Is there a threshold duration of vascular occlusion for hindlimb reactive hyperemia?

Jennifer Rogers and Don D. Sheriff

Department of Exercise Science, The University of Iowa, Iowa City, Iowa

Submitted 15 April 2005; accepted in final form 21 May 2005

Rogers, Jennifer, and Don D. Sheriff. Is there a threshold duration of vascular occlusion for hindlimb reactive hyperemia? J Appl Physiol 99: 1272–1277, 2005; doi:10.1152/japplphysiol.00428.2005.—Relatively brief changes in perfusion pressure and flow through arterioles occur in a number of conditions, such as in the flying environment and during such common everyday activities such as bending forward at the waist. Also, brief periods of negative vertical acceleration (Gz) stress, which reduces perfusion in the lower body, has been shown to impair the regulation of arterial pressure during subsequent positive Gz stress. To examine the contribution that reactive hyperemia makes in these settings, studies on the hindlimb circulation of anesthetized rats (n = 8) were carried out by imposing graded duration vascular occlusion (1, 2, 4, 10, and 30 s) to test the hypothesis that there is a threshold duration of reduction in perfusion that must be exceeded for reactive hyperemia to be triggered. Vascular conductance responses to 1 s of terminal aortic occlusion were no different before and after myogenic responses were blocked with nifedipine, indicating that 1 s of occlusion failed to elicit reactive hyperemia. Two seconds of occlusion elicited a small but significant elevation in hindlimb vascular conductance. The magnitude of the reactive hyperemia was graded in direct relation to the duration of occlusion for the 2-, 4-, and 10-s periods of occlusion and appeared to be approaching a plateau for the 30-s occlusion. Thus there is a threshold duration of terminal aortic occlusion (~2 s) required to elicit reactive hyperemia in the hindlimbs of anesthetized rats, and the reactive hyperemia that results possesses a threat to the regulation of arterial pressure.

METHODS

The following procedures meet National Institutes of Health guidelines and were reviewed and approved by the Institutional Animal Care and Use Committee of The University of Iowa.

Surgical preparation. Eight Sprague-Dawley rats (3 female, 5 male; 270–442 g) were anesthetized with isoflurane and restrained supine on a homeothermically controlled table with body temperature maintained at 37°C. Importantly, myogenic responses have been shown to be preserved under isoflurane anesthesia (15). Catheters were implanted in the left femoral and carotid arteries for pressure measurement and drug infusion. An ultrasonic transit-time blood flow transducer (model 1.5RB, Transonic, Ithaca, NY) was positioned around the terminal portion of the aorta to measure hindlimb blood flow. An occluder cuff (In Vivo Metric, Healdsburg, CA) sized to each animal was positioned around the terminal aorta and vena cava caudal to the flow probe. Both vessels were enclosed within the cuff out of concerns that placing the cuff only around the aorta might cause venous congestion in the deflated state via caval compression, owing to the thickness of the cuff. Importantly, little venous filling is expected when the cuff is inflated for three reasons. First, the cuff was inflated rapidly (~0.2 s) so that arterial inflow and venous outflow ceased simultaneously. Second, discharge of arterial volume from arteries distal to the cuff into the limb venous system is expected to have little effect on venous pressure, because veins are far more compliant than arteries. For example, arterial compliance is reported to be 0.06 ml·mmHg⁻¹·kg body wt⁻¹ (5) and total systemic compliance is reported to be 3.00 ml·mmHg⁻¹·kg body wt⁻¹ (3), a ratio of 50:1, meaning a 100-mmHg decrease in distal arterial pressure would lead to a 2-mmHg rise in venous pressure, assuming a similar ratio for the hindlimbs. Third, collateral paths of venous drainage matters, studies on the hindlimb circulation of anesthetized rats were carried out to test the hypothesis that there is a threshold duration of vascular occlusion that must be exceeded for reactive hyperemia to be triggered. Because even brief (2 s) vascular occlusion has been demonstrated to evoke baroreflex responses in this model (7), studies were repeated after autonomic responses were inhibited with hexamethonium. Finally, studies were repeated after administration of nifedipine to test the degree to which responses were of myogenic origin; nifedipine blocks L-type calcium channels, the entry of calcium into the smooth muscle, which elicits reactive hyperemia (11). Reactive hyperemic responses are responsible for eliciting reactive hyperemia after the release of occlusion. The precise mechanisms responsible for eliciting reactive hyperemia are incompletely understood. Myogenic, metabolic, and endothelial factors, triggered by decreased flow, decreased pressure, and vascular deformation, may all play a role in inducing and/or modifying reactive hyperemia (11). Reactive hyperemic responses are likely to be functionally important in conditions where perfusion is altered, such as during changes in orientation to gravity as well as downstream from sites of vascular constriction. Most studies evaluating the importance of the potential mechanisms of reactive hyperemia have imposed relatively prolonged periods of vascular occlusion (e.g., minutes). Relatively brief (i.e., a few seconds) changes in perfusion occur in the flying environment (2, 13) and during common everyday activities, such as bending forward at the waist. We sought to determine whether reactive hyperemia results from such brief alterations in perfusion, and if so, we sought to determine the role that reactive hyperemia plays in determining the hemodynamic consequences of these changes in perfusion. To address these

A DECREASE IN TISSUE PERfusion imposed by vascular occlusion inhibits vascular smooth muscle, which elicits reactive hyperemia after the release of occlusion. The precise mechanisms responsible for eliciting reactive hyperemia are incompletely understood. Myogenic, metabolic, and endothelial factors, triggered by decreased flow, decreased pressure, and vascular deformation, may all play a role in inducing and/or modifying reactive hyperemia (11). Reactive hyperemic responses are likely to be functionally important in conditions where perfusion is altered, such as during changes in orientation to gravity as well as downstream from sites of vascular constriction. Most studies evaluating the importance of the potential mechanisms of reactive hyperemia have imposed relatively prolonged periods of vascular occlusion (e.g., minutes). Relatively brief (i.e., a few seconds) changes in perfusion occur in the flying environment (2, 13) and during common everyday activities, such as bending forward at the waist. We sought to determine whether reactive hyperemia results from such brief alterations in perfusion, and if so, we sought to determine the role that reactive hyperemia plays in determining the hemodynamic consequences of these changes in perfusion. To address these
from the limbs likely exist. All catheters and cables were exteriorized, and the abdomen was closed.

The occluder cuff was connected to a 1-ml syringe prefilled for each occlusion with the minimum volume of air needed to securely achieve complete occlusion of the underlying vessels. The cuff was manually inflated by rapidly advancing the plunger and was deflated by rapidly retracting the plunger. A 1-s occlusion is illustrated in Fig. 1A (arterial pressure) and Fig. 1B (flow) and demonstrated that occlusion was achieved within a heartbeat and similarly that flow was essentially restored within a heartbeat after deflation.

Experimental procedures. Animals were subjected to complete terminal aortic vascular occlusions lasting 1, 2, 4, 10, or 30 s. Occlusions of each duration were imposed twice in the counterbalanced design illustrated in Fig. 1 to minimize potential confounding effects of repeated occlusion. The recovery times between occlusions were as depicted in Fig. 1C. This protocol was repeated after hexamethonium bromide (10 mg/kg), an inhibitor of autonomic ganglionic neurotransmission, as described previously (7, 9). Finally, studies were repeated after a 10 mg/kg intraperitoneal injection of nifedipine dissolved in dimethyl sulfoxide.

Data collection. The carotid and femoral catheters were secured ~4–5 cm above heart level and connected to pressure transducers (model PE10 EZ, Ohmeda, Madison, WI), which were then connected to a signal conditioner (model 6600, Gould Instrument Systems, Valley View, OH). The flow transducer was connected to a flowmeter (model T106, Transonic). Signals were digitized at 250 Hz and written to a fixed disk of a microcomputer with the use of commercially available software (PONEMAH Physiology Platform, P3, Gould Instrument Systems) for later analysis.

Data analysis. Data analysis was carried out using 1-s averages of the digitized data. Baseline measures for arterial pressure, terminal aortic blood flow, and hindlimb vascular conductance (calculated as hindlimb blood flow divided by femoral arterial pressure using the 1-s average data) were determined by taking an average over the 10-s interval before each occlusion. Also, the peak and/or nadir value of each variable was determined during this 10-s baseline period. The magnitude of the rise in systemic arterial pressure induced by vascular occlusion was calculated as the difference between the peak value of pressure during cuff inflation and the peak value observed during the baseline period. The peak (i.e., flow and vascular conductance) or nadir (i.e., systemic arterial pressure) values of each variable during the reactive hyperemia was identified as the highest (or lowest) value achieved after cuff deflation. The magnitude of the rise in hindlimb blood flow and in hindlimb vascular conductance after cuff deflation was taken as the difference between the highest value observed after cuff deflation and the peak value observed during the baseline period. The magnitude of the fall in systemic pressure was taken as the difference between the nadir value observed after cuff deflation and the nadir value observed during the baseline period. Because the drug treatments induced large changes in baseline arterial pressure, the magnitude of the changes in systemic arterial pressure are expressed as percentage changes.

Statistical analysis. The main effects of treatments were tested by multiple linear regression in which the numeric values of occlusion duration were entered along with dummy variables used to encode drug treatment and to account for interanimal variability (18). For each occlusion duration, the magnitude of the responses were compared using paired t-tests. Adjustments for multiple simultaneous comparisons were done by the Bonferroni procedure. Statistical significance was accepted at P <0.05. Values are presented as means ± SE.

RESULTS

Systemic arterial pressure averaged 99 ± 4 mmHg before hexamethonium, 84 ± 5 mmHg after hexamethonium, and

![Fig. 1. Response of arterial pressure (A) and hindlimb flow (B) to a 1-s occlusion after hexamethonium. Rapid cuff inflation blocked blood flow within 1 heartbeat, and rapid cuff deflation permitted flow restoration within 1 heartbeat. C: time line of vascular occlusion protocol.](http://jap.physiology.org/)
67 ± 3 mmHg after hexamethonium and nifedipine. Hexamethonium and nifedipine exerted significant treatment effects on baseline systemic arterial pressure (P < 0.05). Femoral arterial pressure averaged 86 ± 4 mmHg before hexamethonium, 75 ± 4 mmHg after hexamethonium, and 58 ± 3 mmHg after hexamethonium and nifedipine. Hindlimb blood flow averaged 13.4 ± 1.6 ml/min before hexamethonium, 12.3 ± 1.9 ml/min after hexamethonium, and 11.0 ± 1.3 ml/min after hexamethonium and nifedipine. Neither hexamethonium nor nifedipine treatment exerted a significant effect on baseline blood flow. Hindlimb vascular conductance averaged 0.155 ± 0.016 ml·min⁻¹·mmHg⁻¹ before hexamethonium, 0.169 ± 0.026 ml·min⁻¹·mmHg⁻¹ after hexamethonium, and 0.192 ± 0.025 ml·min⁻¹·mmHg⁻¹ after hexamethonium and nifedipine. Nifedipine exerted a significant treatment effect on baseline vascular conductance.

Figure 2 depicts the responses to 10-s terminal aortic occlusion in the control condition (A and B) and after hexamethonium and nifedipine (C and D). Arterial pressure rose and femoral pressure fell during occlusion, and these variables underwent opposite changes after cuff deflation (A and C). Mean flow dropped from a baseline flow of ~15 to ~0 ml/min during the period of occlusion and rose to a peak of ~25 ml/min soon after cuff deflation (B). Nifedipine reduced arterial pressure (C) and blunted the magnitude of the reactive hyperemia (D).

Figure 3 shows group mean responses of hindlimb blood flow, system arterial pressure, and hindlimb vascular conductance across all conditions. The magnitude of reactive hyperemia was graded to the duration of occlusion in the control (no drug) condition (A) and after hexamethonium (D). Nifedipine treatment after hexamethionol abolished the reactive hyperemia (G).

The effects of vascular occlusion on systemic arterial pressure are shown in Fig. 3 (B, E, and H) and in Table 1. Occlusion duration exerted a significant effect on the rise in systemic arterial pressure under each of the three conditions: the longer the duration, the greater the rise in pressure. Compared with control, the rise in arterial pressure was greater after hexamethonium for all but the 1-s occlusion (Table 1). Nifedipine after hexamethonium reduced the rise in pressure compared with hexamethonium for the 4- and 10-s occlusions.

The delta increase in vascular conductance (peak – highest value during baseline) for the 1-, 2-, 4-, 10-, and 30-s periods of vascular occlusion are shown in Fig. 4. There was no reactive hyperemia after the 1-s period of occlusion, i.e., no difference between the delta conductance before and after nifedipine. The 2-s period of occlusion induced a small but significant reactive hyperemia. The magnitude of the reactive hyperemia was graded in direct relation to the duration of the occlusion for the 2-, 4-, and 10-s periods of occlusion and appeared to be approaching a plateau for the 30-s occlusion.

**DISCUSSION**

The major new findings of the present study are as follows. There is a threshold duration of occlusion required to elicit reactive hyperemia in the hindlimbs of anesthetized, hexamethonium-treated rats, although this duration is relatively short (~2 s). The magnitude of reactive hyperemia is directly proportional to the period of occlusion for periods ranging from 2 to 10 s. Tripling the period of occlusion from 10 to 30 s leads to only a modest further vasodilation.

**Limb vascular conductance.** To study local vascular control mechanisms in isolation, we blocked autonomic function because a previous study demonstrated that brief terminal aortic occlusion activated baroreflex responses in a similar model (7). The response of the arterial baroreflexes to the rise in systemic arterial pressure induced by occlusion is expected to include hindlimb vasodilation elicited by sympathetic withdrawal; thus the baroreflex responses are likely to add to the local responses in the hindlimbs when reflex function is intact. Baroreflex-mediated sympathetic withdrawal could also contribute to the graded nature of the hyperemic response; i.e., the longer

![Figure 2](http://jap.physiology.org/)

**Fig. 2.** Hemodynamic responses from a single rat to 10-s terminal aortic occlusion in the control condition (A and B) and after hexamethonium and nifedipine (C and D). In A and C, the solid black lines depict arterial pressure, the solid white lines depict mean arterial pressure, and the dashed black lines depict mean femoral arterial pressure. Ten seconds of occlusion raised arterial pressure and induced a reactive hyperemia during which arterial pressure fell. Nifedipine reduced baseline arterial pressure and blunted the magnitude of the reactive hyperemia and the associated fall in arterial pressure.
duration occlusions provide a longer time for sympathetic withdrawal to induce vasodilation. Autonomic blockade is likely to alter the distribution of blood flow among hindlimb tissues, inasmuch as different tissues have varying degrees of sympathetic tone; the effect this would have on responses before and after autonomic blockade was not evaluated.

To avoid the potentially confounding influence of the autonomic responses, we have selected to focus on the responses following hexamethonium and again after hexamethonium and nifedipine. Nifedipine appeared to be effective in eliminating the reactive hyperemia induced by vascular occlusion in that there was little reactive hyperemia (Fig. 3G), and the increases in hindlimb conductance after cuff deflation were similar, despite vastly different periods of occlusion (Fig. 3I). The small, residual reactive hyperemia after nifedipine likely reflects refilling of the arterial system distal to the occluder cuff. The consequence of arterial refilling is that it biases the calculation of vascular conductance upward. For example, it is unlikely that 1 s of occlusion elicited any vasodilation under

**Table 1. Percent rise of MAP during graded duration terminal aortic occlusion**

<table>
<thead>
<tr>
<th>Occlusion Duration, s</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>10</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.2±0.8</td>
<td>4.8±0.8</td>
<td>6.1±0.8</td>
<td>7.2±1.7</td>
<td>9.0±2.6</td>
</tr>
<tr>
<td>Hexamethonium</td>
<td>2.1±0.4</td>
<td>6.2±0.8*</td>
<td>9.5±0.9*</td>
<td>12.3±1.3*</td>
<td>15.3±1.9*</td>
</tr>
<tr>
<td>Hex + nifedipine</td>
<td>2.9±0.8</td>
<td>6.1±0.9*</td>
<td>8.0±0.7*†</td>
<td>9.7±1.3†</td>
<td>13.0±0.9</td>
</tr>
</tbody>
</table>

Values are means ± SE given in percent for 8 rats, except for 30 s where n = 6. MAP, mean arterial pressure; Hex, hexamethonium. *P < 0.05 vs. control. †P < 0.05 vs. hexamethonium.
any of the three drug conditions, yet a similar rise of ~0.05 conductance units was seen under all three conditions after 1 s of occlusion (Table 2).

Our results support our hypothesis that there is a threshold duration of occlusion that must be exceeded to elicit reactive hyperemia in the hindlimbs of anesthetized, hexamethonium-treated rats, although this duration is relatively short. We found that brief periods of occlusion led to a detectable reactive hyperemia. Although the 1-s occlusion failed to elicit a reactive hyperemia, the 2-s period of occlusion raised conductance above that seen after nifedipine (Fig. 4). Similarly, the 4-s occlusion induced a modest further increment in conductance beyond that observed after nifedipine (Fig. 4). Although these changes were statistically significant, they do not appear to impose a threat to blood pressure regulation, inasmuch as systemic arterial pressure fell only slightly below baseline after cuff deflation in these trials. The same cannot be said for the 10- and 30-s periods of occlusion, inasmuch as systemic arterial pressure fell more markedly in these trials.

In addition to vascular occlusion, reductions in leg arterial pressure occur during head-down tilt, and 2 s of head-down tilt are sufficient to impair the regulation of eye-level arterial pressure during subsequent head-up tilt in rats (7–9, 17). The findings of the present study would appear to rule out a primary role for tilt-induced local responses in the legs in producing this impairment in the regulation of eye-level arterial pressure. Two seconds of complete vascular occlusion elicited a small reactive hyperemia that reduced systemic arterial pressure by only 1 mmHg, despite the fact that vascular occlusion induced a much larger fall in femoral arterial pressure (75 mmHg) than would occur during head-down tilt (~15 mmHg). The much greater decreases in femoral arterial pressure induced by vascular occlusion compared with tilting in rats better mimics the changes in leg arterial pressure expected in larger species (e.g., dogs and humans) during negative vertical acceleration (Gz) stress, owing to their greater height. The much larger reactive hyperemia observed with the 10- and 30-s occlusions, coupled with the larger fall in arterial pressure that they induced, suggest that local responses could play an important role in the impairment of arterial pressure during head-up tilt after negative Gz stress in dogs (16) or humans (2).

Johnson et al. (10) found that the peak reactive hyperemia increased as the duration of occlusion increased from 5 to 120 s in isolated sartorius muscle of anesthetized cats. Our findings are in agreement with those of Johnson et al., and we expand on their findings in three ways. First, we examined responses to occlusion of <5 s to establish whether there is a threshold duration for eliciting reactive hyperemia vasodilation and directly verified the myogenic contribution of the vasodilation by nifedipine treatment. Second, we imposed reductions in pressure and flow to all hindlimb tissues compared with isolated skeletal muscle, because this approach better mimics the effects of negative Gz stress. Third, we examined the impact of the resulting reactive hyperemia on arterial blood pressure regulation.

**Mechanisms of vasodilation.** Reactive hyperemia elicited by vascular occlusion results from the integrated responses of multiple vascular control mechanisms. As noted above, any rise in upstream pressure could elicit reflex sympathetic withdrawal, which we evaluated by hexamethonium treatment. The fall in downstream pressure will induce myogenic vasodilation and may induce the release of endothelial factors secondary to vascular deformation (11). However, the extent of vascular deformation in the present study may differ from studies on isolated arterioles, because the distending pressure did not fall as much and in situ vessels would be supported (tethered) by the surrounding tissue. The reduction in blood flow may impose competing effects. The associated decrease in shear stress will lead to a reduction in endothelial nitric oxide release and thus vasoconstriction, whereas the reduction in substrate delivery and metabolite washout may lead to metabolic vasodilation. Endothelial factors could thus restrict the initial reactive hyperemia by partially counteracting the vasodilator drive induced by occlusion. Whether this is the case is unclear. For example, nitric oxide inhibition led to no change in the magnitude of reactive hyperemia when the absolute level of blood flow was used to gauge the magnitude of reactive hyperemia, and it led to a small (17%) but statistically significant reduction in peak reactive hyperemia when the absolute level of vascular conductance was used to gauge the magnitude of reactive hyperemia (14). The later observation suggests that nitric oxide may contribute to the peak reactive hyperemia. However, because nitric oxide inhibition greatly (50%) reduced baseline flow and conductance, the magnitude of reactive hyperemia, based on relative measures of flow or conductance (as used in the present study), appears larger after nitric oxide inhibition.
Subsequent to the peak reactive hyperemia, nitric oxide release appears to prolong reactive hyperemia inasmuch as nitric oxide synthase inhibition truncates the duration of reactive hyperemia (14).

Metabolic vasodilation is unlikely to contribute during the relatively brief periods of occlusion employed in the present study. For example, relatively prolonged (minutes) reductions in perfusion imposed by graded partial vascular occlusion fails to elicit vasodilation in resting limbs (4). Furthermore, nifedipine does not impair the mechanisms responsible for the increase in coronary flow that accompanies an increase in cardiac muscle metabolism (1). Thus metabolic regulation should be free to act during vascular occlusion after nifedipine, yet little or no reactive hyperemia was observed. Arteriolar pressure likely remained within the range over which myogenic responses are observed (19) under all flow conditions in the present study (6), i.e., in response to vasodilation proximal arteriolar pressure rises, but distal arteriolar pressure falls. Taken together, the foregoing arguments indicate that the reactive hyperemia observed in the present study stems almost entirely from mechanosensitive elements.

We observed an unexpected pattern of response to graded duration vascular occlusion after hexamethonium and nifedipine (Fig. 4). The greater rise in conductance for the 2- and 4-s occlusions compared with the 1-s occlusion could stem from greater arterial emptying during the longer occlusions and thus greater refilling after cuff deflation. For example, femoral arterial pressure fell much less during the 1-s occlusion (Fig. 1A) compared with the 10-s occlusion (Fig. 2A), signifying less discharge of arterial volume during the shorter occlusion. The apparent reversal to a lower value of conductance after the 30-s occlusion could stem from constriction due to a fall in nitric oxide release during the occlusion or to residual smooth muscle tone.

Regulation of arterial pressure. Vascular occlusion is expected to induce a rise in arterial pressure by imposing a mechanical reduction in total peripheral conductance and thereby simulate the hydrostatic effect on arterial pressure induced by head-down tilt. For example, if the hindlimbs constituted 15% of total peripheral conductance, then complete occlusion would be expected to raise arterial pressure by 15% if all other factors remained unchanged. As shown in Table 1, when the duration of occlusion was >1 s, systemic arterial pressure rose to a greater extent during the occlusion after hexamethonium (Fig. 3E) compared with when reflexes were intact (Fig. 3B). This is consistent with arterial baroreflex buffering of the pressure-raising effects of occlusion when reflexes are intact, as seen in humans by Toska et al. (20). The observation that nifedipine reduced the rise in arterial pressure to the 4- and 10-s occlusions compared with hexamethonium indicates that upper body myogenic constriction contributes to this rise observed after hexamethonium. Release of vascular occlusion is expected to induce a fall in arterial pressure by imposing a mechanical increase in total peripheral conductance and thereby simulate the hydrostatic effect on arterial pressure induced by head-up tilt. The observation that there were larger decrements in systemic arterial pressure after short periods of occlusion (1 and 2 s) when reflex function was intact compared with after hexamethonium (Table 2) indicates that baroreflex responses are important in causing this larger fall in pressure. In contrast, the greater rise in conductance after the 30-s occlusion after hexamethonium compared with control (Table 2) indicates that the baroreflexes normally work to reduce the fall in arterial pressure by restraining the reactive hyperemia.

In summary, there is a threshold duration of occlusion (~2 s) required to elicit reactive hyperemia in the hindlimbs of anesthetized, hexamethonium-treated rats, and the reactive hyperemia that results possesses a threat to the regulation of arterial pressure. The reactive hyperemia appears to result from myogenic (nifedipine-sensitive) responses.

GRANTS

This work was supported by National Heart, Lung, and Blood Institute Grant HL-46314.

REFERENCES


