α₁-Adrenoreceptor activity does not explain lower morning endothelial-dependent, flow-mediated dilation in humans

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The incidence of myocardial infarction, sudden cardiac death, and stroke is up to three times higher between 0600 and 1200, compared with other times of day (23, 16, 38). Given the well-established antiatherogenic functions of the endothelium, including vasodilation, decreased platelet aggregation and adhesion, and foam cell formation (16, 36), variation in endothelial function could play an important role in explaining the elevated cardiovascular risk in the morning.

The flow-mediated dilation (FMD) technique provides insight into endothelial function of conduit arteries (37). When valid and reliable methods are employed, it has been reported that the FMD response is largely nitric oxide (NO) dependent (9, 13, 19, 22). It has been documented, both in asymptomatic subjects and those with established cardiovascular disease (17, 18), that FMD is reduced in the morning compared with other times of day (3, 10, 11, 14, 25, 30).

Recently, our laboratory demonstrated that lower FMD in the morning was not explained by diurnal variation in the eliciting shear rate (SR) stimulus (14). A potential, yet unexplored, explanation of morning reduction in FMD may involve diurnal variation in factors that oppose vasodilation, such as sympathetic nervous system activity (SNA). In support of this possibility, previous researchers have reported circadian variation in SNA (26), although it is unclear whether SNA is higher (26) or not (21) in the morning, and it is also controversial to what extent SNA explains the morning increase in risk of a sudden cardiac event (24). However, it is known that vascular tone in conduit arteries is the product of the competitive balance between NO-mediated vasodilation and α-mediated constriction (12). Therefore, somewhat surprisingly, the effect of SNA on diurnal variation in conduit vessel tone is yet to be determined. To address this question, the present study examined, in a randomized and placebo-controlled design at two times of day, the effect of the α₁-adrenoreceptor blocker, prazosin, on FMD. We hypothesized that blockade of α₁-mediated SNA in the morning would increase FMD, thus diminishing diurnal variation.

METHODS

Participants. Following an estimation of the sample size required for the primary comparisons (see Statistical analysis), 12 normotensive (8 men; 4 women) participants, aged 26 ± 3 yr, with body mass 73 ± 14 kg and height 172 ± 6 cm, participated in the study. All participants were nonsmokers, had no history of cardiovascular disease, and were not taking any medication. Participants were recreationally active, measured via a self-report questionnaire, and typically engaged in low- (e.g., walking) and moderate-intensity (e.g., jogging, stationary bike) aerobic activities (2–3 days/wk). Female participants were tested in the early follicular phase of the menstrual cycle, determined by the first day of menstruation. The study conformed to the standards set by the Declaration of Helsinki and other international standards and was approved by the institutional ethics committee. All participants were informed of the experimental procedures and possible risks involved in the study, and written, informed consent was obtained.

Research design. Participants attended the laboratory on five occasions. The first visit was for familiarization with experimental procedures and possible risks involved in the study, and written, informed consent was obtained.
protocols and for the measurement of resting blood pressure (BP) (three serial measurements with a mercury manual sphygmomanometer), height (m), and body mass (kg). There were then four separate visits for the completion of the main experimental trials. These trials involved FMD measurements following ingestion of placebo or the α1-adrenoceptor blocker prazosin (1 mg/20 kg body mass) in the morning (AM) and the afternoon (PM). The four trials were administered in a counterbalanced order and were separated by ≥36 h. The light intensity in the laboratory was controlled at 200 lux, and temperature maintained at 21°C. In all trials, the protocol began after a 12-h abstinence from caffeine, 24-h abstinence from alcohol and strenuous exercise, and at least a 6-h fast.

**Experimental protocol.** Participants reported to the laboratory at 2230 on the night before the morning trials. They then entered a quiet and temperature-controlled room for overnight sleep. For the afternoon trials, participants reported to the laboratory at 1400. Participants consumed an identical gelatin capsule orally containing either the α1-adrenoceptor blocker prazosin or a placebo with 50 ml of water at 0430 and 1430 for the morning and afternoon trials, respectively. Data collection began at 0600 and 1600 for morning and afternoon trials, respectively. At both times, brachial artery FMD and measurements of systolic and diastolic BP and heart rate (HR) were obtained following ≥30 min of rest in the supine position.

**Measurement procedures.** A 10-MHz multifrequency linear array probe attached to a high-resolution ultrasound machine (Teron 3000, Teratech, Burlington, MA) was used to image the brachial artery in the right arm. Conduit artery endothelium-dependent dilatation was assessed via the response to FMD with the occluding cuff placed distal to the ultrasound probe. The FMD protocol followed the guidelines as described previously (4) and utilized edge detection and wall-tracking software for analysis (15, 39). This software provides continuous and simultaneous diameter, velocity, and SR (four times the velocity divided by the diameter) data. In accordance with recent recommendations (2, 27–29), we also measured the postdeflation area under the SR curve to best interpret any changes in FMD. In light of current debate about the general magnitude of correlation between SR and FMD in different research situations (2), we also calculated the within-subjects correlation coefficient between SR and FMD using appropriate methods that partition the within- and between-subjects influences properly (5).

HR was continuously recorded via a three-lead electrocardiograph (ML132, ADInstruments, Colorado Springs, CO). Beat-to-beat BP was monitored from the finger (Finometer, Biomedical Instruments). In addition, manual BP measurements (mercury sphygmomanometer) were taken to confirm the accuracy of the finger BP measurements during supine rest before any experimental intervention. The continuous finger arterial pressure wave data were analyzed on a beat-to-beat basis using BeatScope pulse contour analysis software (TNO Biomedical Instrumentation). The last 5 min of supine rest before the FMD measurement were used as the baseline measurement for systolic and diastolic BP, as well as HR.

Thirty minutes before data collection, an indwelling 20-gauge (BD, Vialon Material) venous catheter was inserted into the left forearm. Venous blood samples (10 ml) were drawn immediately before FMD measurements. Blood samples were immediately spun at 3,200 rpm, at 4°C, for 10 min, and resulting plasma was separated and stored at −80°C. All samples from each individual were analyzed in the same batch by an experienced technician, who was blinded to the order of samples. Epinephrine and norepinephrine concentrations were detected by high-performance liquid chromatography using electrochemical detection.

**Phenylephrine infusion challenge.** For reliable interpretation of our findings, the dose of prazosin that we prescribed should be capable of significantly inhibiting the pressor effects of α1-adrenoceptor agonists. To explore this issue, BP was also measured on a separate visit in five participants (2 women, 3 men) by a catheter (20-gauge, BD Insyte) placed into the radial artery of the nondominant arm and connected to a transducer (Deltran II, Utah Medical Products, RoI). Following the catheterization (before any data collection), each subject received an intravenous bolus dose of 1.0 μg/kg body mass of the specific α1-adrenoceptor agonist phenylephrine hydrochloride (PE). This exact same bolus dose of PE was injected at 90 min and again 150 min postprazosin ingestion. The PE was diluted with physiological saline to yield a bolus dose equal to 1.0 μg/20 kg body mass. This dose of PE has been demonstrated to evoke an increase in mean arterial pressure (MAP) of ~15–20 mmHg (32). During the three PE infusion protocols (i.e., baseline, 90 min post-, and 150 min postprazosin ingestion), BP was recorded and used to identify the degree of α1-adrenoceptor blockade.

**Statistical analysis.** The primary outcome variable was %FMD, and, according to our hypothesis, the primary analysis was for diurnal variation in FMD to be present in the placebo but not in prazosin condition. Based on previous research (14), we estimated that the morning-afternoon difference in FMD would be 4% in placebo compared with zero in prazosin. Using the NQUERY (Statistical Solutions) software, it was estimated that 12 participants would provide at least 80% power to detect a 4% difference in the diurnal variation of FMD between placebo and prazosin conditions, assuming a standard deviation of differences of ±4.5% and a significance level of 0.05 in a crossover trial. Vascular, BP, and HR measurements were analyzed using a two factor linear mixed model with repeated measures (6). For each dependent variable the linear mixed model has two factors described as main effects: blockade (i.e., overall comparison of placebo vs. prazosin) and time of day (i.e., overall comparison of morning vs. afternoon). Statistically significant interactions between these two factors were followed up with multiple contrasts (i.e., direct comparisons of morning with afternoon in each blockade condition). All data were analyzed using the SPSS 17.0 (SPSS, Chicago, IL) software. Data are presented in the text as means ± SD, and exact P values are cited (values of P of “0.000” provided by the statistics package are reported as “<0.0005”).

**RESULTS**

**Brachial artery FMD and SR stimuli.** A significant interaction between blockade and time of day was evident for brachial FMD (P = 0.02). Follow-up contrast analyses indicated that FMD was lower (8 ± 2%) in the morning compared with the afternoon (10 ± 3%) in the placebo condition (P = 0.04), but not in the prazosin condition (P = 0.20, Fig. 1). Prazosin reduced FMD to 8 ± 2% in the afternoon compared with placebo (P = 0.04). While a small increase in FMD was observed in the morning with prazosin, this was not statistically different compared with placebo (P = 0.24). Similar results were obtained when the absolute (i.e., in millimeters rather than as a percentage of baseline) changes in diameter were analyzed. SR was not influenced by blockade, time of day, nor the interaction between these two factors (P = 0.39). The within- and between-subjects correlations between FMD and SR were not statistically significant (P > 0.05). Baseline and peak artery diameters, as well as the time-to-peak artery diameter, were not influenced by blockade or time of day, nor were there any interaction between these two factors (P > 0.23, Table 1).

**BP and HR.** Both systolic and diastolic BP were not found to be influenced by the interaction between blockade and time of day (P > 0.34; Table 1). Nevertheless, significant main effect of blockade was evident for both systolic and diastolic BP (P < 0.02; Table 1). When averaged across both times of day, systolic BP was 5 ± 6 mmHg and diastolic BP 6 ± 7 mmHg lower following prazosin compared with placebo. A
significant interaction between blockade and time of day was evident for HR ($P < 0.005$; Table 1). HR was elevated in the afternoon (63 $\pm$ 10 beats/min) compared with the morning (58 $\pm$ 12 beats/min) in the prazosin condition ($P = 0.008$), but was not different in the placebo condition ($P = 0.40$). Compared with placebo (57 $\pm$ 13 beats/min), HR in the afternoon increased with prazosin (63 $\pm$ 10 beats/min, $P = 0.005$), whereas no difference was observed in the morning ($P = 0.19$).

**Norepinephrine and epinephrine.** Norepinephrine was not found to be influenced by the interaction between blockade and time of day ($P = 0.62$). There was evidence of a main effect of blockade for norepinephrine ($P = 0.07$) and significant time of day differences ($P < 0.005$), but no difference was observed in the morning ($P = 0.12$). Epinephrine was not found to be influenced by time of day nor the interaction between blockade and time of day ($P = 0.34$). A significant main effect of blockade was evident for epinephrine ($P = 0.03$; Fig. 2).

**PE infusion challenge.** During control rest, PE yielded an increase in MAP of 17 $\pm$ 2 mmHg. At both 90 min and 150 min postprazosin ingestion (i.e., a timeframe within which the FMD tests were conducted), the same dose of PE yielded significantly increases in MAP of 4 $\pm$ 1 and 3 $\pm$ 2 mmHg, respectively ($P < 0.0005$). These results clearly indentify a functional blockade of 78–83% at inhibiting the effects of $\alpha_1$-adrenoreceptor agonists following the prazosin ingestion.

**DISCUSSION**

The major finding of this study was that $\alpha_1$-blockade diminished the diurnal variation in FMD, but, contrary to our hypothesis, this was not achieved by a significant increase in FMD in the morning. This finding indicates that $\alpha_1$-mediated SNA does not explain the morning reduction in endothelium-dependent conduit artery function. Nevertheless, the contribution of SNA in the diurnal variation in FMD cannot be totally excluded. It is possible that $\alpha_2$-mediated SNA, although not assessed in this study, may contribute to lower morning measured FMD.

This is the first study of conduit artery function that has involved examination of differential effects of SNA inhibition in the morning vs. the afternoon. In a previous study, abolition of diurnal variation in vascular resistance following infusion of a nonspecific $\alpha$-blocker, phentolamine, was observed in smaller resistance vessels measured using strain-gauge plethysmography (26). Panza et al. (26) suggested that the morning elevation in vascular resistance was due to increased $\alpha$-sympathetic-mediated vasoconstrictor activity at this time of day. We found abolition of the diurnal variation in FMD following blockade with prazosin. However, despite a small nonstatistically significant increase in morning-measured FMD, this did not explain the abolition of the diurnal variation and thus the morning reduction in FMD.

A number of potential explanations can be postulated for the differences between the current findings and those of Panza et al. (26). First, conduit and resistance vessels are different in terms of artery size and sympathetic regulation (31). Second, it is possible that $\alpha_2$-receptors, although not assessed in the
The present study, still active in the morning, thus potentially contributing to “restraining” vasodilation. This would support the notion from previous studies that acute increases in SNA, induced by interventions such as lower body negative pressure, can decrease FMD. Blockade with a nonspecific α-blocker (phentolamine) prevents the reduction in FMD during such an intervention (12). Furthermore, it has been suggested that postjunctional α2-receptors contribute more to resting vascular tone of resistance vessels than α1-receptors in the forearm of young health men (8). Finally, a related but not mutually exclusive explanation is that the competitive balance between SNA and NO may be the most important influence on the diurnal variation in FMD. Although NO was not measured in this study, circadian variation in NO synthase activity (endothelial NO synthase) has been reported previously in a mouse model, characterized by high endothelial NO synthase activity in the daytime and low in the nighttime, with a morning transition period (35).

In addition, an unexpected finding was that α1-SNA blockade led to a significant reduction in afternoon-measured FMD and thus contributed to the abolition of the diurnal variation. This finding is perplexing and could suggest that the competitive impact of SNA on vasomotor control is less in the daytime and low in the nighttime, with a morning transition period. Alternatively, it is also possible that removal of α1-SNA in the afternoon, i.e., at a time of potentially lower SNA activity (26), may trigger other factors that contribute to vasoconstriction, which can then exert a stronger influence on FMD, e.g., activation or increased contribution of α2-receptors or increase in other factors, such as endothelin (20). Indeed, we observed significantly elevated circulating norepinephrine levels in the afternoon with prazosin; α2-receptors mediate norepinephrine release. Therefore, greater contribution of α2-receptors contributing to “restraining” vasodilation (12) is plausible.

Methodological considerations. The present study has several notable advantages in terms of study design and methodological procedures. The diurnal comparison of conduit arteries using pharmacological blockade is novel, and we employed a randomized and placebo-controlled design on separate days rather than serial FMD measurements during the same day. The diurnal variation in FMD was not explained by systematic differences in the eliciting SR stimulus in placebo, which is in complete agreement with our laboratory’s previous study (14).

We also adhered to the most appropriate FMD methodology on young healthy individuals to suggest a largely NO-mediated response (9, 13) and utilized a specific α1-blocker in an attempt to isolate the activation of α1-adrenoreceptors. Finally, we ensured that the dose of the α1-blocker was functionally effective at inhibiting the effects of α1-adrenoreceptor agonists and that this dose was sufficient to block α1-mediated SNA over the duration of the experimental protocol.

Limitations. Diurnal variation in vascular smooth muscle function (i.e., endothelium-independent vasodilatation) was not measured in the present study. However, we are confident that it is not likely to be a significant contributing factor, since the variation in endothelium-independent, NO-mediated vasodilator function of resistance vessels has been shown not to alter the circadian pattern in vascular resistance (26). In addition, all but one study of the diurnal variation in endothelium-independent function in conduit vessels have demonstrated no diurnal variation in endothelium-independent function (11, 17, 25). The study in which diurnal variation was found reported greater endothelium-independent vasodilation in the morning in conduit arteries (3), similar to the other findings in resistance vessels (26).

As hypothesized, we found that the typical diurnal variation in FMD was attenuated with prazosin. While there was a statistically significant decrease in afternoon-measured FMD with prazosin, we found only a nonsignificant trend for a morning-measured increase in FMD. It is possible that this latter change may have been statistically significant with a larger sample size. Therefore, while our study design and analyses had sufficient power to detect the differences in diurnal variation in the two experimental conditions, there is still some imprecision in what explains this, i.e., a lowered afternoon-measured FMD per se, or this combined with a
higher morning-measured FMD. Future research might focus on this specific aspect. We acknowledge that our study was conducted on young, healthy individuals; therefore, our findings are specific to this population. Nevertheless, previous studies have demonstrated a reduced morning FMD of similar magnitudes in older individuals (25) and patients with hypertension (18) compared with the afternoon. Although a generalization of similar effects of SNA on FMD to these populations is plausible, potential elevations in SNA in these groups (e.g., hypertension) may affect such comparisons and thus warrants further investigation. We also recognize that we did not assess the relative contribution of α1- and α2-receptors to time of day variation in FMD, nor did we employ a NO blocker to explore diurnal variation in the competitive balance between SNA and NO-mediated vasodilation. A carefully designed study examining FMD, while employing a stepwise introduction of α-blockers in the absence and presence of a NO synthase inhibitor, is clearly warranted.

In summary, blockade of α1-adrenoceptors diminished the diurnal variation in endothelium-dependent conduit artery function. Nevertheless, contrary to our hypothesis, lower morning-measured FMD was not explained by α1-mediated SNA. Accordingly, the contribution of α2-adrenoceptors is still a possibility; thus the effect of SNA on diurnal variation in FMD cannot be dismissed.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

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