Oxygen and vascular smooth muscle contraction

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1968—Oxygen tension is an important determinant of the contractile tension developed by isolated helical strips of rabbit aorta. A decrease in PO₂ below 100 mm Hg causes the contractile response to epinephrine (1-3 μg/liter) to diminish linearly to near zero levels at <1 mm Hg. If oxygen tension is rapidly decreased from 100 mm Hg to a lower steady-state value during a sustained contraction produced by epinephrine, the time constant of decreased contractile tension is less than 4 min. If the smooth muscle is stimulated with epinephrine near the end of a 15-min hypoxic period, and contractile tension is allowed to reach a steady state, an increase in PO₂ to 100 mm Hg causes recovery of contractile tension, with a time constant of less than 2.5 min providing the PO₂ during hypoxia is >5 mm Hg. The time constant is approximately 3.5 min after 60 min of hypoxia (>5 mm Hg). At 5 mm Hg or less, however, the time constant is 5.5 min after 15 min hypoxia, and is greater than 15 min after 60 min hypoxia. The immediate dependence of contractile tension on PO₂ is explained on the assumption that the smooth muscle is stimulated with epinephrine near the end of a 15-min hypoxic period, and contractile tension is allowed to reach a steady state, an increase in PO₂ to 100 mm Hg causes recovery of contractile tension, with a time constant of less than 2.5 min providing the PO₂ during hypoxia is >5 mm Hg. The time constant is approximately 3.5 min after 60 min of hypoxia (>5 mm Hg). At 5 mm Hg or less, however, the time constant is 5.5 min after 15 min hypoxia, and is greater than 15 min after 60 min hypoxia. The immediate dependence of contractile tension on PO₂ is explained on the assumption that oxygen plays a metabolic role within the mitochondria of the smooth muscle cells, as the final electron acceptor in the respiratory chain. Such a rate-limiting metabolic device, which is rapidly reversible at PO₂ between 5 and 100 mm Hg, could serve as a control for the production of high-energy intermediates necessary for vascular smooth muscle contraction and provide a means whereby PO₂ could account for local autoregulation of blood flow in situ.


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The rate of change in mechanical tension was computed by measuring the time in minutes from the beginning of the recorded PO₂ change to the point where 63% of the maximal mechanical tension change had
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RESULTS

When a strip of rabbit aorta is placed in a bath of warm PSS and stretched to a rest tension of approximately 600 mg, it undergoes a gradual stress relaxation during which it loses about 100 mg tension over a 30- to 60-min period. Neither this rate of relaxation nor the subsequent equilibrated resting tension is influenced by the P02 over a range of 1 to 675 mm Hg.

Effect of P02 on steady-state tension development in response to epinephrine. Figure 1 is a plot of data accumulated from aortas from 28 rabbits and shows a direct relationship between P02 and the mechanical tension developed by a muscle strip in response to epinephrine (1-3 \(\mu\)g/liter). The tension values represent the tension reached by the tissue after exposure to the designated P02 for 15-30 min, when a steady state had been reached, and are expressed in terms of percent of a control tension developed by the tissue in response to the same concentration of epinephrine at P02 = 100 mm Hg. This P02 was chosen for the control responses since it approximates the highest P02 present under physiological conditions. An elevation of the P02 to values as high as 675 mm Hg produced only about 10 to 15% increase over that attained at 100 mm Hg (Fig. 2).

Effect of P02 on rate of change of active tension. Figure 2 illustrates the change in mechanical tension as a result of lowering the P02. Aortic strips were stimulated with epinephrine (1-3 \(\mu\)g/liter) in the presence of P02 = 100 mm Hg. After a steady state of tension had developed, the P02 of the PSS was reduced rapidly by changing the composition of the aerating gas mixture. In these and subsequent experiments the change in P02 occurred exponentially, requiring approximately 1 min to reach a new steady-state value. The loss of active tension caused by this reduction in P02 was monitored until a second steady state of muscle tension was reached. A time constant was calculated from this new steady state. Data from eight experiments, using paired strips, are presented in Table 1 and indicate that the time constant for the reduction in mechanical tension was less than 4 min, regardless of the new P02 established.

The change in mechanical tension resulting from increasing P02 from previously established hypoxic levels was seen in two types of procedures carried out in 18 experiments using paired strips. First, the effects of several levels of hypoxia on recovery of contractility are illustrated in Fig. 3. The duration of the hypoxia was held constant at 15 min in each case. Prior to the stimulations

<table>
<thead>
<tr>
<th>Level of Hypoxia, mm Hg</th>
<th>Time Constants for Loss of Tension, min</th>
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</thead>
<tbody>
<tr>
<td>&lt; 1</td>
<td>3.8 ± 0.2 (5)</td>
</tr>
<tr>
<td>20-35</td>
<td>3.1 ± 0.1 (5)</td>
</tr>
<tr>
<td>50-75</td>
<td>3.0 ± 0.1 (5)</td>
</tr>
</tbody>
</table>

Values are expressed as means ± se. Numbers in parentheses indicate number of experiments.
depicted in this figure the PO₂ had been reduced from 100 mm Hg to a new stable value; epinephrine (1-3 µg/liter) was added to the bath 1.5 min before the end of the 15-min period of hypoxia and the response of the muscle to epinephrine quickly reached a steady state. Oxygen tension was then increased to 100 mm Hg. The time constants for the recovery of mechanical tension associated with the increased PO₂ (Table 2) were 2.3 (±0.1) min or less after hypoxic levels >5 mm Hg PO₂. After a hypoxic period of 5 mm Hg or less, however, recovery time was considerably longer (3.5 (±0.2) min).

Second, the effects of the duration of hypoxia on recovery of contractility are illustrated in Fig. 4 and the data are summarized in Table 2. It is apparent that as long as PO₂ is maintained above 5 mm Hg, the time constant of recovery is less than 4 min, even after hypoxic periods as long as 60 min. After 30-60 min of 5 mm Hg PO₂ or less the time constant of recovery was markedly prolonged. However, recovery from 60 min of this severe hypoxia was usually complete within 30 min and did not affect subsequent testing.

A game of "auto-regulation" was played (Fig. 5), in which oxygen tension was changed from 100 mm Hg stepwise down to <1 mm Hg and returned stepwise to 100 mm Hg (bottom part of Fig. 5). The associated changes in mechanical tension are recorded in the upper part of Fig. 5. This again demonstrates the dependency of tension development upon PO₂, as well as the complete reversibility of the contractility after the diminished response during severe hypoxia (<1 mm Hg). It becomes apparent from this procedure that the contractile tension in response to epinephrine can be controlled to some extent simply by regulating the PO₂ in the bath.

**DISCUSSION**

Our findings show that in the physiological range low PO₂ is associated with diminished contractility and that contractility returns to normal when control levels of PO₂ are re-established. Possible explanations to account for the dependence of tension development on PO₂ may involve either metabolic or nonmetabolic mechanisms. The nonmetabolic mechanisms are thought of as those in which high-energy phosphate production is not a limiting factor. In this category, one might consider mechanisms which associate reduced oxygen tension with diminished activity of membrane excitation, of excitation-contraction coupling, or of the contractile machinery itself. Since there is no indication at the present time that any of these systems is directly dependent upon oxygen tension except for its energy requirements, nonmetabolic mechanisms are not considered in this discussion.

It appears clear that storage of high-energy phosphate intermediates in vascular smooth muscle is small and that even a single phasic contraction may require that additional energy be made available to the contractile machine (9). In view of this immediate need for energy production, a mechanism involving some type of metabolic control by oxygen tension may best explain the findings of this investigation. The most obvious metabolic

**TABLE 2. Time constants for recovery of contractile response to epinephrine (1-3 µg/liter) following hypoxic periods of several levels and durations**

<table>
<thead>
<tr>
<th>Level of Hypoxia, mm Hg</th>
<th>Time Constants for Recovery of Tension, min</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>7 min</td>
</tr>
<tr>
<td>5 or less</td>
<td>3.2±0.3 (5)</td>
</tr>
<tr>
<td>7-15</td>
<td>2.1±0.1 (4)</td>
</tr>
<tr>
<td>18-55</td>
<td>2.2±0.1 (8)</td>
</tr>
</tbody>
</table>

Values are expressed as means ± se. Numbers in parentheses indicate number of experiments. Recovery was accomplished by raising PO₂ to 100 mm Hg.
role for oxygen in the over-all process of energy production is as the final electron acceptor in the respiratory chain involved in oxidative metabolism. When metabolic processes occur in an environment in which PO₂ is not rate limiting, oxidative phosphorylating substrates such as ADP or Pᵢ, within the mitochondria may serve regulatory roles for high-energy phosphate production (8). On the other hand, as oxygen tension is reduced, a point can be reached where the electrons carried by the respiratory chain are removed at a rate determined by the availability of oxygen. An important consequence of this latter condition is the limitation on metabolic formation of high-energy intermediates necessary for cellular processes, including muscle contraction. In this fashion energy production could become rate limiting for contraction in vascular smooth muscle at PO₂ values less than 100 mm Hg. Howard et al. (5) have found that O₂ consumption of resting vascular tissue is reduced by PO₂ below 12 mm Hg; PO₂ is thought to be rate limiting for oxidative metabolism at this level. These studies were carried out on resting vessels, it is likely that oxygen becomes limiting at much higher PO₂ values in vessels stimulated by epinephrine. This finding would not be inconsistent with the diminished contractility at oxygen tensions below 100 mm Hg observed in the present study.

This interpretation based on a metabolic requirement for oxygen implies that the anaerobic or Embden-Meyerhoff pathway is not able to replace the efficient energy production possible via oxidative means. This is not entirely expected since there are reports (7, 10) which suggest that much of the energy production in vascular smooth muscle may be by anaerobic means. One would have predicted from these reports that the absence of oxygen would not place such severe limitations on contractility. However, the current observations suggest that vascular smooth muscle contractility is largely dependent upon aerobic high energy phosphate production. Other evidence indicating the presence of an oxidative pathway has been supplied by the isolation of specific enzymes involved in Krebs cycle (6). In addition, work in our own laboratory has demonstrated that lactate and pyruvate can serve as substrates for energy production for muscle contraction, (2).

Limitations on high-energy phosphate production not only restrict the amount of ATP available for contraction but may influence other systems as well. Membrane excitation and excitation-contraction coupling are indirectly dependent upon the availability of high-energy intermediates. They, too, may be regulated by variations in energy production related to PO₂ changes, but the consequences of a limited energy supply for these processes is not as relevant to the observed results as is the proposed lack of energy for the contractile process itself. The "time constants" for loss and recovery of contractility on changing from one PO₂ value to another as described above, were all less than 4 min at PO₂ above 5 mm Hg. These short time constants are what would be expected if oxygen tension does determine the over-all rate of energy production and the ability to develop mechanical tension.

If the relationship between PO₂ and tension development which has been observed in our studies exists in situ, oxygen tension would be a rate-limiting factor for contraction of vascular smooth muscle throughout the physiological PO₂ range. The smooth muscle cells of the vascular wall are, no doubt, normally exposed to oxygen tensions below that of arterial blood, allowing for both increase and decrease of vascular tone dependent on local changes in PO₂. It is tempting, therefore, to conceive a built-in control system for vascular tone, in accordance with Guyton's hypothesis (1, 4). The events involved in such a regulatory system might be initiated by a fall in perfusion pressure which would cause a reduced blood flow, and presumably, a lowering of PO₂ in the vicinity of the arterial smooth muscle cells. This, in turn, would be followed by vascular relaxation and a return of local blood flow toward the original level. In this manner autoregulation of local blood flow could be accomplished by alterations in oxygen tensions alone. The observation that there is complete and rapid reversibility of contractility after return to control levels of PO₂ is in accord with the possibility that the mechanism may be of physiological importance. It may be that a metabolic mechanism involving oxygen tension as a key regulator is an important determinant in local autoregulation of blood flow.

REFERENCES