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

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The mechanisms underpinning diurnal variation in FMD are unclear, but potentially relate to a circadian rhythm in sympathetic nerve activity. We hypothesized that blockade of α₁-mediated sympathetic nerve activity would act to attenuate the diurnal variation in FMD. In a randomized and placebo-controlled design, we measured brachial vascular endothelium; high resolution ultrasound; time of day; sympathetic nerve activity; shear rate; blood pressure; heart rate; 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 α₁-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.

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morning (AM) and the afternoon (PM). The four trials were administered in a counterbalanced order and were separated by \(\geq 36\) h. The light intensity in the laboratory was controlled at \(\approx 200\) lux, and temperature maintained at \(21^\circ\)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 saline or the \(\alpha_1\)-adrenoreceptor 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 \(\geq 36\) h. The light intensity in the laboratory was controlled at \(\approx 200\) lux, and temperature maintained at \(21^\circ\)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.

**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 measurements of diameter, diameter, and heart rate (HR) were obtained following \(\geq 30\) min of rest in the supine position.

**Statistical analysis.** The primary outcome variable was \%FMD, and, according to our hypothesis, the primary analysis was for diurnal variation of FMD in each visit for the completion of the main experimental trials. These trials involved FMD measurements following ingestion of placebo or the \(\alpha_1\)-adrenoreceptor blocker prazosin (1 mg/20 kg body mass) in 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 \(\geq 30\) min of rest in the supine position.

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**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 \(\pm 2\%\)) in the morning compared with the afternoon (10 \(\pm 3\%\)) in the placebo condition (\(P = 0.04\)), but not in the prazosin condition (\(P = 0.20\), Fig. 1). Prazosin reduced FMD to 8 \(\pm 2\%\) 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 \(\pm 6\) mmHg and diastolic BP 6 \(\pm 7\) 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 ± 10 beats/min) compared with the morning (58 ± 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 ± 13 beats/min), HR in the afternoon increased with prazosin (63 ± 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 = 0.009), 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 ± 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 smaller increases in MAP of 4 ± 1 and 3 ± 2 mmHg, respectively (P < 0.0005). These results clearly indentify a functional blockade of 78–83% at inhibiting the effects of α1-adrenoreceptor agonists following the prazosin ingestion.

**DISCUSSION**

The major finding of this study was that α1-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 α1-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 α2-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 α-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 α-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 α2-receptors, although not assessed in the

![Fig. 1. Brachial artery flow-mediated dilation (FMD) values in the morning and afternoon in both the placebo and prazosin conditions.](http://ajpregu.physiology.org/)

**Table 1. Vascular, blood pressure, and heart rate values in the morning and afternoon in both the placebo and prazosin conditions**

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Prazosin</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Morning</td>
<td>Afternoon</td>
<td>MF-B</td>
</tr>
<tr>
<td>Shear rate, AU</td>
<td>21.586 ± 6.335</td>
<td>20.012 ± 9.573</td>
<td>0.44</td>
</tr>
<tr>
<td>Baseline artery diameter, mm</td>
<td>3.73 ± 0.63</td>
<td>3.68 ± 0.55</td>
<td>3.88 ± 0.46</td>
</tr>
<tr>
<td>Peak artery diameter, mm</td>
<td>4.02 ± 0.69</td>
<td>4.04 ± 0.55</td>
<td>4.27 ± 0.58</td>
</tr>
<tr>
<td>Time to peak, s</td>
<td>51 ± 21</td>
<td>46 ± 24</td>
<td>49 ± 24</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>122 ± 9</td>
<td>120 ± 6</td>
<td>114 ± 11</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>72 ± 10</td>
<td>73 ± 6</td>
<td>65 ± 7</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>55 ± 7</td>
<td>57 ± 13</td>
<td>58 ± 12</td>
</tr>
</tbody>
</table>

Values are means ± SD. AU, arbitrary units; BP, blood pressure. P values refer to the main effects of blockade (MF-B), time of day (MF-T), and the interaction between blockade and time of day (INT). *Significant effect.
present study, were 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 \(\alpha\)-blocker (phenolamine) prevents the reduction in FMD during such an intervention (12). Furthermore, it has been suggested that postjunctional \(\alpha_2\)-receptors contribute more to resting vascular tone of resistance vessels than \(\alpha_1\)-receptors in the forearm of young healthy 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 \(\alpha_1\)-SNA blockade led to a significant reduction in afternoon-measured FMD and thus contributed to the abolishment 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 \(\alpha_1\)-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 \(\alpha_2\)-receptors or increase in other factors, such as endothelin (20). Indeed, we observed significantly elevated circulating norepinephrine levels in the afternoon with prazosin; \(\alpha_2\)-receptors mediate nor-epinephrine release. Therefore, greater contribution of \(\alpha_2\)-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 \(\alpha_1\)-blocker in an attempt to isolate the activation of \(\alpha_1\)-adrenoreceptors. Finally, we ensured that the dose of the \(\alpha_1\)-blocker was functionally effective at inhibiting the effects of \(\alpha_1\)-adrenoreceptor agonists and that this dose was sufficient to block \(\alpha_1\)-mediated SNA over the duration of the experimental protocol.

**Limitations.** Diurnal variation in vascular smooth muscle function (i.e., endothelium-independent vasodilation) 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 the 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).

REFERENCES


