Plasma from conscious hypoxic rats stimulates leukocyte-endothelial interactions in normoxic cremaster venules

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Orth, Teresa A. Allen, John G. Wood, and Norberto C. Gonzalez. Plasma from conscious hypoxic rats stimulates leukocyte-endothelial interactions in normoxic cremaster venules. J Appl Physiol 99: 290–297, 2005. First published March 3, 2005; doi:10.1152/japplphysiol.00932.2004.—Systemic hypoxia results in rapid increases in leukocyte-endothelial adherence (LEA) and emigration, vascular permeability, and mast cell activation in several microcirculations. Observations in cremaster muscle suggest that this response is initiated by a mediator released from a distant site (Dix R, Orth T, Allen JA, Wood JG, and Gonzalez NC. J Appl Physiol 95: 2495–2502, 2003). The present experiments in rat cremaster muscle tested the hypothesis that, if a circulating mediator triggers hypoxia-induced inflammation, then plasma from hypoxic rats should elicit LEA in normoxic cremaster venules. Plasma from conscious donor rats breathing 10% O2-90% N2 for 5 min was applied topically to the cremaster of normoxic anesthetized rats. In this and all other groups described below, the donor plasma had attained normoxic PO2 when applied to the cremaster. LEA (leukocytes/100-μm venule) increased from 2.7 ± 0.8 to 12.3 ± 2.4, and venular shear rate and arteriolar diameter decreased to 79 ± 9% (P < 0.05, n = 6) and 77 ± 5% of control (P < 0.05, n = 5), respectively, 10 min after application of plasma from hypoxic donors. The decrease in venular shear rate was exclusively due to a reduction of venular blood flow, secondary to the upstream arteriolar vasoconstriction. Plasma from normoxic donors had no effects. Plasma from blood equilibrated in vitro for 5 min with 5% CO2-95% N2 did not alter LEA or shear rate of normoxic cremasters, suggesting that the putative mediator does not originate in blood cells. The effects of plasma from hypoxic rats persisted when the donors were pretreated with the mast cell stabilizer cromolyn, which prevents hypoxia-induced LEA. This suggests that the effects of hypoxic plasma are not due to inflammatory mediators released by adherent leukocytes in the donor rat. There was a positive correlation between LEA and mast cell degranulation observed histologically. These results support the idea that systemic hypoxia produces the release of a substance transported by the circulation that initiates the microvascular inflammation.

leukocyte-endothelial adherence; microvascular inflammation; environmental hypoxia; mast cell activation

SYSTEMIC HYPOXIA, INDUCED BY A DECREASE IN INSPIRED PO2, RESULTS IN A RAPID INFLAMMATORY RESPONSE CHARACTERIZED BY INCREASES IN REACTIVE O2 SPECIES (20), LEUKOCYTE-ENDOTHELIAL ADHESIVE INTERACTIONS (22), LEUKOCYTE EMIGRATION, AND VASCULAR PERMEABILITY (21). THESE PHENOMENA HAVE BEEN OBSERVED IN POSTCAPILLARY VENULES OF THE MESENTERY (20–22), SKELETAL MUSCLE (7, 10, 15), AND BRAIN (13) OF RATS. THE INFLAMMATION INDUCED BY HYPOXIA BECOMES EVIDENT WITHIN 5–10 MIN OF THE REDUCTION IN INSPIRED PO2. MAST CELL ACTIVATION IS AN EARLY FEATURE OF THIS RESPONSE (7, 19), AND LIPID INFLAMMATORY MEDIATORS, WHICH ARE KNOWN TO BE RELEASED BY ACTIVATED MAST CELLS, SUCH AS PLATELET-ACTIVATING FACTOR AND LEUKOTRIENE B4, PLAY A ROLE IN THE HYPOXIA-INDUCED INFLAMMATION (4, 17).

The rapid and widespread nature of the response to hypoxia suggests that it may be triggered by the reduction in microvascular PO2, which also shows a rapid time course after the decrease in inspired PO2 (7, 15). However, in studies in which we were able to alter cremaster PO2 independently of systemic arterial PO2, we observed a dissociation between hypoxia-induced inflammation and cremaster microvascular PO2 (7, 15). When the reduction in PO2 was restricted to the cremaster microcirculation and systemic arterial PO2 was maintained within normoxic values, neither leukocyte endothelial adherence (15) nor mast cell degranulation (7) were observed. Conversely, systemic hypoxia, induced by 10% O2 breathing, resulted in leukocyte-endothelial adherence and mast cell degranulation, even though cremaster microvascular PO2 was maintained above normal levels (7, 15).

When only the cremaster is made hypoxic, exposure of leukocytes to low PO2 is limited to their transit through the hypoxic cremaster. This short time may not be enough to fully activate the leukocytes and initiate their adherence to cremaster venules. If this is the case, this would mean that exposure to hypoxia of endothelial cells, mast cells, and other microvascular elements is insufficient to induce leukocyte-endothelial interactions, as long as leukocytes are not also activated by hypoxia. An alternative explanation for these results is that the leukocyte-endothelial adherence observed during systemic hypoxia is initiated by a mediator released from a distant site that triggers the microvascular inflammation independent of the local PO2. The time course and wide distribution of the inflammatory response suggest that the putative mediator is a preformed or rapidly synthesized substance released from a remote site that reaches the peripheral tissues rapidly, probably via the circulation.

In these experiments, we further explore the possibility that systemic hypoxia produces the release of a circulating mediator from a remote site that triggers the microvascular inflammation, independent of the local microvascular PO2. We tested the hypothesis that, if a circulating mediator is involved in the initiation of hypoxia-induced inflammation, then plasma obtained from hypoxic rats should initiate cremaster microvascular inflammation under normoxic conditions.

METHODS

All procedures were approved by the Animal Care and Use Committee of the University of Kansas Medical Center, an institution

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accredited by the American Association for the Accreditation of Laboratory Animal Care.

Surgical Preparation

Male Sprague-Dawley rats, 175–225 g, were anesthetized with urethane (1.5 g/kg im) after an overnight fast with free access to water. Body temperature was maintained at 36–38°C by using a homeothermic blanket system connected to a rectal probe. PE-50 catheters were inserted in the jugular vein and in the carotid artery. Lactated Ringer solution was infused continuously via the jugular vein at a rate of 2 ml/h. Arterial blood pressure was continuously measured with a digital blood pressure monitor connected to the carotid artery catheter. A tracheotomy was performed, and a PE-240 catheter was connected to a rodent non-rebreathing two-way valve. The animals breathed spontaneously throughout the experiment.

Intravital Microscopy

The right cremaster muscle was prepared for intravital microscopy as described previously (1). The rat was placed on the platform of a Nikon E600 FN microscope, and the cremaster was spread over a hollow Lucite cylinder, the top of which was sealed with a glass slide. Water was circulated through the cylinder to maintain the muscle temperature at 37°C. Temperature was monitored continuously via a thermistor placed underneath the muscle. The cremaster was covered with Saran Wrap throughout the experiment.

Images of the cremaster microcirculation (×40 objective) were recorded on a videocassette recorder with a time-date generator. Straight, unbranched venules of 100 μm in length, 20–40 μm in diameter, and no adjacent lymphatics were selected for microscopic observation. Venular diameter was measured using a video caliper. An optical Doppler velocimeter was used to measure venular centerline red blood cell velocity. Average red blood cell velocity was calculated as centerline velocity/1.6 (6). Wall shear rate (s⁻¹), which represents the force generated at the vessel wall by the movement of blood, was calculated as 8 × [average red blood cell velocity (mm/s)/venular diameter (mm)] (11). Adherent leukocytes were defined as those leukocytes that remained stationary for 30 s or longer. Leukocyte adherence was expressed as the number of adherent leukocytes per 100 μm of vessel length.

In some experiments, arterioles were visualized to determine the effect of the various experimental interventions on arteriolar diameter and to establish the possible role of arteriolar vasoconstriction on venular shear rate changes. Second- or third-order arterioles (15- to 30-μm diameter) and nonadjacent veins (20– to 40-μm diameter) were observed alternately every 3 min. Observation started 10 min before/after measurement. No adjacent lymphatics were selected for microscopic observation. Venular diameter was measured using a video caliper.

Histological Visualization of Mast Cells

In three experiments of each of the groups described below, the cremaster was excised and fixed in Zamboni’s solution for 24–48 h. The muscle was transferred to a 30% sucrose solution, and five circular muscle samples of 3-mm diameter were obtained. Fifteen slices of ~20-μm thickness were obtained from each sample. The tissue slices were mounted on glass slides and stained with 1% toluidine blue solution. Mast cells were counted in five slides from each of the samples. An average of 177 ± 15 (mean ± SE) mast cells was counted in each muscle.

Exposure of Conscious Donor Rats to Hypoxia

Male Sprague-Dawley rats, 250–300 g, were anesthetized with pentobarbital sodium (35 mg/kg ip). A PE-50 catheter was placed in the carotid artery, tunneled subcutaneously, exteriorized at the back of the neck, and flame-sealed. Two to three hours after complete recovery from anesthesia, the rats were placed into a Lucite chamber where 10% O₂-90% N₂ was circulated. At 5 min of exposure to hypoxia, a 3-ml blood sample was obtained, and the blood centrifuged for 10 min. Plasma was separated and applied topically onto the cremaster of a rat prepared for intravital microscopy as described above. Approximately 12–15 min elapsed between blood withdrawal from the donor rat and the first topical application of plasma onto the cremaster. During this time, no attempts were made to avoid equilibration of plasma gas tensions with ambient air and the resulting increase in donor plasma PO₂.

Experimental Protocol

Effects of application of donor plasma to normoxic cremaster muscles. After a 30-min stabilization period postsurgery, the cremaster microcirculation was observed for 10 min. The Saran Wrap covering the muscle was then removed, and ~1 ml of donor rat plasma was distributed evenly over the entire surface of the cremaster. The muscle was covered again with Saran Wrap, and the microcirculation observed for 10 min. An additional 1 ml of plasma was applied, and the microcirculation observed for another 10 min.

The following groups of experiments were performed on animals breathing room air throughout the experiment.

GROUP A: EFFECT OF PLASMA OBTAINED FROM HYPOXIC DONOR RATS (N = 6). After a 10-min control period, plasma from a hypoxic donor rat (5-min hypoxia) was applied twice, at 10-min intervals as described above, onto the cremaster of a normoxic rat. Five additional experiments using this protocol were carried out to measure arteriolar diameter, as described above.

GROUP B: EFFECT OF PLASMA OBTAINED FROM NORMOXIC DONOR RATS (N = 5). These experiments served as control for group A. The same protocol was followed, except that the donor rat was not exposed to hypoxia.

GROUP C: EFFECT OF PLASMA OBTAINED FROM BLOOD EQUILIBRATED IN VITRO WITH HYPOXIC GAS MIXTURES (N = 6). Blood obtained from normoxic donor rats was placed in a tonometer and equilibrated with 5% CO₂-95% N₂. After 5 min of equilibration, the blood was removed from the tonometer, centrifuged for 10 min, and the plasma applied onto the cremaster of a normoxic rat.

GROUP D: EFFECT OF PLASMA OBTAINED FROM HYPOXIC RATS PRETREATED WITH CROMOLYN (N = 7). In this protocol, the donor rats were pretreated with the mast cell stabilizer cromolyn (35 mg/kg iv). Cromolyn was administered ~3 h after the donor rats had recovered from anesthesia. Thirty minutes after cromolyn injection, the conscious donor rats were placed in the hypoxic chamber, and a blood sample withdrawn 5 min later. Plasma was separated and applied onto the cremaster of a normoxic rat as described above. Five additional

Table 1. Arterial blood pressure values of conscious plasma donor rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
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<tbody>
<tr>
<td>I</td>
<td>109±2</td>
<td>103±5</td>
<td>103±5</td>
<td>112±5</td>
</tr>
<tr>
<td>II</td>
<td>99±5*</td>
<td>98±5*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>93±4*</td>
<td>97±5</td>
<td>98±3</td>
<td>93±7*</td>
</tr>
</tbody>
</table>

Values are means ± SE in mmHg. Blood pressure values of conscious plasma donor rats. I, control normoxic period; II, values after 5 min of hypoxia (groups A and D only); III, values immediately after withdrawal of 3 ml of blood. *P < 0.05 vs. I.
Table 2. Po2 values of donor plasma

<table>
<thead>
<tr>
<th>Group</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>37±1</td>
<td>93±3</td>
<td>8±2</td>
<td>36±3</td>
</tr>
<tr>
<td>II</td>
<td>116±8*</td>
<td>132±7*</td>
<td>95±7*</td>
<td>109±12*</td>
</tr>
</tbody>
</table>

Values are means ± SE in Torr. I, Po2 values at the time of blood withdrawal from the animal (groups A, B, and D) or from the equilibrating tonometer (group C); II, plasma Po2 values after 10–15 min of centrifugation and separation of blood cells, immediately before application on the normoxic cremasters. *P < 0.05 vs. I.

RESULTS

Experiments using this protocol were directed to measure arteriolar diameter.

Effects of systemic hypoxia on cremaster microcirculation. Two additional series of experiments, which did not involve application of donor plasma to normoxic cremasters, were carried out.

GROUP E: EFFECT OF INTRAVENOUS CROMOLYN ON SYSTEMIC HYPOXIA-INDUCED LEUKOCYTE-ENDOTHELIAL ADHERENCE (N = 6). These experiments served as control for group D to determine the effectiveness of intravenous cromolyn administered 30 min before hypoxia in blocking hypoxia-induced leukocyte adherence. In this case, the animals were prepared for intravitral microscopy examination of the cremaster as described above. Cromolyn (35 mg/kg) was injected intravenously, and 30 min later systemic hypoxia was induced by having the anesthetized rat breathe 10% O2 for 10 min, after which the animal was returned to normoxia for an additional 10 min. In five additional experiments using the same protocol, arteriolar diameter was measured.

GROUP F: EFFECT OF SYSTEMIC HYPOXIA ON LEUKOCYTE-ENDOTHELIAL ADHESION IN UNTREATED RATS (N = 5). In this group, the animals were prepared for observation of the cremaster microcirculation as described. After a stabilization period of 30 min, systemic hypoxia was induced for 10 min by 10% O2 breathing, after which the animal was returned to normoxia for an additional 10-min period. Arteriolar diameter was measured in five additional experiments using this protocol.

Data Analysis and Statistics

The effect of a given treatment on leukocyte endothelial adherence, venular shear rate, or arteriolar diameter was assessed using a t-test for paired samples. Each experimental value was subtracted from the control value, with the latter being calculated as the average of the values obtained every minute during the 10-min control period. The experimental-control differences of all animals in the same group were averaged, and a t-test was applied to determine whether the average difference was significantly different from zero. Venular shear rate and arteriolar diameter were expressed as experimental/control value. This was done to minimize the effect of variations in the control values of individual animals. The control value was calculated by averaging the values obtained in each measurement of the 10-min control period. The effect of interventions was assessed as described for leukocyte adherence. Intergroup comparisons were made using a one-way analysis of variance followed by the Bonferroni test for multiple comparisons. A P value of <0.05 was considered to indicate a significant difference.
3 ml of blood was not accompanied by further significant decreases in blood pressure in either of these groups or in groups B and C, which remained under normoxic conditions (Table 1).

Breathing 10% O₂ for 5 min lowered arterial plasma PO₂ of conscious donor rats to <40 Torr (groups A and D; Table 2). In vitro equilibration of blood with 95% N₂-5% CO₂ reduced PO₂ even further (group C; Table 2). Nevertheless, by the time it was applied onto the normoxic cremaster, plasma PO₂ had increased substantially in all groups as a consequence of exposure to room air during blood centrifugation and blood cell separation.

Topical application of plasma obtained from conscious hypoxic rats onto the normoxic cremaster (group A) was followed by a rapid and sustained increase in leukocyte-endothelial adherence (Fig. 1, top), which became statistically significant 3 min after application of plasma. This was accompanied by a decrease in venular shear rate (Fig. 1, middle), which also became statistically significant 3 min after plasma application. A second application of plasma did not appear to have additional effects (Fig. 1). The reduction in venular shear rate was entirely due to a decrease in blood velocity, since venular diameter remained unchanged. The decrease in venular blood velocity with an unchanged vessel caliber reflects reduced blood flow. This, in turn, was the result of upstream arteriolar vasoconstriction, as indicated by the decrease in arteriolar diameter that followed application of plasma from hypoxic rats (Fig. 1, bottom).

Plasma obtained from normoxic rats (group B) did not have any noticeable effect on either leukocyte adherence (Fig. 1, top) or shear rate (Fig. 1, middle) when applied to the cremaster of normoxic rats.

Topical application of plasma obtained from blood made hypoxic in vitro (group C) did not produce changes in either leukocyte-endothelial adherence (Fig. 1, top) or in shear rate (Fig. 1, middle) of normoxic cremaster venules. In vitro equilibration of normoxic donor blood with 5% CO₂-95% N₂ for 5 min lowered blood PO₂ to 8 ± 2 Torr (Table 2). This value is not different from the cremaster microvascular PO₂ value previously obtained in anesthetized rats breathing 10% O₂ (7 ± 2 Torr) (15).

Plasma obtained from donors treated with cromolyn before the hypoxic exposure (group D; Fig. 1) resulted in changes in leukocyte-endothelial adherence, venular shear rate, and arteriolar diameter that were similar in extent and time course to those produced by plasma from hypoxic, untreated rats (compare open circles with shaded circles in Fig. 1).

Application of plasma to the cremasters of the rats of groups A–D was not accompanied by changes in systemic blood pressure of these animals (Table 3). This suggests that the hemodynamic changes observed in the cremaster microcirculation of the rats of groups A and D are not the reflection of systemic cardiovascular changes induced by plasma application.

Figure 2, top, shows that systemic hypoxia (group F) produced the expected increase in leukocyte-endothelial adherence in untreated rats. This was blocked by intravenous administration of cromolyn 30 min before breathing 10% O₂ (group E; Fig. 2, top). Both groups showed a decrease in venular shear rate (Fig. 2, middle). With the exception of the values at 1 min of hypoxia, there were no significant differences in venular shear rate between groups E and F. In both cases, shear rate decreased via a reduction in venular blood velocity, without changes in diameter, indicating a reduction in venular blood flow. The blood flow decrease in group F may have been due in part to the transitory arteriolar vasoconstriction observed (Fig. 2, bottom). Cromolyn blocked the vasoconstriction (group E, Fig. 2, bottom), and this may account for the small initial difference in venular shear rate between groups (Fig. 2, middle). It is clear, however, that the main reason for the reduction in venular shear rate in both groups was the arterial hypotension associated with systemic hypoxia (Table 3).

Application of plasma from hypoxic rats was accompanied by a large number of degranulated mast cells (Fig. 3A). Mast cell degranulation was significantly lower in groups B and C. Mast cell activation in these cases may have resulted from non-specific stimuli associated with the dissection of the muscle. Group D, in turn, showed mast cell degranulation frequency similar to that of group A (Fig. 3). Figure 4 shows that, as expected, systemic hypoxia resulted in a high mast cell degranulation count (group F), which was significantly attenuated by cromolyn administration 30 min before hypoxia (group E).

Table 3. Arterial blood pressure values of anesthetized rats used for intravital microscopy of the cremaster microcirculation

<table>
<thead>
<tr>
<th>Condition</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
<th>Condition</th>
<th>Group E</th>
<th>Group F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>105 ± 6</td>
<td>108 ± 4</td>
<td>110 ± 4</td>
<td>112 ± 3</td>
<td>Normoxia</td>
<td>116 ± 3</td>
<td>113 ± 8</td>
</tr>
<tr>
<td>Plasma 1</td>
<td>104 ± 7</td>
<td>103 ± 4</td>
<td>105 ± 5</td>
<td>107 ± 4</td>
<td>Hypoxia</td>
<td>64 ± 7*</td>
<td>65 ± 3*</td>
</tr>
<tr>
<td>Plasma 2</td>
<td>103 ± 8</td>
<td>104 ± 4</td>
<td>103 ± 3</td>
<td>109 ± 3</td>
<td>Recovery</td>
<td>117 ± 5</td>
<td>115 ± 5</td>
</tr>
</tbody>
</table>

Values are means ± SE in mmHg. Plasma 1, first topical application of 1 ml of donor plasma; Plasma 2, second topical application of 1 ml of donor plasma. Rats from groups A–D breathed room air throughout the experiment. Plasma from donor rats was applied twice (Plasma 1 and Plasma 2) with a 10–min interval. Groups E and F breathed 10% O₂ for 10 min (Hypoxia), followed by 10 min of room air breathing (Recovery). No significant changes in systemic blood pressure occurred after application of plasma to the normoxic cremaster (groups AD). In contrast, systemic hypoxia was accompanied by a substantial hypotension (group F), which was not affected by pretreatment with intravenous cromolyn (group E). *P < 0.05 vs. Normoxia.

DISCUSSION

The major observation of this study is that topical application of plasma obtained from conscious hypoxic rats onto normoxic cremasters has the same effect on leukocyte-endothelial adherence as that produced by systemic hypoxia. The responses to plasma were not due to a reduction in the cremaster PO₂, since, by the time it was applied, plasma PO₂ (Table 2) had increased well above the microvascular PO₂ values observed in the normoxic cremaster (7, 15). The increased leukocyte-endothelial adhesive interactions were not the result of a non-specific effect of plasma, since they were not observed
when plasma obtained from normoxic rats was applied to the cremaster (group B; Fig. 1).

Plasma obtained from blood equilibrated in vitro with hypoxic gas mixtures did not elicit an inflammatory response when applied to the normoxic cremaster (group C; Fig. 1). This lack of effect cannot be attributed to an insufficient hypoxic stimulus: in vitro blood PO2 was reduced for the same time as in the conscious hypoxic donors and reached a value that was similar to that observed in the cremaster microcirculation of intact rats breathing 10% O2 (7, 15). Thus the results of group C suggest that the effects of plasma obtained from hypoxic rats are not due to substances generated in blood cells during the hypoxic exposure.

Blood was withdrawn from the donor rats 5 min after the onset of hypoxia. Because the inflammatory response develops rapidly, a circulating mediator should be present in plasma at that time. On the other hand, at 5 min of hypoxia, a substantial increase in leukocyte-endothelial adherence has already occurred (group F; Fig. 2). Given that hypoxia-induced inflammation appears to be ubiquitous (10, 13, 20–22), inflammatory mediators released by adherent leukocytes in the donor rats may reach plasma concentrations sufficient to elicit leukocyte-endothelial adherence when applied onto the normoxic cremaster. In other words, the effect of plasma obtained from hypoxic donor rats may be the result of the inflammation elicited by hypoxia rather than of the putative mediator. This possibility was tested in the experiments of group D, in which the hypoxic donor rats were pretreated with cromolyn, a mast cell stabilizer that blocks the mast cell degranulation and increased leukocyte-endothelial adherence of hypoxia (7, 19). Leukocyte-endothelial adherence, venular shear rate, and arteriolar diameter changed in a manner similar to that seen in the experiments of group A, where plasma from untreated hypoxic donors was applied on the normoxic cremaster (compare open circles and shaded circles in Fig. 1).

The experiments of group E (Fig. 2) show that cromolyn, administered in the same amount and time frame as in the experiments of group D, effectively prevented the hypoxia-induced increase in leukocyte-endothelial adherence. Accordingly, it is reasonable to assume that, when cromolyn was administered in the same manner to the conscious donor rats of group D, hypoxia in these animals did not result in leukocyte-endothelial adherence. The donor plasma in the experiments of group D may have contained cromolyn; however, 30 min after intravenous administration, the plasma concentration of cromolyn, which distributes throughout total body water, is likely to be low. More importantly, cromolyn present in donor plasma would tend to attenuate the inflammatory response in the normoxic cremaster, i.e., the opposite of the effect observed in these experiments. In conclusion, the combined results of groups D–F suggest that the effects of plasma from hypoxic rats are not due to inflammatory mediators released by adherent leukocytes into the donor plasma.

Fig. 2. Effect of systemic hypoxia (breathing 10% O2–90% N2) for 10 min on leukocyte-endothelial adherence (top), venular shear rate (middle), and arteriolar diameter (bottom) of the cremaster of anesthetized rats. ▲, Rats pretreated with cromolyn intravenously 30 min before systemic hypoxia (group E; n = 6 for top and middle; n = 5 for bottom); ◦, untreated systemic hypoxia (group F; n = 5). Data are means ± SE.
Venular shear rate also decreased in the anesthetized rats breathing 10% O₂ (groups E and F; Fig. 2). In this case, the major factor responsible for the reduced shear rate is likely to be the marked arterial hypotension occurring in both groups (groups E and F; Table 3), with arteriolar vasoconstriction making a minor contribution in group F (Fig. 2, bottom). The data of group E agree with observations in the mesentery in which several interventions, including administration of cromolyn (19), antioxidants (20), and nitric oxide donors (18, 22), block hypoxia-induced leukocyte endothelial adherence but do not prevent the decrease in shear rate.

Cremaster samples obtained from animals that responded with increased leukocyte-endothelial adherence to application of plasma (groups A and D) showed a large percentage of degranulated mast cells (Fig. 3). On the other hand, when leukocyte-endothelial adherence did not develop in response to plasma application (groups B and C), the incidence of mast cell degranulation was substantially lower (Fig. 3). Mast cell acti-

Fig. 3. Microphotographs of representative cremaster muscles showing examples of mast cell degranulation in groups A (A) and D (D). Groups B (B) and C (C) showed a significantly smaller incidence of degranulation. Data under the photographs are means ± SE of 3 cremaster muscles each. Average number of mast cells counted per muscle: 177 ± 15.

Fig. 4. Representative microphotographs of cremaster muscles of rats exposed to systemic hypoxia. A large number of degranulated mast cells were observed in the rats exposed to systemic hypoxia (group F; F). Cromolyn significantly attenuated hypoxia-induced mast cell degranulation (group E; E).
viation is a key early event in systemic hypoxia-induced inflammation (7, 19). Cromolyn blocks systemic hypoxia-induced mast cell degranulation (Fig. 4) and the accompanying increases in reactive O₂ species-dependent fluorescence activity and leukocyte-endothelial interactions in cremaster and mesenteric microcirculations (Fig. 2; Refs. 7, 19). Cromolyn also attenuates the increases in leukocyte emigration and vascular permeability of the mesenteric microcirculation observed in conscious hypoxic rats (19). Mast cell degranulation is observed during systemic hypoxia in cremaster muscles maintained at normal microvascular PO₂ (7). Conversely, selective cremaster hypoxia, in the presence of normal systemic arterial PO₂, does not induce mast cell degranulation (7) or leukocyte-endothelial adherence (7, 15). The dissociation between local microvascular PO₂ and mast cell degranulation, together with the effects of plasma from hypoxic rats applied to normoxic cremasters (Fig. 1), indicate that the mast cell degranulation observed during systemic hypoxia is not the result of reduction in local tissue PO₂.

The combined data presented in this study support the notion that systemic hypoxia-induced inflammation is independent of the local PO₂ and is initiated, at least in part, by a mediator released into the circulation from a remote site. The effects of plasma from hypoxic rats on mast cell degranulation suggest that the putative mediator, directly or indirectly, leads to mast cell activation, which, in turn, is necessary for leukocyte-endothelial adherence to occur.

The nature of this putative mediator cannot be established from the present data. The rapid development of the response to hypoxia suggests that it is a preformed or rapidly synthesized substance. Its diffusion characteristics allow it to increase leukocyte-endothelial adhesive interactions in superficial cremaster venules with a time course similar to that of systemic hypoxia. It also must be a relatively stable substance, since its effects are still present ~15 min after blood withdrawal and are not eliminated by the increase in plasma PO₂ that follows blood withdrawal and separation of blood cells.

Application of plasma from hypoxic rats was accompanied by local arteriolar vasoconstriction. Whether the vasoconstriction is directly or indirectly initiated by the mediator, or is the result of a substance present in plasma from hypoxic rats that is unrelated to the initiation of inflammation cannot be ascertained from the present data. Answering this question should provide important clues concerning the nature of the mediator. The observation that both systemic hypoxia (Fig. 2) and application of plasma from hypoxic rats produced arteriolar vasoconstriction (Fig. 1) leads to the question as to whether the same agent is responsible for the vasoconstriction in both cases. The difference in time courses would argue against a common mechanism; however, systemic hypoxia is a complex condition that includes major hemodynamic changes and local and systemic neurohumoral responses not present in the normoxic animals receiving donor plasma. These factors could influence the patterns of responses to the same vasoconstrictor. The attenuation by cromolyn of the vasoconstriction induced by systemic hypoxia (group E; Fig. 2, bottom) points to an involvement of mast cells in the arteriolar response. The fact that mast cell activation also occurred after application of plasma from hypoxic rats suggests the possibility that similar mechanisms may underlie the arteriolar vasoconstriction to both systemic hypoxia and plasma application. Mast cell-dependent vasoconstriction has been observed in the skeletal muscle microcirculation. Adenosine and its metabolite inosine stimulate adenosine A₃ receptors and produce mast cell degranulation and arteriolar vasoconstriction (8) mediated via histamine and thromboxane release (5, 9). Vasoconstriction secondary to mast cell stimulation of A₃ receptors was observed after skeletal muscle ischemia-reperfusion (12). Mast cells could be involved in the generation of another vasoconstrictor and proinflammatory agent, angiotensin II (14). Angiotensin I can be converted to angiotensin II at the tissue level through angiotensin-converting enzyme located in the endothelium or the interstitium of several tissues or by the action of mast cell chymase (2, 3). The possible role of these or other agents in hypoxia-induced inflammation cannot be determined from the present data and should be established by further research.

In summary, the results of the present study add more evidence in support of the idea that systemic hypoxia, induced in intact animals by reducing inspired PO₂, acts via the release of a circulating mediator that initiates the inflammatory response. The evidence presented here suggests a role of the mediator in the activation of mast cells, increased leukocyte-endothelial adhesive interactions, and arteriolar vasoconstriction. The relative contribution of the events triggered by the putative mediator to the overall response of intact animals to environmental hypoxia cannot be adequately assessed from these studies, since additional mechanisms may come into play at different times of hypoxic exposure. Nevertheless, the present data suggest that a circulating mediator is a key factor in the early events elicited by systemic hypoxia in the microcirculation of skeletal muscle.

GRANTS

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REFERENCES


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