressure, heart rate, diameter, and flow velocity were measured before bolus injection and at 0, 0.5, 1, 1.5, 2, 3, 4, and 5 min (n = 5).

Flow increase with vehicle perfusion. To mimic the fluid shear stress of blood flow increase during reactive hyperemia, we infused vehicle vehicle for 20 s at 1.5, 3, and 4.5 ml/min via the intra-aortic catheter.

Fig. 1. Measurement of femoral artery (FA) diameter. A: longitudinal sections of the rat FA were acquired with a high-resolution ultrasound probe placed on the upper thigh. Right: representative ultrasound image with automatically detected vessel walls. B: ECG, flow velocity, diameter, and instantaneous wall shear stress (WSS) tracings over 4 heart cycles. Dotted lines enclose 1 heart cycle. bpm, Beats/min.
FA diameter and flow were monitored before, during, and until 3 min after infusion \((n = 3\) animals). Physiological vasodilation during reperfusion after temporary hindlimb ischemia. Ultrasound diameter and Doppler-flow measurements were obtained from longitudinal sections of the FA before and after 5 min of hindlimb ischemia \((n = 5)\). Reproducible ischemia and reperfusion of the hindlimb were achieved with an arterial loop occluder that was positioned upstream of the site to be visualized, around the common iliac artery, through a transabdominal incision. This approach was chosen to minimize movement artifacts and to ensure complete ischemia of the hindlimb. The loop occluder consisted of a 5-0 prolene filament around the artery and passed through a 15-cm PE-90 tubing, which was externalized and skin closed with clips. Hindlimb ischemia was achieved by pulling on the filament through the tubing and clamping with a hemostat clamp. After a 15-min equilibration period, baseline readings were taken and the common iliac artery was occluded with the loop occluder. Flow arrest was confirmed by abrogation of the Doppler signal. After 5 min of ischemia, the hindlimb was reperfused by release of the occluder. Reactive hyperemia was monitored by flow velocity and diameter of the FA at 0, 0.5, 1, 1.5, 2, 3, 4, and 5 min. To determine the contribution of NOS, we injected \(N^\bullet\)-monomethyl-l-arginine (L-NMMA, 8 mg/kg in 0.1 ml/100 g; Sigma), a competitive NOS inhibitor, and repeated measurements following the occlusion protocol outlined above.

Mechanistic Inhibitor Studies in Younger and Older Animals

To study the mechanisms involved in flow-mediated vasodilation, we measured FMD in 9- to 10-wk- and 20–24-wk-old animals \((n = 4\) to 5 per group) before and after injection of saline, L-NMMA, indomethacin, charybdotoxin + apamin, and L-NMMA + charybdotoxin + apamin (\(n = 4\) to 5 per group). NOS inhibition was accomplished by injection of L-NMMA (8 mg/kg in 0.1 ml saline/100 g; Sigma). To study the impact of EDHF, the calcium-activated channel was inhibited by charybdotoxin (stock solution, 5 \(\mu\)mol/l in 0.1 ml/100 g; Sigma) and apamin (stock solution, 5 \(\mu\)mol/l in 0.1 ml/100 g; Sigma) at doses previously shown to completely inhibit EDHF-mediated vasodilation \((17)\). COX was blocked by indomethacin \((0.2\ mg/kg\ in 0.1\ ml/100\ g;\ Merck,\ Whitehouse\ Station,\ NJ)\). Endothelium-independent vasodilation was also measured at 2 min following NTG \((8.8 \times 10^{-5}\ mol/l\ in\ 0.1\ ml/100\ g\ bolus\ iv)\).

Reproducibility of Arterial Diameter and FMD Measurements

Reproducibility of FA diameter and vasodilation following 5 min of ischemia in rats was confirmed by three repeated measurements on the same day with 10-min breaks between measurements \((n = 5)\). The ultrasound probe remained fixed in a stereotactic stand, and image settings were not changed throughout the investigation. In another set of rats we performed a baseline FMD and repeated measurements after 1 mo. Following the baseline measurements, we removed the occluder and closed the skin with clips \((n = 5)\). All measurements were performed off-line from recorded cine loops using the automated edge detection system.

Statistical Analyses

Data are presented as means (SD). The \(n\) values refer to number of individual animals in which the experiments were performed. The primary test for an effect was a test of the interaction in a two-way repeated-measures ANOVA (where the 2 factors were time and intervention). The family of pairwise comparisons were conducted using the Holm-Sidak method. \(P\) values of \(<0.05\) were regarded as significant. Correlations were Pearson \(R\) correlation. Graphs and sigmoid fit of dose responses were performed with Origin 7 (Origin-Lab, Northhampton, MA); all other analyses, with SigmaStat 3.5 (Systat Software, San Jose, CA). Statistical power analyses were performed post hoc using PS Power and Sample Size Calculations 2.1.30 (4).

RESULTS

Dose-Dependent Pharmacological Vasodilation Induced by NTG and Acetylcholine

To determine whether this approach can detect physiologically relevant changes in FA diameters in living rats, we established dose-response curves for two different, well-characterized, pharmacological agents mediating vasodilation via endothelium-independent and -dependent mechanisms using NTG and acetylcholine, respectively.

Systemic injection of NTG bolus doses at \(8.8 \times 10^{-4}–8.8 \times 10^{-6}\ mol/l\) led to significant dose-dependent vasodilation of the femoral artery (Fig. 2A). Peak vasodilation was \(21.2\%\) (SD 3.8) at \(\geq8.8 \times 10^{-7}\ mol/l\), and the concentration to exert half maximal effects \((EC_{50})\) was calculated to be \(3.3 \times 10^{-6}\ mol/l\) (Fig. 2B). Each bolus at \(8.8 \times 10^{-6}–8.8 \times 10^{-8}\ mol/l\) was followed by a gradual diameter increase that peaked at \(-2\ min\) and then gradually decreased. Noninvasive blood pressure and heart rate, 123 (SD 6)/71 (SD 3) mmHg and 399 beats/min (SD 2), respectively, were not significantly affected by NTG at the applied doses, confirming that the observed endothelium-independent vasodilation was independent of hemodynamic changes.

The endothelium-dependent vasodilator acetylcholine caused a dose-dependent vasodilation of the FA at \(10^{-6}–10^{-4}\ mol/l\) when injected as 200 \(\mu\)l bolus locally into the iliac artery (Fig. 2C). Maximal vasodilation was \(26.8\%\) (SD 4.0), and \(EC_{50}\) was \(1.3 \times 10^{-6}\ mol/l\) acetylcholine (Fig. 2D). There was no change in heart rate [327 beats/min (SD 37)]. The systolic [89 mmHg (SD 13)] and diastolic [67 mmHg (SD 12)] blood pressure remained unaffected at doses \(\leq10^{-6}\ mol/l\). A transient decrease in systolic blood pressure was observed at 0.5–1 min following the \(10^{-4}\ mol/l\) bolus \([80\ (SD\ 11)\ and\ 84\ mmHg\ (SD\ 6)]\). All parameters remained unaffected after vehicle injection. The peak acetylcholine-induced vasodilation was not significantly different from the NTG response.

FA Vasodilation Following Resistance Artery Dilation with Adenosine and After Physical Shear Stress

To further characterize the flow dependence of vasodilation, we induced vasodilation in the hindlimb downstream resistance vessels to increase flow velocity in the upstream femoral artery (Fig. 3A). This was accomplished by a bolus injection of adenosine into the distal aorta with the catheter tip at the iliac bifurcation. A \(10^{-5}\ mol/l\) adenosine bolus led to an immediate flow increase, which was followed by a diameter increase that mimicked ischemia-induced reactive hyperemia (compare Figs. 3B and 4). Maximal vasodilation of 10\(\Delta\%)\ (SD 2)\) was achieved between 1–1.5 min of postinfusion.

To further confirm flow dependence, we induced temporal WSS in the FA by vehicle injection via a distal intra-aortic catheter using increasing infusion rates (Fig. 3C). Injection of vehicle at 1.5, 3.0, and 4.5 ml/min over 20 s led to an increase in flow velocity (Fig. 3D) in the downstream FA, which in turn led to an increase in WSS from 143 (SD 8) to 160 (SD 2), 211 (SD 3), and 283 dyn/cm\(^2\) (SD 16), respectively (Fig. 3E).
Increases in WSS corresponded to 11%, 48%, and 97%. After cessation of injection, flow and WSS returned to baseline values. Whereas infusion at 1.5 ml/min did not significantly alter FA diameter, 3.0 and 4.5 ml/min led to significant FA vasodilation with maximal values of 10\% (SD 0.1) and 14\% (SD 0.5), respectively, at 2.0 and 2.5 min. This dependence on flow and associated shear stress is illustrated in Fig. 3F.

**Transient Hindlimb Ischemia Leads to NOS-Dependent Vasodilation of the FA**

Five minutes of hindlimb ischemia (see Fig. 4) led to an approximate twofold increase in flow velocity and WSS compared with baseline and was followed by a rapid decay to baseline values at \textasciitilde3 min. This was associated with a delayed increase in FA dilation that peaked at 1 to 2 min and then slowly decayed. Together, these changes resulted in a significant increase in blood flow for 2 min. NOS inhibition with l-NMMA completely blocked FA dilation, but it did not affect the peak flow velocity and WSS increase (Fig. 4; see closed symbols). As a result, WSS was significantly higher at 1 min.

**Age-Dependent Impairment of FMD**

Aging is a biological factor known to be associated with a gradual decrease in endothelium-dependent vasodilation (8). To demonstrate the physiological relevance of this approach, we compared FMD measurements from young and older rats as a model of endothelial dysfunction. FMD was measured in a young group of rats at 9 to 10 wk of age (n = 10) and an older group at 20–24 wk of age (n = 10). FMD was significantly lower in the older group [16.0\% (SD 2.1) vs. 12.6\% (SD 0.9), \(P < 0.001\); Fig. 5]. The two groups did not differ significantly in terms of either endothelium-independent vasodilation following NTG [20.7\% (SD 2.7) vs. 19.7\% (SD 1.8), \(P = 0.593\)] or baseline diameter [0.39 (SD 0.12) vs. 0.37 mm (SD 0.04), \(P = 0.308\)] of the FA, suggesting that the observed differences in FMD were specific to the endothelium.

To study the mechanisms underlying FMD in both groups, FMD was measured before and after the injection of inhibitors of NOS (l-NMMA), EDHF (charybdotoxin + apamin), and COX (indomethacin; Fig. 5; \(n = 4\) to 5 per group). In confirmation of our results (as described in the previous section), FMD was completely abolished by l-NMMA in younger rats [15.4\% (SD 3.1) vs. 0.4\% (SD 0.4), \(P < 0.001\)], suggesting NOS dependence. In contrast, FMD in older rats was blocked by only \textasciitilde50% following infusion of l-NMMA. Charybdotoxin + apamin had no effect in the younger rats, but FMD in older rats was partially blocked by these inhibitors. The combination of l-NMMA + charybdotoxin + apamin completely blocked FMD in both groups of rats. Whereas FMD in the older rats was affected by EDHF inhibitors that did not affect the younger rats, neither group was affected by the COX inhibitor indomethacin.

**Reproducibility of Arterial Diameter and FMD Measurements**

Repeated measurements of FA diameters in individual rats on the same day had a SD of 1.8\% (intra-animal variation). The variation of repeated FMD measurements on the same day in individual rats had an average SD of 1.7\% and an averaged difference between repeated readings of 0.9\% (SD 0.6). At a sample size of 4 and this SD of 1.7\%, a change of 3.4\% (paired tests for FMD) can be detected at an alpha probability of 0.05 and power of 0.8. The variation of repeated FMD measurements (\(n = 5\)) after 1 mo in individual rats had a SD of 0.7\% and an averaged difference between repeated readings of 0.4\% (SD 0.2). The readings before and after 1 mo were
highly correlated ($r = 0.98, P = 0.004$). When averaging baseline FMD measurements from 20 different animals, we observed a mean FMD of 14.3% with a slightly greater SD of 2.4% (between animal variation). At a sample size of 5 and a SD of 2.4%, a change of 4.8% (independent tests for FMD) can be detected at an alpha probability of 0.05 and power of 0.8.

DISCUSSION

In this study, we show that it is feasible to use the human FMD approach to measure endothelial vasodilator function in living rats. The key findings can be summarized as follows. First, we can detect different degrees of vasodilation in the FA of living rats using both endothelium-dependent and -independent pharmacological vasodilators. Second, the conduit artery vasodilation can also be triggered by pharmacologically and mechanically induced increases in WSS. Third, transient ischemia of the hindlimb leads to reactive hyperemia with an instantaneous increase in WSS and a subsequent NOS-dependent conduit artery dilation. Fourth, biological relevance is underscored by the demonstration of age-dependent impairment of FMD and a shift in underlying mechanisms from NOS-dependent to -independent effects, potentially EDHF dependent. Fifth, this vasodilation is highly reproducible on the same day and even after 1 mo.

In human cardiovascular research, the measurement of flow-mediated vasodilation using ultrasound represents a widely applied and prospectively validated clinical tool to assess vascular health. Until now, there has been no integrative model for FMD in rats that is concordant with the human method and would, therefore, allow the direct comparison of results. This was in part due to technical limitations of relatively low-frequency ultrasound in the context of small peripheral vessels in rodents. Recent development of high-frequency transducers (25) together with the automated edge detection and analysis software that we employed for human trials (8) allowed us to reproducibly measure vessel diameters and flow even in peripheral arteries of rats with small diameters of 0.2–0.5 mm. To test whether the ultrasound system with an axial resolution of 50 μm is able to detect physiologically relevant diameter changes, we performed dose-response experiments with established and characterized vasodilators. Classically, aortic rings or strips were isolated from animals after death, maintained in an organ bath, and preconstricted with phenylephrine. The vasodilation of larger vessels was determined as a decrease in tension or increase in diameter during the addition of vasoactive substances (6, 11, 31). This methodology was applied to other vessels including femoral (26), iliac (3), mesenteric (1), and renal arteries (23) from various species. The results for these conduit arteries were similar and showed predominantly endothelium- and NOS-dependent vasodilation in response to acetylcholine, as well as similar dose-response curves for endothelium-independent vasodilators (e.g., NTG) (29). The other frequently used method to determine vascular reactivity is the determination of changes in perfusion pressure or flow that occur with vasoactive substances. A decrease in perfusion pressure or increase in flow predominantly reflects dilation of the resistance arteries and is largely independent of NOS (29). These in vitro experiments have enabled very powerful reductionist approaches to the study of vascular reactivity. However,
these isolated vessel systems lack the contact of the endothelium with the blood that normally bathes it, and they are removed from the context of the whole organism (e.g., endocrine factors, hemodynamics, or nervous system). In the present study, we show in living rats that intravenous injection of the endothelium-independent vasodilator NTG showed dose-dependent FA vasodilation at 1 to 2 min after injection. At dose of $10^{-10}$–$10^{-9}$ g, maximal vasodilation was achieved without affecting systemic hemodynamics, suggesting that this dose could be used as a positive control to determine endothelium-independent vasodilation in these experiments, similar to the generally applied NTG in human trials. Regional intraarterial injection of acetylcholine led to instantaneous dose-dependent vasodilation similar to that reported in human coronaries, the forearm in vivo, and in animal experiments with aortic rings or strips ex vivo. Our results demonstrate that the high-resolution ultrasound system can detect pharmacologically induced, physiologically relevant changes in FA diameters in living rats.

In the human forearm, reperfusion after brief ischemia is characterized by two phases: an early and a delayed phase (21). Ischemia induces an initial vasodilation of resistance arteries, which leads to a delayed flow- and NOS-mediated vasodilation of the upstream conduit artery (3, 16, 18, 20–22). In the present study, we show analogous vasodilator responses in the FA of rats. The onset of reperfusion is accompanied by an initial flow velocity increase ($E$), leading to a significant increase in wall shear stress ($F$), which is followed by a prolonged dilation of the conduit artery ($G$). Whereas hyperemic flow velocity and WSS increases were unaffected by $N^\bullet$-monomethyl-L-arginine (L-NMMA), a competitive nitric oxide synthase (NOS) inhibitor, FA vasodilation was completely blocked by L-NMMA, suggesting NOS dependence. Symbols are means (SD). *P < 0.05 vs. baseline; #P < 0.05 vs. vehicle.
In 9- to 10-wk-old animals, FMD was completely blocked by L-NMMA, an inhibitor of EDHF. In older animals, FMD was only partly blocked by either L-NMMA and CTx apamin, whereas the injection of all 3 inhibitors led to complete block of FMD. (Each intervention with pre- and postinhibitor FMD; n = 10 animals per group). Bars are means (SD). *P < 0.05 vs. saline; #P < 0.05 vs. L-NMMA and CTx + apamin; †P < 0.05 vs. 9- to 10-wk group.

Fig. 5. Age-dependent impairment of endothelial function and change in mechanisms mediating flow-mediated dilation (FMD). A: whereas conduit artery vasodilation (FMD) is lower in 20–24-wk-old animals, NTG-mediated endothelium-independent vasodilation (NTG) and baseline diameter (B) were not significantly different between groups (A + B; n = 10 animals per group). C: in 9- to 10-wk-old animals, FMD was completely blocked by L-NMMA, a NOS inhibitor, and not affected by the charybdoxotoxin (CTx) and apamin-inhibiting EDHF. In older animals, FMD was only partly blocked by either L-NMMA and CTx + apamin, whereas the injection of all 3 inhibitors led to complete block of FMD. (Each intervention with pre- and postinhibitor FMD; n = 4 to 5 animals; therefore, baseline FMD column represents n = 22 animals in each group.) Bars are means (SD). *P < 0.05 vs. saline; #P < 0.05 vs. L-NMMA and CTx + apamin; †P < 0.05 vs. 9- to 10-wk group.

be a result of flow increases ex vivo. We provide further evidence for flow dependence of FA vasodilation in this model. We show that an increase in flow experimentally induced by two different stimuli is followed by FA dilation: 1) adenosine, a vasodilator predominantly but not exclusively acting on the resistance arteries, mimics this pattern of reactive hyperemia, and 2) physical perfusion of the hindlimb with vehicle (saline) at similar flow rates as observed during the onset of reactive hyperemia leads to similar degrees of vasodilation of the FA (15%). Several limitations apply to these approaches. Adenosine can also cause vasodilation of the conduit artery, and, therefore, the vasodilation cannot exclusively be attributed to the effects on the microcirculation. However, the time course of vasodilation is similar to FMD, with maximal dilation occurring later than that observed after local injection of acetylcholine. The WSS increase exerted by adenosine fully accounts for the observed ~10% FA vasodilation, judging from the results of the perfusion experiments with 1.5 ml/min. Another limitation of this approach is that saline, which is virtually free of calcium, may lead to falling intravascular calcium concentrations during infusion, which may hypothetically impair FMD. Furthermore, saline has lower viscosity than blood and would, therefore, be expected to exert lower shear stress as would be expected from blood at similar flow rates. Nevertheless, the maximally achieved vasodilation following local saline perfusion was similar in magnitude with that observed after transient hindlimb ischemia.

We and others have shown in humans that FMD is impaired in different vascular disease states and that this may be, at least in part, due to an impaired NO metabolism (10, 15, 20). Although FMD was primarily used as a read out of NO bioavailability, newer literature suggests that under certain disease conditions, other NOS-independent mechanisms may become more dominant in FMD. In our present study, we show a decreased FMD response in older adult animals (20–24 wk) compared with younger animals (9 to 10 wk), despite similar endothelium-independent vasodilation (NTG) and baseline FA diameter. These results are similar to findings in humans that also show a gradual reduction of FMD from childhood over adulthood to senescence with FMD responses of 11% at 3 yr (Natarajan et al., unpublished data), 7% at 25 yr, and 5% at (61 yr) (8). One limitation of the present study is that we cannot exclude body size as a major contributor to endothelial dysfunction since we also observed significantly greater body weight in 20–24-wk-old animals besides the greater age, but arterial baseline diameters did not differ significantly between groups. Interestingly, our present studies show that the mechanistic contribution of NOS is decreased in older animals and that part of the vasodilator response can be blocked with charybdoxotoxin + apamin, suggesting that part of the FMD response is attributable to EDHF. It is tempting to speculate that EDHF-dependent vasodilation represents an alternate pathway of vasodilation associated with aging.

Taken together, we present the proof of principle of a new vasodilation model in living rodents that yields results concordant with techniques measuring vasodilator function in humans and ex vivo models. Our observation of age-related impairment of FMD not only shows physiological relevance but also demonstrates that this approach can be used to assess vascular dysfunction in a range of genetic and induced rat disease models, exposure to environmental toxins, and beneficial effects of potential therapies. The major limitations of this technique include the need for invasive placement of an arterial occluder, the use of anesthesia, and, at this point, a lack of application in transgenic mouse models due to size-related technical limitations. However, this system will provide a useful tool for cardiovascular research, especially in cases that require repeated measures of vascular function in animal models that can be easily translated to humans.

GRANTS

This work was supported by an award from the American Heart Association (to C. Heiss) and awards from the American Heart Association and the UCSF Academic Senate Committee on Research (to M. L. Springer) and the Wayne and Gladys Valley Foundation.
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