Contribution of prostaglandins to the dilation that follows isometric forearm contraction in human subjects: effects of aspirin and hyperoxia

Thet Su Win and Janice M. Marshall
Department of Physiology, Division of Medical Sciences, The Medical School, Birmingham, United Kingdom

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Win, Thet Su, and Janice M. Marshall. Contribution of prostaglandins to the dilation that follows isometric forearm contraction in human subjects: effects of aspirin and hyperoxia. J Appl Physiol 99: 45–52, 2005. First published March 3, 2005; doi:10.1152/japplphysiol.01289.2004.—In 11 healthy volunteers, we evaluated, in a double-blind crossover study, whether the vasodilation that follows isometric contraction is mediated by prostaglandins (PGs) and/or is O2 dependent. Subjects performed isometric handgrip for 2 min at 60% maximal voluntary contraction (MVC), after pretreatment with placebo or aspirin (600 mg orally), when breathing air or 40% O2. Forearm blood flow was measured in the dominant forearm by venous occlusion plethysmography. Arterial blood pressure was also recorded, allowing calculation of forearm vascular conductance (FVC; forearm blood flow/arterial blood pressure). During air breathing, aspirin significantly reduced the increase in FVC that followed contraction at 60% MVC: from a baseline of 0.09 ± 0.011 [mean ± SE, conductance units (CU)], the peak value was reduced from 0.24 ± 0.03 to 0.14 ± 0.01 CU. Breathing 40% O2 similarly reduced the increase in FVC relative to that evoked when breathing air; the peak value was 0.24 ± 0.03 vs. 0.15 ± 0.02 CU. However, after aspirin, breathing 40% O2 had no further effect on the contraction-evoked increase in FVC (the peak value was 0.15 ± 0.02 vs. 0.16 ± 0.02 CU). Thus the present study indicates that prostaglandins make a substantial contribution to the peak of the vasodilation that follows isometric contraction of forearm muscles at 60% MVC. Given that hyperoxia similarly reduced the vasodilation and attenuated the effect of aspirin, we propose that the stimulus for prostaglandin synthesis and release is hypoxia of the endothelium.

vasodilation; hyperemia; hypoxia; hyperoxia

There is controversy over the extent to which prostaglandins (PGs) contribute to the hyperemia and vasodilation that are associated with muscle contraction in human subjects. Consistent with a proposed dilator role, Nowak and Wennmalm (26) showed that the venous efflux of PGE increased during dynamic leg exercise, whereas Wilson and Kapoor (38) showed the venous efflux of 6-keto-PGF1α, the stable metabolite of PG12 (prostacyclin), and PGE2 increased during dynamic exercise of the wrist. More recently, others showed by using microdialysis that muscle interstitial concentration of 6-keto-PGF1α (10) and PGE2 (2, 20) increased during dynamic leg exercise.

The first functional evidence that PGs may contribute to exercise hyperemia was provided by Kilbom and Wennmalm (21), who reported that the cyclooxygenase (COX) inhibitor indomethacin reduced the hyperemia that followed low-level dynamic exercise of the forearm and that occurred during and after isometric contraction of the forearm by ~50%. However, these results are difficult to evaluate because recordings were made before and after indomethacin in the same experimental session with no reported time controls. The conclusion drawn for isometric contraction is particularly unsafe because blood flow was recorded by venous occlusion plethysmography, which is unreliable when applied during isometric contraction (see Ref. 38).

More recently, both indomethacin and aspirin substantially reduced the increase in calf blood flow and decrease in vascular resistance that occurred following a 30-min period of treadmill exercise (4). In this study, a placebo or one of the COX inhibitors was given on different experimental days in randomized order. However, these indomethacin also potentiated the increase in systolic pressure that occurred during exercise, and both COX inhibitors facilitated an increase in blood flow that occurred in the nonexercised forearm. Thus interpretation of the effects of COX inhibition on the local response to muscle contraction was complicated by its effect on the systemic cardiovascular response to exercise.

In a further study, infusion of indomethacin into the forearm reduced the increase in forearm blood flow (FBF) and the decrease in forearm vascular resistance (FVR) measured by plethysmography in transient breaks during 5-min periods of dynamic wrist exercise (38). However, indomethacin produced a decrease in baseline FBF and an increase in baseline FVR that were similar in magnitude to the effects it had on FBF and FVR values recorded during exercise. Thus there was no clear evidence that indomethacin affected the changes in FBF or FVR induced by exercise. The study of Duffy et al. (6) is more conclusive in that infusion of aspirin into forearm reduced the increase in FBF measured by plethysmography and the decrease in FVR that followed a 2-min period of dynamic wrist exercise: they corrected for the change in baseline FBF and FVR when calculating the effects on the postexercise hyperemia but acknowledged that the change in baseline per se may have limited the hyperemia.

By contrast, Nowak and Wennmalm (27), who used plethysmography, reported that indomethacin had no effect on the hyperemia that occurred in the calf during dynamic leg exercise. Moreover, Shoemaker et al. (34), who used the ultrasonic Doppler technique, which can be used effectively during muscle contraction, showed that COX inhibition had no effect on the increase in FBF recorded during dynamic wrist exercise.

To summarize, the one study so far performed on the role of PGs in the hyperemia associated with isometric contraction (21) produced inconclusive results, and the various studies performed on dynamic exercise allow only the proposal that...
PCI2 production in cultured endothelial cells (25), and COX inhibition attenuated hypoxia-induced dilation of muscle arterioles and small arteries in vitro (3, 12, 24) and in vivo (31). Moreover, shear stress stimulated PG synthesis from cultured endothelial cells (11, 14), and COX inhibition attenuated shear stress-induced dilation of muscle arterioles in vivo and in vitro (22, 23). We, therefore, hypothesized that PGs make a substantial contribution to the hyperemia that follows a period of isometric contraction, given that PO2 falls in muscle during contraction (13) and that the increase in blood velocity that occurs when muscle contraction ceases (19) is likely to increase endothelial shear stress.

To test this hypothesis, we investigated the effect of aspirin on the hyperemia that followed isometric contraction of the forearm at 60% maximum voluntary contraction (MVC) by using a double-blind crossover study design. To make an indirect test of the role of hypoxia in this hyperemia, we used a comparable study design in which subjects were given supplementary O2 (40% O2) to breathe with the aim of alleviating the fall in PO2 that occurs during contraction. In a final protocol, we tested the combined effect of aspirin and breathing 40% O2 on hyperemia. Our results allow the novel proposals that PGs a main contributor to the hyperemia that follows isometric contraction and that this contribution is O2 dependent.

METHODS

Subjects

Eleven healthy, recreationally active male subjects of age 21–28 yr (21.45 ± 0.8 yr, mean ± SE) participated in the present study. After full details of the experimental protocol, which was approved by the University of Birmingham Ethics Review Committee, consent forms were signed by the subjects. All subjects denied taking aspirin or any other nonsteroidal anti-inflammatory drugs for 10 days before the experiment. The subjects also refrained from smoking or drinking alcohol within 24 h of the experiment and were asked not to eat a heavy meal, consume caffeine, or take vigorous exercise for at least 2 h before the experiment. In practice, none of the subjects consumed caffeine or undertook any exercise other than that required to walk to the laboratory on the day of the experiment. Each subject attended a temperature-controlled laboratory (18–20°C) on six occasions, each on a different day. The first visit served to familiarize the subjects with the study protocol. They filled in a questionnaire concerning their age, weight, height, exercise status, smoking and dietary habits, alcohol intake, and history of any cardiovascular disease. In addition, the MVC of each subject was determined.

MVC

Each subject was asked to perform a maximal strength hand grip with his dominant hand, using a hand-grip dynamometer (TKK 5001, Takei, Japan) and was told before he began that he would have to maintain the contraction for 10 s. Each subject was given strong verbal encouragement during the contraction. The force reading at the end of the 10 s was taken as 100% MVC: in each subject, it was no more than 2% lower than the force measured in the first 1 s of the contraction. The 100% MVC was measured in this way to avoid the subject using muscle in addition to those of the forearm to generate an unrealistically large “snap” contraction.

Forearm Exercise

During the protocols (see below), subjects performed an isometric contraction of forearm at 60% MVC with the dominant hand for 2 min. The force reading was displayed by the deflection of an arrow on the dynamometer so that the force exerted was accurately monitored by the subject and checked by the experimenter.

Recordings

FBF was measured from the dominant forearm, which was arranged on foam cushions at heart level, by venous occlusion plethysmography (37) by using a dual strain-gauge plethysmograph (Lectromed, Letchworth Garden City, UK). The strain gauge was connected via a Wheatstone bridge circuit and amplifier to an Apple Macintosh computer (Power Macintosh 7100/66, Apple Computer). The output of the strain gauge was displayed on the computer screen using MacLab hardware (MacLab/400; AD Instruments, Hastings, UK). For each recording of FBF, the wrist cuff was inflated to ~200 mmHg, and then, when the output of the strain gauge was stable, the arm cuff was inflated to ~40 mmHg. The pressure in the arm cuff was maintained for ~4–5 s and that in the wrist cuff for no more than 10 s for each recording of FBF. Throughout the experiment, a continuous recording of cutaneous red cell flux (cRCF) of dominant forearm was made by using the laser Doppler perfusion monitor (DRT-4, Moor Instruments, Axminster, UK), which was connected to a probe (PF-308). The probe was positioned just distal to the plethysmograph at the medial, hair-free aspect of the half-pronated forearm. The laser Doppler flowmeter was also connected to the computer. Measurements of cRCF for analysis were taken immediately before each measurement of FBF since inflating the arm cuff affected forearm cRCF.

Arterial blood pressure (ABP) was measured from the nondominant arm, using a semiautomatic sphygmomanometer (Omron, M4, Omron Healthcare, Hamburg, Germany) that also provided a measure of heart rate. Mean ABP (MABP) was calculated as one-third the pulse pressure plus diastolic pressure. Vascular conductance in the forearm and cutaneous circulations (FVC and CVC, respectively) were calculated as FBF or cRCF divided by MABP.

Delivery of Hyperoxic Gas or Atmospheric Air

Delivery of hyperoxic gas was achieved by the use of an O2 concentrator (O2 Live Machine, Oxygen Leisure Products, London, UK) that delivered atmospheric air concentrated to 95–100% O2. An intussusceptible face mask (Intersurgical Wokingham) was fastened on the subject, with the mask covering his nose and mouth. The mask was connected via a Venturi valve (Intersurgical Wokingham) and tubing to the O2 concentrator. This allowed 95–100% of O2 to be forced at 2.5 l/min through the Venturi valve, which entrained air from the atmosphere and reduced the final concentration to ~40% O2. The gas delivered to the subject was within the range of 39 to 41% O2. The O2 concentration of the gas delivered by the O2 concentrator and by the mask was checked at regular intervals by passing a sample through a gas analyzer (IL 1640). In other experiments, delivery of atmospheric air through the mask was achieved by the use of a Reciprocator Pump (Stanhope Seta), which was connected via tubing to the mask via the Venturi valve. This provided a control for the experience of breathing via a mask through which gas was delivered.
The equipment was screened so that the subject could not see which gas he was breathing.

**Drug Administration**

Aspirin (Aspro Clear, Laboratories Roche Nicholas) was given orally (600 mg): this dose produces maximal inhibition of the synthesis of PGs and thromboxane A2 after 30 min, and PGI2 synthesis is still inhibited by 70% after 90 min (17). To disguise the taste and appearance of aspirin, it was dissolved in 450 ml of diluted orange juice (200 ml of Sainsbury’s Pure Orange Juice diluted with 250 ml of water). For placebo intervention, 450 ml of diluted orange juice was given. Placebo or aspirin was given in a double-blind randomized fashion.

**Protocols**

**Protocol 1.** On the second visit, when the subject was seated comfortably in the laboratory, he was given either placebo or aspirin. The subject then rested for at least 35 min, and during this period he was instrumented to record FBF, ABP, and cRCF (see above). During the last 5 min of the resting period, FBF and ABP were measured four times to ensure that a stable baseline had been achieved. The subject then performed an isometric contraction at 60% MVC with his dominant hand for 2 min. Immediately after the contraction, FBF and ABP were measured (time 0), and these measurements were repeated at 30 s, 1 min, 2 min, and 3 min after the contraction: to achieve the FBF measurements at these times, the cuff on the wrist was inflated 3–4 s before the end of the contraction and before the 1-, 2-, and 3-min readings (see above). On the third visit, the subject received the alternative intervention (i.e., either aspirin or placebo). The protocol described above was then repeated.

**Protocol 2.** This protocol was conducted on the fourth and fifth visits. It was similar to protocol 1, with the subjects being given aspirin or placebo at the beginning of each session, except that from the end of the 35-min rest period (see above), the subject breathed 40% O2 for 5 min before, during, and after the 2-min period of contraction at 60% MVC.

**Protocol 3.** In this protocol, which was undertaken on the sixth visit, the subject was given aspirin on arrival at the laboratory. Then, the first day’s session of protocol 2 was performed, but with the subject breathing atmospheric air via the intersurgical mask instead of hyperoxic gas. The subject was unaware of which gas he was breathing and did not know whether he had taken aspirin. This protocol was not performed at a second session.

**Statistical Analysis**

Data are expressed as means ± SE. Each subject served as his own control. Repeated-measures ANOVA was used to compare values recorded at different times after isometric contraction with those recorded at rest within one condition. Factorial ANOVA was used to detect differences between conditions. If a difference was found at time 0, Bonferroni/Dunnett’s post hoc tests were performed to locate the differences, with significance again being set at P < 0.05.

Data were also analyzed as described above using change from baseline rather than absolute values. The outcome of these analyses were the same, and thus we have not considered them separately in the description below.

### RESULTS

**Protocol 1**

The weight and height of the subjects were 71.09 ± 1.8 kg and 1.71 ± 0.034 m, respectively. The forearm circumference was 24–29 cm (25.73 ± 0.52 cm). The baseline values for MABP, heart rate, FBF, and cRCF after placebo or aspirin are shown in Table 1. There were no significant differences between the baselines after placebo or aspirin treatment.

After placebo treatment, isometric contraction produced the expected increase in MABP; it reached a value that was different from baseline immediately after the release of contraction and then swiftly returned toward baseline (Fig. 1). There was also an increase in FBF, this being significantly different from baseline at time 0 and throughout the 3-min recovery period. Accordingly, FVC was significantly increased, indicating vasodilation, at all time points during the recovery period (Fig. 1). There was no significant increase in cRCF. Furthermore, CVC did not change significantly from the baseline values (Fig. 1). In fact, cRCF and CVC showed no significant change after contraction in any of the protocols. Moreover, baseline rCRF and CVC were not affected by aspirin or by breathing 40% O2 or air via the mask. Thus these variables are not given any further consideration below.

After aspirin treatment, isometric contraction again increased MABP, with the values being comparable to those recorded after placebo treatment (Table 2). However, after aspirin treatment, the increase in FBF from baseline was significant only at time 0 and 0.5 min after the isometric contraction, and by ANOVA, FBF was lower after aspirin than placebo treatment over the full 3-min recovery period. The increase in FVC was also substantially reduced after aspirin treatment over the whole time period after contraction, with this effect being particularly pronounced at time 0 (Fig. 2A). Because changes in the effect of isometric contraction on FVC demonstrate changes in the effect on vascular tone, we have concentrated on FVC when describing the results in the text below and in Figs. 1 and 2. The change in MABP evoked by isometric contraction was not altered by any of the experimental conditions. Thus the changes evoked in FBF reflected the changes in FVC (see Table 2).

### Table 1. Baseline values after placebo and after aspirin when subjects breathed air and when they breathed 40% O2 with or without a mask

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th></th>
<th>Aspirin</th>
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<tbody>
<tr>
<td></td>
<td>Air</td>
<td>40% O2</td>
<td>Air</td>
<td>40% O2</td>
</tr>
<tr>
<td></td>
<td>Without mask</td>
<td>With mask</td>
<td>Without mask</td>
<td>With mask</td>
</tr>
<tr>
<td>MABP, mmHg</td>
<td>79.6±1.6</td>
<td>80.1±2.3</td>
<td>82.5±1.8</td>
<td>80.3±2.1</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>64.2±2.0</td>
<td>64.7±2.0</td>
<td>64.2±1.7</td>
<td>64.6±2.3</td>
</tr>
<tr>
<td>FBF, ml·100 ml tissue · min⁻¹</td>
<td>6.8±0.6</td>
<td>5.6±0.6</td>
<td>5.4±0.5</td>
<td>6.0±0.5</td>
</tr>
<tr>
<td>cRCF, Pu</td>
<td>10.3±1.3</td>
<td>9.4±0.7</td>
<td>10.7±1.6</td>
<td>9.9±0.9</td>
</tr>
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</table>

Values are means ± SE. Pu, perfusion units; MABP, mean arterial blood pressure; HR, heart rate; FBF, forearm blood flow; cRCF, cutaneous red cell flux.
Protocol 2

There were no significant differences between the baseline values for MABP, FBF, and FVC after placebo and aspirin treatment when breathing 40% O₂ (Table 1 and Fig. 2C). Thus, by contrast to the results obtained when subjects breathed air, when they breathed 40% O₂ there were no differences between the changes evoked in FVC after aspirin and after placebo (Fig. 2C, cf. Fig. 2A).

Protocol 3

There were no differences between the baseline values recorded when breathing air without a mask after aspirin treatment (protocol 1) and those recorded when breathing air via the mask after aspirin (Table 1). Furthermore, the values of FVC recorded after isometric contraction when the subjects breathed air via the mask after aspirin were fully comparable to those recorded when they breathed air without a mask after aspirin treatment in protocol 1 (Fig. 2D).

DISCUSSION

In the present double-blind crossover study, the hyperemia and vasodilation that occurred in forearm after a 2-min period of isometric contraction at 60% MVC was substantially reduced by the COX inhibitor aspirin, and a similar reduction occurred when the subjects breathed 40% O₂ rather than air. By contrast, in the presence of aspirin, breathing 40% O₂ had no effect on the postcontraction vasodilation.

The pattern of response evoked by 60% MVC was as expected from many previous studies. There was an increase in MABP that waned over the 3 min after the end of the contraction, and this was associated with an increase in FBF that waned from the cessation of contraction but was still significantly above control level at 3 min. FVC showed an increase of ~3.5-fold at the end of contraction that persisted at ~3-fold above control at 3 min, indicating maintained vasodilation in the forearm. Because cRCF in the forearm and CVC showed no significant change, we can assume, as others have done (see Refs. 18, 35), that the increase in FVC primarily reflected postcontraction vasodilation in skeletal muscle.

Effects of Aspirin

Aspirin had no effect on the baseline levels of any of the recorded variables, including FBF and FVC. This is consistent with the findings of other studies in which a COX inhibitor (aspirin, indomethacin, or ibuprofen) was given systemically (4, 21, 34). By contrast, when indomethacin or aspirin was directly infused into the forearm circulation, baseline FVC decreased and venous efflux of PGI₂ and PGE₂ decreased, indicating a tonic dilator influence of PGs (6, 38). The reason for this disparity is not clear: it may be that the local effect of PG inhibition that is evident after infusion of a COX inhibitor into the forearm (6, 38) is obscured by other systemic and compensatory effects when recordings are made after a single systemic dose of the antagonist or when repeated doses have been given for 24 h (Refs. 4, 21, 34, and present study).

The fact that aspirin had no effect on baseline FBF or FVC in the present study makes the interpretation of our results straightforward (cf. Refs. 6, 38). The dose of aspirin we used produces near-maximal inhibition of COX for 0.5–1.5 h (17). Thus our finding that aspirin attenuated the whole period of postcontraction hyperemia and vasodilation (increase in FVC),
with a particularly pronounced effect on the peak hyperemia and peak increase in FVC, which were reduced by ~35%, strongly suggests that PGs make a major contribution to this vasodilatation, especially to the early part of the response. As far as we are aware, the only previous study on the effect of a COX inhibitor on the hyperemia that follows isometric contraction is that of Kilbom and Wennmalm (21). Our results are consistent with their findings. However, we suggest that our experimental design was better, and thus our conclusion is more secure. The particularly pronounced effect of aspirin on the peak vasodilatation immediately after contraction is reminiscent of the finding that COX inhibition decreased the peak reactive hyperemia recorded in the forearm after a period of arterial occlusion (8). Given that the peak of reactive hyperemia is attributed at least in part to mediators that accumulate during the period of ischemia (33), our finding is consistent with the proposal that PGs accumulate in muscle when PO2 falls as a consequence of the increase in O2 demand caused by muscle contraction (see Combined Effects of Aspirin and Hypoxia).

Effects of Hyperoxia

It is reasonable to assume that when 40% O2 is breathed, the PO2 of the arterial blood increased to ~240 Torr from ~98 Torr (see Ref. 7). There must have been a consequent increase in the dissolved O2 in plasma and a small increase in the arterial saturation of hemoglobin from ~98 to ~100%. In other words, we must have produced a state of hyperoxia. This condition produced no change in baseline FBF or FVC. In previous studies, subjects breathing 100 or 200% O2 showed a decrease in baseline forearm or calf blood flow (1, 16). However, consistent with our finding, Pedersen et al. (28) showed no change in baseline FBF when subjects breathed 60% O2. Thus it seems that inspired O2 concentrations of >60% may be required to reveal the recognized vasoconstrictor effect of a rise in PO2 (29). Given that baseline FBF did not change in our study, we can conclude that, under resting conditions, any increase in O2 delivery to the forearm was small and mainly the consequence of an increase in the O2 content of the plasma.

The second new finding of the present study was that the postcontraction hyperemia and increase in FVC were reduced when breathing 40% O2, with the peak increase in FVC being reduced by ~35%. The experiments in which comparisons were made between responses evoked during air breathing with and without the mask (see Protocol 3, RESULTS) indicated that the mask per se did not affect the response. Therefore, the reduction in the vasodilation was due to breathing 40% O2 rather than to the mask. Others have shown that breathing 100 or 60% O2 reduced the increase in leg blood flow that occurred during dynamic leg exercise (28, 36). As far as we are aware, our study is the first to show that hyperoxia can reduce the vasodilation that follows isometric contraction of the forearm. The most obvious explanation for this finding is that breathing 40% O2 increased the O2 supply during the period of contraction and thereby reduced the accumulation of vasodilator substances that caused vasodilatation when contraction ceased. This proposal obviously requires that blood flow through the forearm continued during isometric contraction at 60% MVC.

The most comprehensive study on blood flow in the forearm during isometric contraction was performed by Humphreys and Lind (18), who measured FBF by venous occlusion plethysmography. They argued, from the effects of arterial occlusion on time to physical exhaustion on forearm contractions at different percentages of MVC, from measurements of intramuscular temperature in active and inactive muscles of the forearm when the forearm had been immersed in warm or cool water before contraction and from measurements of FBF made during isometric contraction, that FBF increases above control during isometric contractions to >60% MVC. Their results indicated that the increase in blood flow was through the contracting muscles and not through inactive muscles or skin. Moreover, their findings suggested that >70% MVC is required to produce total occlusion. Consistent with these conclusions, more recent ultrasonic Doppler recordings from the brachial artery indicated that FBF increased to similar levels, by 85–95% from control, during isometric forearm contraction at all intensities from 10 to 70% MVC: higher percent MVC values were not tested (19).

Table 2. FBF and MABP values recorded at rest and at intervals after isometric contraction under different conditions

<table>
<thead>
<tr>
<th>Condition</th>
<th>FBF, ml/100 ml tissue⁻¹min⁻¹</th>
<th>MABP, mmHg</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>0</td>
</tr>
<tr>
<td>Placebo</td>
<td>6.8 ± 0.6</td>
<td>20.7 ± 3.0*</td>
</tr>
<tr>
<td>Aspirin</td>
<td>5.4 ± 0.5</td>
<td>13.1 ± 1.1†</td>
</tr>
<tr>
<td>Placebo + 40% O2</td>
<td>7.0 ± 0.6</td>
<td>16.5 ± 1.2*</td>
</tr>
<tr>
<td>Aspirin + 40% O2</td>
<td>6.0 ± 0.5</td>
<td>13.6 ± 1.4</td>
</tr>
<tr>
<td>Aspirin + air with mask</td>
<td>6.5 ± 1.2</td>
<td>12.6 ± 1.2*</td>
</tr>
</tbody>
</table>

Values are means ± SE at times corresponding to the FVC values shown in Figs. 1 and 2. Within each condition, the change in FVC and MABP over time was significant (P < 0.05) by ANOVA. *Significant increase from resting value within condition. Values obtained after aspirin and after placebo + 40% O2 were significantly different by ANOVA from those obtained after placebo alone. †Significant difference between one of these conditions and placebo at a given time point, by post hoc test. There was no significant difference by ANOVA between placebo + 40% O2 and aspirin + 40% O2 or between placebo alone (with mask) and aspirin + air with mask; see text and cf. Fig. 2.

PROSTAGLANDINS AND POSTCONTRACTION DILATION
Combined Effects of Aspirin and Hyperoxia

Our third new finding was that, in the presence of COX blockade with aspirin, hyperoxia had no further effect on the postcontraction vasodilation, not even on the peak of the response. This argues against endothelial shear stress being a major stimulus for PG synthesis. Indeed, the more likely explanation for this finding is that hyperoxia and aspirin acted on the same dilator pathway(s). In other words, hyperoxia prevented the accumulation of vasodilator PGs that contributed to the postcontraction vasodilation. This interpretation is fully consistent with the substantial evidence that vasodilator PGs are released from the endothelium of muscle arterioles in response to hypoxia, a fall in PO2, and cause arteriolar dilation (3, 12, 24, 31). It is also consistent with evidence that, during muscle contraction, the decrease in venular PO2 causes the release of PGs from venular endothelial cells, possibly via the action of ATP released from red blood cells, and that these PGs then cause the dilation of nearby arterioles (15, 31). If PGs are released into the interstitial fluid during isometric contraction as they are in dynamic exercise (see Refs. 2, 10, 20) and if this is due to a relative hypoxia of the muscle fibers, it seems very unlikely that this was counteracted by the small increase in O2 delivery to muscle produced by breathing 40% O2. In other words, supplementary O2 breathing is much more likely to have affected PG production by endothelium than by muscle fibers.

The pathways that mediate vasodilation are known to be interdependent. For example, inhibition of the cAMP pathway that mediates the dilation to PGs can inhibit dilation via cGMP, the second messenger for nitric oxide (NO) (5). Furthermore, we recently showed that, when arterial PO2 is reduced during dynamic exercise of the forearm, the decrease in venular PO2 causes the release of PGs from venular endothelial cells, possibly via the action of ATP released from red blood cells, and that these PGs then cause the dilation of nearby arterioles (15, 31). If PGs are released into the interstitial fluid during isometric contraction as they are in dynamic exercise (see Refs. 2, 10, 20) and if this is due to a relative hypoxia of the muscle fibers, it seems very unlikely that this was counteracted by the small increase in O2 delivery to muscle produced by breathing 40% O2. In other words, supplementary O2 breathing is much more likely to have affected PG production by endothelium than by muscle fibers.

Thus it is very unlikely that FBF was fully occluded in the present study during isometric contraction at 60% MVC; indeed, blood flow to the contracting muscles probably increased to some extent. This proposal is consistent with the fact that the circumstance of the forearm was relatively small for male subjects (see RESULTS), reflecting their lack of regular participation in sport involving the use of forearm muscles and by the fact that they were able to sustain contraction at 60% MVC for as long as 2 min: muscle mass determines the intramuscular pressure developed at a given percent MVC and, in turn, time to exhaustion.

Thus we can conclude that breathing 40% O2 produced its effect on postcontraction dilation by increasing the PO2 of the blood supplied to the muscle during contraction rather than by increasing the bulk delivery of O2 and that the increase in PO2 reduced the accumulation of a vasodilator substance(s) whose formation is O2 dependent. This accords with our recent finding that, when 40% O2 was breathed only during the period of isometric contraction, the postcontraction increase in FBF was 18.64 ± 1.95 ml·100 ml tissue⁻¹·min⁻¹ at peak rather than 24.11 ± 2.13 ml·100 ml tissue⁻¹·min⁻¹ (Ref. 9; Fordy G and Marshall JM, unpublished observations). It is also consistent with evidence that PO2 is an important signal for the regulation of skeletal muscle blood flow (29).
whereas the hyperemia induced by dynamic exercise of knee extensors was inhibited by combined blockade of PG and NO synthesis but not by single blockade of either (2). Such results have led to the idea that blockade of one pathway involved in generating the dilation results in a compensatory increase in the role of other factors. Thus the combined effect of COX inhibition and hyperoxia on the postcontraction dilation of the present study may have been similar to their independent effects, not because they acted on the same dilator pathway but because, in the presence of hyperoxia and COX inhibition, there was a compensatory increase in the contribution of factors that are independent of COX and PO2. Such factors may include those released by hypoxia of the muscle fibers, including K+, lactate, and H+ ions (13) and the myogenic response to the fall in intravascular pressure that occurs during contraction (33). Assay of PGE2, PGL2, adenosine, NO, K+, etc. in venous plasma and interstitium, with and without aspirin and/or supplementary O2, are required to investigate these possibilities (see Refs. 2, 10, 20).

In summary, the present study has provided compelling evidence that PGs make a major contribution to the vasodilation that occurs in forearm muscles after isometric contraction at 60% MVC, particularly to the peak of that dilation. Furthermore, because a similar reduction in this postcontraction vasodilation was produced by breathing 40% O2, the study also provided strong evidence that this dilation is O2 dependent. Because the effect of 40% O2 did not occur in the presence of COX inhibition, it is likely that they interfere with the same dilator pathway. We propose that the stimulus for the synthesis of vasodilator PGs during isometric contraction is a fall in PO2 of the endothelium.

REFERENCES


