Impact of combined NO and PG blockade on rapid vasodilation in a forearm mild-to-moderate exercise transition in humans

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Saunders, Natasha R., Frank A. Dinanno, Kyra E. Pyke, Anna M. Rogers, and Michael E. Tschakovsky. Impact of combined NO and PG blockade on rapid vasodilation in a forearm mild-to-moderate exercise transition in humans. Am J Physiol Heart Circ Physiol 288: H214–H220, 2005. First published September 2, 2004; doi:10.1152/ajpheart.00762.2004.—We tested the hypothesis that nitric oxide (NO) and prostaglandins (PGs) contribute to the rapid vasodilation that accompanies a transition from mild to moderate exercise. Nine healthy volunteers (2 women and 7 men) lay supine with forearm at heart level. Subjects were instrumented for continuous brachial artery infusion of saline (control condition) or combined infusion of Nω-nitro-L-arginine methyl ester (L-NAME) and ketorolac (drug condition) to inhibit NO synthase and cyclooxygenase, respectively. A step increase from 5 min of steady-state mild (5.4 kg) rhythmic, dynamic forearm handgrip exercise (1 s of contraction followed by 2 s of relaxation) to moderate (10.9 kg) exercise for 30 s was performed. Steady-state forearm blood flow (FBF; Doppler ultrasound) and forearm vascular conductance (FVC) were attenuated in drug compared with saline (control) treatment: FBF = 196.8 ± 30.8 vs. 281.4 ± 34.3 ml/min and FVC = 179.3 ± 29.4 vs. 277.8 ± 34.8 ml·min⁻¹·100 mmHg⁻¹ (both P < 0.01). FBF and FVC increased from steady state after release of the initial contraction at the higher workload in saline and drug conditions: ΔFBF = 72.4 ± 8.7 and 52.9 ± 7.8 ml/min, respectively, and ΔFVC = 66.3 ± 7.3 and 44.1 ± 7.0 ml·min⁻¹·100 mmHg⁻¹, respectively (all P < 0.05). The percent ΔFBF and ΔFVC were not different during saline infusion or combined inhibition of NO and PGs: ΔFBF = 27.2 ± 3.1 and 28.1 ± 3.8%, respectively (P = 0.78) and ΔFVC = 25.7 ± 5.2 and 26.0 ± 4.0%, respectively (P = 0.94). The data suggest that NO and vasodilatory PGs are not obligatory for rapid vasodilation at the onset of a step increase from mild- to moderate-intensity forearm exercise. Additional vasodilatory mechanisms not dependent on NO and PG release contribute to the immediate and early increase in blood flow in an exercise-to-exercise transition.

In separate experiments, Shoemaker et al. observed that the magnitude and time course of the increase in forearm blood flow (FBF) that occurs in the transition from rest to exercise was not affected under conditions where PG (29) or NO production (27) was inhibited before the onset of exercise. This suggests that NO or PGs in isolation may not be essential in regulating the blood flow adaptation to dynamic exercise in humans. However, observations that blockade of NO or PGs results in the upregulation of the other vasodilator (2) may explain the lack of single-blockade effect on the adaptation of exercise hyperemia. In contrast, combined blockade of NO and PGs has consistently demonstrated a blunting of steady-state exercise hyperemia (3, 22) and reactive hyperemia (10). This is in agreement with suggestions that NO and PGs may act synergistically in contributing to steady-state exercise-induced vasodilation (3). Whether this is the case at the onset of exercise is unknown.

The use of a rest-to-exercise transition to evaluate the hyperemic response has limited previous approaches to examining factors contributing to the immediate blood flow response to a change in exercise intensity (27, 29, 30, 37). In this transition, the muscle pump and vasodilatory contributions to the rapid hyperemia may be activated. An important aspect of investigating vasodilatory contributions is determining whether a given dilator is responsible for all or part of the observed vasodilation. The potential for a muscle pump-mediated increase in blood flow at exercise onset prevents this distinction from being made. In a recent study, we used strain-gauge plethysmography to measure forearm volume as

**WITH EXERCISE ONSET, muscle blood flow increases rapidly in a biphasic manner to a steady-state level that matches metabolic demand (25, 28, 30). Characteristic of the first phase is an immediate and very rapid increase in flow on release of the first contraction, with an initial plateau of the response in the first 5–7 s of exercise (19, 25, 27, 28, 30). The factors responsible for the early exercise hyperemia are thought to include the muscle pump effect (23, 25) and, more recently, rapid vasodilatory mechanisms (17, 21, 35, 37). However, the substances that mediate the rapid vasodilation with exercise onset are unknown. Furthermore, it is not clear whether some of the factors that may act to sustain blood flow during steady-state exercise, such as nitric oxide (NO) (9, 41) and vasodilator prostaglandins (PGs) (29), are involved in the rapid vasodilation at exercise onset.**

PGs released from the endothelium during exercise have been shown to contribute to steady-state exercise hyperemia in some (13, 40), but not all (29, 42, 43), experimental models. Sudden increases in PGs in response to increased shear stress (a form of mechanical distortion of the endothelium) have been observed (12) and have a marked impact on increasing blood flow in rat arterioles (14). Thus, because mechanical distortion of the vessel with contraction at exercise onset may cause a similar release of PGs, it is plausible that PGs contribute to rapid vasodilation. Similarly, NO is released in response to mechanical distortion of the endothelium (7) and, therefore, may also contribute to rapid vasodilation at exercise onset.

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an estimate of forearm venous volume during rhythmic, dynamic forearm exercise (21). We observed that the minimum venous volume during the relaxation phase between contractions at 10% of maximal voluntary contraction (MVC) intensity was not further reduced when contraction intensity was increased to 20% MVC. This indicated that, in this type of forearm exercise, there is no additional contribution of the muscle pump in a transition from 10% to 20% MVC contractions. Therefore, we applied this exercise protocol to evaluate vasodilatory contributions to the immediate increase in blood flow with an increase in exercise intensity, independent of any muscle pump contribution.

With this as a background, the purpose of this study was to investigate the influence of combined inhibition of PGs and NO on the time course and magnitude of the rapid change in forearm vascular conductance (FVC) in the transition from mild to moderate forearm exercise in humans. We hypothesized that combined blockade of these vasodilators would act to delay and/or attenuate the expected vasodilator response immediately after the exercise transition.

METHODS

Subjects

Nine healthy young subjects (2 women and 7 men) volunteered for the study. The mean age of the subjects was 24.8 ± 1.6 yr, height was 179.9 ± 2.0 cm, and weight was 77.9 ± 3.6 kg. The subjects had no known cardiovascular or musculoskeletal disease and were not taking any medications. Each woman had a negative pregnancy test within 24 h of the study. All protocols and procedures were approved by the Institutional Review Board at the Mayo Clinic and met the requirements for human studies outlined in the Declaration of Helsinki. Each subject provided his or her written informed consent before participation in the study.

Experimental Design

Before testing, a 20-gauge, 5-cm Teflon arterial catheter for forearm administration of study drugs was placed in the brachial artery of the nondominant exercising arm under aseptic conditions after local anesthesia (1–2 ml of 1% lidocaine). A three-port connector was placed in series with the catheter-transducer system to allow for drug infusions and continuous measurement of mean arterial pressure (MAP). Infusion does not affect blood pressure measurement, as evidenced by no change in blood pressure readings in our laboratory when infusion is initiated or stopped. When it was not being used for drug infusion, the catheter was flushed continuously with heparinized saline (2 U/ml, 3 ml/h).

Exercise protocol. The study was conducted as part of the pilot work for a larger study investigating the roles of NO and PGs during steady-state forearm exercise (22). Each subject assumed a supine posture, with the arm extended laterally at heart level. Handgrip exercises involved raising and lowering a weight of 5.4 kg [mild exercise, equivalent to ~10% MVC (21)] or 10.9 kg [moderate exercise, equivalent to ~20% MVC (21)] through a vertical distance of 5 cm by squeezing a handgripping device connected to a pulley system. Exercise duty cycle was 1 s of contraction and 2 s of relaxation, and a signal light with a metronome was used to ensure the correct timing of contractions.

The exercise protocol consisted of a 1-min resting baseline followed by 5 min of forearm exercise at mild exercise intensity (5.4-kg weight). Three 30-s step increases to 10.9-kg weight were then performed, each separated by 2 min of exercise at 5.4-kg weight (Fig. 1). After the last 30-s exercise bout at 10.9-kg weight, the weight was lowered to 4.1 kg, and the subject exercised for 20 min at this workload. After 30 min of rest, the above-mentioned exercise protocol was repeated, this time under combined NO and PG blockade. For each subject, responses to the three step increases in workload in each condition were averaged to yield a mean response for that subject after it was confirmed that there was no systematic trial-to-trial variation in the response.

NO synthase and cyclooxygenase blockade. The saline condition was always carried out first with saline infused at 1 ml/min during rest and exercise, because the drugs used to block NO synthase (NOS) and cyclooxygenase (COX) have long-lasting effects. Nω-nitro-L-arginine methyl ester (L-NAME, drug 1) and ketorolac (drug 2) was counterbalanced between subjects. Times indicate start of changes in exercise intensity. Note: 4.1 kg exercise intensity was used for exercise drug infusions as part of pilot work for the study of Schrage et al. (22).

Fig. 1. Experimental protocol. Three 30-s exercise bouts at 10.9 kg were separated by 2 min at 5.4 kg. Order of infusion of Nω-nitro-L-arginine methyl ester (L-NAME, drug 1) and ketorolac (drug 2) was counterbalanced between subjects. Times indicate start of changes in exercise intensity. Note: 4.1 kg exercise intensity was used for exercise drug infusions as part of pilot work for the study of Schrage et al. (22).

Throughout each trial, beat-by-beat heart rate (HR, by ECG), MAP (by arterial catheter), and brachial artery mean blood velocity (MBV, by pulsed Doppler velocimetry; model 500V, Multigon Industries, Mt. Vernon, NY) data were collected continuously on a computer-based system. Brachial artery MBV was measured from the spectra of a pulsed Doppler ultrasound signal. A flat probe with an operating frequency of 4 MHz was fixed to the skin over the brachial artery, proximal to the catheter insertion site, as previously described (38). The ultrasound gate was set to insonate the total width of the artery lumen. With this setup, a clear Doppler signal was maintained during steady-state mild exercise and the transition from mild to moderate exercise. Beat-by-beat MBV was calculated as the average of the instantaneous MBV values over each cardiac cycle, with the R-R
interval used to signal the end of one blood pulse wave and the beginning of the next.

Baseline MBV during mild exercise was taken as the mean of 10 contraction-free full cardiac cycles before the addition of weight. With the transition, the onset of vasodilation was assessed by evaluating the MBV for a complete cardiac cycle during relaxation. Assessment of MBV using this section of the profile allows vasodilatory onset to be assessed without the impedance of contraction on arterial blood flow (Fig. 2). A linear 7.0-MHz echo Doppler ultrasound probe (model 128XP, Acuson, Mountain View, CA) was placed in a holder securely fixed to the skin sited over the brachial artery, proximal to the pulsed Doppler probe. This probe was used to measure arterial cross-sectional area at rest and during steady state (30 s before each exercise transition). Pilot work for a previous study (21) demonstrated that diameters remain stable through these transitions. Vessel diameter was estimated as the average of three online measurements from frozen screen images during diastole. All measures were made by the same operator.

Forearm blood flow (FBF) was calculated as follows

\[ \text{FBF} = \frac{\text{MBV} \times 60 \, \text{min}^{-1} \times \pi \times (\text{brachial artery diameter}/2)^2}{100} \]

where FBF is measured in milliliters per minute, MBV in centimeters per second, and brachial artery diameter in centimeters.

Forearm vascular conductance (FVC) was calculated as follows

\[ \text{FVC} = \frac{\text{FBF}}{\text{MAP}} \times 100 \]

where FVC is measured in milliliters per minute per 100 mmHg. Flow per 100 mmHg was used so that FVC was quantitatively similar to the units for FBF.

**Statistical Analysis**

Initial comparisons of the main effects of drugs and time on FBF, FVC, MAP, and HR were analyzed for each time point by repeated-measures two-way ANOVA (SigmaStat 2.03). The level of significance was set at \( P < 0.05 \), and any differences were further assessed with Tukey’s post hoc tests. Values are means ± SE.

**RESULTS**

Table 1 summarizes HR, MAP, FBF, and FVC responses during the transition from mild to moderate exercise intensity for the first relaxation (R1) after contraction at the higher work intensity for the control (saline) and drug (\( \mathrm{iNOS} \) and \( \mathrm{K2P} \)) trials. HR and MAP increased immediately \((-0–1 \, \text{s after contraction})\) in the drug and saline trials \((P < 0.05)\). FBF and FVC also increased immediately after the transitions \((P < 0.05 \text{ for both})\).

Figure 3 shows the steady-state mild-exercise MAP, FBF, and FVC and the responses over the first 30 s after the exercise-to-exercise transition. The values for each relaxation represent the measured variable in the first full cardiac cycle after contraction. More specifically, R1 represents \(-0–1 \, \text{s after the first contraction at the new exercise intensity} \) R2 represents \(-3–4 \, \text{s after transition}\), and so on. MAP was elevated during steady-state mild exercise by \(-10 \text{ mmHg}\) in the drug compared with the saline condition \((P < 0.001)\). This difference remained during the first 30 s of moderate-intensity exercise. FBF and FVC were reduced in the drug compared with the saline condition during steady-state mild exercise, and this difference remained throughout the first 30 s of moderate-intensity exercise.

Figure 4 illustrates the percent change in MAP, FBF, and FVC with a transition from mild to moderate exercise intensity. The percent change in MAP over the first 30 s was not different between conditions \((P = 0.332)\). FBF and FVC increased immediately after the exercise-to-exercise transition, and there were no differences in the immediate percent increase \((R1)\) for both variables \((P = 0.847 \text{ and } P = 0.955, \text{ respectively})\). However, by R7, the percent change in FBF and FVC was significantly elevated in the drug compared with the saline condition.

**DISCUSSION**

This study tested the hypothesis that combined blockade of NO and PGs would delay and/or blunt the rapid vasodilation that accompanies the transition from mild to moderate exercise in the human forearm. The important novel findings of this study were that combined NO and PG blockade 1 failed to delay the onset of increases in FVC with an increase in exercise intensity and 2) did not affect the immediate or continued early percent increase in FVC. This suggests that NO and PGs do not play an essential role in the rapid vasodilation at exercise onset. It also suggests that a separate dilatory mechanism(s), not dependent on the release of NO or PGs, is responsible for the initial exercise hyperemia. In addition, in agreement with Boushel et al. (3), the present results demonstrate that combined blockade of PGs and NO substantially attenuates blood flow during steady-state exercise.

**Rationale: Use of an Exercise-to-Exercise Transition**

One of the inherent difficulties in evaluating the factors involved in blood flow regulation at exercise onset is the inability to separate muscle pump and vasodilatory contributions to the initial hyperemia. During such a transition, one or
both of these mechanisms may be activated to produce the observed increase in flow. In the present study, we sought to isolate the vasodilatory response by evaluating the blood flow response to the addition of weight in an already-contracting muscle, a condition whereby muscle pump contribution is already maximized. In our laboratory, we observed that veins are maximally emptied at mild exercise intensities during dynamic and isometric handgrip exercise (21, 35). With an increase in workload, there is no further emptying of the veins and, hence, no further increase in the arteriovenous pressure gradient to augment muscle pump effectiveness (see Ref. 21 for a full rationale of this methodology).

Given that the muscle pump effect via contraction intensity is already maximized at mild work intensities (21, 23, 26), changes in blood flow after an exercise-to-exercise transition at the same contraction frequency should expose the action of local vasodilators. Our previous observation of an immediate increase in FBF in an exercise-to-exercise transition has demonstrated the existence of rapid vasodilatory mechanisms activated with increases in contraction intensity (21). In this context, if NO and PGs were contributors to the rapid hyperemia at the onset of a change in exercise intensity, we would expect the FBF to be delayed and/or blunted with an exercise-to-exercise transition during L-NAME and ketorolac administration.

**PGs and NO in Immediate Exercise Hyperemia: Single Blockade**

Previous studies investigating the role of endothelium-derived PGs and NO in the adaptation of blood flow to a change in exercise intensity have examined these factors in isolation. In human models, local forearm blockade of NO (4, 27) or systemic blockade of PGs (29) alone did not affect the early adaptation of muscle blood flow. In contrast, in exercising dogs, systemic NO inhibition impaired the increase in blood flow from 3 to 7 s at exercise onset (23), and the time course of vascular conductance increases was substantially slowed with systemic NO blockade (24). The reason for these contrasting results is not clear but may reflect an impact of substantially elevated systemic blood pressure during systemic NO inhibition (6) on vasoregulation in exercising muscle. Under substantially elevated systemic blood pressure, a greater underlying myogenic vasoconstriction may be expected and could, potentially, modify the early vasodilatory response. Furthermore, the characteristics of the adaptation of blood flow in dogs and humans differs markedly (27, 36). It is therefore quite possible that the mechanisms contributing to the early blood flow adaptation may also be species dependent.

**Combined PG and NO Blockade in Immediate Exercise Hyperemia**

Although it may be true that previous findings showing no effect of localized NOS inhibition alone (4, 27) or PG inhibi-
it is possible that one vasoactive substance may act to compensate for the diminished formation of the other during inhibition, thereby masking the true effect of the blocked vasodilator. Supporting this possibility is the observation that, during NOS blockade, PG synthesis is augmented (2, 18) and vice versa (2). Additionally, evidence suggesting that, under certain conditions, NO and PGs interact positively to enhance vasodilation has been supported by the work of Engelke et al. (10), which demonstrated that the effects of NOS inhibition alone during reactive hyperemia were potentiated by simultaneous COX inhibition. More recently, Boushel et al. (3) showed that combined inhibition of NO and PGs established before the onset of progressive increases in exercise intensity reduced muscle blood flow during dynamic exercise in humans.

Our observation of a substantially blunted steady-state exercising FBF (~30% decrease) and FVC (~35% decrease) during mild forearm exercise under combined NO and PG blockade is consistent with a role for these vasodilators in regulation of steady-state exercise hyperemia. This observation also demonstrates that we achieved a physiologically significant blockade of the NO and PG vasodilator systems. Under this condition, we observed an immediate increase in FBF and FVC with an increase in exercise intensity where the muscle pump is already maximized. Furthermore, the immediate (R1) and continued (R2–R9) percent increase in FBF and FVC in response to an increase in contraction intensity were virtually identical under combined NO and PG blockade and control conditions (Fig. 4). However, because steady-state FBF and FVC were lower during combined inhibition, absolute changes from mild to moderate exercise (FVC and FBF) were ~30% less with NO and PG blockade.

The use of percent vs. absolute change to represent vasoregulation has been encountered in studies examining the effectiveness of sympathetic vasoconstriction in resting (low blood flow) vs. exercising (high blood flow) muscle, i.e., the issue of functional sympatholysis. It has been clearly demonstrated that the effectiveness of a given level of sympathetic vasoconstriction under conditions of different baseline blood flows is reflected in the percent change in vascular conductance, not the absolute change (5, 31, 33, 38). This is illustrated by the observation that stimulation of sympathetic nerves innervating a muscle vascular bed results in the same percent reduction in vascular conductance at resting (lower flow) and vasodilator infusion-induced (higher flow) baseline blood flows (31, 33). This issue has been further addressed and summarized in a recent review (32). Unfortunately, to our knowledge, similar studies to determine the expected effect of a given vasodilator influence on vascular conductance under different conditions of baseline vascular conductance have not been performed.

However, on the basis of the information described above, we believe that percent change is the appropriate index of comparison in the present study. The examination of percent change indicated identical degrees of rapid vasodilation between control and blockade conditions. However, given that there was a blunted absolute change in FVC with combined NO and PG blockade, the possibility that NO and PGs may play a limited role in the magnitude of the rapid hyperemia must be acknowledged. Regardless of which index is used, these data clearly demonstrate that 1) NO and PGs cannot, by themselves, account for the immediate and continued early hyperemia after an increase in exercise intensity and 2) a rapid vasodilatory mechanism(s) exists that does not require NO or PG release.

Advantages and Limitations

An important advantage to the use of an exercise-to-exercise transition in this study is the ability to isolate the vasodilatory contribution to the initial exercise hyperemia, allowing unambiguous interpretation of the magnitude of change in blood flow with changes in exercise intensity. Also, this study is the first to explore the effects of combined blockade of these vasodilators on immediate exercise hyperemia. Furthermore, although previous investigations evaluated the blood flow response using 1-s time averages (17, 26, 39), the present experiment used the first full cardiac cycle unaffected by contraction. With muscle contraction, arterial inflow can be impeded because of the associated mechanical compression of the arteries (16). Incorporating the contraction and relaxation phases of the flow profile underestimates the actual vasodilatory response, because it includes the section of the flow profile that is reduced from arterial compression.

A potential limitation of this study is our ability to ensure that infusion of L-NAME and ketorolac was able to completely inhibit NO and PGs, respectively. The local dose of L-NAME (25 mg total, or 17–35 mg/l forearm volume and 2.5 mg/min maintenance dose) was similar to doses that have previously been used and demonstrated to show obvious increases in resting vascular tone and reductions in the vasodilator responses to brachial artery infusions of acetylcholine (8).

In addition, the local dose of ketorolac was equal to or higher than those used previously locally or systemically with related compounds that have demonstrated an effective reduction in PG production (13, 40). Therefore, although we are unable to definitively validate the complete blockade of NO and PGs, the local doses were likely more than adequate to produce the desired blockade and should not limit the interpretation of our findings.

Furthermore, we were unable to examine NO or PG blockade in isolation in this study, inasmuch as the intervention of intermittent elevations in exercise intensity after each subsequent drug infusion could have confounded the pilot data for the experimental design of the study of Schrage et al. (22). However, as we stated in the introduction, the isolated contributions of NO and PG to a transition from rest to exercise in humans have been examined previously (27, 29), and no contribution was evident under those conditions. Thus the combined-blockade approach of this study examines an important new aspect of NO and PG contribution to early exercise hyperemia beyond the established lack of effect of establishing isolated NO or PG blockade before a change in exercise intensity.

Finally, we were unable to counterbalance the drug and saline conditions, because L-NAME and ketorolac have prolonged effects. Thus testing conditions could not be randomized. However, to reduce any residual fatigue or metabolites from the previous exercise bout, ≥30 min were allowed between conditions; therefore, the effects of this limitation are expected to be insignificant.
Conclusions

This study has provided evidence that the rapid vasodilation observed in the transition from mild to moderate forearm exercise in humans is not delayed under conditions of combined NO and PG blockade. Furthermore, the relative immediate increase in FVC is also not affected. Thus a rapid vasodilatory mechanism(s) not dependent on NO and PG release appears to be activated with an increase in contraction intensity. Future investigations will need to focus on the nature of this mechanism(s).

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