Effect of cardiac pacing on forearm vascular responses and nitric oxide function

DANIEL GREEN,1,2,3 CRAIG CHEETHAM,1,3 CHELSEA HENDERSON,1 RUKSHEN WEERASOORIYA,1,3 AND GERARD O’DRISCOLL2,3
1Department of Human Movement and Exercise Science, The University of Western Australia, Nedlands 6907; and 2Cardiac Transplant Unit, 3Department of Cardiology, Royal Perth Hospital, Perth, Western Australia 6000

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Green, Daniel, Craig Cheetham, Chelsea Henderson, Rukshen Weerasooriya, and Gerard O’Driscoll. Effect of cardiac pacing on forearm vascular responses and nitric oxide function. Am J Physiol Heart Circ Physiol 283: H1354–H1360, 2002. First published May 23, 2002; 10.1152/ajpheart.00050.2002.—We examined the hypothesis that changes in heart rate at rest influence bioactivity of nitric oxide (NO) in humans by examining forearm blood flow responses during cardiac pacing in six subjects. Peak forearm and mean forearm blood flows across the cardiac cycle were continuously recorded at baseline and during pacing, with the use of high-resolution brachial artery ultrasound and Doppler flow velocity measurement. The brachial artery was cannulated to allow continuous infusion of saline or N(G)-monomethyl-L-arginine (L-NMMA). As heart rate increased, no changes in pulse pressure and mean or peak blood flow were evident. L-NMMA had no effect on brachial artery diameter, velocity, or flows compared with saline infusion. These results contrast with our recent findings that exercise involving the lower body, associated with increases in heart rate and pulse pressure, also increased forearm blood flow, the latter response being diminished by L-NMMA. These data suggest that changes in blood pressure, rather than pulse frequency, may be the stimulus for shear stress-mediated NO release in vivo.

blood flow; high-resolution ultrasound; Doppler velocity; shear stress

THE VASCULAR ENDOTHELIUM was originally considered to be an inert barrier between the circulating blood and vascular wall. The endothelium is now known to have numerous antiatherogenic and regulatory functions, including maintenance of vascular tone, through the production and release of modulating factors, including nitric oxide (NO) (19).

The location of the endothelium between the blood and vascular smooth muscle makes it strategically placed to transduce the effects of mechanical forces and it has been known for some time that increased shear stress and pulsatile flow provide a stimulus to NO production in vivo (22, 24). In humans, flow-mediated dilatation is attenuated by coinfusion of N(G)-monomethyl-L-arginine (L-NMMA), suggesting that conduit vessel dilatation in response to this stimulus may, at least in part, be NO dependent (11, 13). During exercise, both pulse pressure and heart rate increase, and a number of studies in animals (9, 10, 14, 21) and humans (3, 4, 6, 23) indicate that NO contributes to exercise hyperemia. In addition, in subjects with cardiovascular disease or risk factors, improvements in upper limb NO-mediated vascular function occur in response to lower limb exercise training programs that exclude physical conditioning of the forearm musculature, suggesting that exercise increases NO bioactivity on a chronic basis in vascular beds distant from the exercising musculature (16–18).

We (7) recently demonstrated that the increase in blood flow to the resting forearm during incremental cycle ergometer exercise is partly dependent on increased NO bioactivity, suggesting that systemic release of NO occurs during exercise in a nonexercising vascular bed. The mechanism responsible for this finding may be related to shear stress-mediated NO production as a result of hemodynamic changes that occur during exercise. This, in turn, may be related to the change in systolic, diastolic, or pulse pressure during exercise, or changes in heart rate or pulse frequency. The aim of this study was to investigate whether increases in heart rate in the absence of exercise stimulate NO production and regulate blood flow.

METHODS

Subjects and Screening Measures

Six subjects with symptomatic bradyarrhythmias and cardiac pacemakers implanted in situ for a minimum of 4 wk were recruited. The following were subjects were excluded: 1) premenopausal or postmenopausal females on cyclical hormone replacement therapy (due to the effects of cyclical estrogen levels on vascular function); 2) individuals with atrial fibrillation, valvular or congenital heart disease, or ventricular dysfunction (ejection fraction <40%); 3) subjects likely to have impaired endothelial function, including smokers, insulin and non-insulin-dependent diabetes mellitus; 4)

Address for reprint requests and other correspondence: D. Green, Dept. of Human Movement and Exercise Science, The Univ. of Western Australia, Parkway Entrance No. 3, 35 Stirling Hwy., Crawley, Western Australia 6009.
those with systolic blood pressure (SBP) >160 mmHg or diastolic blood pressure (DBP) >100 mmHg; 5) those with hypercholesterolemia (total cholesterol >6.5 mmol/l); and 6) those with known or suspected clinical vascular disease. Five subjects were implanted with pacemakers due to intermittent or constant sick sinus syndrome and one following post-atrivoventricular node ablation. The mode of pacing was as follows: 1) one dual-chamber paced, sensed, and demand; 2) three dual paced, sensed and demand, and rate responsive; 3) one ventricular paced, sensed, and inhibitory; and 4) one ventricular paced, sensed and inhibitory, and rate responsive. The study procedures were approved by the Ethics Committee of Royal Perth Hospital and all subjects gave written consent before commencement of the study.

**Experimental Design**

To determine the effect of cardiac pacing on forearm vascular responses and NO bioactivity, vascular function was assessed during two separate visits, randomized in their order of administration. Before attendance at either session, subjects received an information sheet instructing them to abstain from food within 4 h of the testing and alcohol and/or caffeine within 12 h. Conducting each study for any given subject at the same time of the morning controlled for circadian variation. Each session contained two identical cardiac pacing protocols separated by a 30-min rest period. One of the sessions involved brachial artery cannulation and infusion of physiological saline or l-NMMA (Clinalpha), the other session being undertaken to control for the possibility of an order effect during the session described above.

**Experimental Procedures**

**l-NMMA infusion session.** Investigations were conducted in a quiet, temperature-controlled laboratory on supine subjects. A 20-gauge arterial cannula (Arrow; Reading, PA) was introduced into the brachial artery of the nondominant arm under local anesthesia with <2 ml of 1% lidocaine (Astra Pharmaceuticals) to transduce pressure for the infusion of l-NMMA or physiological saline. Intra-arterial pressure was measured continuously (Transpac, Abbott Laboratories) throughout the study. l-NMMA and saline infusions were administered using an infusion pump (model 770, IVAC) at a constant rate of 60 ml/h.

After cannulation, saline was infused to maintain patency throughout a 30-min stabilization period. After infusion, a 2-min baseline recording of brachial artery diameter and velocity of flow was undertaken, followed by five 2-min epochs of cardiac pacing at 80, 90, 100, 110, and 120 beats/min. Brachial artery diameter and velocity were continuously recorded throughout the pacing protocol, which was followed by a 30-min recovery period during which pacemakers were set to 80 beats/min to facilitate the return of parameters to baseline levels. The infusion of saline was then replaced with l-NMMA, at a constant dose of 8 μmol/ml, and a second resting baseline was recorded. This was followed by a repeat of the pacing protocol detailed above, in the presence of continuous l-NMMA infusion to inhibit NO production.

**Control session.** During the l-NMMA infusion session outlined above, the order of saline and l-NMMA administration was not randomized due to the prolonged vasoconstriction that follows brachial artery l-NMMA infusion (26). The possibility of an order effect was therefore investigated by repeating the above pacing protocols on a separate day, in the absence of l-NMMA or saline infusions. This enabled an assessment of the effect of repeated cardiac pacing protocols, separated by a 30-min rest period, on forearm vascular responses.

**Experimental Measurements**

**Forearm blood flow assessment.** Blood flow was assessed using high-resolution vascular ultrasonography with synchronized Doppler velocity assessment. A 12–15 MHz multifrequency linear array probe attached to a high-resolution ultrasound machine (Aspen, Acuson) was used to visualize the brachial artery in the distal third of the upper arm. Ultrasonic parameters were then set to optimize longitudinal, B-mode images of the lumen/arterial wall interface. Once set, these parameters remained constant throughout the session. The probe was held in a constant position for the duration of the study with the use of a stereotactic clamp, and its precise location was recorded and standardized for the repeat session by measurement of the proximal and distal distance of the probe from the radiale. Continuous Doppler velocity was also recorded with the ultrasound at an inclination angle of ~60°.

Posttest analysis of brachial artery diameter was performed using custom-designed edge-detection and wall-tracking software that is independent of investigator bias (27). Briefly, B-mode images were either recorded on a S-VHS tape inside the ultrasound machine and then played back on a separate S-VHS video recorder for analysis, or, alternatively, the video signal was taken directly from the ultrasound machine and, using an Imaq Pci-1407 card, was directly encoded and stored as a digital Dicom file on the personal computer. Subsequent software analysis of this data, at ~20–30 frames/s, was performed using an icon-based graphical programming language (LabView version 6.02, National Instruments; Austin, TX) and toolkit (Imaq, National Instruments), in which developers build software programs called virtual instruments. Data collected from all regions of interest are used in subsequent acquisition of S-VHS or Dicom image files from which synchronized diameter and velocity measures are stored for each analyzed frame, at 20–30 Hz (8).

**Display and analysis of results.** Once the study has been acquired, a data display virtual instrument plots a graph of the arterial diameter and flow velocity against time. In addition, these synchronized velocity and diameter measurements are used to calculate and display the volume rate of blood flow as a continuous plot across the cardiac cycle. The volume rate of blood flow was calculated as the product of cross-sectional area (CSA) and velocity, where CSA was calculated from the software-derived arterial diameter measurements using the equation \( CSA = \pi \cdot \text{radius}^2 \). Operator-controlled cursors can be used to select and zoom in on sections of the data set that are of interest (e.g., cardiac pacing epochs), and clearly erroneous data points may be manually removed by the observer or a smoothing algorithm applied if required. Finally, data displayed between the cursors is analyzed and presented in a number of formats. Mean forearm blood flow rate (MFBF), velocity, and diameter are calculated as the algebraic mean of all data points between the cursors, which may be placed on either side of a discrete cardiac cycle or at the beginning and end of an array of such cycles. Area under the curve data for blood flow was calculated as the integral of each trace over time, yielding the volume of flow per minute. A peak forearm blood flow (PFBF), diameter, and velocity detection virtual instrument was used to identify and display the maximum data point within each cardiac cycle, and to subsequently calculate the average of these peaks. Finally, the area under the curve of all positive and all

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negative blood flow data points that lie between the cursors was presented to provide a global index of antegrade and retrograde blood flow. In the present study, the final 20 s of each pacing epoch were used to calculate all measures, including blood pressures and the antegrade and retrograde flow data, the latter being related to values per minute. All data are plotted across the cardiac cycle for the baseline and each of the pacing heart rates. PFBF and MFBF measures for the baseline and pacing heart rates were calculated across the final 20 s of each period.

**Treatment and analysis of data.** Results are expressed as means ± SE. The responses of variables to pacing at increasing heart rate were compared with the use of one-way ANOVA with post hoc t-tests where indicated. The contribution of NO to forearm vascular responses was determined by comparing saline and L-NMMA responses at each heart rate using two-way ANOVA with post hoc t-tests. P < 0.05 was considered significant.

**Results**

The subjects had the following characteristics: age, 58.2 ± 3.8 (SE) yr, height, 177 ± 3 cm, weight, 87.2 ± 5.3 kg, body mass index, 24.6 ± 1.2, total cholesterol, 5.1 ± 0.3 mmol/l, low-density lipoprotein cholesterol, 1.6 ± 0.5 mmol/l, high-density lipoprotein cholesterol, 1.3 ± 0.1 mmol/l. Baseline characteristics preceding the commencement of pacing included a SBP of 121 ± 7 mmHg, DBP of 78 ± 4 mmHg, and resting heart rate of 69 ± 4 beats/min.

**Effect of pacing on blood pressure responses.** When examined with the use of one-way ANOVA, increasing levels of cardiac pacing did not significantly influence SBP, DBP, or pulse pressure under either the saline infusion or L-NMMA conditions although there was a tendency for pulse pressure to fall slightly (Fig. 1).

**Effect of pacing on mean and peak brachial artery velocity, diameter, and blood flows.** Cardiac pacing did not influence either peak (Fig. 2A) or mean (Fig. 3A) brachial artery diameter compared with baseline (one-way ANOVA). Peak flow velocity tended to decrease with pacing, although not significantly, during both saline and L-NMMA infusion (one-way ANOVA, Fig. 2B). Mean flow velocity did not differ relative to base-
line under either the saline or L-NMMA condition, at any heart rate (Fig. 3B).

The tendency for PFBF to fall with increasing heart rate during saline and L-NMMA infusion was not significant (Fig. 2C). Similarly, MFBF did not differ as pacing rate increased under either the saline or L-NMMA condition (one-way ANOVA, Fig. 3C).

**Effect of L-NMMA on peak and mean forearm vascular responses to cardiac pacing.** When saline and L-NMMA conditions were compared at baseline, MFBF was, on average, lower during L-NMMA, but not significantly so (84 ± 24 vs. 61 ± 21 ml/min, P = 0.2), consistent with only a slight NO-induced vasodilation. Compared with saline infusion, L-NMMA did not significantly alter peak brachial artery diameter, velocity, or blood flow over the heart rates studied (two-way ANOVA, Fig. 2). Similarly, mean diameter, velocity, and flow were not altered by L-NMMA infusion at any heart rate (Fig. 3). These data contrast with the effect of L-NMMA observed in a recent study (7) of the effect of lower limb exercise on forearm vascular function in which L-NMMA substantially reduced flow rates and volumes during exercise. L-NMMA did not influence blood pressure data over the range of heart rates studied.

**Antegrade and retrograde brachial artery flows during cardiac pacing.** Figure 4 presents area under the curve data for retrograde and antegrade flows at baseline and during each pacing heart rate under the saline and L-NMMA conditions. No significant changes in either retrograde or antegrade flows were evident with
increasing pacing rate, under either the saline or 1-NMMA condition (one-way ANOVA; see Fig. 4). When saline and 1-NMMA infusion data were compared, no differences were evident in antegrade or retrograde flows at any heart rate (two-way ANOVA; see Fig. 4).

Comparison of pacing-induced changes in vascular function to exercise responses. We recently completed a study (7) investigating the effect of incremental cycle ergometer exercise, that is, lower limb exercise, on resting forearm vascular responses in healthy subjects. In these subjects, heart rate at baseline was 79 ± 4 beats/min; at 40 W, 104 ± 6; at 60 W, 113 ± 6; and at 80 W, 124 ± 6 beats/min. As these heart rates approximate those achieved by pacing in the present study (80, 100, 110, and 120 beats/min), we present data from the two studies together in Fig. 5. As lower limb exercise intensity increased, pulse pressure increased with significance achieved at 110 and 120 beats/min (P < 0.001). This was associated with increases in peak blood flow at 100, 110, and 120 beats/min (*P < 0.01). In contrast, cardiac pacing at similar heart rate to those achieved during exercise was not associated with increased blood flow responses (one-way ANOVA). When exercise and pacing data were compared, both pulse pressure and peak blood flow responses to exercise were significantly greater than those to pacing at 110 and 120 beats/min (P < 0.01). These data suggest that changes in pressure, rather than heart rate, are responsible for the increase in MFBF during lower limb exercise.

Fig. 4. Retrograde and antegrade brachial artery flow to the resting forearm across the cardiac cycle at baseline and during incremental cardiac pacing heart rates in the presence of saline (open bars) and NO blockade with 1-NMMA (solid bars). Values are means ± SE, and data have been normalized to 1 min. Relative to preceding baseline data, no significant changes in either antegrade or retrograde flows were evident at any heart rate, under either the saline or 1-NMMA condition. When saline and 1-NMMA infusion data were compared, no differences were evident in antegrade or retrograde flows at any heart rate. AUC, area under the curve.

Fig. 5. Peak brachial artery blood flow (A) and pulse pressure (B) to the resting forearm at baseline and during pacing-induced increments in heart rate (•) and matched heart rates induced by cycle ergometer exercise (○) (7). Exercise was associated with increased pulse pressure at 110 and 120 beats/min (†P < 0.001) and increased peak blood flow at 100, 110, and 120 beats/min (*P < 0.01). Cardiac pacing did not increase pulse pressure or blood flow responses. Pulse pressure and peak blood flow responses to exercise were significantly greater than those to pacing at 110 and 120 beats/min (†P < 0.01). These data suggest that changes in pressure, rather than pulse frequency, are responsible for the increase in forearm blood flow during lower limb exercise.
Effect of repeated cardiac pacing protocols on MFBF responses. Repeated cardiac pacing protocols, separated by a 30-min rest period, were undertaken to examine the possibility of an order effect on forearm blood flow responses. No significant differences were evident between the initial and repeat bouts of pacing for either MFBF or PFBF or for any other measured variables.

DISCUSSION

We (17, 18) recently demonstrated that improvements in upper limb NO-mediated vascular function could occur as a result of lower limb exercise training programs that excluded physical conditioning of the forearm musculature. These findings raised the possibility that exercise, possibly via a hemodynamic-mediated shear stress phenomenon, increases NO bioactivity in vessel beds distant from the exercising musculature. Further studies indicated that, in response to cycle ergometer exercise during which the upper limbs were at rest, PFBF increased incrementally with exercise intensity (8), a response partly inhibited by L-NMMA (7), confirming that NO is released from vascular beds other than those that feed metabolically active skeletal muscle during exercise. Because it is well established that shear stress and pulsatile flow provide a physiological stimulus to endothelial NO production (21, 22, 24, 25), and incremental exercise was associated with increases in blood pressure, pulse pressure, and heart rate (7), we postulated that the increased blood flow and NO bioactivity observed in these studies may have resulted from hemodynamic changes in blood pressure or heart rate associated with exercise. The purpose of the present study, therefore, was to investigate whether increases in heart rate, in the absence of exercise and the increase in blood pressure and pulse pressure associated with it, stimulate NO production and regulate blood flow.

The principal finding of the present study is that cardiac pacing at heart rates similar to those observed during exercise (7) is not associated with increased forearm dilatation, blood flow, or NO bioactivity. The only effect L-NMMA had on any of the measured variables was to increase retrograde flow in the brachial artery at baseline and the lowest pacing rate, consistent with slightly greater vascular resistance in the perfused vascular bed. This is the first study to our knowledge that has investigated the effect of cardiac pacing on peripheral vascular responses and NO function in humans, and the findings, taken together with those of a recent exercise study (7), suggest that changes in blood pressure, rather than heart rate, may be the more important stimulus for shear stress-mediated NO release in vivo. Whereas several animal (22, 24, 25) and human studies (13, 15) have established that flow-mediated arterial dilator responses are endothelium dependent, few have determined which of the physical characteristics of the flow stimulus may be important in determining the nature of the vascular response. In a recent study, Mullen et al. (20) found that vasodilatation in response to a brief episode (5 min) of reactive hyperemia was largely NO dependent, whereas sustained hyperemia in response to prolonged ischemia or hand warming was not attenuated by L-NMMA. The authors concluded that a physiological role exists for NO-mediated dilatation in limiting the degree to which shear stress is elevated in response to rapid changes in flow, such as those that may occur in response to exercise, and that conduit artery dilatation in humans is dependent on the physical and dynamic characteristics of the flow stimulus. Similarly, studies (5, 23) of the effects of cardiac pacing on coronary artery diameter and flow suggest that a complex interplay exists between the effects of pulse frequency and amplitude on flow-mediated dilatation in vivo. The present study, when taken together with the results of our recent exercise study (7), extends these results by suggesting that peripheral conduit artery endothelium-dependent responses may also be dependent on the nature of central hemodynamic changes.

Our results suggest that changes in pulse frequency, when they occur independent of changes in blood pressure, are not associated with significant flow-mediated dilatation through conduit arteries in vivo. This finding contrasts with that of Hutcheson and Griffith (12), who employed a cascade bioassay system and peristaltic pumps to dissociate the effects of frequency and amplitude of pulsatile perfusion on rat aortic segments. Whereas increases in pulse pressure amplitude, at a constant frequency, were associated with NO-dependent vasodilatation, they also found that, when perfused at low pulse pressure amplitudes, increases in pulse frequency were associated with detector vessel dilatation, which was attenuated by the NO synthase inhibitor L-NAME. Whether the disparity between these findings and those in the present study relates to species or experimental differences is unclear, but our findings (7) suggest that under in vivo conditions, pulse pressure rather than pulse rate may be a more important determinant of flow-mediated dilatation.

A limitation of the present study is the possibility that comparison of data to that obtained from our recent study (7) may be limited by differences in the subjects studied. For example, the subjects studied here (age 58 ± 4 yr) were significantly older than those in whom exercise responses were studied (age 22 ± 4 yr), raising the possibility that endothelial function was impaired in the present group. However, flow-mediated dilatation (FMD) in response to a 5-min ischémic stimulus, a widely accepted measure of endothelial function (1, 13), was normal in our subjects (7.0 ± 1.1%) compared with gender-matched healthy controls aged 48 ± 7 yr in whom FMD was 7.1 ± 0.6%. The value is also similar to FMD data reported by others in younger subjects (2). We therefore think it is unlikely that the lack of NO contribution to BF responses with pacing-induced increases in heart rate was due to impaired endothelial function in the subjects studied here.

In summary, this study investigated the effect of changes in heart rate on peripheral artery conduit...
vessel function in vivo. Cardiac pacing at increasing heart rates was not associated with an increase in pulse pressure and did not induce changes in arterial diameter or flow. In addition, L-NMMA had no effect of brachial artery diameter, velocity, or peak or mean flow compared with saline infusion during pacing. These results contrast with our recent findings, which indicated that exercise-mediated increases in heart rate together with pulse pressure also increased MFBF, the latter response being diminished by infusion of L-NMMA. These data suggest that changes in blood pressure, rather than pulse frequency, may be the more important stimulus for shear stress-mediated NO release in vivo.

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